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Original Article

Detecting genetic similarity and genetic divergence between the male and female flour beetle *Tribolium confusum* and the male and female puzzling flour beetle *Tribolium castaneum* through the use of molecular primers within the RAPD technique

Salim Younis Ahmed [a, *], Ibrahim Kh. Ibrahim [a]

[a] Department of Biology, College of Education for Pure Sciences, University of Mosul, Mosul 41002, Iraq.

Corresponding: Salim Younis Ahmed, Email: salem.22esp14@student.uomosul.ed

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ABSTRACT

The two species, the similar flour beetle Tribolium confusum and the red flour beetle Tribolium castaneum, belong to the Coleoptera order, the Tenebrionidae family. They are similar in appearance and it is difficult to distinguish them. In this study, 12 random primers were used, 6 of which were used in the RAPD method. The DNA was extracted, and then the purity of the samples used was confirmed by using the Bio Drop device. The purity of the samples used ranged between values 1.8-2 nanometers.Use a 1-kb DNA ladder. RAPD-PCR analysis yielded 64 molecular bands divided into 49 unique bands and 15 divergent bands. The random primer OPE-19 gave the highest number of total packets, about 19 packets. The random primer OPL-07-K gave the lowest number of total bands of 5 bands. The random primer OPA-11 gave the highest number of unique packets of 13 packets. The random primer OPL-07-K gave the lowest number of unique bands of 3. Random primer OPA-15 gave the highest number of divergent bands of 8. Random primer OPE-19 gave the highest efficiency value of 29.687%. The random primer OPL-07-K and OPA-15 gave the lowest efficiency value of 7.812%. The OPE-19 random primer gave the highest estimated discriminative value of 53.33%. The random primer OPA-15, OPA-11, and OPA-13 gave the lowest discriminatory ability of 6.66%. To find out the genetic matrix and the dendrogram, the MVSP program was used, and the genetic convergence matrix showed the presence of genetic differences between the studied samples. Genetic difference values were extracted by using a mathematical equation.

Keywords: *Tribolium castaneum*, *Tribolium confusum,* RAPD, PCR, Genetics Similarity

Introduction

Flour beetles belong to the insects of stored products, as they have a high ability to reproduce and infect stored materials when conditions are favourable. Losses of stored materials infected with this insect reach about 15%. In addition to contaminating the rest of the materials and becoming unfit for human and animal use due to the unacceptable odors that are emitted as a result of the infection, it also affects public health through defensive secretions, causing strong irritation and itching of the skin and may cause severe disturbances to the respiratory system [1]. These pests affect stored goods by increasing their temperature and humidity,

encouraging microorganisms such as fungi to grow in them and causing seeds to germinate [2]. and then causing major economic losses in these goods because of their contamination and reducing their quality and quantity [3].

Tribolium castaneum and *Tribolium confusum*

These two species belong to the flour beetles of the order Coleoptera, and they are economically important pests of stored products. They have the ability to find and infect scattered stored goods, as the infestation can lead to a rapid spread of damage throughout the stored materials [4,5,6], They have the ability to destroy stored grains in all countries of the Earth and are the main pests in various countries [7,8]. These two insects can live in most climatic conditions [9] and infect flour and its products and are considered secondary pests in Stored products [10] Because they are secondary pests, they only infect damaged grains, leading to their destruction [11], and flour is one of the most favorite foods for the red beetle and the similar beetle [12]. These two pests cause significant losses and decline in flour quality [13,14]. They are found in mills, bakeries, pet stores, and storage units [15].

Molecular genetics

It is one of the important branches of biology that studies living organisms at the molecular level for the purpose of identifying their components. A single gene either contains deoxyribonucleic acid, which is found in eukaryotic organisms, or it contains ribonucleic acid It is found in organisms with primitive nuclei. The functions of the DNA molecule differ depending on its location in the cell, and the molecule's size is found in the nucleolus region within the nucleus and contains all the important genetic information and requirements for organisms to grow and develop [16].

