Genetic diagnosis of the the rust-red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera:) and its Mortality, attraction and repelling effect of some vegetable oils

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Abstract

The study was conducted in the laboratories of the Plant Protection Department, College of Agriculture, Basra University in 2019-2020. The study aimed to genetically diagnose the rust-red flour beetle. The study proved that the insect belongs to the genus *Tribolium* and *castaneum* species according to the genetic diagnosis and according to the region of S rRNA28 and recorded in the NCBI gene bank for the first time and given Accession number and recorded as UB-Jin1 and gene sequence number in GenBank MW512269.1,The genetic tree was drawn using NCBI's BLAST program to know which insects are genetically close to them. By comparing the results of the evolutionary tree branching, it was shown that *Tribolium castaneum*, which was registered in our study in the NCBI Gene Bank under No. MW512269.1, participated in one secondary branch when compared to a group of the same species that has genetic distances Few of the samples used are under accession numbers JX412253.1, HM156703.1, EU048301.1, 8.1EU67767 and 1KP419677 and depending on the location of the branches of the phylogenetic tree of different insect species and the genetic distances that follow the genus *Tribolium* among themselves with the specie *Tribolium castaneum* registered with accession number 1MW512269. It was noted that the nearest species *Tribolium freemani* and its accession number is EU048302.1, while the type of *Tribolium confusum* registered with accession number JX412254.1, It possessed the highest genetic distance with *Tribolium castaneum* and therefore it is considered the farthest type compared to *Tribolium castaneum*, and to know the effect of some vegetable oils on some aspects of the life performance of the insect and its preparation as arable seeds.The results of the study showed the the Mortality, attraction and repelling effect of Cactus oil , *Cinnamomum camphora* (L.), *Mentha piperth* L. and *Watercress sativa* had the highest average lethal effect of camphor oil 80.03%, and the least effect of Watercress oil 40.74% for adults of the flour beetle, and camphor oil showed excelled on the rest of the oils in the average of its insect repelling effect and amounted to 58.25%, while Watercress oil had an attraction effect and the average of repelling was -6 1%.

Key words: Genetic diagnosis ,*Tribolium castaneum, Aloe vera , Cinnamomyum camphora , Mentha piperth ,Watercress sativa*

Introduction

Grains and their products are the main and important source of human food, where they constitute the main part as a main material for many peoples (Alonso, 2011). The rust – Red flour beetle (*Tribolium castaneum* (Herbst) caused severe losses and damage to many grains and their products during storage operations in stores and shops, ranging between 5-10% and may reach more than 20-30% in some tropical areas, and the percentage of damage may reach approximately 10-40% of the crops stored in the world (Al-Jaber, 2006). The insect causes great damage to stored materials in addition to unacceptable and foul-smelling secretions, which are benzoquinoes. These compounds are carcinogenic and cause a number of allergic diseases in humans (Lu *etal*., 2012).In the animal kingdom, insects are the largest qualitative structure, where they have a huge undetected genetic diversity that can be detected using the technology of molecular markers. Insect communities, even within the same species, differ morphologically and behaviorally because of their complex interactions and relationships with their environment and differences in size (Petrson *etal*., 2000).Therefore, the use of DNA Markers technology in insects and other living organisms has given focused and clear information to researchers about genetic diversity and gene flow between different species and has an important role in diagnosing the multiple morphological manifestations of DNA in the genome in a fast and highly efficient manner in distinguishing between species in very close traits Which live together in the same environment and on the same plant host (Haymer and Lnnis, 1994). (Behura et al., 2001).To diagnose different insects, the (PCR-RAPD) DNA technique was used, which is efficient and has useful results in the field of detecting differences between insect species and strains (Hoy, 2003). The PCR - RAPD technique was also used in genetic mapping and the reasons for the emergence of resistance, so many studies were conducted to detect the maps of some stored insects, including the Confused flour beetle *Tribolium confusum* (Boyer *etal*., 2012). PCR-RFLP analysis was used for genetic diagnosis between *T. castaneum* and *T. confusum* (Ming *etal*., 2014). Molecular genetics research proved that the variation in DNA arrangement represents the basis of changes that lead to the evolution of species such as phenotypic differences, so the PCR technique makes a comparison Gene sequencing is a practical method for studying the emergence of species and as an accurate taxonomic tool for diagnostic purposes (Ahmed, 2020). The wrong, increasing and repeated use of pesticides caused many negative effects in addition to environmental pollution, in

