Molecular Profiling and Disease Management of Musca Domestica in Salah Al-Din Governorate: A Comprehensive Study on House Fly Populations

Salam jasim mohammed Al-kalash[1] , Liqaa hussain Alwan[2] , Husham najy Hameed[3]
[1,3]Department of Biology, College of Education, University of Samarra
[2]Department of Chemistry, College of Education, University of Samarra

Abstract
The present study was conducted in the Al-Alam district of Salah Al-Din Governorate, with the aim of identifying the types of Musca domestica present in the region. The house fly (Musca domestica L.) is a common and widespread insect that has coexisted with humans since ancient times. It is known for its role as a household, veterinary, and medical pest, as well as a vector for various pathogens affecting humans, poultry, and livestock. In this study, the house fly specimens were registered in the World Genome Bank under the accession number op975717.1.
The significance of this research lies in understanding the role of house flies in disease transmission and the spoilage of food. House flies are found in both urban and rural areas, particularly in polluted environments and waste disposal sites, where they can contaminate human food, excrement, animal dung, and decomposing organic matter. As a result, they contribute to the spread of diseases such as typhoid, cholera, dysentery, paratyphoid, and diarrhea, as well as the transmission of intestinal worms, smallpox, and cutaneous anthrax.
Given the importance of studying the symbiotic relationship between microbes and insects in disease transmission, researchers have explored various strategies to combat house fly infestations. This study emphasizes the need for effective molecular diagnosis techniques to identify and control Musca domestica populations, thereby mitigating the risks associated with vector-borne diseases. By understanding the biology and behavior of house flies, it becomes possible to implement targeted measures to reduce their impact on public health and hygiene.

Keywords: house fly, molecular diagnosis, Musca domestica, vector-borne diseases, pathogen transmission, insect symbiosis, disease control.

- Introduction

The house fly (Musca domestica L. (Diptera: Muscidae) is one of the most common and widespread types of flies in the world, as it appeared with the beginning of human life (2015, Hussein and John, 2014; Ommi et al), and it is considered one of the main household, veterinary and medical pests And it plays as a vector for many pathogens for humans, poultry, and livestock (Tsagaan et al., 2015). The house fly, Musca domestica, is one of the insects that adhered to humans and coexisted with them wherever they are. House flies are among the most numerous and widespread insect species in most parts of the world, especially indoors. The housefly is found in villages and cities, especially in polluted areas and waste dumping sites, on human food and excrement, and is also found on animal dung and decomposing organic matter, as well as in kitchens and restaurants (Nylon et al, 2002). Thus, it causes disease epidemics by transmitting many pathogens such as typhoid, cholera, dysentery, paratyphoid, and diarrhea. It

(28 ± 2) C and relative humidity (70 ± 5)% until a colony is obtained. of the insect for the purpose of the study, and then the medium on which the eggs are to be laid was prepared .

1. Ground chicken droppings (600 grams)
2. yeast (22 grams)
3. . wort (200 grams)
4. Sodium hydroxide 5 gauge (20) ml
5. 1200ml of distilled water

These components were mixed well, then placed in glass dishes with a capacity of 1000 ml, and then placed in breeding cages in order for flies to lay eggs to produce new generations of the insect for the purpose of the study.), the samples were transferred to the insect laboratory, also transmits some intestinal worms, smallpox, and cutaneous anthrax.

(Wilson, 2016 & Jabbar, Burns, 2017), and due to the prominent role of microbes associated with insects in transmitting diseases and spoiling food, many researchers have studied the symbiotic relationship between microbes and different types of insects (Abdul Samad, 2004). Therefore, there was a need to search for ways to combat this insect.

Materials and methods

Insect collection

Adults of houseflies were collected from Al-Alam district in Salah Al-Din Governorate during the period from

Company	Origin	Apparatus	No

Department of Life Sciences / College of Education for Girls, and all information was recorded on the place, date, method of collection, and method of treatment, after which they were sent to the molecular diagnosis in the DNA Glow laboratories in Baghdad .

laboratory tests

1/9/2022 to 1//11 2022. The insect was fed a diet consisting of milk and sugar in a ratio of (1:1) dissolved in 20 ml of distilled water and placed in dishes. Petri and put a piece of cotton in the middle of the dish in order to prevent the insect from sticking (Hazfez, 1949; Abdel-Fattah, 1989), and put the breeding cage in appropriate laboratory conditions with a temperature

