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Comparative molecular study between two species of flour beetle *Tribolium castaneum* and *Tribolium confusum* by using RAPD-PCR

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ABSTRACT

This research was carried out for molecular comparison between two species of flour beetle T. castaneum and T. confusum in Iraq by using RAPD-PCR technique, because the difficulty of distinguishing between the two types in morphology, In this research used seven primers and genetic population analysis programs for calculates the percentage efficiency and discriminatory ability for each primer , As well as it is used genetic identity for Nei's. The results obtained show that the primers Number 3 , 4 and 6 were given the highest number of amplified polymorphic fragments and the highest percentage of efficiency and the ability of discriminatory it is 7, 7, 20 and 21.2 on the sequentially ., While primer number 2 gave the lowest number of amplified polymorphic fragments and the lowest percentage of efficiency and the ability of discriminatory it is 2 , 2 , 5.71 and 6.06 on the sequentially ., When comparing between two types T. castaneum and T. confusum based on genetic identity it was observed that T. castaneum close genetically to T. confusum, the degree of genetic convergence is 0.111.

Keywords: Tribolium castaneun , Tribolium confusum , RAPD-PCR , IRAQ

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INTRODUCTION

T. castaneum and ***T. confusum*** (Coleoptera: Tenebrionidae) are very similar in morphologically and revers distributed stored crop pests. It was named last because of confusion about his identity because they are very similar (**1**). These are two global brands in flour mills where grain and other dried foods are processed or stored and are classified as major pests in flour mills and grain stores. (**2**). In laboratory, both the red and confused flour beetle males responded towards their conspecific females and completed the whole courtship sequence (contact, mount and copulation) in a mating area, whereas some males of both species mounted and attempted copulation to heterospecific females (**3**). Moreover, if confined in vials the two species can hybridize and produce offspring. Therefore, the genetic relationships between ***T. castaneum*** and ***T. confusum*** are of interest as both species show great morphological similarities and incomplete reproductive isolations. The genetic relationships among ***Tribolium*** beetle species were investigated by electrophoretic analysis of isozymes (**4**), and the results showed that ***T. castaneum*** and ***T. confusum*** were closely associated with the North American flour beetle ***Tribolium brevicornis*** than they were with each other, which were in disagreement with the morphological data. At the DNA level, relationships between some species of ***Tribolium*** were derived using highly repetitive DNA properties, whose sequence symmetry is abundant (**5**), in contrast to the chemotaxonomic phylogeny proposed by (**6,7,8**) inferred phylogenies of ***Tribolium*** species based on DNA sequence variations, and their results gave moderate support to taxonomic development based on morphological characteristics.. At the same time, their studies suggested that additional sequence data were needed to further resolve the genetic relationships between ***Tribolium*** species. Due to the importance of economic flour beetles and the difficulty of distinguishing between the two species morphology, this research suggested to study the degree of genetic convergence between the two species using the RAPD-PCR technique because it is easy to use and does not require large amount of DNA .

MATERIALS AND METHODS

This research was carried out in the Molecular Genetics Laboratory of the University of Diyala - College of Education for Pure Sciences - Department of Biology. Specimens of ***T.castaneum*** and ***T.confusum*** were collected from grain stores belonging to Diyala province with 20 samples per 10 males and 10 females both tow species. The specimens of the flour beetle were stored in 70% ethanol in glasses tube. The DNA was extracted using the extraction kit (Genomic DNA Mini Kit (Tissue) Protocol), Equipped by Pioneer company in Korea. The purity and concentration of extracted DNA was measured using a spectrophotometer and by analysing optical density ratio at 280 nm and 260 nm. The quality of DNA extracted was assessed by running 5µl of DNA per specimen on 1% agarose gel. The extracted DNA was stored at -20 ° C until use in amplification processes. The primer sequences used in this research were designed by Pioneer company- Korea, as shown in Table 1. The reaction mixture is prepared at a temperature of 4C° and the quantity is 20µl, consisting of 5 µl from PCR PreMix, 4 µl of primer 5 µl DNA and 6 µl Deionized water. The amplification process was carried out in PCR device American-made. The amplification conditions for all primers were shown in Table 1. In order to a negative control reaction was performed without the DNA template to detect any DNA contamination for each primer. The amplification reaction was repeated twice per sample and per primer to determine the range of

