The use of Some Forensic Medical Insects to Reveal the Causes of Death Resulting from Sexual Harassment

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Abstract

The study was conducted in Al-Nahrain sub-district of Al-Dhuluiya district, south of Salah Al-Din Governorate, for the period from 2021/10/7 to 2022/4/12. Two types of insects were used during the study, the larvae of the green metal fly Chrysomya albiceps and Leather beetle larvae Dermestes maculactus To carry out the experiment by feeding these larvae on minced sheep meat ovis aries supplemented with human semen simulating sexual harassment of three volunteers separately by three replications and determining the duration of human semen DNA in larval tissues for different periods, The presence of DNA of human semen was detected in the extract of the bodies of insect larvae fed on the treated sheep Ovis aries with a volume of 2 ml of human semen for 24 hours. The experiment simulates the cause of death resulting from sexual harassment in four periods of time (1,5,10,15) days, and the presence of DNA of human semen in the larval tissues was proven in all periods at a rate of 100% using the initiator (rs 6259).

Keywork: Forensic medical insects; sexual harassment; Dermestes maculalctu; Oris Aries; human semen DNA; AL-Naharin.

1. Introduction

Forensic entomology is a field of forensic medicine that specializes in determining the post-mortem period, and the continuous use of forensic entomology has led to the emergence of modern techniques at the molecular level, such as identifying the criminal or identifying the victim using evidence related to insects and arthropods. (Sundus *et al*; 2019). One of the most important fields of forensic entomology is forensic medicine, which deals with criminal cases, such as knowing the post-mortem period (Huntington and Hall,2019). Studies and reports from the United Nations Office in 2020 showed that sexual harassment causes more than 81,000 deaths each year, and the presence of semen in the victim's body is one of the most important evidence used in identifying cases of sexual harassment (rape) (Maria *et al*;2010). Insects feeding on the bodies of victims reveal the criminals in sexual harassment cases by retaining the offender's genetic fingerprint for a long period of time, and the analysis of these insects reveals the genetic fingerprint of the semen, allowing specialists to identify the criminal (AL Mansour,2021). Sexual harassment is defined as a type of unwanted behavior and is a criminal behavior that causes many physiological changes (Muhammad,2022). Sexual assault behavior can cause many harassments in many places such as home, work and school, which pushes the victim to leave the workplace or the job (5). In most cases, sexual assaults are negative, leading to many harms, up to suicide, among most of the victims (Elli *et al*;2009). The study aimed to detect the semen in the tissues of the larvae of leather beetles and the green metal fly feeding on corpses contaminated with semen as a simulation of death crimes. Resulting from sexual harassment and considering the case as one of the causes of deat, the ami of the study is dectect the presence of human DNA in the extiacts of insect larvae bodies and as one of the causes of death 2.

2. Materials and Method

Samples of whole and larvae of the green metal fly *Chrysomya albicepas* and leather beetles *Dermestes maculactus* were collected from the agricultural areas of Al-Nahrain district, south of Salah Al-Din Governorate. Which is 91 km away from the governorate center during the period 2021/7/3 until 2021/10/2 at a temperature ranging between 45-29°C. Rabbits were also collected from the local markets of the Al-Nahrain to be used as a laboratory animal to conduct experiments on them, While green metal flies and leather beetles were collected from the carcass of rabbits used as a primary source for the whole insects under study to provide insect larvae and carry out the experiment, net traps were used to collect adults, while the larvae were collected using small mattresses and then placed in plastic containers with perforated cover for protection and breathing and then reared on Sheep meat ovis aries and studies on them, while some of them were sent to the Natural History Research Center and Museum at the University of Baghdad / Department of Insects and Invertebrates for the purpose of diagnosis

Raising insects on tissues treated with semen

The method of (Chamoun and olivera,2019). was adopted in carrying out the experiment with some

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modifications. Where lamb ovis aries weighing (100 g) were used after contaminating it with a volume of 2 ml of human semen from three volunteers, each separately, after placing the mixture in a plastic container with dimensions 15×15×10 cm, as 150 larvae of *Ch. albiceps* green metal flies were used at an average weight of 12g for all of them and 100 larvae of leather beetles *D. maculactus* at an average weight of 8g. The containers were covered with a piece of tulle cloth to prevent the larvae from escaping and at the same time allow air to enter the larvae, the larvae were starved. The larvae were starved for 24 hours, to ensure their feeding, and then the third instar larvae of the green metallic flies *Ch. albiceps* and fifth instar larvae of leather beetles *D. maculaetus* were fed on minced meat contaminated with human semen for 24 hours, after which the larvae were prevented from feeding on meat treated with human semen for different periods of (1, 5, 10, 15) days, after which the larvae were killed For each period, using 70% ethanol alcohol, then transferred to the Molecular Biology Laboratory – Department of Life Sciences, College of Science, Tikrit University for the purpose of extraction and examination.