	Material	Cat #	company
1	Agarose	8100.11	Conda / USA
2	Red safe staining souluion	21141	Intron / Korea
3	6X Loading dye	21161	Intron / Korea
4	Ladder 1000 plus bp	24075	Intron / Korea
5	Pre mix pcr	25025	Intron / Korea
6	TBE buffer 10 X	IBS.BT004	Conda / USA
7	Primer	---	Integrated DNA technologies /USA
8	G- spin DNA extraction kit	17045	intron biotechnology/Korea

	Italy	AURA TM PCR Cabinet	**1**
Bio San	Germany	Microspin 12, High-speed Mini-centrifuge	**2**
Digsystem	Germany	V-1 plus, Personal Vortex for tubes	**3**
Bio San	Germany	Bio TDB-100, Dry block thermostatbuilt	**4**
	Germany	Biopette Variable Volume 2-20 µl 0.5-10 µl 20-200 µl 10-100 µl 100-1000 µl	**5**
	Chain	Mini-Power Supply 300V, 2200V	**6**
Labnet	USA	MultiGeneOptiMax Gradient Thermal Cycler	**7**
CBS, Scientific	USA	Electrophoreses	**8**
Labnet	USA	Document system	**9**
Vilber lourmat	Farance	UV.transmission	**10**
Biosan	Lativa	Microspin	**11**
Biosan	Lative	Combi-spin	**12**
Kernpfb	Germany	Balance	**13**
Jrad	China	Incubation	**14**
Gosonic	China	Microwave	**15**
	China	Water distilater	**16**

DNA extraction

G- spin DNA extraction kit , intron bio echnology , cat.no. 17045

Kit Contents

Label	Contents 50 Columns
Buffer CL	25 ml
Buffer BL	25 ml
Buffer WA	40 ml
Buffer WB	10 ml
Buffer CE	20 ml
Spin Column / Collection Tube	50 ea
RNase A (Lyophilized powder)	3 mg x 1 vial
Proteinase K (Lyophilized powder)	22 g x 1 vial

Collection Tube) without wetting the rim, close the cap, and centrifuge at 13,000 rpm for 1 min. Discard the filtrate and place the Spin Column in a 2 ml Collection Tube (reuse).

8- Add 700 µl of Buffer WA to the Spin Column without wetting the rim, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and reuse the Collection Tube.

9- Add 700 µl of Buffer WB to the Spin Column without wetting the rim, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and place the Column into a 2.0 ml Collection Tube (reuse), Then again centrifuge for additionally 1 min to dry the membrane. Discard the flow-through and Collection Tube altogether.

10- Place the Spin Column into a new 1.5 ml tube (not supplied), and 30 - 100 µl of Buffer CE directly onto the membrane. Incubate for 1 min at room temperature and then centrifuge for 1 min at 13,000 rpm to elute.

Agarose gel electrophoresis of DNA

Electrophoresis has been done to determine DNA pieces after the process of

Protocol

1- Measure 25 mg of ground tissue sample, and then transfer into 1.5 ml tube using a spatula .

2- Add 200 µl Buffer CL, 20 µl Proteinase K and 5 µl RNase A Solution into sample tube and mix by vortexing vigorously.

3- Incubate the lysate at 56°C (preheated heat block or water bath) for 10 ~ 30 min.

4- After lysis completely, add 200 µl of Buffer BL into upper sample tube and mix thoroughly. Then incubate the mixture at 70°C for 5min.

5- Centrifuge the sample tube at 13,000 rpm for 5 min to remove un-lysed tissue particles. Then carefully transfer 350 ~ 400 µl of the supernatant into a new 1.5 ml tube (not provided).

6- Add 200 µl of absolute ethanol into the lysate, and mix well by gently inverting 5 - 6 times or by pipetting. DO NOT vortex. After mixing, briefly centrifuge the 1.5 ml tube to remove drops from inside of the lid**.**

7- Carefully apply the mixture from step 6 to the Spin Column (in a 2 ml

for detecting nucleic acid in agarose gels. It emits green fluorescence when bound to DNA or RNA. This new stain has two fluorescence excitation maxima when bound to nucleic acid, one centered at 309nm and another at 419nm. In addition, it has one visible excitation at 514nm. The fluorescence emission of Red Safe bound to DNA is centered at 537nm. RedSafe Nucleic Acid Staining Solution (20,000x) is as sensitive as EtBr. The staining protocol for Red Safe Nucleic Acid Staining Solution (20,000x) is similar to that for EtBr. Compared toEtBr, known as astrong mutagen, Red Safe Nucleic Acid Staining Solution (20,000x) causes much fewer mutations in the Ames test. In addition, Res Safe Nucleic Acid Staining Solution (20,000x) has an negative result in mouse marrow chromophilous erythrocyte micronucleus test and mouse spermary spermatocyte chromosomal aberration test. So it is wise to choose RedSafe Nucleic acid Stainig Solution (20,000x) in stead of EtBr for detecting nucleic acid in agarose gels. (Cat. No. 21141)

SiZer DNA Markers (intron)

extraction or to detect the result of the interaction of PCR during the presence of the standard DNA to distinguish the bundle size of the outcome of the interaction of PCR on the Agarose gel.