Polymerase chain reaction (PCR)

It is one of the techniques of molecular biology through which DNA sequences can be copied [17], and it serves as a standard tool in the fields of biology and various fields [18]. This technique is characterized by accuracy and sensitivity, which makes it essential in study laboratories. Molecular [19], and the oligomerization reaction technology is one of the important and necessary technologies in molecular biology laboratories It depends on the manufacture of large numbers of DNA pieces in the laboratory (in vitro) and through a Thermo cucler device, which performs the process of denaturation of the DNA molecule, and its working conditions range from a temperature of 95-98 degrees Celsius, which causes it to disintegrate into two single strands, after which the enzyme is added. Polymerase Taq and adding other materials to it, resulting in the manufacture of large numbers of original copies [20].

RAPD-PCR

This reaction works to replicate DNA segments and relies on short random primers of about 10 nitrogen bases [21]. These primers find complementary sites on the DNA strand, and the product of replication consists of (2-10) segments of different lengths and numbers. The presence of replication fragments is detected by transferring the reaction product onto an agarose gel and exposing it to ultraviolet light, which shows the DNA replication fragment in the form of bands of varying lengths. Using several primers, it ensures great efficiency in examining the target DNA fragment to estimate the total variation between models. This method has been used to find genetic variations between different creatures belonging to the same species and even other species [22].

RAPD is one of the most widely used techniques for molecular and taxonomic study in entomology and is a widely used technique [23,24]. This technique uses random primers that bind to deoxyribonucleic acid, as amplified random DNA is simple, cost-effective, and can be performed in the laboratory [25]. Nano-gram quantities of genomic DNA are amplified at a low fusion temperature, which becomes possible to separate them and stain them with the required dye, as species that are genetically close to each other show similar bands, while species that are different and far apart from each other show different banding patterns. In this way, it is possible to obtain different types of DNA [26]. This technology relies on the use of primers and a single primer instead of using cutting enzymes, as is the case in the RFLP technology. This technology is easy, quick to perform, and inexpensive compared to other methods. It can also be used with any genome, unlike the RFLP technology, which is used with closely related species. In this method, we need to small amounts of DNA and polymorphisms resulting from this method are larger than those resulting from RFLP technology [27].

Genetic similarity matrix

This matrix is a triangular chart in the form of a table through which genetic similarity values and genetic dimension values are extracted between the types of studied organisms based on DNA packages. The studied samples are distributed in it along both rows and columns. This chart is made using programs and equations, especially for genetic analysis. [28-31].

Dendrogram

Dendrogram is a chart that shows the relationship between genetic groups in terms of the percentage of genetic difference and genetic closeness of the groups on which the study was based. The MVSP program was used to obtain cluster tree diagrams and tables of genetic closeness values by using Computer. [28,29,30].

Due to the great similarity between the two insects in appearance, the study aimed to examine the genetic differences between males and females of the two species using random primers within the RAPD method.

Methods

Table 1. Shows the names of random primers used in the RAPD-PCR technique

Number	Name Primer	Sequences 5 ➡ 3	length	Company and origin
1	OPE-19	ACG GCG TAT G	10	**Bioneer -Korea**
2	OPA-18	AGG TGA CCG T	10	**Bioneer -Korea**
3	OPA-15	TTC CGA ACC C	10	**Macrogen -Korea**
4	OPA-13	CAG CAC CCA C	10	**Macrogen -Korea**
5	OPA-11	CAA TCG CCG T	10	**Macrogen -Korea**
6	OPL-07-K	AGG CGG GAA C	10	**Bioneer -Korea**

This research was conducted in the laboratories of the University of Mosul, including the Embryology and Tissue Laboratory in the Department of Life Sciences in the College of Education for Pure Sciences and the Central Laboratory for Molecular Genetics in the College of Agriculture. Samples of the type *T. castaneum* were collected from the College of Science, University of Mosul, and samples of the type *T. confusum* were collected from the College of Education for Girls. University of Mosul, with 5 samples for males and 5 samples for females for each type. The samples were diagnosed based on distinctive phenotypic characteristics for both types. The DNA of the studied samples was extracted using the Favor Prep Tissue Genomic DNA Extraction Mini Kit 50 preps and following the steps of the animal tissue extraction protocol of Korea Bioneer in Korea. After successfully extracting DNA from each sample, the purity and quality of the DNA of the samples studied were measured using the Biodrop device, which is characterized by accuracy. Purity is measured at a wavelength of 260 nm and a wavelength of 280 nm. The value (1.8-2) is considered the ideal value for DNA purity, and all values . The purity of the samples is as follows.

