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The First Record of The Insect *Tanaecia Pelea* on The Artemisia Plant in Nineveh

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Abstract

The study included diagnosing and recording an insect *Tanaecia pelea.* On Artemisia plants in Nineveh Governorate, which was attacking Artemisia plants in Nineveh Governorate for the year 2022, and the results showed the presence of the insect in nurseries in Nineveh Governorate from the date of 3/1/2022, in numbers that ranged from 1 larva on 3/1/2022, and its numbers increased on 5/20. In 2022, there were 60 larvae compared to the number of adults, which ranged from 4 to 25 adults, depending on the temperature and relative humidity. This was confirmed by the correlation values between temperature and relative humidity, as the values for larvae reached 0.70 for temperature and relative humidity -0.78, for adults 0.79 and -0.65, respectively. In addition to studying the diagnosis of the insect based on molecular characteristics, the results were shown when using polymerase chain technologyPCR in diagnosing the insect on Artemisia plants in Nineveh Governorate, as the match rate reached 99%, according to the tree branch of that insect.

Key words: *Tanaecia pelea*, Insects, First record, Artemisia.

Introduction

Moth returns *Tanaecia pelea* to the scale of the wings Lepidoptera, the Nymphalidae family, is a widely distributed family, as it includes a large number of small to medium-sized species, numbering several thousand, and they have different colors ranging from blue to violet, copper, and black (Smart, 1976).

The *T. pelea* species of butterflies has a wingspan of about 65-70 mm and the wings are pale brown to black with bluish edges that have dark brown markings on the basal area. These butterflies are found in Singapore, mainland Malaysia, Urnio, Nias and Pulaar (Myers and Epinosa, 2023).

Tan et al. (2015) emphasized the importance of *Tanaecia pelea* as one of the most economically important insects in the world and gave a clear description of these butterflies.

Kirton (2014) explained the nature of the *Tanaecia pelea* insect and gave a list of butterfly species in Malaysia. Likewise, Zarim and Ahmad (2014) explained the importance of the *Tanaecia pelea* insect and gave a complete description of butterflies in Malaysia.

While Praveena and Maria (2013) provided a complete description, facts, types and shapes of butterflies, including *T. pelea*, in regions of Malaysia.

Chey and Lim (2008) pointed out the importance of *T. pelea* among the butterflies found in the world.

Corbet and Pendlebury (1993) gave a general description of butterfly species, especially *T. pelea* butterflies, and mentioned their economic importance, types, shapes, and colors, and the nature of their nutrition, harms, and behavior.

Artemisia is a genus of perennial herbaceous plants from the Asteraceae family, and its types are widespread in the world, including wild, medicinal, and industrial. The plant's leaves are alternate, its flowers are delicate and predominantly green in color, and all of them are medicinal plants (Abdelkader, 1997; Sharifa, 2021).

Hamid et al. (2018) also emphasized the importance of the Artemisia plant in the anatomical and chemical study and the biological effects of the Artemisia plant growing in western Anbar, Iraq, and they gave a clear and complete description of the Artemisia plant in all morphological, medical, and chemical aspects.

Many researchers in the field of insects have resorted to finding more accurate indicators in determining insect diversity and using modern techniques that rely on genetic makeup, which is distinguished from phenotypic studies by not being affected by environmental conditions (Yeu et al., 2006).

Molecular indicators are of great importance in plant breeding in light of opening new horizons in studying genetic diversity and relationships between various plants. These indicators help in selection processes and thus reduce the incidence of agricultural pests (Al-Yaqoubi, 2017).

The aim of the research was to study the *Tanaecia pelea* insect on the Artemisia plant in Nineveh Governorate, and to study the molecular indicators of the insect and partially diagnose it using modern techniques, with the aim of recording it for the first time in Nineveh Governorate.

Materials and Methods

Study of the sensitivity of Artemisia plants to insects *Tanaecia pelea*

To implement the study, random samples were taken semi-monthly of Artemisia plants from nurseries in the city of Mosul, starting from 3/1/2023 until 6/30/2023, at a rate of ten plants from each nursery, to calculate the number of larvae and adults and to calculate simple correlation values for the relationship between the population fluctuation of the insect stages and the average scores. Temperature and relative humidity obtained from the weather station in Nineveh Governorate.