Prepare of the Agarose gel

According to Sambrook *et al* (1989), the agarose gel has been made in 1.5% condensation by melting 1.5 g of agarose in 100 ml of previously made TBE buffer. Agarose has been heated to boil then left to cool down at (45-50°C). The gel has been poured in the pour plate in which the plate of agarose support has been prepared after fixing the comb to make holes that would hold the samples. The gel has been poured gently not to make air bubbles and left 30 minutes to cool down. The comb has been removed gently of the solid agarose. The plate has been fixed to its stand in the Electrophoresis horizontal unit represented by the tank used in the Electrophoresis. The tank has been filled with TBE buffer in which it covers the gel surface.

Preparation of sample

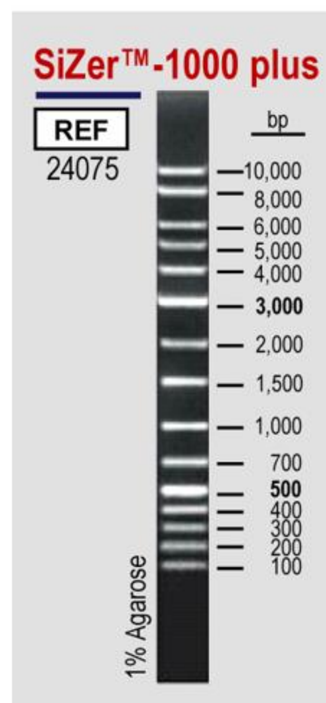
3 µl of the processor loading buffer (Intron / Korea) has been mixed with 5 µl of the supposed DNA to be electrophoresis (loading dye), after the mixing process, the process of loading is now to the holes of the gel. An Electric current of 7 v\c2 has been exposed for 1-2 h till the tincture has reached to the other side of the gel. The gel has been tested by a source of the UV with 336 nm after put the gel in pool contain on 30µl Red safe Nucleic acid staining solution and 500 ml from distilled water.

Red safe Nucleic acid staining solution

RedSafe Nucleic Acid Staining Solution (20,000x) is anew and safe nucleic acid stain, an alternative to the traditional ethidium bromide (EtBr) stain

The primers used in the interaction

The primers were lyophilized, they dissolved in the free ddH2O to give a final concentration of 100 pmol/µl as stock solution and keep a stock at -20 to prepare 10 pmol/µl concentration as work primer suspended, 10 µl of the stock solution in 90 µl of the free ddH2O water to reach a final volume 100 µl, was investigated by IDT (Integrated DNA Technologies company, Canada).



1) The specific primer COX of gene

Primer	Sequence	Product size	Ref.
Forward	5'- GGTCAACAAATCATAAAGATATTG - 3'	720 base pair	Folmer et al., 1994
Reverse	5'- TAAACTTCAGGGTGACCAAAAAATCA - 3'		

Biol. Biotechnol. 3 (1994) 294e299.

http://dx.doi.org/10.1371/journal.pone.0013102.

Maxime PCR PreMix kit (i-Taq) 20µlrxn (Cat. No. 25025)

iNtRON's *Maxime* PCR PreMix Kit has not only various kinds of PreMix Kit according to experience purpose, but also a 2X Master mix solution. *Maxime* PCR Pre Mix Kit (*i*-Taq) is the product what is mixed every component: *i*-Taq DNA Polymerase, dNTP mixture, reaction buffer, and so on-in one tube for 1 rxn PCR. This is the product that can get the best result with the most convenience system. The first reason is that it has every components for PCR, so we can do PCR

The optimal condition has identified for (Initial denaturation and annealing) after a work several experiments to gain for this condition, the temperature has changed through the work of (Gradient PCR) for all samples to select the optimal condition, and also changed the concentration for DNA template between (1.5-2µl) where is considered these two factors from important factors in primer annealing with complement.

O. Folmer, M. Black, W. Hoeh, R. Lutz, R. Vrijenhoek, DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates, Mol. Mar.

