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Seasonal Abundance and Molecular Diagnosis of the Leafhopper *Amrasca biguttula* (Hemiptera: Cicadellidae) on Okra plant.

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Abstract:

Amrasca biguttula is a significant pest affecting okra crops. This study investigated the seasonal population dynamics of both nymphs and adults, alongside the molecular identification of the species. Results indicated that the highest densities of nymphs and adults reached 29.83 and 8.19 insects per leaf, respectively, recorded during the last week of October and the first week of. November. Conversely, the lowest densities were recorded in the first week of September, with 0.88 nymphs and 0.15 adults per leaf. For molecular diagnosis, A. biguttula specimens collected from okra plants across seven different locations in Basra Governorate were genetically characterized by sequencing the mitochondrial cytochrome oxidase I (COI) barcode region. PCR amplification and sequencing confirmed that all isolates belong to the same species, with accession numbers ranging from LC843363 to LC843369 in the GenBank database. These isolates exhibited 100% sequence identity with previously reported A. biguttula isolates from Pakistan and India.

Keywords*: leafhopper A. biguttula, density of insect. Molecular identification.*

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الوفرة الموسمية والتشخيص الجزيئي لنطاط الأوراق *Amrasca biguttula* (رتبة نصفية الأجنحة: فصيلة النطاطات الورقية) على نبات الباميا

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الخلاصة

تُعَدُّ *Amrasca biguttula* آفةً مهمةً تؤثر في محاصيل الباميا. هدفت هذه الدراسة إلى دراسة الديناميكية الموسمية لتعداد كلٍّ من الحوريات والحشرات الكاملة، إلى جانب التشخيص الجزيئي لهذا النوع. أظهرت النتائج أن أعلى كثافة للحوريات والحشرات الكاملة بلغت 29.83 و8.19 حشرة/ورقة على التوالي، وسُجِّلت خلال الأسبوع الأخير من شهر تشرين الأول والأسبوع الأول من شهر تشرين الثاني. في المقابل، سُجِّلت أدنى الكثافات في الأسبوع الأول من شهر أيلول، إذ بلغت 0.88 حورية و0.15 حشرة كاملة/ورقة.

وفيما يخص التشخيص الجزيئي، جرى التوصيف الوراثي لعينات *A. biguttula* التي جُمعت من نباتات الباميا في سبعة مواقع مختلفة ضمن محافظة البصرة، وذلك من خلال تسلسل منطقة الباركود لجين السيتوكروم أوكسيداز I (COI) في الميتوكوندريا. وأكد تضخيم تفاعل البلمرة المتسلسل (PCR) وتسلسل الحمض النووي أن جميع العزلات تعود إلى النوع نفسه، مع أرقام إيداع تراوحت بين LC843363 و LC843369 في قاعدة بيانات GenBank. كما أظهرت هذه العزلات تطابقًا تسلسليًا بنسبة 100% مع عزلات *A. biguttula* المُسجَّلة سابقًا من باكستان والهند.

الكلمات المفتاحية: *نمط الأوراق A. biguttula، كثافة الحشرة، التشخيص الجزيئي.*

1. Introduction

Okra (*Abelmoschus esculentus*) L. (Monenth) is a hairy herbaceous plant belonging to the Malvaceae family, native to Ethiopia. It was cultivated by the ancient Egyptians around the 12th century BC, and its cultivation spread throughout the Middle East and into medieval Africa (Swamy, 2023). Okra seed oil contains calcium and iron, and okra is a high-vitamin E food. It is an important food for promoting nutritional health because it contains antioxidants (Kanani *et al*., 2019). Okra is a plant vegetable rich in fiber and contains many nutrients that detect insect germs, which cause serious damage. The insect pests are: bud and fruit worm, whitefly, jassids, and red cotton bug (Adetuyi *et al*., 2012 and Dube *et al*., 2023). Nymphs and adults infect plants mainly from the surface of the leaves and cause toxic phytochemical burns known as hopper burn, which leads to complete desiccation and has become a limiting factor for the economic productivity of okra (Patel *et al*., 2024). When infested with the leafhopper, the leaves become mottled, brown, and curl downwards, which is a symptom of hopper burn. The leaf margins break and crumble into pieces when crushed. The leaves dry up and fall off, and stunted growth occurs (Devi *et al*., 2018). The leafhopper (*A. biguttila*) is one of the most harmful infestations of okra as it infects the anterior side of the leaves and causes upward curling along the leaf margins, ultimately reducing the yield (Bhandari *et al*., 2022). Okra is subject to a wide range of insect pests, including the leafhopper, which is one of the most important pests affecting the crop (Maqbool *et al*., 2024). The group comprises the largest group of species in the group, possessing a wide diversity of undiscovered knowledge. New knowledge can be better explored using molecular marker techniques. The goodness of DNA (mt DNA) and RNA amplified polymorphic DNA (RAPD) have contributed to understanding the basis of insect diversity (Behura, 2006). Morphological identification of organisms is labor-intensive, and DNA characterization has the potential to be an effective and accurate complement to morphological methods (Nogashi *et al*., 2011). Determining the biodiversity characteristics of the species living within ecosystems is of great scientific importance for understanding the mechanisms of operation of these systems, the need to study molecular mechanisms in living organisms has made the pcr technique an indispensable tool for understanding the functions of this biological system (Kadri, 2019). the

