

RAPD-PCR analysis and gene expression of Cytochrome P450 in *Tribolium castaneum* adults in response to different insecticides

Shymaa H. Mahmoud^{1*} and Aida S. Kamel²

¹ Zoology Department, Faculty of Science, Menoufia University, Menoufia, Egypt

² Entomology Department, Faculty of Science, Benha University, Benha, Egypt

ABSTRACT

Random amplified polymorphic DNA analysis by polymerase chain reaction (RAPD-PCR) was used as a tool to screen genetic variation in *Tribolium castaneum* adults treated by LC₅₀ of *Bacillus thuringiensis*, *Beauveria bassiana*, teflubenzuron and spinetoram using ten random oligonucleotide primers for amplification. The results of cluster analysis using Dice coefficient among treated and untreated *T. castaneum* adults revealed that adult beetles were grouped into 2 main clusters. One contains control adults. The other cluster was divided into 2 main subclusters; one includes adults treated with LC₅₀ of *B. thuringiensis* and teflubenzuron while the other subcluster contains adult beetles treated with LC₅₀ of *B. bassiana* and spinetoram. Furthermore, the transcriptional response of Cytochrome P450 gene to each of the used insecticides in *T. castaneum* was examined by qRT-PCR. The results revealed that *CYP450* was up-regulated by all the four insecticides tested. The highest level of up-regulation was recorded in adult beetles treated with LC₅₀ of teflubenzuron (25.9-fold) followed by *B. thuringiensis* treated adults (7.6-fold), and spinetoram (6.8-fold). The least level of up-regulation was recorded in adults exposed to LC₅₀ of *B. bassiana* (2.9-fold) as compared to control group.

Keywords: Bio-insecticides, RAPD-PCR, qRT-PCR, CYP450 *Tribolium castaneum*.



INTRODUCTION

Red flour beetle, *T. castaneum* is a major global pest of stored agricultural products causing damage to flour, cereal, beans, and nuts (Weston and Rattlingourd, 2000; Phillips and Throne, 2010). The management of red flour beetle heavily depends on synthetic insecticides and the insect has been reported to be resistant to all five classes of insecticides and fumigants used against it in many areas (Athie and Mills, 2005). In addition to acquired resistance of chemical insecticides, there are adverse effects of these insecticides on non-target mammals, birds, beneficial insects leading to environmental pollution, so the concept of chemical control should be replaced by other eco-friendly control methods (Fields, 1998). Microbial control agents with selectivity and a low environmental impact could become ideal components of integrated pest management programs (IPM) (Lacey and Goettel, 1995). Entomopathogenic fungi were among the first organisms to be used for biological control of pests. More than 700 fungal species from about 90 genera are pathogenic to insects (Hong, 2003). *Beauveria bassiana* is the most important entomopathogenic fungus from phylum Ascomycota (Hypocreales: Cordycipitaceae).

Bacillus thuringiensis (Bt) is an entomopathogenic Gram-positive, rod-shaped and sporulating bacterium that has been isolated globally from a great diversity of environment including soil, water, dead insects, leaves from deciduous trees, diverse conifers, and insectivorous mammals, as well as from human tissues with severe necrosis (Roh *et al.*, 2007). Bt strains produce a wide variety of insecticidal proteins which are active against larvae of very diverse insect orders; Lepidoptera, Coleoptera, Hemiptera and Diptera (Palma *et al.*, 2014 and Nair *et al.*, 2018).