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	5 min.	1 cycle
2-	Denaturation -2	95°C	45sec	35 cycle
3-	Annealing	58°C	45sec	
4-	Extension-1	72°C	45sec	
5-	Extension -2	72°C	7 min.	1 cycle

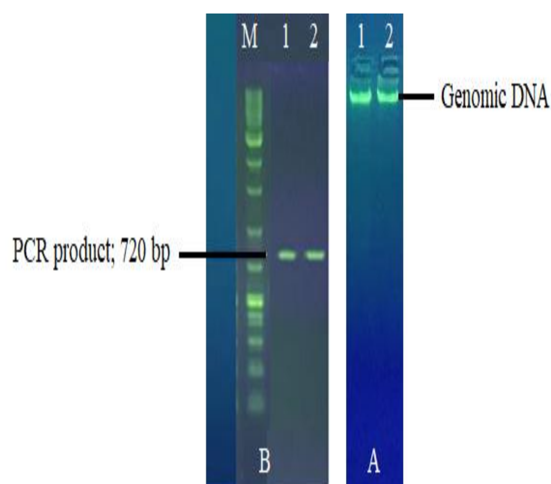


Figure 1(A) : Gel electrophoresis of genomic DNA extraction , 1% agarose gel at 5 vol /cm^2 for 1:15 hour.
(B) : PCR product the band size 720 bp. The product was electrophoresis on 2% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours.
(1 and 2= Musca domestica)

Table (1) Molecular diagnosis of the insect *Musca domestica* based on the percentage matching of the cytochrome c oxidase subunit I (COX1) gene sequences

just add a template DNA, primer set, and D.W.. The second reason is that it has Gel loading buffer to do electrophoresis, so we can do gel loading without any treatment. It is suitable for various sample's experience by fast and simple using method.

Table: The Components of the Maxime PCR PreMix kit (i-Taq)

Material	Concetration
i-Taq DNA Polymerase	5U/ µl
DNTPs	2.5mM
Reaction buffer (10X)	1X
Gel loading buffer	1X

Diagnosis of Gene

Table: Mixture of the specific interaction for diagnosis gene

Components	Concentration
Taq PCR PreMix	5µl
Forward primer	10 picomols/µl (1 µl)
Reverse primer	10 picomols/µl (1 µl)
DNA	1.5µl
Distill water	16.5 µl
Final volume	25µl

Table: The optimum condition of detection gene

recorded in the World Gene Bank. with the highest similarity of the insects

The world record for the registered breed	The insect strain diagnosed in this study and registered in the International Gene Bank	similarity %	world number	The type and strain of the insect is the most closely related
OP975717.1	Musca domestica isolate Sal-1	100	OM541938.1	Musca domestica isolate TRMdom7 (Turkey)

Information (NCBI), which enters all the nucleotide sequences in the BLAST field to perform alignment, sequence comparison and matching of the genetic sequence of the two insects to be diagnosed and to know its type and sex with the sequences in the database Known and previously diagnosed strains were aligned and matched with the strains registered in the Global Genetic Bank.

Molecular diagnosis of the two insects

Figure (1) shows the electrophoresis of the genomic DNA of the two insects in question. It is clear from the figure that there is one single band for each insect, and this indicates the accuracy of the DNA extraction and its non-disintegration. As for Figure ((2), the electrophoresis of the PCR results using the insect primer is evident from the figure, and it is clear from the figure that there is a single band Each insect has a molecular size of 720 base pairs, which is the standard molecular size that indicates that the PCR results are a microduplication of the COXl gene.

The complete classification of the insect:

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Subclass: Pterygota
Order: Diptera
Family: Muscidae
Genus: *Musca*
Species: M. *domestica* (Linnaeus,1758)

Results and discussion.

Sequencing and Sequence Alignment

Determine the nucleotide sequence of the amplified gene of the COXI gene immediately after obtaining the gene duplication product by sending 25 µl of the PCR product and 100 µl of each primer to the Korean company Macrogen, then the results were compared through a computer program on the Internet (a research tool). A basic website for the nucleotide sequence (BLAST) with the database in the National Center for Biotechnology