mitochondrial COI region is a standard barcode for identifying the great diversity of insect species, isolating high quality DNA is an essential step in the insect DNA barcoding (Suganthi, *et al.*, 2023).

2. Materials and Methods

2.1. Seasonal presence of the leafhopper

The study was conducted at the Agricultural Research Station of the College of Agriculture, University of Basra, on July 10, 2024. After preparing the land by plowing and loosening the soil, the field was divided into four rows, with a distance of 50 cm between each row and 25 cm between each plant. Drip irrigation was used. A local okra variety was planted, and crop maintenance, including fertilization and weed removal, was carried out. The seasonal presence of the leafhopper *A. biguttula* was studied weekly. Samples were taken, and the population density of leafhoppers nymph and adults was calculated from the first week of September (September 1, 2024) until the first week of December (December 1, 2024). Three plants were randomly selected for each replicate, and three leaves were taken from each of the different plant growth levels (lower, middle, and upper). The insect density was calculated directly in the field.

2.2 Molecular identification

Leafhopper insects were collected from a field of okra grown at the Agricultural Research Station of the University of Basra (fixed station), in addition to six areas in Basra governorate such as Al-mdaina, Al-hartha, Shatt Al- Arab, Al- deir, Abi khasseb and Al-Huwer morphologically identified at the Natural History Museum of the University of Basra by Professor Dr. Muslim Ashour Abdul Wahid. As for molecular diagnosis the insect bodies sectioned under a dissecting microscope, with only the thorax and head regains removed selected sections, were placed in Eppendorf tubes and stored in a freezer until experiments were conducted, DNA was extracted from 20 by insects, using an extraction kit provided by Geneaid (Korea), according to the manufacturer's instructions. , amplification products were detected using electrophoresis on a 1.5% agarose gel after electrophoresis the gel was examined using gel documentation device to determine band size ,estimation quantity and quality of mtDNA by using Nan Drop((Thermo-scientific) at wavelengths of 260-280 nm according to the method of (lima and Scarpaassa, 2009) . polymerase chain reaction (PCR). The COI gene region in the mitochondrial genome using the primers detailed in Table (1) (Macrogen, Korea), as shown in table (1). The reaction was performed using PCR thermocycler according to thermal cycling program shown in the corresponding table (2). The PCR products were sent to Macrogen in south korea for Sanger sequencing of the targeted gene segments of the COI regain.

Table 1. Primers used in molecular identification of leafhoppers *A. biguttula*

Fragment Code	Primer Sequence	Fragment Size	Annealing Temperature (Ta °C)	Source
HCO2198	GGTCAACAAATCATAAAGATATTGG	700 bp	48	Folmer et al., 1994
LCO1490	TAAACTTCAGGGTGACCAAAAAATCA			

Table 2 Thermal cycling program for PCR reactions of the COI gene region

NO	PHASE	Tm(C)	Time(min)	NO.of cycle
1	Initial Denaturation	95 C	4	1
2	Denaturation	95 C	0.30	35
3	Annealing	48C	0.45	
4	Extension	72 C	1.00	
5	Extension final	72	7	1

2.3. Statistical analysis

Complete Randomized Block Design was to perform felid experimental, **statistically** analyzed using GenStat procedure Library Release PL18.2. The significance of differences was determined using the least significant difference (L.S.D) at the probability level of $p<0.05$(Al-rawi and khalaf Alla, 1980).