Teflubenzuron is an insect growth regulator of the

Benzoyl urea group; it is a stomach poison and disrupts chitin formation, and therefore, control mainly the immature stages but also has species specific ovicidal action. Insect growth regulators (IGRs) are attractive to be used as alternatives to wide spectrum insecticides since they are more selective, less harmful to environment and more compatible with pest management systems including biological controls. Because IGRs act on systems unique to insects, or shared with close relatives, they do not affect other organisms. (Krysan and Dunley, 1993). Spinosyns group insecticides exhibit low mammalian toxicity and are considered harmless for the environment since they degrade to simpler fragments containing only carbon, oxygen, nitrogen, and hydrogen (Dripps *et al.*, 2011). Recently, spinetoram that is a mixture of two synthetically modified spinosyns (spinosyn J and spinosyn L), which are metabolites of the bacterium *Saccharopolyspora spinosa* (Bacteria: Actinobacterida), was introduced as a new spinosyn insecticide with greater potency and faster speed of action in comparison with spinosad (Sparks *et al.*, 2008 and Dripps *et al.*, 2008). Recently, spinetoram has been tested and found to be effective for the control of several stored grain beetle species (Vassilakos *et al.*, 2012; Isikber *et al.*, 2013) while its efficacy was practically not affected by temperature and relative humidity (RH) (Vassilakos and Athanassiou, 2013). Spinetoram has some surface treatment studies against all life stages of *Tribolium confusum*. (Saglam *et al.*, 2013).

RAPD-PCR involves the amplification of random segments of genomic DNA, using short primers without the requirement of previous knowledge of genomic DNA (Williams *et al.*, 1990), and has been used in species and strain identification (Bardakci and Skibinski, 1994; Cocconcelli *et al.* 1996), DNA finger-

* Corresponding author e-mail: drshaymaahusseini@gmail.com

rinting offers a useful biomarker assay in ecotoxicology (Savva, 1998; Rocco *et al.*, 2010, 2012).

Cytochrome P450 (*CYP*) gene family constitutes one of the largest gene super families, with representatives in all living organisms, including bacteria, fungi, plants, and animals (Werck-Reichhart and Feyereisen 2000). Cytochrome P450 monooxygenases were reported to be a major metabolic factor responsible for pyrethroid and other organophosphates insecticide resistance (Schenkman and Jansson, 2003). The present study tested the applicability of RAPD-PCR to screen genetic variation in *T. castaneum* adults treated by LC₅₀ of *B. thuringienensis*, *B. bassiana*, teflubenzuron and spinetoram using ten random oligonucleotide primers for amplification. Furthermore, the transcriptional response of *CYP450* gene to each of the used insecticides in *T. castaneum* by qRT-PCR was evaluated.

MATERIALS AND METHODS

Insect culture

T. castaneum, were collected from stored flour, identified according to Anderson (1987) and Bousquet (1990) then maintained in the insectary of the Zoology Department, Faculty of Science, Menoufia University, on a mixture of wheat flour and brewer's yeast. The cultures were maintained in an incubator at 27 °C with 70 % relative humidity (Singh and Lakhota 2000). Adult beetles were collected and placed in petri dishes containing flour mixed with each used insecticide. The percentage of mortality (Data not published here) and resultant LC₅₀ was calculated according to Finney (1971), using "Ldp line" software by (Bakr, 2000). After 72 hours of treatment, adult beetles were separated and used to subsequent analysis.

The insecticide used were *B. thuringienensis*, *B. bassiana*, teflubenzuron and spinetoram and obtained as commercial formula from Plant Protection Research Institute, Dokki, Giza, Egypt.

Extraction of genomic DNA.

DNA extraction was performed using QIAamp DNA Mini Kit (QIAGEN, Cat. No. 51304) according to manufacturer's instructions. The quality and concentration of DNA solution were determined by A260/A280 ratio of diluted DNA solution in a UV spectrophotometer. Ten decameric oligonucleotides of arbitrary sequence were tested; OP-A4, OP-A8, OP-A15, OP-B9, OP-B14, OP-B18, OP-C6, OP-C10, OP-C15 and OP-C18 (Table 1). RAPD-PCR reactions were accomplished with all the samples, including the controls, essentially as described by Atienzar and Jha (2006).

RAPD PCR amplification

Polymerase chain reactions for random amplified polymorphic DNA (RAPD) analysis were carried out in 25 µl volume, according to Williams *et al.* (1990). Each reaction tube contained 20 ng of genomic DNA, 12.5 µl master mix (Fermentas), and 10 pmol of primer (Operon Technologies, Alameda, CA). The amplifications were carried out by using a thermal cycler (TC96K, Acculab, USA) programmed at 94°C

for 4 min, followed by 40 cycles of 94°C for 1 min, 36°C for 1 min, 72°C for 2 min, and final extension at 72°C for 5 min. The amplified products were visualized on 2% agarose gel and photographed with Gel Doc XR system (Bio-Rad, USA).