Table 2. Shows the DNA concentrations and purity values for the samples used in the study

Number	Sample	Purity at wave lengths 260nm/280nm
1	*T . castaneum* Male	**1.858**
2	*T . castaneum* Female	**1. 981**
3	*T. confusum* Male	**1.808**
4	*T. confusum* Female	**1.804**

PCR mixture

The master reaction mixture consists of 11ml of nuclease-free water, 1ml of Primers, and 3ml of DNA and 10 ml of Master Mix and the total sum of the mixture components is 26 ml. These ingredients were mixed using a fine glass pipette tube by withdrawing it several times and then placed in the Vortex shaking device to mix the ingredients together well. Distribute the mixture into 4 sterile tubes of 0.2 ml and mark them according to the type of sample to be studied, at a rate of 5 microlitres of the mixture for each tube. 3 microliters of DNA was added to each sample in its designated tube, then the reaction mixture was distributed on the tubes before adding the DNA to avoid contamination, and the tubes were placed in the thermal polymerase device [32].

Table 3. Shows the thermal stages of the RAPD-PCR reaction, the temperature and time for each stage.

Nomber	Reaction stages	temperature	Time
1	Initial Denaturation	98	**minute6**

2	Denaturation	96	**30 sec**
3	Primer Annealing	35	**30 sec**
4	Primer Extension	72	**minute1**
5	Final Extension	72	**minute6**

Gel Electrophoresis

The gel was prepared by weighing 2 grams of agarose powder using a sensitive electric balance. The weighed amount of agarose was placed inside a 500 ml graduated glass beaker containing a solid capsule. For the purpose of mixing the gel components during heating, 130 ml of TBE Buffer solution, at a concentration of 10X, was added to the glass beaker. It contains agarose powder. The solution was placed in the microwave until it dissolved completely, for two minutes. Leave the gel to cool to a temperature of 40 to 50. Add 5 microliters of Safe stain to the solution. The basin prepared for pouring the gel was prepared, followed by the pouring of the spread gel gently, with complete care, and continuously, to prevent any bubbles from occurring. It was then left to solidify. Plastic combs were placed in the basin to make holes in the gel in which to place the samples. After the gel had hardened, the comb was removed from it, then the gel was gently removed from the pouring basin. With complete care, it was placed in the basin of the Gel Electrophoresis device, which was immersed in a 10X strength TBE Buffer solution. The RAPD-PCR samples were added in their final form into the holes in the gel, taking into account that the sample does not come out of the hole, and the DNA marker prepared from 20 microliters of Free Water solution, ul3 from DNA Ladder, and ul3 from Safe stain were added. The electrodes of the electrical relay device were connected, and the relay process was carried out at 100 volts for a period of 60-80 minutes for random starters after the completion of the relay process. Completing the gel, the gel is transferred to the UV unit to see and read the beams (33).

The percentage efficiency of each starter was calculated using the following equation: Total number of primer packets/total number of packets of all primers x 100. The discriminatory ability of each primer was calculated using the following equation: At primer divergent packets / number of divergent packets for all primers x 100. [34]. Genetic similarity values and cluster dendrograms were extracted using a program Multi Variate Statistical Package (MVSP) by installing the application on the computer. Genetic variation was calculated through the following equation: Genetic difference = 1-genetic similarity [28, 30,31].