3. Results and Discussion

3.1. Seasonal Presence of the Leafhopper (*A. biguttula*) During the Season

The results of Figures (1,2) show that the highest population densities of leafhopper nymphs and adults reached 29.83 and 8.19 insects per leaf, respectively, during the last week of October and the first week of November, respectively. In contrast the lowest population density of nymphs and adults reached 0.88 and 0.15 insects per leaf, respectively, during the first week of September. This disparity in population densities is attributed to plant age. in the early plant age. in the early stages of growth, leaves have thinner veins and thinner leaf surfaces, which discourages insect colonization. over time, the thickness of the veins and leaf lamina increases, and the leaf hair content decreases, providing more favorable conditions for egg laying and insect settlement, thus increasing population, (Rugumoorthi and Kumar, 2000). On the other hand, the population density of the leafhopper *A. biguttula* is affected by environmental factors, particularly temperature and humidity. Numerous studies have shown that these factors have a direct impact on the insect's activity and presence during different months of the year (Akhlia, *et al.*, 2020;

al., 2020; Srinivas and Kumar, 2020).

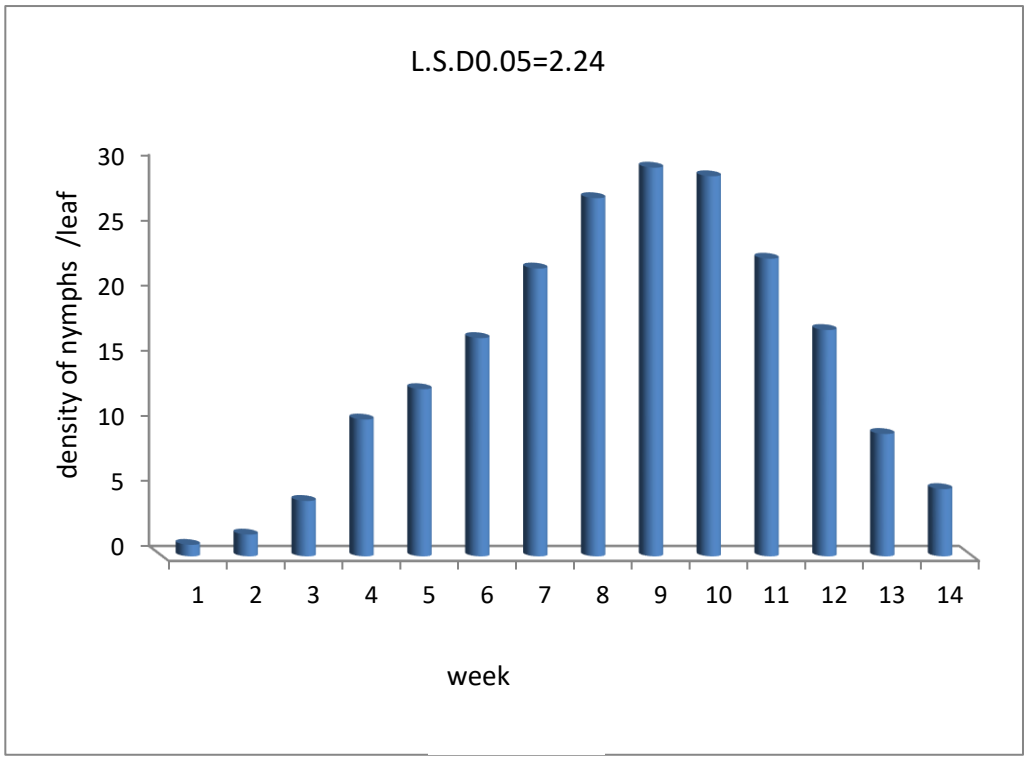

Figure 1. The population density of leafhopper *A. biguttula* nymphs on Okra

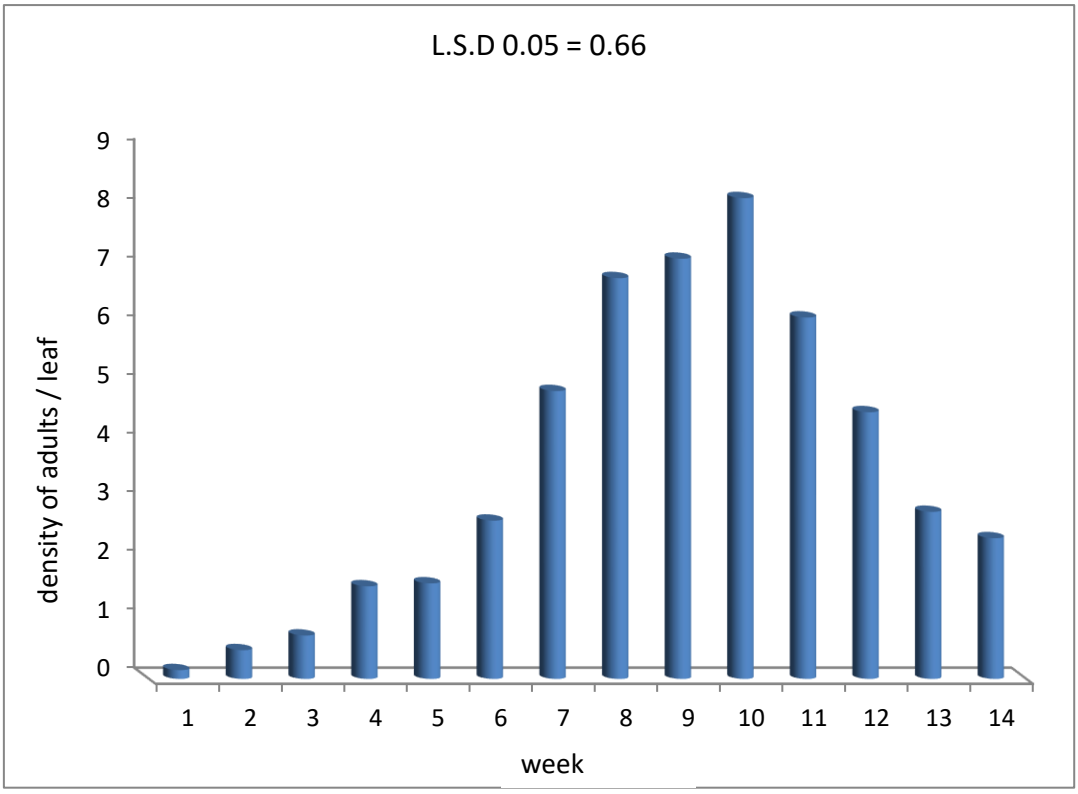

Figure 2. The population density of adult leafhopper *A. biguttula* on okra.

3.2. Detection of genetic amplification DNA

Electrophoresis results on 1.5 % agarose gels demonstrated successful amplification using the LCO1490) producing clear bands approximately 700 COI gene specific primer (HCO2198and, base pairs long in all isolates from the studied areas. this identical product size indicates that all samples belong to the same genus and species the leafhopper (3). This genetic homogeneity reflects the low molecular variation among the collected individuals, indicating that the insect population in these areas belong to a single genetically stable lineage