The stages of insect development were also studied in order to identify the stages of the insect and the periods of each stage by taking ten Artemisia plants, making a number of artificial plants, infecting the plants with the insect, and monitoring the plants and insects to calculate the number of larvae during the implementation of the experiment and by direct examination, as for calculating the number of adults using light traps (Al-Abada, 2022).

To study the genetic diagnosis of the insect, the study was carried out in the biotechnology laboratories at Jisr Al-Musayyab Company / Baghdad / Iraq, as follows:

Instruments and Equipment

Table (1) shows the laboratory instruments and equipment that were used in this study and their sources

Table (1): Equipment & Instruments.

Equipment & instruments	Company / Country
Centrifuge	(DLAB /Ghain)
Sensitive balance	(Sartorius /Germany)
Electrophoreses	Clarivate /UK

Cabinet hood	(BioLAB/ Korea)
Water Distillatory	(GFL/ Germany)
Micropipettes (Different sizes)	(Eppendroff/ Germany)
Refrigerator	(Al Balsan / Turkey)
Tips (Different sizes)	(Jippo / Japan)
1.5 ml Eppendorf tubes	(Sigma-Aldrich /USA)
UV.transmission	Vilber Lourmat Sté /Farance
Vortex	Fisher Scientific/ USA
Thermostatic Incubator	Zxinstrument/Chain
Power Supply	Biorad/ USA
Applied Biosystems™ ProFlex™ PCR System	Fisher Scientific/USA

Biological Materials and Chemicals

The chemical and biological elements utilized in the current investigation, together with their respective manufacturers, were documented in Table 2.

Table (2): Biological Materials & Chemicals.

Biological Materials & Chemicals	**Company / Country**
Agarose	Carl Roth/Germany
Red safe staining souluion	Intron / Korea
FavorPrep insects Genomic DNA Extraction Mini Kit	Korea
6X Loading dye	Intron / Korea
TBE buffer10 X	Intron / Korea
Pre mix pcr	Intron / Korea
primer	Macrogen/ Korea
Ladder100bp	Kapa /USA

Extraction DNA

Before using EasyPure® Genomic DNA Kit

1. Using liquid nitrogen, grind up 20 mg of the sample until it forms a powder.

2. Include one hundred microliters of LB2 in the cell pellet. Pipette or use a vortex to completely combine the ingredients.

3. To the lysate, add 20 l of RNase A, and then incubate the mixture at room temperature for 2 minutes.

4. To the lysate, add twenty microliters of proteinase K. After thoroughly combining the ingredients by vortexing them, the mixture is left to incubate for two minutes at room temperature.

5. Pour 500 microliters of BB2 into the container, and quickly mix it by vortexing it for five seconds. Maintain at room temperature for the 10 minute incubation period.

6. Place the entire volume of lysate into a spin column. Perform a 30-second centrifugation at a speed of 12,000 g. Put the flow through in the trash.

7. Add 500 l of CB2 and centrifuge at 12,000 g for 30 seconds. Before using, check to make sure that you have already added ethanol. Put the flow through in the trash.

8. Add 500 uL of WB2 and centrifuge at 12,000 g for 30 seconds. Before continuing, double confirm that ethanol was included in the initial preparation. Put the flow through in the trash.

9. Perform step 5 once more.

10. Centrifuge at 12,000g for two minutes in order to thoroughly remove any residual WB2.

11. Transfer the spin column to a clean microcentrifuge tube that has a capacity of 1.5 milliliters. In the middle of the column matrix, add 50-200 l of elution buffer that has been preheated to 60-70°C or sterile, deionized water that has a pH greater than 7.0 and has been preheated to the same temperature. At room temperature, let the mixture to incubate for one minute. To elute the DNA, centrifuge the sample at 12,000 g for one minute. 9. If you want a better yield, go back and do step 8 once more. Keep the isolated DNA at -20 degrees Celsius.

Determination of DNA Concentration:

Gel Electrophoresis to Analyze DNA Quality:-

1.An agarose solution was made by dissolving one gram of agarose powder in one hundred milliliters of one times TBE in a flask with a capacity of one hundred milliliters. Then, the agarose was melted in a hot block until the solution became transparent.