addition to the emergence of resistance in many insects (Sutton *etal*., 2011).Therefore, it was necessary to search for safe and environmentally friendly alternatives, including the use of natural products that have biological activity and work as highly efficient pesticides (Benner, 1993). Daiz *et al*. (2010) indicated that extracts of ethanol, hexane, petroleum ether, plant extracts, vegetable oils and secondary compounds of many plants act as insecticides.Ibrahim and AL-Naser (2009) confirmed that sesame, olive and sunflower oils at a concentration of 7.5% gave a high repellency rate to the cowpea beetle *Callosobruchus maculatus* Fab and prevent her from laying eggs.The natural plant products of plants have been used as a successful and effective natural means of control. They are quick-degrading compounds that are highly effective against insects and are less harmful to humans, animals, and the environment. They are considered nutritional inhibitors, repellents or growth regulators (Petson *etal*., 2000). AL-Bayati *etal*. (2013) used ginger oil to control some insect pests such as the rust – Red flour beetle *Tribolium castaneum* (Herbst), and the killing average was 78.4%. AL-Alan *etal* (2017) tested the effect of oil for mustard, Camphor, Mentha, cinnamon, clove and ginger on the viability of the pupal phase of the flour moth *Ephestia kuehniella*. Saker *etal.* (2018) used some extracts and vegetable oils of thyme leaves, hot pepper fruits, olive fruits and garlic cloves against the rice weevil *Sitophilus oryzae* (L.) and adults of *Trogoderma granarium* (Everst). The study aimed at genetic diagnosis of the rust – red flour beetle *Tribolium castaneum* (Herbst) due to the importance of the insect being one of the main insects that infest stored grain and cause great damage to stored materials and due to its presence throughout the year on grain, its products and the diversity of its families.The multiplicity of its generations in the year and its phenotypic similarity with other types of insects that infect wheat grains, and to ensure the genetic diagnosis of it, where there is no genetic diagnosis for it in Basra governorate and its widespread and in large numbers in most stores and local markets and knowing the Mortality, attraction and repelling effect of some vegetable oils on the adults of the insect to preserve the grains as seeds suitable for cultivation.

Materials and methods

molecular study

Molecular diagnosis of flour beetle adults insects was coundected using polymerase chain reaction (PCR) technique.

The DNA was extracted according to the protocol of the Korean producing company, Genomic TM DNA Extraction Kit. The samples were sent to the specialized company for the purpose of conducting sequence analysis.

Oil extraction

To implement this study, vegetable oils were extracted from the leaves of eucalyptus, Peppermint, Cactus oil and Watercress , and it was estimated using Soxhlet device by Al-Fawaz method (2008) with a weight of 5 gm of leaf powder for each plant separately after drying them in the shade and placing them in the extraction flask. The sample was then covered with a quantity of glass wool. Put the beaker inside the extraction unit to rotate the solvent ten times over the sample.Then the flask containing the sample was placed in an oven at a temperature of 105 °C for 5 minutes, with the oven door slightly opened to allow the ether to evaporate, then cool the sample and weigh it. The difference in weight before and after extraction represents the amount of oil in the sample. The oil percentage was calculated according to the following equation:

$$\text{crude oil\%} = \frac{oil\ weight}{sample\ weight} \times 100$$

Mortality, attraction and repelling effect for oil on adults of *Tribolium castaneum*