consistency and frequency in the bands. The amplification result was then loaded in parallel with the volumetric guide of DNA on the 1% agarose gel and electrophoresed (**9**). Gels were filmed under ultraviolet radiation; band and their molecular weights were determined using a gel analysis program. Based on the appearance and absence of bands in each specimen for each insect species, the data matrix was then prepared. Randomly Amplified Polymorphic DNA is the marker of dominant expression, and thus is detected dominant allele in a certain position by the presence of the band while the absence of the band is the appearance of a recessive allele symmetric in this place. Genetic software analysis tools were used to calculate the genetic identity (**10,11**). average heterozygosity and clustering of the samples. The following equation was used to calculate the primer efficiency = Number of bands per primer / number of bands for all primers X 100 , While the primer discriminatory ability was calculation by the following equation = number of polymorphic bands per primer / number of polymorphic bands for all primers X 100 (**12**).

Table 1. The random primers used in research with their sequences and amplified reaction conditions

Primer No.	Primers sequence (5-3)	Amplified reaction conditions	Reference
1	CCAGCCGAAC	Initial denaturation at 94c° for 5 min (1 cycle) , 45 cycles of denaturation at 94c° for 1 min annealing at 36C tor I min , extension at 72c° for 2 min and a final extension at 72c° for 7 min (1 cycle)	(**13**)
2	GACTAGGTGG		
3	GGGACGTTGG		
4	AGGGTCGTTC		
5	TGCGTGCTTG		
6	GTCCCGACGA		
7	TGATCCCTGG		

RESULTS

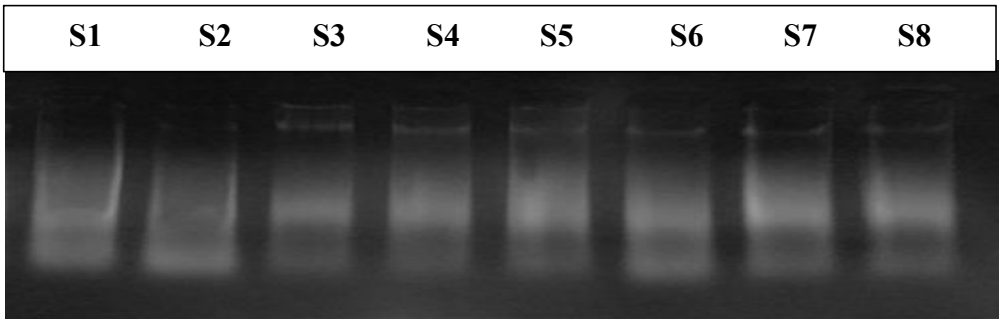


Fig.1. The product of DNA isolated from the flour beetle both tow species ***T. castaneum and T. confusum*** S1 , S2 , S3, S4 Specimens of ***Tribolium castaneum*** S5 , S6 , S7, S8 Specimens of ***Tribolium confusum***