2.The agarose solution was allowed to cool to between 50 and 550 degrees Celsius, with the flask being stirred every so often to ensure that it cooled uniformly.

3.A three-microliter droplet of red stain was poured into the warm gel before the ends of the casting tray were taped shut with two layers of tape.

4.The combs were put in the tray that was used for gel casting.

5.An agarose solution that had been melted was put into the casting tray.

6. After allowing the agarose to cool to ambient temperature, the comb was carefully removed, and the tape was taken off. After the TBE (1x) buffer in the electrophoresis chamber had been poured in, the gel was then placed on top of the chamber.

7.Agarose gel wells were loaded with DNA samples that were five microliters in volume and included three microliters of DNA loading buffer.

8.The agarose gel electrophoresis was finished at a voltage of 70V and a current of 65Amp for one hour.In order to study the DNA, we looked at it under a UV trans illuminator.

The primers preparation

After the primers were lyophilized, they were dissolved in the free ddH2O to give a final concentration of 100 pmol/l as a stock solution. A stock was then stored at a temperature of -20 degrees Celsius in order to prepare a work primer concentration of 10 pmol/l by suspending 10 l of the stock solution in 90 l of the free ddH2O water to reach a final volume of 100 l.

Statistical Analysis

DNA which was produced in light of the application of partial indicators and reliance on them to determine the type of insect.

Finding the genetic relationship and spacing values between plants and calculating the correlation between temperatures, relative humidity, and insect numbers in infected plants (Antar and Adnan, 2017).

Results and Discussion

The results of Tables (3) showed a clear variation in the numbers of larvae and adults of the insect with variation in temperature and relative humidity and according to the date of sampling. The numbers of larvae were highest on 5/20/2022, reaching 60 larvae, compared to the adults, which were 25 insects. Adult on 10/5/2022. The numbers of larvae ranged between (0-60) larvae and the numbers of adults ranged between (0-25) adults, depending on the environmental conditions of temperature and humidity. This was also confirmed by the existence of a wave correlation between the number of insects and the temperature, as the correlation values reached 0.70 for temperature and the number of larvae, and were -0.78 for humidity and the number of larvae, and for adults they were 0.79 and -0.65, respectively.

As for the coefficient of determination R^2, it appeared that there was a clear variation in this value depending on the temperature, humidity, and stages of the insect, as it reached 0.60 and 0.55 for both the adult larvae with temperature, and with regard to relative humidity, this value reached 0.53 and 0.60 for both the larvae and the adult, respectively (Table 4).

We conclude that the presence of insects has varied according to environmental conditions, such as temperature and humidity, and according to the date samples were taken from the plants, since there are no previous studies available on this insect and its effect on the Artemisia plant, its numbers, its relationship to heat and humidity, and its harm to the Artemisia plant.

Figure (1) shows the stages of development of the T. insect and the symptoms of infection by this insect on the Artemisia plant, which varied according to temperature and relative humidity.

Figure (1): Showing the stages of the insect *Tanaecia pelea* and the symptoms and manifestations of the infection on the Artemisia plant.

Table (3): The effect of sampling, temperature, and relative humidity on the average numbers of larvae and adults of an insect *Tanaecia pelea* On Artemisia plants in Nineveh Governorate.

Date of sampling	Average temperature °M	Average relative humidity %	Larvae	Complete ones
9/3	19	57	Zero h	4 h
10/3	20	55	Zero h	7 f
20/3	18	59	1 g	3 g
30/3	19	56	4 f	5 f g
1/4	17	58	17 d	9 e
10/4	18	60	13 e	15 d
20/4	17	62	27 c	20 b
30/4	20	58	45 a b	30 a

Date of sampling	Average temperature °M	Average relative humidity %	Larvae	Complete ones
10/5	27	56	40 a b	25 a b
20/5	34	42	60 a	20 b
30/5	33	40	45 a b	19 b c
10/6	29	35	30 c	15 d
20/6	27	36	19 d	Zero i
30/6	31	37	4 f	Zero i
Total	23.5	50.78	21.78	12.28

Means with similar letters are not significantly different according to Duncan's multinomial test above the 5% probability level.