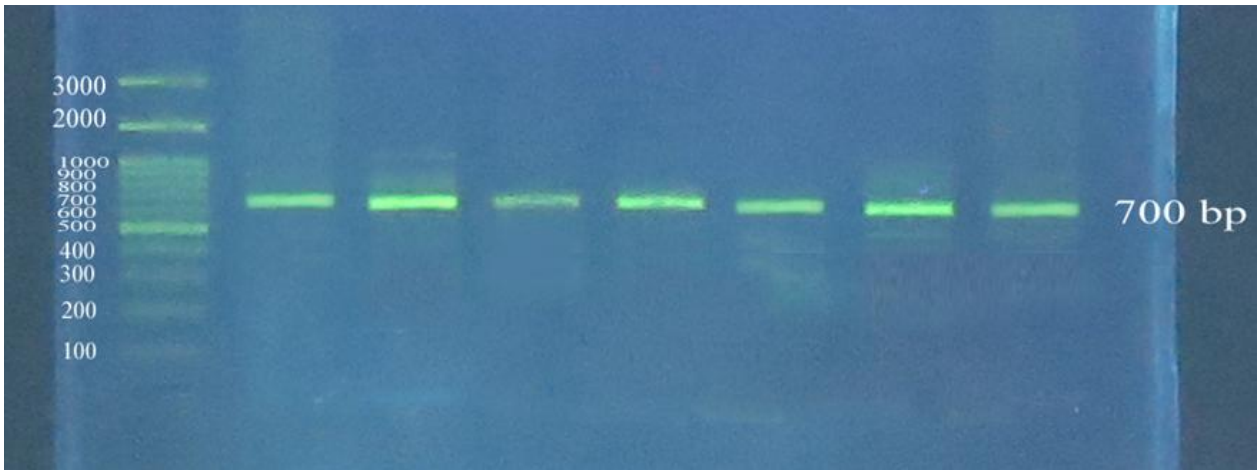

Figure 3. Amplification of the mtCOI primer on 1.5% gelatin of the leafhopper *A. biguttula*

from seven areas in Basra governorate where Okra is grown.

3.3. Molecular Diagnosis

The results of blast matching in the base sequences of the study samples and comparing it with the standard copies deposited in the US national center for biotechnology information (NCBR) showed 100% matching and which bear the registration number in the GenBank as shown in the Table (3).