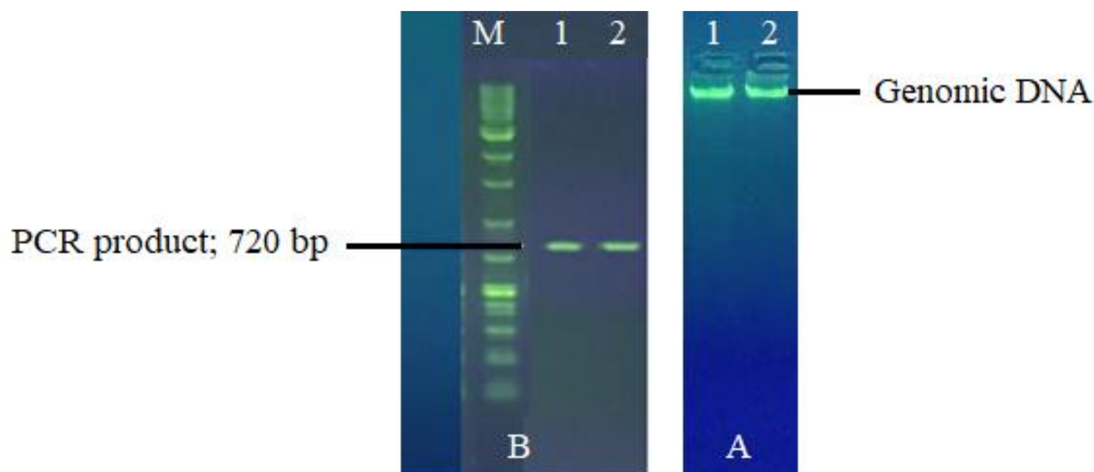


Figure 1(A) : Gel electrophoresis of genomic DNA extraction , 1% agarose gel at 5 vol /cm^2 for 1:15 hour.
(B) : PCR product the band size 720 bp. The product was electrophoresis on 2% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours.
(1 and 2= Musca domestica)

mitochondrial Query ID: OP975717.1
Length: 609

>Sequence ID: Query_53745 (Treated fly)
Length: 490
Range 1: 22 to 490

Score:841 bits(455), Expect:0.0,
Identities:465/469(99%),
Gaps:0/469(0%), Strand: Plus/Plus

Query 1
AGCATGATCTGGTATAGTAGGAACA
TCATTAAGAATTTTAATTCGAGCTG
AATTAGGACA 60
||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||
Sbjct 22
AGCATGATCTGGTATAGTAGCAACA
TCATTAAGAATTTTAATTCGAGCTG
AATTAGGACA 81

Query 61
CCCTGGTGCTCTAATTGGAGACGAT
CAAATTTATAATGTTATTGTAACAG
CTCATGCTTT 120
|||||||||||||||||||||||||||||||||||||||||||||||||||||| |||||
Sbjct 82
CCCTGGTGCTCTAATTGGAGACGAT
CAAATTTATAATGTTATTGTAACAG
CTCAAGCTTT 141

Determine the sequences of nitrogenous bases

The sequences of the nitrogenous bases of the PCR product of the gene (cytochrome oxidase secondary unit 1 - in mitochondria) of the insect to be diagnosed for the PCR reaction were determined.
Sequences obtained from the Korean company Bioneer were analyzed using the National Center Biotechnology Information (NCBI) website within the Blast sub-window, then the secondary sub-window was selected as Nucleotide blast.
The insect was recorded in the global gene bank, GenBank, with the global numbers listed in Table No. (1).

Query: *Musca domestica* isolate Sal-1 (Untreated fly) cytochrome c oxidase subunit I (COX1) gene, partial cds;

TAGCAGGAATTTCTTCAATTTTAGG
AGCAGTAAA 420
||||||||||||||||||||||||||||||||||||||||||||||| |||||||||||
Sbjct 382
TGATTTAGCTATTTTCTCTCTTCACT
TAGCAGGAATTTCTTCAATTTAAGG
AGCAGTAAA 441

Query 421
TTTTATTACAACTGTTATTAACATAC
GATCAACAGGAATTACATTCGAT
469
||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 442
TTTTATTACAACTGTTATTAACATAC
GATCAACAGGAATTACATTCGAT
490

Figure (1) Alignment of the nucleotide sequence of the cytochrome c oxidase subunit I (COX1) gene of the insect *Musca domestica* isolate Sal-1 (untreated) with the same treated insect (match 99%).

Query: Musca domestica isolate Sal-1 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial Query ID: OP975717.1 Length: 609
>Musca domestica isolate TRMdom7 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial
Sequence ID: OM541938.1 Length: 658
Range 1: 22 to 630
Score:1122 bits(607), Expect:0.0,
Identities:609/609(100%),
Gaps:0/609(0%), Strand: Plus/Plus

Query 1
AGCATGATCTGGTATAGTAGGAACA
TCATTAAGAATTTTAATTCGAGCTG
AATTAGGACA 60
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 22
AGCATGATCTGGTATAGTAGGAACA
TCATTAAGAATTTTAATTCGAGCTG
AATTAGGACA 81

Query 121
TATTATAATTTTCTTTATAGTAATAC
CTATTATAATTGGAGGGTTTGGAAA
TTGATTAGT 180
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 142
TATTATAATTTTCTTTATAGTAATAC
CTATTATAATTGGAGGGTTTGGAAA
TTGATTAGT 201