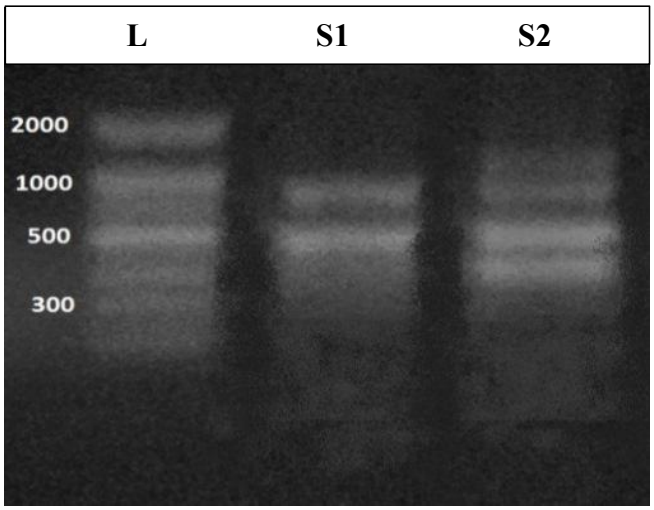
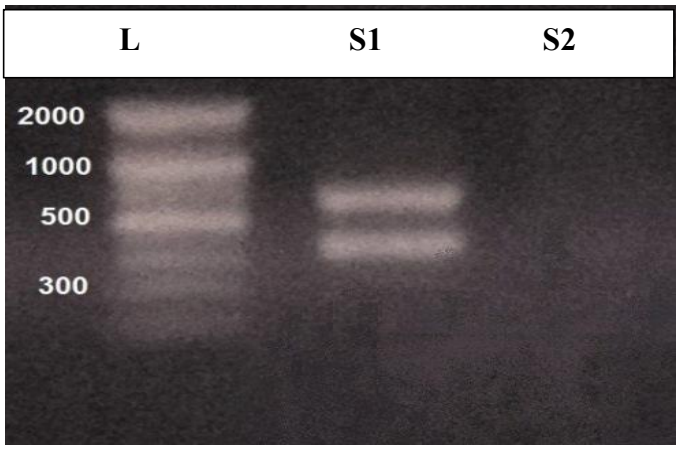


Fig. 2. Amplification PCR product from primer 1(5" CCAGCCGAAC 3") Track 1 : Ladder DNA (100 – 2000 bp) , Track 2 : ***T.castaneum*** sample , Track 3 : ***T.confusum*** sample



L.	M.W	S1	S2
1	710	1	0
2	475	1	0

Fig. 3. Amplification PCR product from primer 2(5" GACTAGGTGG 3") Track 1 : Ladder DNA (100 – 2000 bp) , Track 2 : ***T.castaneum*** sample , Track 3 : ***T.confusum*** sample.

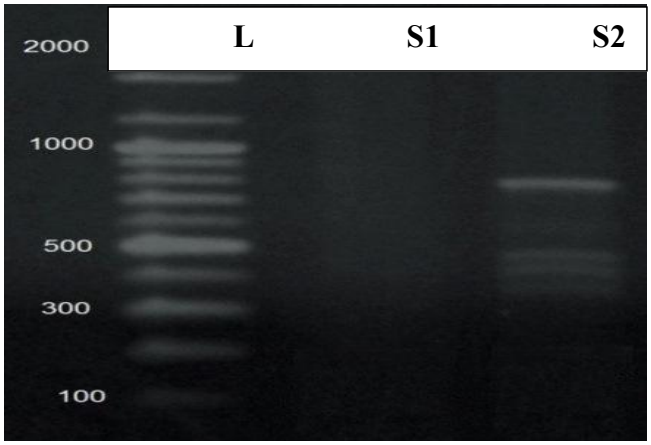


Fig. 4. Amplification PCR product from primer 3(5" GGGACGTTGG 3") Track 1 : Ladder DNA (100 – 2000 bp) , Track 2 : ***T.castaneum*** sample , Track 3 : ***T.confusum*** specimen.

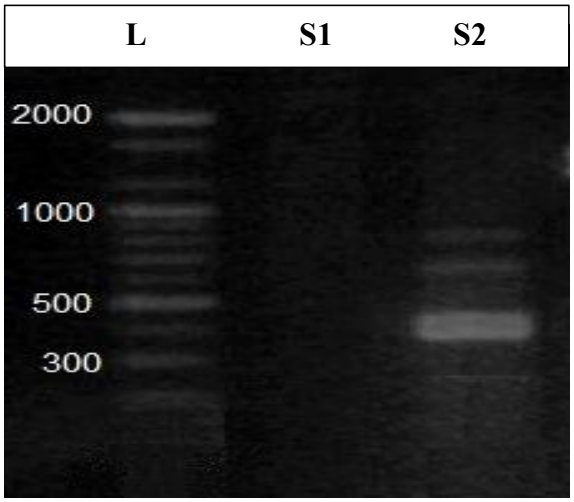


Fig. 5 Amplification PCR product from primer 4(5" AGGGTCGTTC 3") Track 1 : Ladder DNA (100 – 2000 bp) , Track 2 : ***T.castaneum*** sample , Track 3 : ***T.confusum*** sample