Picture (1) a box for the larvae of the green metallic fly Ch. albiceps used in the experiment

Picture (2) Green metal fly larvae Ch.albiceps are used in the experiment.4X

Picture (3) D. muculatus beetle larvae used to carry out the experiment.4X

2.2Extraction method

The process of extracting the DAN was conducted by the use of the several extraction (Qlamb Corex coming,inc QA03), After collecting the larvae fed on food contaminated with semen for each period separately, they were ground with liquid nitrogen using a ceramic mortar to obtain a fine powder. 0.5 g was placed in a 2 ml Eppendorf tube and 700 µl of Lysis buffer-1 solution was added to it. Then the samples were incubated in a water bath. For an hour, with stirring every 10 minutes, and after removing the samples from the water bath, they were left at room temperature to cool for 5 minutes, with the addition of 200 microliters of the protease enzyme Analysis (protease). The sample was mixed well and kept in the refrigerator for 5 minutes, then the samples were placed in a centrifuge and centrifuged at 14,000 rpm for 5 minutes, then kept in the filter tube with 600 µl of the sample added into a 2 ml Eppendorf tube with the addition of 750 µl of Binding buffer solution (DNA binding solution) and the samples were left for 5 minutes at room temperature with the sample mixed well and then 600 µl of the sample was transferred to a Klum tube with the solution poured at an angle of 45 on the wall of the filter tube to avoid puncturing the filter, The samples were centrifuged at 10,000 rpm for one minute, with the precipitate removed and 500 µl added to the sample from the first washing solution buffer-1 and the sample was centrifuged at 10,000 rpm for one minute, the precipitate was removed and 500 µl was added to the sample from the second Wishing buffer-2 Centrifuge the sample at 10,000 rpm for a minute, then the sample was dried in a centrifuge at 13,000 rpm for 3 min, and the sediment filter was removed and placed in a 2 ml Eppendorf tube with the addition of 150 µl of Elushtion DNA The samples are left vertically for 5 minutes, then centrifuged at 10,000 rpm for 2 minutes, after which a DNA sample is obtained. The sample was then left at room temperature for 24 hours and then kept in the refrigerator.

PCR reactions

The reaction mixture for DNA amplification of human semen was prepared in a test tube before transferring to the thermopolymer device. The preparation process was summarized as follows:
1- 10 µl of the reaction mix (master mix) was put into 0.2 ml tubes.
2-To the mixture, 5 µl of DNA was added with 1 µl of the Main initiator for all tubes.
3-4 µl of distilled water was added to complete the volume to 20 µl, provided that the reaction components are mixed well.
4-The samples were placed in the thermometer, and the program was set, as shown in Table (1).

Table 1: program of samples placed

Temperature	94°C	94°C	63°C	72°C	94°C	60°C	72°C	94°C
Time	2m	30s	1m	1m	30s	1m	1m	5m
Number of cycles	15				20			

3. Results

The samples were diagnosed in the Natural History Research Center and Museum, University of Baghdad / Department of Insects and Vertebrates, according to book No. 16 on 2022/3/17, and as shown in Table (2).

Table2: the insects used to conduct the experiment.

No.	The scientific name	The scientific name
1	Green metal fly	Chrysomya albiceps
2	Leather beetle	Dermestes maculactus

Detection of the DNA of the semen in the extracts of the bodies of the insect larvae under study:

The results of the study showed that the process of extracting DNA from human semen from the extracts of the larvae of each of the green metal flies Ch. albiceps leather beetles D. maculatus was successful, as the study revealed the presence of human DNA in the extracts of the bodies of insect larvae fed on sheep meat Ovis aries contaminated with human semen for all periods after they were prevented from feeding, which are (1, 5, 10, 15) days. With a percentage of 100%, DNA bundles were obtained in the samples of leather beetle larvae D. maculatus and larvae of the green metallic fly Ch. albiceps and as shown in Figure (1).