Results and Discussion

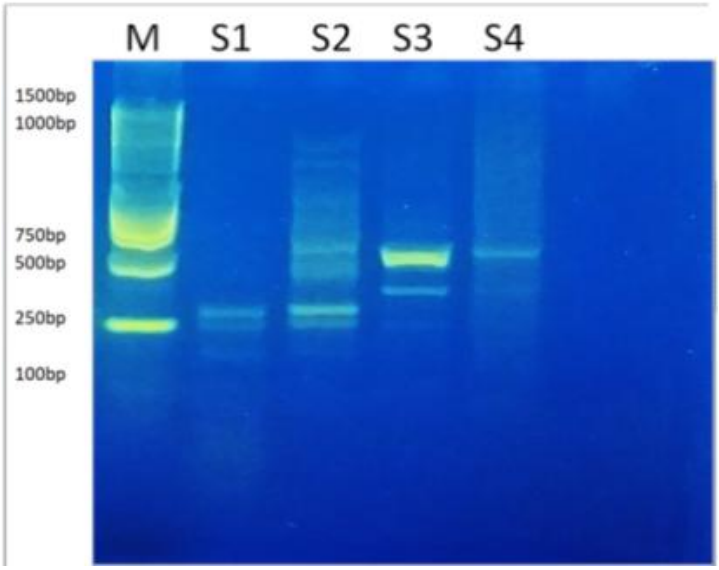

Figure 1. Results of OPE-11 primer analysis on gel electrophoresis

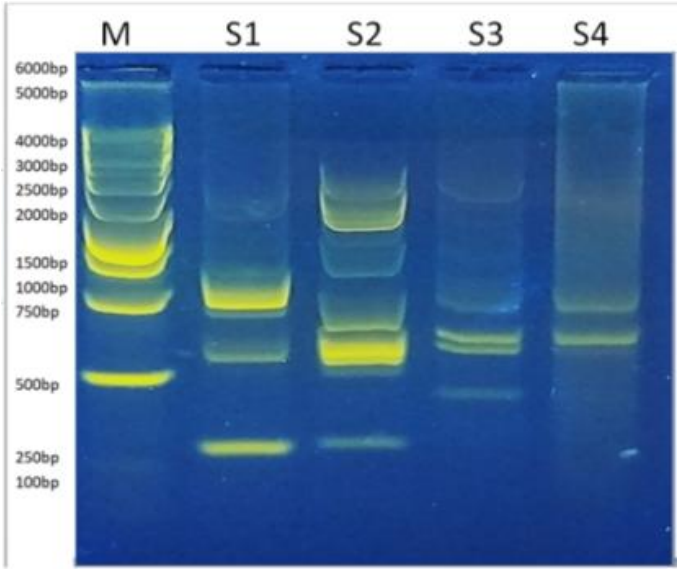

Figure 2. Results of OPE-19 primer analysis on gel electrophoresis

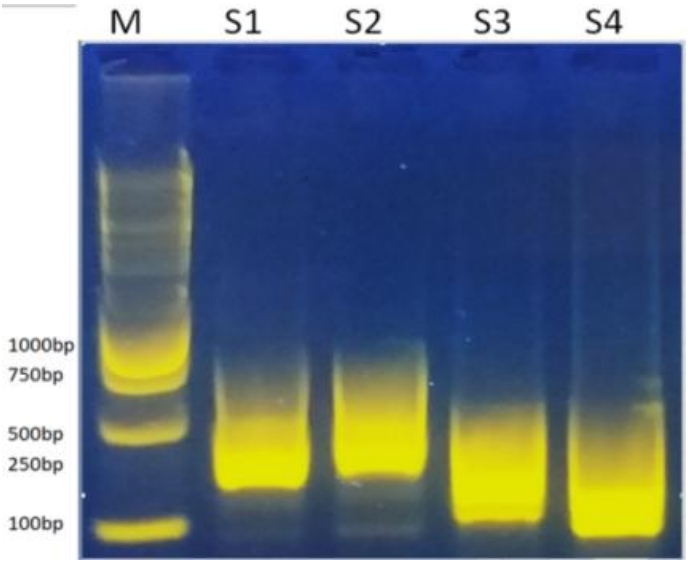

Figure 3. Results of OPE-15 primer analysis on gel electrophoresis

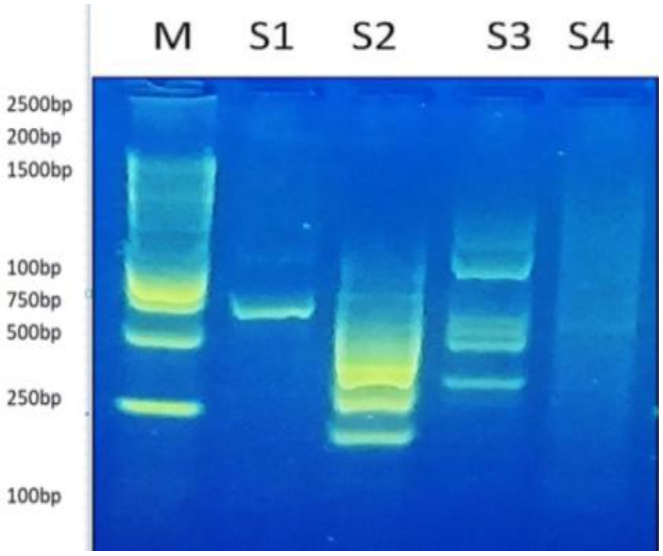

Figure 4. Results of OPE-13 primer analysis on gel electrophoresis