This study was conducted after the extraction of vegetable oils and the Mortality effect of oils on the insect was calculated by using test tubes, where 1 ml was placed in each test tube with three replicates of the four concentrations of oils 2, 4, 6, 8%. The tubes were moved to distribute the oil on its inner surface. As for the control treatment, the tubes were treated with acetone (Daoud *etal*., 1991).The tubes were left to dry and 2 gm of wheat flour was added to it as food for the insect as food for each tube, and then 10 adults were added to each replicate.It was taken from the permanent farm of insects raised at a temperature of 27±2 C and a humidity of 70 ± 5%, then the tubes were covered with a piece of cotton, and the percentage of Mortality was calculated after 24 and 48 hours of treatment.To study the attraction and repelling effect for the vegetable oils used in the study, the Helen method modified by Talukder and Howse (1993) was applied by placing a sterile plastic dish with a diameter of 4 cm and a height of 0.5 cm and fixed in the middle of a Petri dish with a diameter of 9 cm and a height of 1 cm, and 10 adults were placed inside the small dish After the small dish was treated with 1 ml of oils and with four concentrations and three replicates separately, the dish was left to dry and the adults were placed in it.As for the control treatment, the small dish was treated with acetone only. The numbers of adults outside the small dish were calculated within 24 hours of the treatment, and the repelling percentage was calculated according to the equation:

PR=2(C-50)

Where PR = percentage of repelling , C = percentage of adults outside the small plate

Note that if C is more than 50%, PR becomes positive and oils have a repellent effect

But if C is less than 50%, PR becomes negative and oils have an attraction effect

Results and discussion

molecular study

Genetic diagnosis is the accurate methods to distinguish closely related species of insects and through the use of an insect primer to confirm the genetic diagnosis of it and differentiate it from other species in the Genebank in Korea after DNA was extracted from the insect and sent to the specialized company to decode the genetic code of the 28S rRNA gene.Molecular sequencing of the gene from the insect *T.castaneum* from Basra province was documented for the first time in the National Center for Biotechnology Information (NCBI) genebank database. The insect isolate was recorded in the gene bank with the name UB-Jin1 and the molecular sequence number in the genebank 1.MW512269, and the genetic tree was drawn with the specialized program BLAST using the NCBI website.The Neighbor-joining tree method was chosen by drawing this tree, and the evolutionary tree was based on the sequence of the nitrogenous bases of the 28S ribosomal RNA gene for a group of insects based on the reference transcript data recorded in the NCBI Gene Bank for different species of the insect genus *Tribolium* and by comparing the results of the branching of the evolutionary tree observed. *Tribolium castaneum* recorded in our study in the NCBI Gene Bank under the number MW512269.1 shared one sub-branch when compared with a group of the same species with low genetic distances. and 1KP419677 and depending on the location of the branches of the phylogenetic tree of different insect species and the genetic distances that follow the genus *Tribolium* among themselves with the type *Tribolium castaneum* registered with accession number 1MW512269. It was noted that the closest species *Tribolium freemani* with accession number EU048302.1. As for *Tribolium confusum* registered with accession number JX412254.1, it had the highest genetic distance with *Tribolium castaneum* and therefore it is considered the farthest species compared to *Tribolium castaneum,*It was found through the genetic tree that the *castaneum* is isolated in a cluster alone and that the distances mentioned in the genetic affinity tree for the isolate of the insect under study differed from the rest of the isolates genetically according to the difference in the genetic distances shown in the tree.The results show that in the genetic affinity tree there are two main branches, and the first main branch included each of the two isolates JX412254.1 and EU048304.1. The second main branch was divided into 4 secondary branches as shown in Figure (1) and

the fourth secondary branch was divided into several other branches and they matched The isolate under study with isolate Eu048301.1 and differed from the rest of the isolates found in the genebank.The reason for the difference may be due to the presence of a discrepancy in the sequence of the nitrogenous bases. The genetic diagnosis of insect pests and the presence of a difference or variation in the sequence of the nucleotide bases of the DNA indicates the presence of a difference or change in the morphological characteristics (Sarvananda, 2018).The nucleotide sequences of the Iraqi isolate under study were compared with the species in the gene bank, and the percentage of compatible between them was as shown in Table (1).