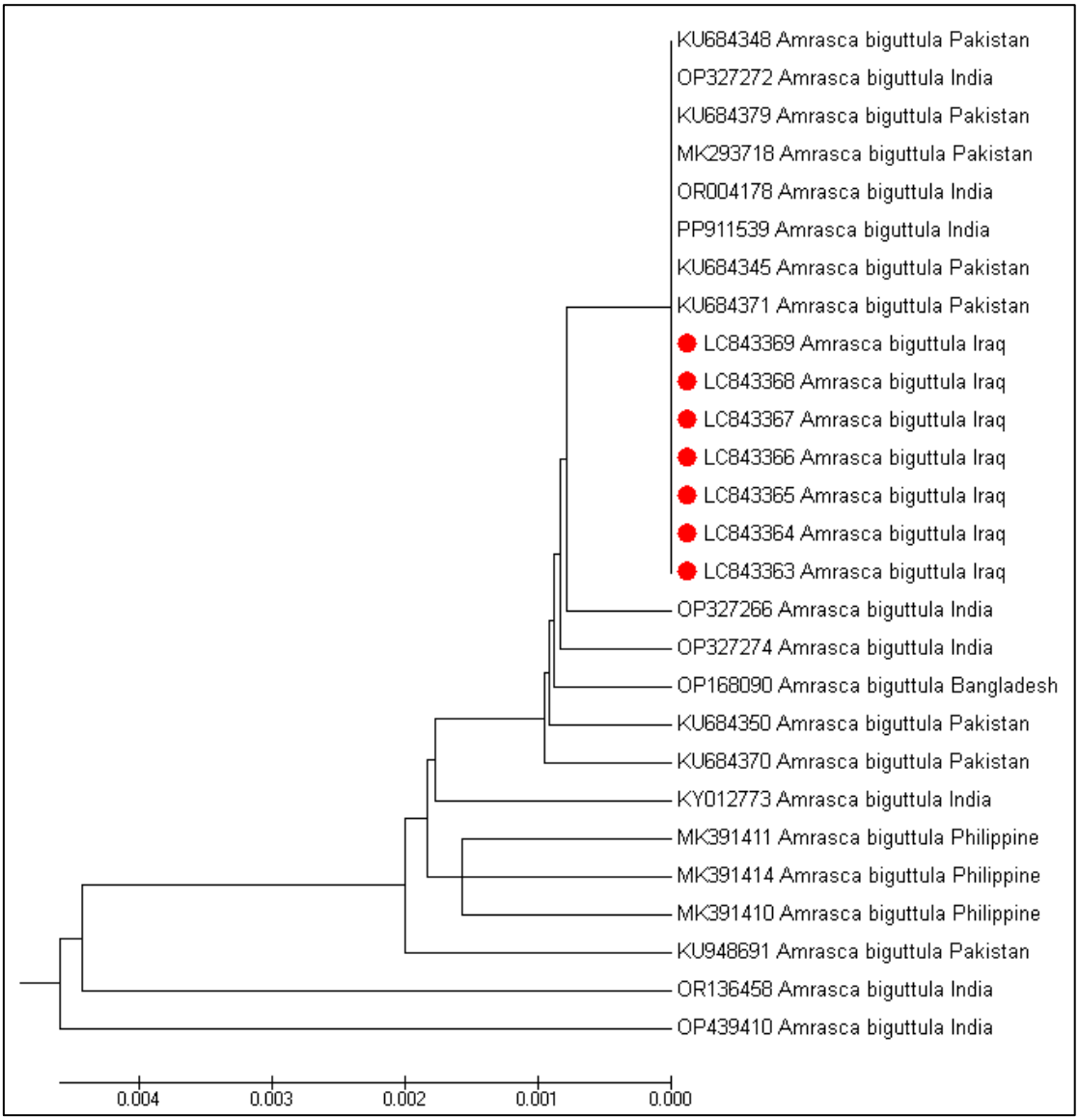

Figure 4. The phylogenetic tree of the *A. biguttula* leafhopper and its comparison with the other leafhoppers registered in the GenBank.

Table 3 molecular diagnosis of the leaf hopper *A. biguttula* using primers HCO2198 and, LCO1490) and comparison with standard clones deposited in the GenBank

3.4. phylogenetic tree and Genetic Relationship

No.	Accession Number (Our Study)	Query caver	Identification Percent.	Science Name	Reference Copy (NCBI)
D1	LC843363	100%	100%	*Amrasca biguttula*	KU684371
D2	LC843364	100%	100%	*Amrasca biguttula*	KU684371
D3	LC843365	100%	100%	*Amrasca biguttula*	KU684371
D4	LC843366	100%	100%	*Amrasca biguttula*	KU684371
D5	LC843367	100%	100%	*Amrasca biguttula*	KU684371
D6	LC843368	100%	100%	*Amrasca biguttula*	KU684371
D7	LC843369	100%	100%	*Amrasca biguttula*	KU684371

The phylogenetic tree of *A. biguttula* was drawn for the studied isolates using MEGA 7 software and compared with the isolates registered in the NCBI gene bank. The results showed 100% similarity with both the Pakistani Ku684371and the Indian isolate Op168090 shown in as Figure (4). Jaod and Nawar, (2023) reported that the *A. biguttula* on cowpea registered in the GenBank was genetically identified using the same primer used in this study.

4. Conclusion

The leafhopper, *A. bigutulla*, is one of the most dangerous pests that infect okra plants. It is recommended to conduct control when the insect *A.biguttula* reaches the highest population density, which recoded during the last week of October and beginning the first week of November, to