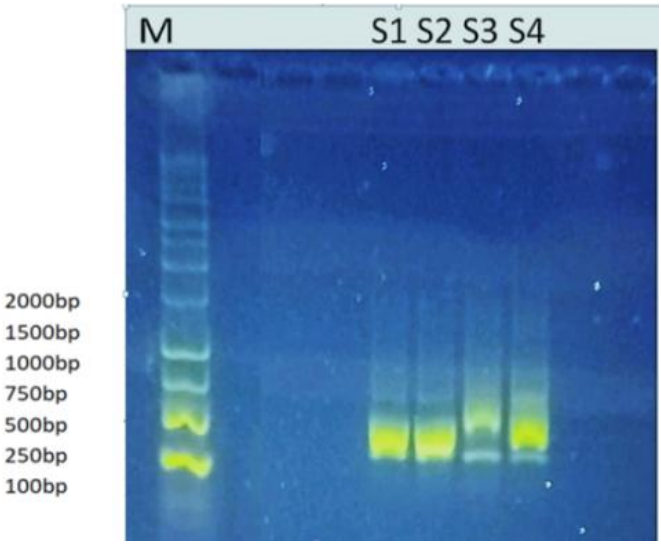

Figure 5. Results of OPE-18 primer analysis on gel electrophoresis

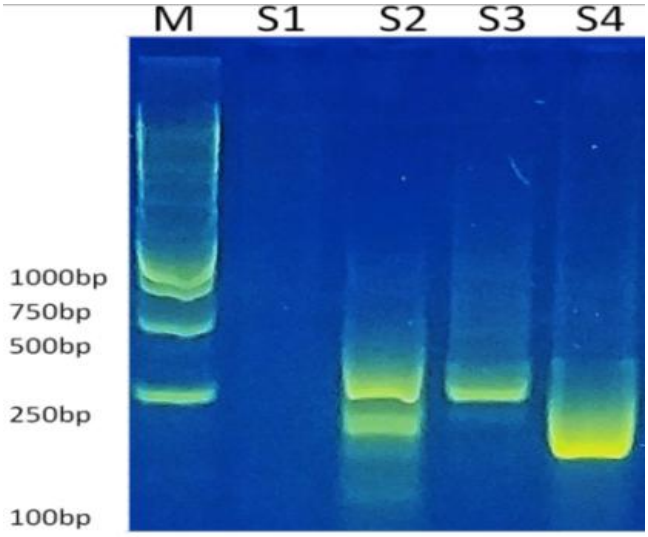

Figure 6. Results of OPL-07-K primer analysis on gel electrophoresis

M=Marker

S1=Male *T . castaneum*

S2=Female *T . castaneum*

S3=Male *T. confusum*

S4=Female *T. Confusum*

With the number of unique and different RAPD packages,

Table 4. Shows the random primers used for the technique and their efficiency and discriminatory ability rates.