Table (1) The percentage of compatible between the Iraqi isolate and its related species recorded in the gene bank

No.	species	Accession Number	Country	compatible%
1	*Tribolium confusum*	JX412254.1	China	% 92.08
2	*Tribolium confusum*	EU048304.1	USA	% 91.75
3	*Tribolium brevicornis*	EUO48305.1	USA	% 92.07
4	*Tribolium madens*	EU048303.1	USA	%94.85
5	*Tribolium freemani*	EU048302.1	USA	% 98.10
6	*Tribolium castaneum*	JX412253.1	China	%99.60
7	*Tribolium castaneum*	HM156703.1	USA	%100
8	*Tribolium castaneum*	EU048301.1	USA	%100
9	Tribolium castaneum	EU677678.1	USA	% 99.84
10	Tribolium castaneum	KP41677.1	USA	%100

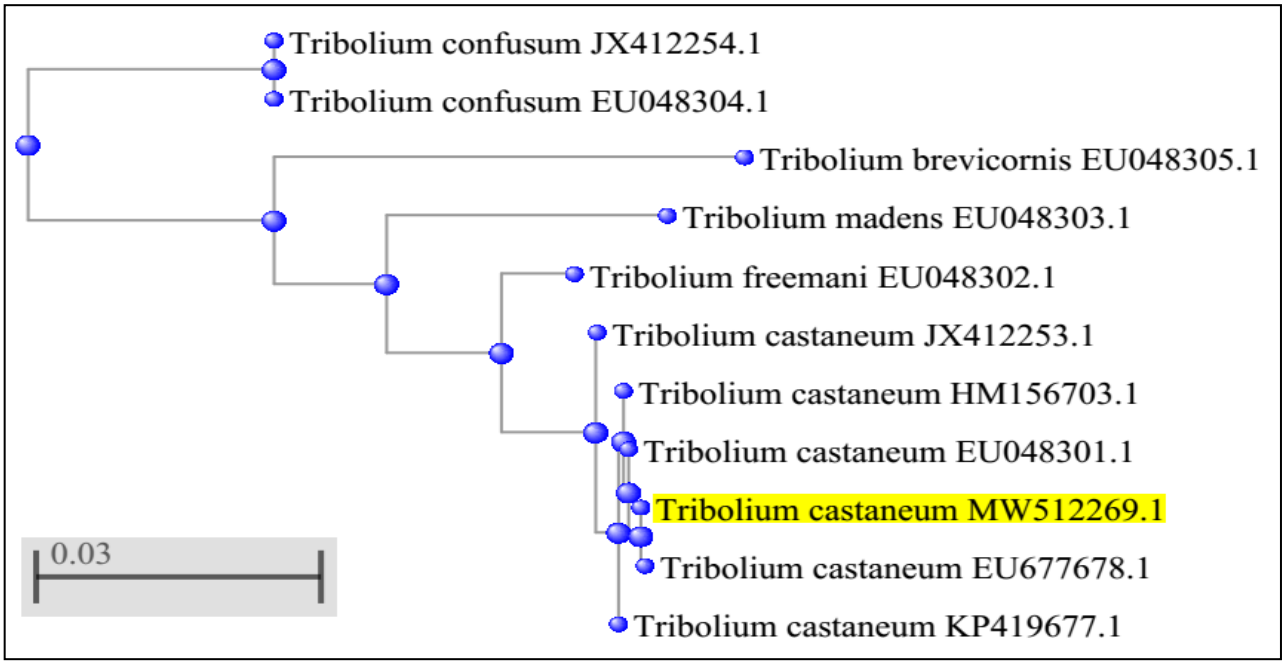

Figure (1) Genetic relationship tree among the beetle*Tribolium castaneum* and its comparison with Isolates found in the genebank

The Mortality, attraction and repelling effect for oil on adults of *Tribolium castaneum* beetle

The results in Table (2) showed the excelled of camphor oil in its Mortality effect, where the percentage of Mortality percentage was 80.03%, and the lowest effect of Watercress oil was 40.74%. 95% for concentrations 2, 4, 6 and 8%, respectively. The results of the statistical analysis showed a significant difference in the time effect average, where the percentage of Mortality increased when the exposure period was increased, reaching 53.03 and 64.66%, respectively, within 24 and 48 hours.The effect of camphor oil is due to the fact that it contains large amounts of the compound Safrole, which makes it toxic when entering the digestive system and causes serious side effects, including death, and its toxicity appears quickly within 5 – 90 minutes. It also causes symptoms that appear on the nervous system such as muscle spasms (Boyers,2014 ; Abu Khalif, 2019). While Saljoqi *et al*. (2006) confirmed that the extract of Peppermint leaves had a lethal effect on some insects such as the rice weevil *Sitophilus oryzae L.* . Gonzalesz *et al*. (2014) confirmed that oils do not change in size significantly after six months of storage and that they decompose slowly to release effective turbines in the control.