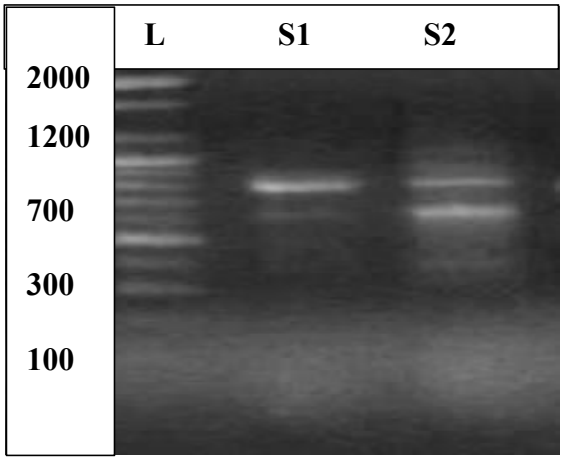


Fig. 6. Amplification PCR product from primer 5(5" TGCGTGCTTG 3") Track 1 : Ladder DNA (100 – 2000 bp) , Track 2 : ***T.castaneum*** sample , Track 3 : ***T.confusum*** sample

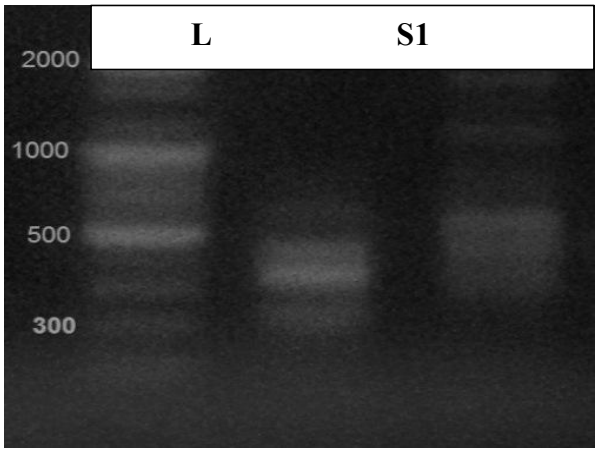


Fig. 7. Amplification PCR product from primer 6(5" GTCCCGACGA 3") Track 1 : Ladder DNA (100 – 2000 bp) , Track 2 : ***T.castaneum*** sample , Track 3 : ***T.confusum*** sample.

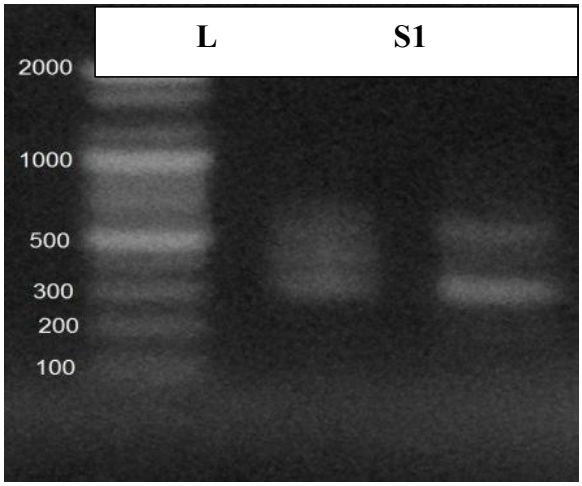


Fig. 8. Amplification PCR product from primer 7(5" TGATCCCTGG 3") Track 1 : Ladder DNA (100 – 2000 bp) , Track 2 : ***T.castaneum*** sample , Track 3 : ***T.confusum*** sample polymorphic fragments , percejntage efficiency primer and percentage of discriminatory of ability of primer between ***T.castaneum*** and ***T.confusum***