Table (4): Correlation values and coefficient of determination for the relationship between the number of insect larvae *Tanaecia pelea* average temperatures and relative humidity.

The whims	Correlation values and coefficient of determination			
	Average temperatures °M		Average relative humidity %	
	R^2	r	R^2	r
Larvae	0.60	0.70	0.53	-0.78
Adult females	0.55	0.79	-0.6	-0.65

Table (4) shows the effect of the correlation and the coefficient of determination for the relationship between the numbers of larval and adult insects and the average temperature and relative humidity. The results showed that there was a significant correlation between the number of larvae and temperature, reaching 0.70, while the correlation was insignificant and negative with the relative humidity, reaching -0.78. As for adult females, the correlation reached 0.79 for temperature and -0.65 for relative humidity, respectively.

Molecular diagnosis of *Tanaecia pelea*

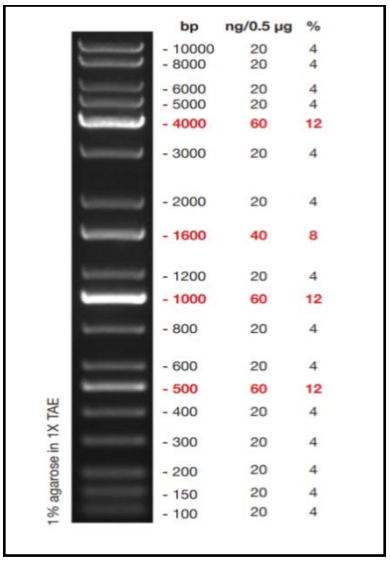
The primers for gene cox 1 for recording insects in this research were designed based on the genetic sequence of gene cox 1 found on the NCBI Gen Bank website, using the primers design program. These primers were prepared by the Korean company Macrogene.

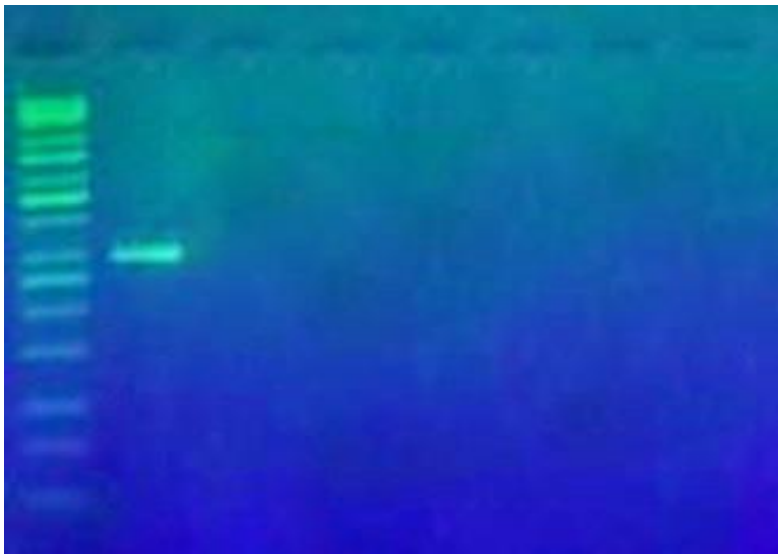
Table (5): The sequence of primers that used this study.

The specific primer Cox1 of gene.

Primer	Sequence	Product size
Forward	5'-ATTCAACCAATCATAAAGATATTGG- 3'	600
Reverse	5'-TAAACTTCTGGATGTCCAAAAAATCA- 3'	base pair

Figure (2) shows the yield of DNA replicated using polymerase chain reaction (PCR) from the insect in this study using the Cox 1 primer, which contains all components of the PCR extracted from the insect.





SiZer 100bp DNA Markers (Kapa /USA). **PCR product the band size. The product was electrophoresis on 1.5% agarose at 5 volt/cm². 1x TBE buffer for 1:30 hours. M: DNA ladder (100).**

Figure (2): It represents an electrophoresis image of an agarose gel containing the results of a PCR test for diagnosing the insect *Tanaecia pelea*

Table (6) which was sent through the global genetics website NCBI-Gen bank, shows the percentages of identity between the global isolates and the local isolates obtained in the current study. It is noted that the percentage of identity is very high for the local sample and reached 99%, and thus it was considered a new local species.