Table (2) The Mortality effect of vegetable oils on the adults of the *Tribolium castaneum* beetle

Oils	Consintration	Mortality % / h		Effect of oils	Effect of onsintration
		24	48		

Camphor oil	**2**	**58.66**	**64.00**	**80.03**	**41.43**
	4	**60.66**	**86.66**		**52.41**
	6	**88.00**	**92.33**		**64.41**
	8	**90.00**	**100**		**78.45**
Peppermint oil	**2**	**30.33**	**40.00**	**52.95**	
	4	**40.66**	**44.33**		
	6	**52.66**	**60.00**		
	8	**70.00**	**85.66**		
Watercress oil	**2**	**20.33**	**35.66**	**40.74**	
	4	**30.33**	**40.66**		
	6	**33.66**	**50.00**		
	8	**50.00**	**65.33**		
Cactus oil	**2**	**40.33**	**42.33**	**62.99**	
	4	**50.33**	**65.66**		
	6	**60.66**	**78.00**		
	8	**72.66**	**94.00**		
Control	**0**	**0**	**0**		
time effect		**53.07**	**64.66**		

RLSD 0.01 for the effect of oils = 8.95, for the effect of concentrations = 7.84, for the effect of time = 4.93, for the effect of interaction= 8.42

The study in Table (3)showed that the three oils, camphor, Peppermint and Cactus oil, had a repelling effect on the adults of the Tribolium castaneum. The average repelling percentage was 58.25, 17.80 and 41.0%, respectively, while Watercress oil had an attraction effect on the insect. 16 ,The results in Table (3) showed that the concentrations of oils are directly proportional to the percentage of centrifugal, which amounted to 2.65, 19.65, 41.30 and 47.95 percent for 8,6,4,2 percent concentrations, respectively. The percentage of vegetable oils varied depending on what they contain of different compounds, which differ in their effect as repelling or disinfectants. AL-Yahya (2007) found that eucalyptus oil contains a group of compounds, including:

Aromadendren 1-h-cycloprop, Alpha-Gurjnene 1h-cycloprop, Cineole, Cineol Eucalyptol1,8 and Ledeen 1-h-cyclopropazulene. Therefore, camphor oil was used as an insect repellent and flea killer, in addition to its toxicity to insects and bacteria (Chen *etal*., 2013). As for the Peppermint plant, it is rich in menthol compounds, limonene, pine nuts, eucalyptol and tannic acid (Al Taie, 2018),AL-Bayati (2013) also revealed the compounds present in the essential oil of Peppermint, which is one of the secondary metabolites of the plant and is rich in terpenes, Di-terpenes, Tri-terpenes, Tetra-terpenes, Hemi-terpenes and Sesqui-terpenes. The effect of oils in combating many storehouse insects is due to their having a deadly, repellent or attractive effect for many insect species, where they are characterized by their ability to prevent insects from feeding or

touching the insect's body and their high permeability inside the insect's body or preventing the insect from moving freely (Sharshi *et al*., 2000).

Table (3) The the Mortality, attraction and repelling effect of some vegetable oils on adults of the *Tribolium castaneum* beetle

Oils	Concentration%	Expulsion %	Effect of oils	Effect of concentration
Camphor oil	2	20	58.25	2.65
	4	46.6		19.65
	6	73.2		41.30
	8	93.2		47.95
Peppermint oil	2	6.6	17.8	
	4	20		
	6	40		
	8	46.6		
Watercress oil	2	- 28	- 16	
	4	- 20		
	6	- 8		
	8	- 8		
Cactus oil	2	12	41.0	
	4	32		
	6	60		
	8	60		
Control	--	0		

RLSD 0.01 for effect of oils=7.46 , for effect for concentration = 3.75 , for effect for interaction =6.99

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