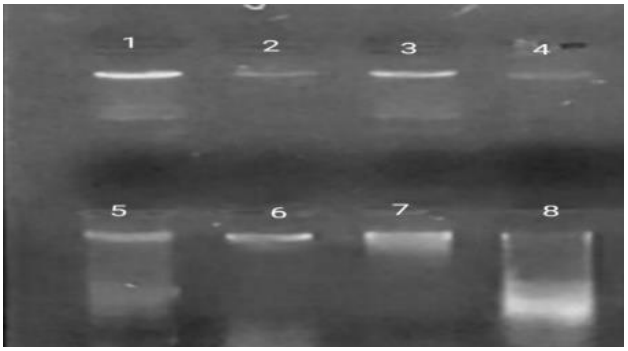

Figure (1) shows the electrophoresis of genomic DNA on an agarose gel

bundle No. (1) represents the genomic DNA of human semen in the extract of the bodies of the larvae of green metallic flies Ch.albiceps after being prevented from feeding for one day from their first feeding on meat contaminated with human semen, while bundle (2) represents the DNA in the extract of The bodies of green metallic flies larvae after 5 days of feeding, while bundle (3) represented the DNA obtained after the larvae were prevented from feeding for 10 days, while bundle (4) represented the DNA bundle in the extract of green metallic flies larvae that was prevented from feeding for 15 days, and in general, the DNA bundles of the human semen were more clear in bundles (3, 1) than in bundles (4, 2). The reason may be due to the use of fresh samples and direct extraction of samples after feeding, while the picture was less clear In bundles (4, 2), the reason is due to the preservation period in 70% ethanol alcohol, where the results of the study showed that keeping insects in ethanol alcohol for a long time can affect their DNA, and this is consistent with the study of (AL Jourani et al; 2014). Or the reason is due to the long period of time during which the insects were left before the extraction process, as the time period between extracting samples (4, 2) and samples (3,1) is 15 days, which may affect the image and quality of the bundle, and this is consistent with the study of (Mostafa et al 2019). As for bundle No. (5), it represents the DNA bundle in the extract of the bodies of leather beetle larvae D. maculaetus after one day of feeding them, while bundle (6) shows the DNA in the extract of the same larvae after they were prevented from feeding for 5 days of feeding, Also, bundle (7) appeared in the extract of the bodies of beetle larvae that were prevented from feeding for 10 days, and finally bundle (8) represented the DNA in the same larvae after they were prevented from feeding for 15 days of feeding. The results of the study, which were obtained from the samples represented by the periods (8, 7, 6, 5) which represent the samples of the larvae of flies, leather beetles D. maculatus, through which the DNA was obtained clearly in the samples (5, 6, 7), while the image of the bundle was weak in period (8), perhaps for the same reason above, as the study showed that the bundles of DNA obtained from the extract of larvae of green metallic flies Ch. Albiceps was more clear than the bundles obtained from the larvae of leather beetles D. maculatus. The reason may be due to the nature of their nutrition, as the larvae of flies prefer to feed on liquids and in very large quantities In contrast to the larvae of beetles that prefer dry tissue (Lindal et al 2020), the results of the study agreed with the results of the study of (Dansky et al; 1997). During the study, larvae of the green metal fly Chrysomya albiceps fed on treated beef with 2 ml of human semen were used to detect human DNA, whereby the larvae were prevented from feeding for several periods of time as they are (48,72,120,144,192 hours) The results of the study (Donia,2022). revealed the detection of DNA of human semen from the extract of Green adenium fly larvae Ch. albiceps in all time periods and at 100%. The results of the study also differed with the results of the study of (Metwally et al., 2020), Through which the DNA of mice and rats was detected from extracts of larvae of each of the green metal flies Ch. albiceps and Musca domestica. After feeding for the period (24,48,72,96,120) hours, DNA was detected in the third instar larvae of both insects for the periods (24,48,72) only, while no results were found in the periods (96,120) and the results were in agreement with the results of the study (Sambrook et al; 1989). with the different type of feeding was used during the study the larvae of each Ch. albiceps and Sarcophaga carnaria fed pork at intervals of (24,48,72,96) hours to detect DNA. The results of the study revealed the presence of DNA of laboratory animals from the extract of Ch. albiceps larvae And Sar. Carnaria in all periods and at 100%.

Results of DNA amplification (PCR)

The initiator (rs6259) showed one general bundle with a molecular size of 120 bp for all periods, as shown in Figure (2), where the bundle was visible in periods (1, 2, 3, 4), which represents the extract of metallic fly larvae bodies *Ch. albiceps* in all stages, while the bundle was weak in periods (5,6,7,8), which represents the extract of the bodies of the larvae of leather beetles *D. maculatus*, especially in the period (8), the bundle was almost non-existent. The reason may be due to the long period or due to the metabolism process that It occurs in insect larvae or because of the nature of the feeding, so it is recommended to use fresh samples and not to agitate with 70% ethanol alcohol for long periods.

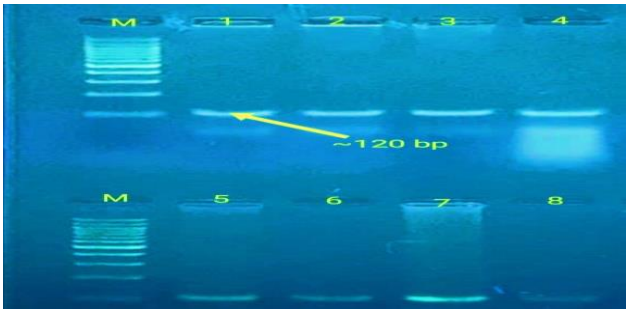

Figure (2) shows the results of electrophoresis on 1.5% agarose gel (PCR)

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