Table (6): Genetic matching between local insects and species registered globally on the Gen Bank website.

	Accession	Country	Source	Isolation source	Compatibility
1.	ID: MN993719.1	Switzerland	*Tanaecia pelea*	----------	99%
2.	ID: MN993718.1	Switzerland	*Tanaecia pelea*	----------	99%
3.	ID: MG741082.1	Sweden	*Tanaecia pelea*	----------	97%
4.	ID: AB511421.1	Malaysia	*Tanaecia pelea*	----------	97%
5.	ID: KF226635.1	Malaysia	*Tanaecia pelea*	----------	97%
6.	ID: HQ962240.1	Thailand	*Tanaecia pelea*	----------	95%

It can be seen in Figure (3) the analysis of the genetic tree of the insect *Tanaecia pelea*. In the current study, the genetic tree was analyzed and the results of the analysis showed a clear affinity of the studied insect with the species registered on the NCBI Gen bank website. The registration code for the species diagnosed in the study was also obtained for the *Tanaecia pelea* species. Which was sent from the International Gene Bank website, in addition to the official registration document for type *Tanaecia pelea*, which was recorded in this study on the International Gene Bank website ON989344.1.

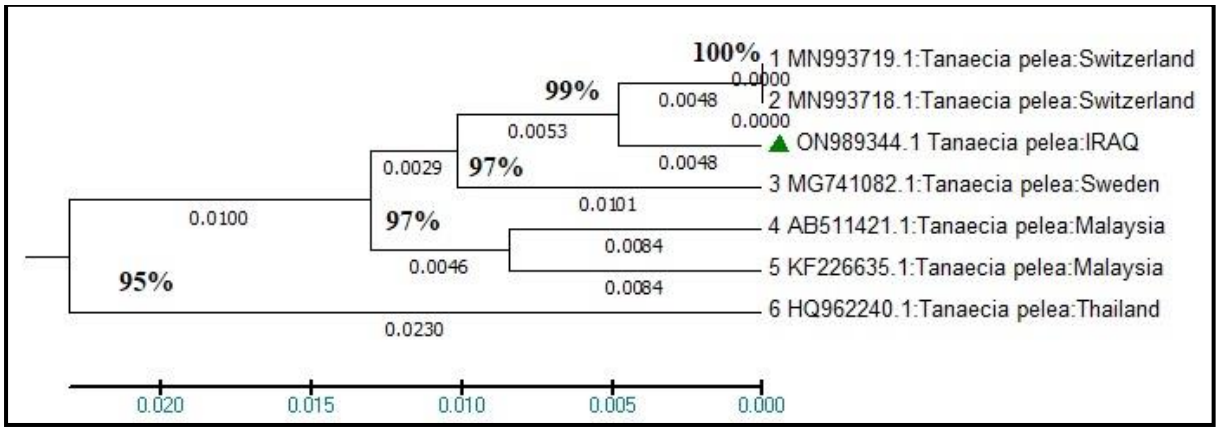


Figure (3): Dendritic diagram of the degree of similarity and difference using the polymerase chain reaction (PCR) technique.

Evolutionary relationships of taxa

The UPGMA method, which was developed by Sneath and Sokal in 1973, was used to deduce the evolutionary history. The optimal tree is illustrated above, with the total of the branch lengths equaling 0.08244280. The phylogenetic tree has been drawn to scale, with branch lengths represented in the same units as the evolutionary distances that were used to infer the tree. The evolutionary distances were measured in terms of the number of base substitutions that occurred at each site and were calculated using the Maximum Composite Likelihood approach (Tamura et al., 2004).

Conclusion

Seven different nucleotide sequences were used in the analysis. Included codon positions were the first, second, third, and noncoding locations. We got rid of every single slot that had blanks and information that was lacking. The completed dataset contained 420 locations in total over its entirety. MEGA6 was used to do the evolutionary analyses that were described in Tamura et al. (2013).

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