Query 181
TCCTTTAATATTAGGAGCTCCAGAT
ATAGCATTCCCTCRAATGAATAATA
TAAGTTTTTG 240
|||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||
Sbjct 202
TCCTTTAATATTAGGAGCTCGAGAT
ATAGCATTCCCTCRAATGAATAATA
TAAGTTTTTG 261

Query 241
ATTATTACCTCCTGCATTAACTCTAT
TATTAGTAAGAAGTATAGTAGAAAA
GGGAGCTGG 300
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 262
ATTATTACCTCCTGCATTAACTCTAT
TATTAGTAAGAAGTATAGTAGAAAA
GGGAGCTGG 321

Query 301
AACAGGTTGAACTGTTTATCCACCT
TTATCATCAATTATTGCTCATGGTG
GAGCTTCAGT 360
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 322
AACAGGTTGAACTGTTTATCCACCT
TTATCATCAATTATTGCTCATGGTG
GAGCTTCAGT 381

Query 361
TGATTTAGCTATTTTCTCTCTTCACT

TTATCATCAATTATTGCTCATGGTG
GAGCTTCAGT 360
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 322
AACAGGTTGAACTGTTTATCCACCT
TTATCATCAATTATTGCTCATGGTG
GAGCTTCAGT 381

Query 361
TGATTTAGCTATTTTCTCTCTTCACT
TAGCAGGAATTTCTTCAATTTTAGG
AGCAGTAAA 420
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 382
TGATTTAGCTATTTTCTCTCTTCACT
TAGCAGGAATTTCTTCAATTTTAGG
AGCAGTAAA 441

Query 421
TTTTATTACAACTGTTATTAACATAC
GATCAACAGGAATTACATTCGATCG
AATGCCTTT 480
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 442
TTTTATTACAACTGTTATTAACATAC
GATCAACAGGAATTACATTCGATCG
AATGCCTTT 501

Query 481
ATTTGTTTGATCAGTTGTAATTACTG
CattattattattattatCTCTTCCTGTTCTTGC
540
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 502
ATTTGTTTGATCAGTTGTAATTACTG
CATTATTATTATTATTATCTCTTCCT
GTTCTTGC 561

Query 541
TGGAGCTATTACTATACTATTAACT
GATCGAAATTTAAATACTTCATTTTT
TGACCCAGC 600
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Query 61
CCCTGGTGCTCTAATTGGAGACGAT
CAAATTTATAATGTTATTGTAACAG
CTCATGCTTT 120
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 82
CCCTGGTGCTCTAATTGGAGACGAT
CAAATTTATAATGTTATTGTAACAG
CTCATGCTTT 141

Query 121
TATTATAATTTTCTTTATAGTAATAC
CTATTATAATTGGAGGGTTTGGAAA
TTGATTAGT 180
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 142
TATTATAATTTTCTTTATAGTAATAC
CTATTATAATTGGAGGGTTTGGAAA
TTGATTAGT 201

Query 181
TCCTTTAATATTAGGAGCTCCAGAT
ATAGCATTCCCTCRAATGAATAATA
TAAGTTTTTG 240
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 202
TCCTTTAATATTAGGAGCTCCAGAT
ATAGCATTCCCTCRAATGAATAATA
TAAGTTTTTG 261

Query 241
ATTATTACCTCCTGCATTAACTCTAT
TATTAGTAAGAAGTATAGTAGAAAA
GGGAGCTGG 300
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 262
ATTATTACCTCCTGCATTAACTCTAT
TATTAGTAAGAAGTATAGTAGAAAA
GGGAGCTGG 321