Number	Initiator name	Number of unique packages	Number of different packages	Total number of packages	%starter efficiency	Discrimination ability of the primer%
1	OPE-19	11	8	19	%29.687	**%53.33**
2	OPE-11	13	1	14	%21.875	**%6.66**
3	OPE-15	4	1	5	%7.812	**%6.66**

4	OPE-13	12	1	13	%20.312	**%6.66**
5	OPE-18	6	2	8	%12.5	**%13.33**
6	OPL-07-K	3	2	5	%7.812	**%13.33**
7	Grand total	49	15	64	%99.998	**%99.97**

The results of Table 27 showed that the OPE-19 primer had a total number of bands that it showed, which was 19 molecular bands, divided into 11 unique molecular bands and 8 different molecular bands. The efficiency of the primer was about 30.15%, while the discrimination ability of the primer was about 57.41%. While the initiator OPA-11 gave about 14 molecular bands distributed among 13 unique molecular bands and one different molecular band, the efficiency of the initiator was about 22.2%, while the discriminatory ability was about 7.14%. As for the primer OPA-15, it gave 5 molecular bands, one different band and 4 unique molecular bands. The efficiency of the primer was about 7.93%, while the discrimination ability of the primer was about 7.14%. As for the primer OPA-13, it gave about 13 bands divided into 12 unique molecular bands and one different band. The efficiency of the primer was about 20.6%, while the discriminatory ability of the primer was about 7.14%. As for the primer OPA-18, it gave 8 molecular bands divided into 6 unique bands and 2 different bands. The efficiency of the primer was 12.6%, while the discriminatory ability of the primer was about 14.28%, while the primer OPL-07-K gave about 5 molecular bands divided into 2 different bands and 3 unique molecular packages and the initiator efficiency reached 6.34% and the capacity Discrimination of Badi 7.14%. The total number of primer bands included in the analysis was about 64 molecular bands, divided into 49 unique bands and 15 different bands, while RAPD-PCR did not give any primer a single molecular band.

The result of the combined genetic array for the two techniques RAPD-PCR

Table 5. Genetic similarity matrix result for the two techniques RAPD-PCR

Samples	Male *T. castaneum*	Female *T. castaneum*	Male *T. confusum*	Female *T. confusum*
Male *T. castaneum*	1			
Female *T. castaneum*	0.255	1		
Male *T. confusum*	0.161	0.118	1	
Female *T. confusum*	0.133	0.047	0.242	1

Table 6. Results of the combined genetic matrix for genetic variation for the two techniques: RAPD-PCR

Samples	Male *T. castaneum*	Female *T. castaneum*	Male *T. confusum*	Female *T. confusum*
Male *T. castaneum*	0			
Female *T. castaneum*	0.745	0		
Male *T. confusum*	0.839	0.882	0	
Female *T. confusum*	0.867	0.953	0.758	0

Through Tables 30 and 31, the values of the genetic matrix o from the RAPD-PCR analysis showed that the degree of genetic closeness between the male insect *T. castaneum* and the male insect *T. castaneum* and another T. castaneum is 1. Also, the degree of genetic divergence is 0, and of closeness between the male insect *T. castaneum*. *T castaneum* and the female insect T. castaneum is 0.255. In addition,the degree of divergence between them is 0.745 The degree of closeness between the male *T. castaneum* and the male *T. confusum* was 0.162. Also, the degree of divergence between them was 0.838, and the degree of closeness between the male *T. confusum* is 0.162. *T castaneum* and the female *T.confusum* is 0.133 and the degree of divergence is 0.867, while the degree of closeness between the female *T. castaneum* and the female *T. castaneum* is 1.000 and the divergence is 0 ..The degree of closeness between the female *T. castaneum* and the male *T.confusum* is 0.118 with a degree of divergence which is 0.882. TbBtween the female *T. castaneum* and the female *T.confusum*. the closenessis 0.047 and divergence 0.953. The male *T.confusum* closness to the male *T.confusum* is 1.000, and the male *T.confusum* and the female *T.confusum* of is 0.242 with a divergence of 0.758. The female *T.confusum* is close to female *T.confusum* insect at 1,000 with a divergence of 0. Dendritic diagram of RAPD-PCR technology Dendrogram 1 for samples S1 S2 S3 S4 was constructed from RAPD-PCR data analysis using UPGMA and genetic affinity matrix according to Nei & Lis coefficient. Dendrogram of RAPD-PCR technique. As shown in figure 7.