reduce potential economic damage and prevent the infestation from worsening during the advanced stages of plant growth. DNA analysis is an essential tool for confirming to phenotypic diagnosis the leafhopper, given the presence of several morphologically similar species that are difficult to distinguish based on morphological characteristics alone. molecular analysis, particularly COI gene sequencing, contributes to accurate and reliable taxonomic identification.

References

Adetuyi, F., Ajala, L., & Ibrashim, T. (2012). Effect of the addition of defatted okra seed (*Abelmoschus esculentus*) flour on the chemical composition, functional properties, and Zn bioavailability of plantain (*Musa paradisiaca* Linn.) flour. *Journal of Microbiology, Biotechnology and Food Sciences (JMBFS)*, 2(1), 69–82.

Akhila, E., Upendhar, S., Sree, K. V., & Sagar, B. V. (2020). Population dynamics of leafhopper *Amrasca biguttula* (Ishida) (Homoptera: Cicadellidae) on popular cotton hybrids in Telangana. *Journal of Entomology and Zoology Studies*, 8(5), 511–515. http://www.entomoljournal.com/

Al-Kanani, E. A. S., Al-Hilifi, S. A. H., & Al-Kareem, A. H. (2019). The nutritional composition and vitamin E of three Iraqi okra (*Abelmoschus esculentus* L.) seeds oil. In *IOP Conference Series: Earth and Environmental Science* (Vol. 388, No. 1, p. 012058). IOP Publishing. https://doi.org/10.1088/1755-1315/388/1/012058

Al-Raawi, K. M., & Khalf Allah, A. M. (1980). *Design and analysis of agricultural experiments*. Dar Al-Kotob for Printing and Publishing, University of Mosul, Iraq. 488 pp.