Table (1).List of the RAPD primer names and their nucleotide sequences

No	Name	Sequence 5' → 3'
1	OP-A04	5' AATCGGGCTG 3'
2	OP-A08	5' GTGACGTAGG 3'
3	OP-A15	5' TTCCGAACCC 3'
4	OP-B09	5' TGGGGGACTC 3'
5	OP-B14	5' TCCGCTCTGG 3'
6	OP-B18	5' CCACAGCAGT 3'
7	OP-C06	5' GAACGGACTC 3'
8	OP-C10	5' TGTCTGGGTG 3'
9	OP-C15	5' GACGGATCAG 3'
10	OP-C18	5' TGAGTGGGTG 3'

Gene expression of *CYP 450*

RNA extraction and cDNA synthesis

Analysis was carried out at Biotechnology Lab at Cairo University Research Park, Faculty of Agriculture (CURP). RNA extraction from adult beetles was performed using Gene Jet RNA purification Kit (Thermo Scientific, Cat. no. K0731). Reverse transcription was done using Revert Aid First Strand cDNA synthesis Kit (Thermo Scientific, Cat. no. K1621) according to manufacturer's instructions.

Quantitative real time PCR (qRT-PCR)

Real-time PCR was performed using Maxima SYBR Green/ROX qPCR Master Mix Kit (Thermo Scientific, Cat. no. K0251) in a Biosystem step one plus instrument. Reaction mixtures were incubated for 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, 60 s at 58 °C, and finally 60 s at 72 °C, melting curve from 70.0 °C to 95.0 °C, read every 0.3 °C, held for 10 s. The entire analysis was performed using a *T. castaneum* actin as an internal standard, which showed constant expression in *T. castaneum*. Each group test was performed using three replicates. Sequence of *CYP450* forward primer was (5' TCAACCGACTGCACCTGTAT'3), and reverse primer was (5'GCGTATCTATCAGGGCGACT'3). Sequence of actin forward primer was (5'ACACACCAAAAATGTGCGACG'3) and reverse primer was (5'CGGTGGTGGTGAACGAGTAA'3).

Statistical analysis

The amplification profiles for all the primers were compared with each other and the bands of DNA fragments were scored as present (1) or absent (0) generating the 0, 1 matrices. The clustering was done and the dendrogram was drawn by unweighted pair group with arithmetic mean (UPGMA) using SPSS software (Rohlf 1998). Data analysis of qRT-PCR was achieved by using relative quantification (Livak and Schmittgen 2001).

RESULTS

In the present results, total DNA extracted from

control and treated *T. castaneum* adults analyzed on 2 % agarose gel represented a high molecular weight DNA. It was seen as a single intact condensed band free from degradation. DNA concentration as determined by 260 A / 280 A was 20 ng and its purity was 1.8. RAPD- PCR results of control and treated adult using ten decameric arbitrary primers were illustrated in Figs. (1, 2 and 3). The data in (Table 2) showed that Primer OP-A04 generated one band in control and all treated adults with molecular size of 487.50 bp. Primer OP-A08 generated one band in control adults and in those treated with LC₅₀ of *B. thuringienensis*, teflubenzuron and spinetoram with molecular size of 962.51bp and completely disappeared from adults treated with LC₅₀ of *B. bassiana*. In case of adults treated with LC₅₀ of *B. thuringienensis*, a unique band with molecular size of 814.27 bp was detected. Primer OP-A15 generated two bands in adults treated with LC₅₀ of *B. bassiana* with molecular size 1702.60 and 475.97 bp and only one band in adults treated with LC₅₀ of teflubenzuron with molecular size 1702.60 bp. In case of primer OP-B09, three bands were observed in adults treated with LC₅₀ of *B. thuringienensis* and teflubenzuron with molecular size of 1164.19, 631.97 and 492.22 bp. Also the two first bands were detected in case of treatment with *B. bassiana*. Primer OP- B14