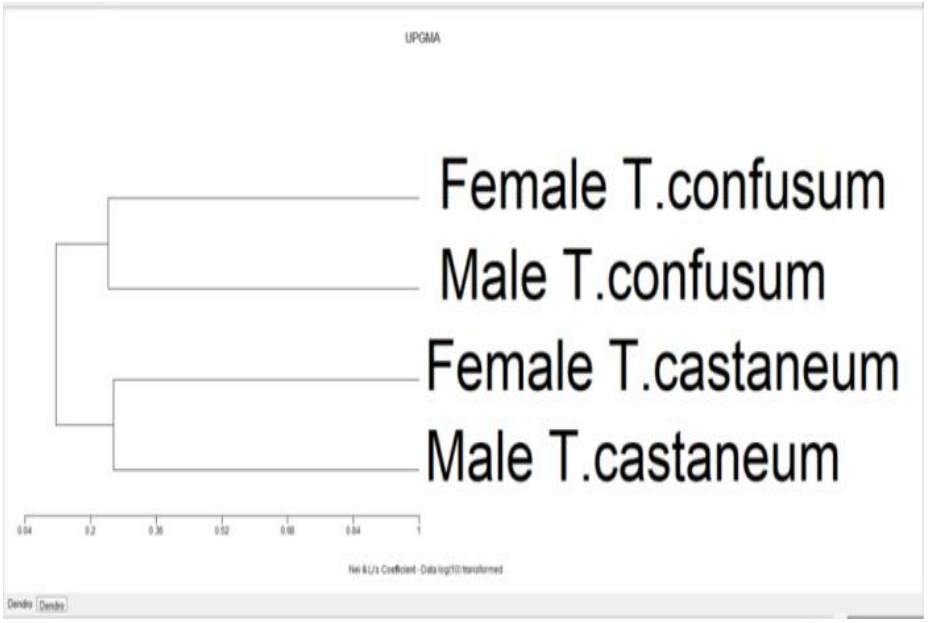

Figure 7. Dendrogram of samples S1 S2 S3 S4 was constructed from RAPD-PCR data analysis using UPGMA and Nei & Lis genetic affinity matrix.

The degree of convergence and divergence between genetic tree groups

Table 7. The degree of convergence and divergence of the genetic tree groups according to the results of RAPD-PCR analysis using the MVSP program.

Cluster number	Sample 1	Sample 2	Degree of similarity	Degree of spacing	Number of samples
1	T.castaneum Male	T.castaneum Female	0.255	0.745	**2**
2	T.confusum Male	T.onfusum Female	0.242	0.758	**2**
3	Cluster 1	Cluster 2	0.116	0.884	**4**

According to the results of Table 36 and the results of the RAPD-PCR analysis using the MVSP program, the degree of similarity of the genetic tree groups for the first cluster, which includes the male insect *T. castaneum* and the female insect *T. castaneum.* is 0.255 and the degree of divergence is 0.745. The second cluster of the diagram includes the male insect *T.confusum* and the female insect *T.confusum* whose similarity is 0.242 and divergence of 0.758. The third cluster of the genetic tree diagram brings together the first cluster and the second cluster of the diagram with a similarity of a genetic analysis between the two groups of the first cluster (a male *T. castaneum* and a female *T. castaneum* insect). The two groups of the second cluster (a male insect *T. confusum* and the female insect *T. confusum*) reaches 0.116 whose divergence is 0.884.

Conclusion

The observed seasonal peak in urinary tract infection (UTI) cases during the summer months indicates a strong correlation between elevated temperatures and increased UTI incidence, particularly among females. *Escherichia coli* was identified as the primary pathogen, followed by *Klebsiella* spp., *Proteus mirabilis*, and *Staphylococcus aureus*. Further research into the mechanisms by which temperature influences UTI prevalence, as well as the dynamics of pathogen distribution, is warranted to improve understanding and inform effective preventive strategies against UTIs.

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Conflict of Interest

The authors declare no competing interests.

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