Query 301
AACAGGTTGAACTGTTTATCCACCT

Riyadh, Saudi Arabia, first edition, pages 12-13.
3. Abdulazeez, M. I., Hamdi, A. Q., Mohammed, H. Y., & Ahmed, M. (2020). Dental trauma of permanent incisor teeth in children/Kirkuk city. studies, 22, 23.
4. **Alhudaib. K (1, 2), Ajlan.A.(1) and Faleiro.J,2,3.(2016-2017).** Genetic Diversity among *Rhynchophorus ferrugineus* Populations from Saudi Arabia and India. Scientific Journal of King Faisal University (Basic and Applied Sciences) Vol.19 (1) June 2018 (1439H).
5. **Alhussaini, M. S.; Moslem, M. A.; Alghonaim, M. I.; Al-Ghanayem, A. A.; AL-Yahya, A. A. I.; Hefny, H. M. and Saadabi, A. M. (2016)** Characterization of Cladosporium species by internal transcribed spacerPCR and microsatellites-PCR. Pakistan Journal of Biological Sciences, 7: 143-157.
6. Ali, A. H., Ahmed, H. S., Jawad, A. S., & Mustafa, M. A. (2021). Endorphin: function and mechanism of action. Sci Arch, 2, 9-13.
7. **Ali, S. H., Armeet, H. S., Mustafa, M. A., & Ahmed, M. T. (2022, November).** Complete blood count for COVID-19 patients based on age and gender. In AIP Conference Proceedings (Vol. 2394, No. 1, p. 020044). AIP Publishing LLC.
8. **Al-Yaqoubi, Muhammad Salih، Hilal, Saadi Muhammad، Announ, Muhammad Rida. (2017).** Molecular diagnosis of the genus *Cotesia spp* in Al-Diwaniyah city using polymerase chain reaction (PCR) technique. Al-Qadisiyah Journal for Pure Sciences, Volume 22, Issue 3.

Sbjct 562 TGGAGCTATTACTATACTATTAACT GATCGAAATTTAAATACTTCATTTTT TGACCCAGC 621

Query 601 TGGAGGAGG 609
|||||||||
Sbjct 622 TGGAGGAGG 630

Figure (2) Alignment of the nucleotide sequence of the cytochrome c oxidase subunit I (COX1) gene of the insect Musca domestica isolate Sal-1 with the most closely matched insect Musca domestica isolate TRMdom7 (match 100%) .

Molecular diagnosis is one of the accurate means in diagnosing living organisms, especially insects, as the COX gene is one of the important genes whose genetic sequence is fixed because it is a gene that encodes for the production of the enzyme cytochrome oxidase from mitochondria, and its presence in all types of insects made it an accurate tool for diagnosis by comparing its nucleotide sequences. Gene identification of insects by several studies (Al-Yaqoubi *et al.*, 2017; Giantsis *et al.*, 2017; Alhudaib *et al.*, 2018) .

References

1. **Abdel Fattah, Nihad Mostafa (1989).** The effect of mutual constant temperatures and relative humidity on the growth, survival and reproduction of the housefly (*Musca domestica* L. (Diptera: Muscidae), Master Thesis, College of Science - University of Baghdad, Iraq.
2. **Abdel Samad, Mohamed Kamel (2004).** Scientific miracles in Islam and the Sunnah of the Prophet, disease and medicine in flies. Dar Al-Qibla,

15. **Govindarajan, S., Mustafa, M. A., Kiyosov, S., Duong, N. D., Raju, M. N., & Gola, K. K. (2023).** An optimization based feature extraction and machine learning techniques for named entity identification. Optik, 272, 170348.
16. **Hazfez , M .(1949).** A simple method for breeding the house fly *Musca domestica* (Diptera : Muscidae) . in the laboratory . Bull . Entomol . Res . 39 :385- 386 .
17. **Hegde , k.; C.M. kalleshwaraswamy and V. Venkataravanappa .2019.** Evaluation of insecticides against aphid, *Myzus persicae* in potato and their cost economics. Pest Management in Horticultural Ecosystems. 25(2): 156-164.
18. **Hussein, S , and John , L.(2014).** Housefly, Musca domestica Linnaeus (Insecta: Diptera: Muscidae). Inst. Food Agric. Scie.,47:1–7
19. **Jabbar, Aseel Karim (2017).** Studying the role of the house fly, *Musca domestica,* in the transmission of Giardia lamblia and Entamoeba histolytica parasites that cause diarrhea in Babylon province. Karbala University Journal, Volume Fifteen, Issue One, Scientific.
20. Kadham, S. M., Mustafa, M. A., Abbass, N. K., & Karupusamy, S. (2022). IoT and artificial intelligence–based fuzzy-integral N-transform for sustainable groundwater management. Applied Geomatics, 1-8.
21. **Karupusamy, S., Mustafa, M. A., Jos, B. M., Dahiya, P., Bhardwaj, R., Kanani, P., & Kumar, A. (2023).** Torque control-based induction motor speed control using Anticipating Power Impulse Technique. The