Table 2 . The primers used, total number of amplified fragments , range of amplified fragments , number of

Primer no.	Sequence 5"---3"	Total number of amplified fragment	Range of amplified fragments in (bp)	Number of polymorphic fragments	Percentage efficiency of primer	Percentage of discriminatory ability of primer
1	CCAGCCGAAC	4	407 – 990	4	11.42	12.12
2	GACTAGGTGG	2	475 – 710	2	5.71	6.06
3	GGGACGTTGG	7	200 – 1000	7	20	21.2
4	AGGGTCGTTC	7	410 – 1590	7	20	21.2
5	TGCGTGCTTG	4	365 – 800	3	11.42	9.09
6	GTCCCGACGA	7	300 – 1959	7	20	21.2
7	TGATCCCTGG	4	300 - 1000	3	11.42	9.09

Table 3. Genetic identity of Neis depending on the comparison of RAPD patterns between ***T.castaneum*** and ***T.comfusum*** .

	T.castaneum	***T.confusum***
T.castaneum	0.00	
T.confusum	0.111	0.00

DISCUSSION

RAPD-PCR technique is an efficient method for analysing genetic polymorphisms among diverse group of organisms (**14,15,16,17**) . The technique utilizes random decamer sequences as a single primer that anneals and primes at multiple locations throughout the genome of an organism. The patterns of amplicons produced are characteristic of the template DNA (**18**). The presence and absence of a specific PCR product is diagnostic for the primer binding sites on genomic DNA, therefore, can serve as useful molecular markers for genetic characterization as well as assessment of genetic relationships (**19**). The advantages of RAPD-PCR include its cost effectiveness, small DNA sample requirements, no requirement of prior knowledge of the sequence of DNA and the ability to identify hundreds of new markers in a short time **(20**). Results shown in Table 2 that the primers No. 3 , 4 and 6 gives the highest of amplified fragment which amounted to7 bands and it gave the highest of the polymorphic fragments which amounted to 7 bands and it gave the highest of the percentage efficiency and percentage of discriminatory ability which amounted to 20 , 21.2 respectively. While the primer No. 2 gave the lowest of amplified fragment which amounted to 2 bands and it gave the lowest of the polymorphic fragments which amounted to 2 bands and it gave the lowest of the percentage efficiency and percentage of discriminatory ability which amounted to 5.71 and 6.09 respectively . When the comparison between ***T.castaneum*** and ***T.confusum*** through genetic identity, as is evident in Table 3 was observed that ***T.castaneum*** is the nearest to ***T.confusum*** and valued at 0.111 . The cause of heterogeneity between ***T.castaneum*** and ***T.confusum*** may be ascribed to the fact that RAPD loci have higher mutation rates, hence are more polymorphic than that of other markers (**21**). Different regions in the genome evolve at different rates and single-copy genes have relatively low substitution rates because some alteration in a coding sequence may cause a severe effect on the coded protein product. However, changes in repetitive DNA apparently do not result in these consequences and thus the polymorphism in these regions is greater than coding regions (**22**). The genetic identity values between the ***T.castaneum*** and ***T.confusum*** was low, this could be ascribed to the fact that The genetic variation depends on colonization, host and reproductive pressures such that any species distributed over a great variety of environmental conditions would be genetically more heterozygous as compared to the species of restricted distribution (**23,24**).

CONCLUSION

T. castaneum and ***T. confusum*** are genetically distinct from each other even though their morphology and size are very similar . The RAPD technique is constant marker or polymorphic diagnostic marker used in the diagnosis variety of genus belonging to the one order as well as used in the diagnosis variety of species belonging to one genus and find the genetic relationship between them. The data were also used to clarify the taxonomic relationship between the two species of insect. In this study, it was clear from the results of the RAPD analysis between the two species of insect that are closed to each other .

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