Behura, S. K. (2006). Molecular marker systems in insects: Current trends and future avenues. *Molecular Ecology*, 15(11), 3087–3113. https://doi.org/10.1111/j.1365-294X.2006.03014.x

Bhandari, S., Thakuri, L. S., Rimal, S., & Bhatta, T. (2022). Management of okra jassid (*Amrasca biguttula*) through the use of botanicals and chemical pesticides under field conditions in Chitwan, Nepal. *Journal of Agriculture and Food Research*, 10, 100403. https://doi.org/10.1016/j.jafr.2022.100403

Dubey, V. K., Sahoo, S. K., Sujatha, B., & Chowdhury, S. (2023). Integrated pest management strategies for okra, *Abelmoschus esculentus* (L.) Moench. *AgriCose e-Newsletter*, 4(12), 36–38.

Devi, Y. K., Pal, S. U. M. A. N. T., & Seram, D. E. V. I. N. A. (2018). Okra jassid, *Amrasca biguttula* (Ishida) (Hemiptera: Cicadellidae): Biology, ecology, and management in okra cultivation. *Journal of Emerging Technologies and Innovative Research*, 5(10), 332–343. http://www.jetir.org/

Jaod, A. S., & Nawar, M. H. (2023). Molecular identification and field control of cowpea jassid *Amrasca biguttula* (Hemiptera: Cicadellidae) infecting cowpea in Baghdad, Iraq. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1225, No. 1, p. 012084). IOP Publishing. https://doi.org/10.1088/1755-1315/1225/1/012084

Kadri, K. (2019). Polymerase chain reaction (PCR): Principle and applications. In *Synthetic Biology: New Interdisciplinary Science* (pp. 1–17).

Lima Júnior, R. S., & Scarpassa, V. M. (2009). Evidence of two lineages of the dengue vector *Aedes aegypti* in the Brazilian Amazon, based on mitochondrial DNA ND4 gene sequences. *Genetics and Molecular Biology*, 32, 414–422. https://doi.org/10.1590/S1415-47572009005000036

Maqbool, M. E., Mushtaq, M. N., Tamathues, K. T. K., Zulfiqar, A., Sarwar, M. S., Yousaf, Z., & Nosheen, F. (2024). Assessing the impact of insect pest biodiversity on the ecological footprint of okra (*Abelmoschus esculentus* L.) cultivation in Faisalabad, Punjab, Pakistan. *Punjab University Journal of Zoology*, 39(2), 169–175. https://doi.org/10.17582/journal.pujz/2024/39.2.169.175

Nagoshi, R. N., Brambila, J., & Meagher, R. L. (2011). Use of DNA barcodes to identify invasive armyworm *Spodoptera* species in Florida. *Journal of Insect Science*, 11(1), 154.

Patel, Z. N., Pathan, N. P., & Chachpara, P. P. B. (2024). Seasonal incidence of leafhopper, *Amrasca biguttula* (Ishida), infesting summer okra. *International Journal of Advanced Biochemistry Research*,

8(8), 24–27. https://doi.org/10.33545/26174693.2024.v8.i8Sa.1726

Ragumoorthi, K. N., & Kumar, S. (2000). Biophysical factors of bhendi, *Abelmoschus esculentus* (L.) Moench, offering resistance to leafhopper *Amrasca biguttula* (Ishida) (Homoptera: Cicadellidae). *Pestology*, 23, 12.

Srinivas, G., & Kumar, S. (2020). Incidence of major pests of okra in relation to weather factors and cropping systems. *Pest Management in Horticultural Ecosystems*, 26(1), 48–54. https://doi.org/10.5958/0974-4541.2020.00008.9

Suganthi, M., Abirami, G., Jayanthi, M., Kumar, K. A., Karuppanan, K., & Palanisamy, S. (2023). A method for DNA extraction and molecular identification of aphids. *MethodsX*, 10, 102100.

Swamy, K. R. M. (2023). Origin, distribution, taxonomy, botanical description, cytogenetics, genetic diversity and breeding of okra (*Abelmoschus esculentus* L. Moench). *International Journal of Development Research*, 13(3), 62026–62046. https://doi.org/10.37118/ijdr.26440.03.2023