9. Badi, S., Hamed, A., Abualama, M., Mustafa, M., Abdulraheem, M., & Yousef, B. (2021). Knowledge, attitude, and practice of sudanese pharmacists toward COVID-19 in Khartoum State, Sudan: An online-based cross-sectional study. Libyan International Medical University Journal, 6(01), 19-26.
10. **Basha, K.; P. N. Ewang and E. N. Okoyo .2017**. Factors affecting productivity of smallholder potato growers in Bore district, Guji Zone, Oromia Regional State, Ethiopia. Developing Country Studies. 7(9): 18-26.
11. **FAOSTAT .2020**. Available at http://www.fao.org/faostat/ en/ #compare (accessed on 14 January 2020).
12. **Frechette, B.; M. Bejan ; É. Lucas ; P. Giordanengo and C. Vincent .2010.** Resistance of wild Solanum accessions to aphids and other potato pests in Quebec field conditions. Journal of Insect Science. 10(1): 1-16.
13. **Geiser DM, Jimenez-Gasco MD, Kang SC, Makalowska I, Veeraraghavan N, Ward TJ, Zhang N, Kuldau GA, O"Donnell K .(2004).** FU.S.A.RIUM-ID v. 1.0: A . DNA sequence database for identifying Fusarium. European Journal of Plant Pathology 110:473 -479.
14. **Giantsis, I. A. ; Chaskopoulou, A. and Bon, M. C. (2017).** Direct Multiplex PCR (dmPCR) for the Identification of Six Phlebotomine sand fly species (Diptera: Psychodidae), including major leishmania vectors of the mediterranean. Journal of economic Entmology, 110 (1), 172-182.

and New Zealand, Epidemiol. Infect.,128(3): 383-390.

28. **Ommi, D., Hashemian, S., Tajbakhsh, E., and Khamesipour F.(2015).**Molecular detection and antimicrobial resistance of Aeromonas from houseflies (Musca domestica) in Iran. Revista MVZ Córdoba.,20(Suppl):4929–36.
29. **Sambrook, J. ; Fritschi, E.F. and Maniatis, T. (1989**). Molecular cloning, a laboratory manual 3rd (ed.). Cold spring Harbor laboratory press, New York.
30. **Sudha, I., Mustafa, M. A., Suguna, R., Karupusamy, S., Ammisetty, V., Shavkatovich, S. N., ... & Kanani, P. (2023).** Pulse jamming attack detection using swarm intelligence in wireless sensor networks. Optik, 272, 170251.
31. **Tsagaan, A., Kanuka, I., and Okado, K.(2015).** Study of pathogenic bacteria detected in fly samples using universal primer-multiplex PCR. Mongolian J Agricultural Scie.,15(2):27–32.
32. **West , L. S. (1951).** The house fly , its Natural History . Medical important , and Control .Comstock Publ Co . Ithaca , New York 584 pp.
33. **Wijesinha-Bettoni, R. and B. Mouillé .2019.** The contribution of potatoes to global food security, nutrition and healthy diets. American Journal of Potato Research. 96(2): 139-149.
34. **Wilson, B.H, Burns, E.C. (2016).** Induction of Resistance to Bacillus thuringiensis in laboratory strain of house flies . Journal of Economic Entomology. 6:1747-1748.

International Journal of Advanced Manufacturing Technology, 1-9.

22. **Keith A. S., Robert A. S., Jeremy R. d., Jos H., C. Andre, Jean-Marc M.,Gerry L., and Paul D. N. H. .(2007).**Prospects for fungus identification using CO1 DNA barcodes, with Penicillium as a test case. PNAS . 104(10): 3901–3906.
23. **Kreuze, J. F.; J. A. C. Souza-Dias ; A. Jeevalatha ; A. R. Figueira ; J. P. T. Valkonen and R. A. C. Jones .2020.** Viral diseases in potato. In : Campos H., Ortiz O. (eds). The potato crop Springer. 389-430.
24. **Mahdi, E. M., & Mustafa, M. A. (2022).** Effect of different concentrations of extract of Urtica dioica and Cladosporium cladosporiodes on Tribolium castaneum or: Coleoptera after 24-48 hours of exposure in Samarra City/Iraq. HIV Nursing, 22(2), 3207-3210.
25. **Mujica , N. and J. Kroschel .2013.** Pest intensity-crop loss relationships for the leafminer fly *Liriomyza huidobrensis* (Blanchard) in different potato (*Solanum tuberosum* L.) varieties. Crop Protection. 47: 6-16**.**
26. **Mustafa, M. A., Kadham, S. M., Abbass, N. K., Karupusamy, S., Jasim, H. Y., Alreda, B. A., ... & Ahmed, M. T. (2023).** A novel fuzzy M-transform technique for sustainable ground water level prediction. Applied Geomatics, 1-7.
27. **Nylon , G.; Dunstan, F. ; Palmer, S.; Ersson, Y.; Bager, F.; Cowden, J.; Rel, G.; Galloway,Y.; Kapper,G.; Megaud, F.; Mol back,K.; Peterson, R. and Runntu, P.(2002),** The seasonal distribution of campylobacter infection in nine European countries