generated a common band in control and all treated bands were generated in adults treated with *B. thuringienensis* and teflubenzuron with molecular size of 310.12 and 284.73 bp, respectively. Primer OP- B18 generated two similar bands in control and adults treated with *B. thuringienensis* with molecular size of 405.13 and 287.57 bp. The latter band is also observed in adults treated with *B. bassiana*, teflubenzuron and spinetoram. Two unique bands were recorded in adults treated with teflubenzuron with molecular size of 1464.92 and 361.39 bp. Primer OP-C06 generated two similar bands in control and adults treated with *B. thuringienensis* with molecular size of 952.14 and 739.23 bp. A relatively unique band with molecular size of 941.61 bp appeared in adults treated with *B. bassiana* and spinetoram. Primer OP- C10 generated a unique band in each of control, adults treated with *B. thuringienensis* and *B. bassiana* with molecular size of 758.54, 808.57 and 891.33 bp, respectively. Primer OP-C15 generated two bands with molecular size of 623.12 and 313.70 bp in all treated adults and disappeared in control group. Primer OP-C18 generated one band with molecular size of 1451.12 bp in adults treated with *B. bassiana*, teflubenzuron and spinetoram and disappeared in control group as well as *B. thuringienensis* treated adults.

Table (2): Molecular size analysis generated by primers A4, A8, A15, B9, B14, B18, C6, C10, C15 and C18. Lane 1: control adults, lane 2: adults treated with LC₅₀ of *B. thuringienensis*, lane 3: adults treated with LC₅₀ of *B. bassiana*, lane 4: adults treated with LC₅₀ of teflubenzuron, lane 5: adults treated with LC₅₀ of spinetoram.

primer	Band number	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5
OP-A04	1	487.50	487.50	487.50	487.50	487.50
OP-A08	1	962.51	962.51	---	962.51	962.51
	2	---	814.27	---	---	---
	3	---	664.67	---	664.67	664.67
OP-A15	1	---	---	1702.60	1702.60	---
	2	---	---	475.97	---	---
OP-B09	1	---	1164.19	1164.19	1164.19	---
	2	---	631.97	631.97	631.97	---
	3	---	492.22	---	492.22	---
OP-B14	1	548.05	548.05	548.05	548.05	548.05
	2	---	284.73	---	310.12	---
OP-B18	1	405.13	405.13	287.57	1464.92	287.57
	2	287.57	287.57	---	361.39	---
	3	---	---	---	287.57	---
OP-C06	1	952.14	952.14	941.61	---	941.61
	2	739.23	739.23	---	---	---
OP-C10	1	758.54	808.57	891.33	---	---
OP-C15	1	---	623.12	623.12	623.12	623.12
	2	---	313.70	313.70	313.70	313.70
OP-C18	1	---	---	1451.12	1451.12	1451.12

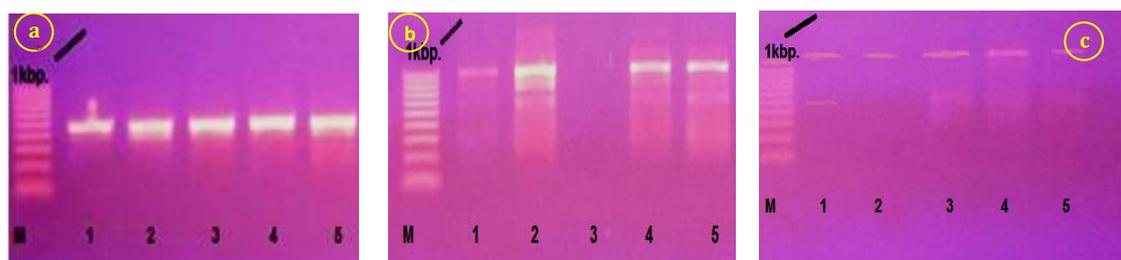


Figure.(1). (a) ,(b) & (c): RAPD-PCR profiles of control and treated adults of *T. castaneum* generated by A4 , A8 and A15 primers, respectively . Lane M: marker, lane 1: control adults, lane 2: adults treated with LC₅₀ *B. thuringienensis*, lane 3: adults treated with LC₅₀ *B. bassiana*, lane 4: adults treated with LC₅₀ teflubenzuron, lane 5: adults treated with LC₅₀ spinetoram.

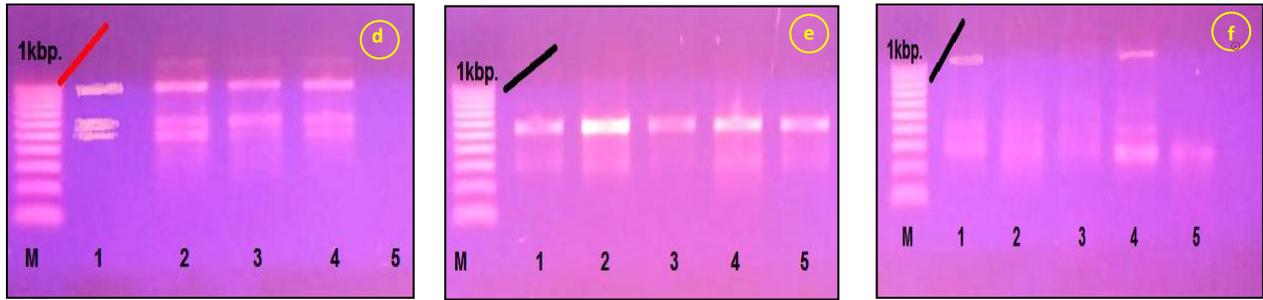


Figure (2): (d),(e) &(f), RAPD-PCR profiles of control and treated adults of *T. castaneum* generated by B9,B14 and B18 primers, respectively . Lane M: marker, lane 1: control adults, lane 2: adults treated with LC₅₀ *B. thuringiensis*, lane 3: adults treated with LC₅₀ *B. bassiana*, lane 4: adults treated with LC₅₀ teflubenzuron, lane 5: adults treated with LC₅₀ spinetoram.

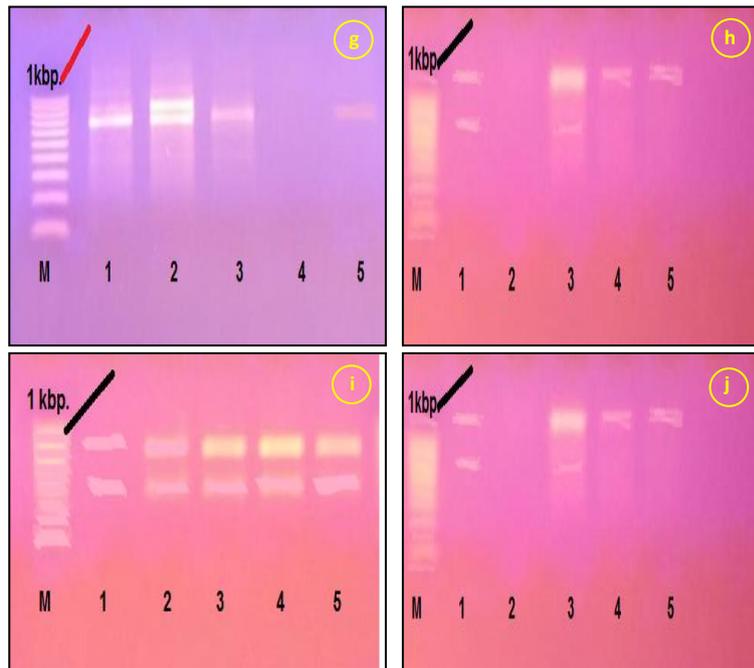


Figure (3): RAPD-PCR profiles of control and treated adults of *T. castaneum* generated by C6 ,C10, C15 & C18 primers, respectively. Lane M: marker, lane 1: control adults, lane 2: adults treated with LC₅₀ *B. thuringiensis*, lane 3: adults treated with LC₅₀ *B. bassiana*, lane 4: adults treated with LC₅₀ teflubenzuron, lane 5: adults treated with LC₅₀ spinetoram.

Cluster analysis using Dice coefficient as a measure of similarity among *T. castaneum* treated and untreated adults were displayed in dendrogram in (Fig. 4). The tree revealed that adult beetles were grouped into 2 main clusters. One contains control adults while,

another cluster divides into 2 main subclusters; one of which includes adults treated with LC₅₀ of *B. thuringiensis* and teflubenzuron and the another subcluster contains adult beetles treated with LC₅₀ of *B. bassiana* and spinetoram

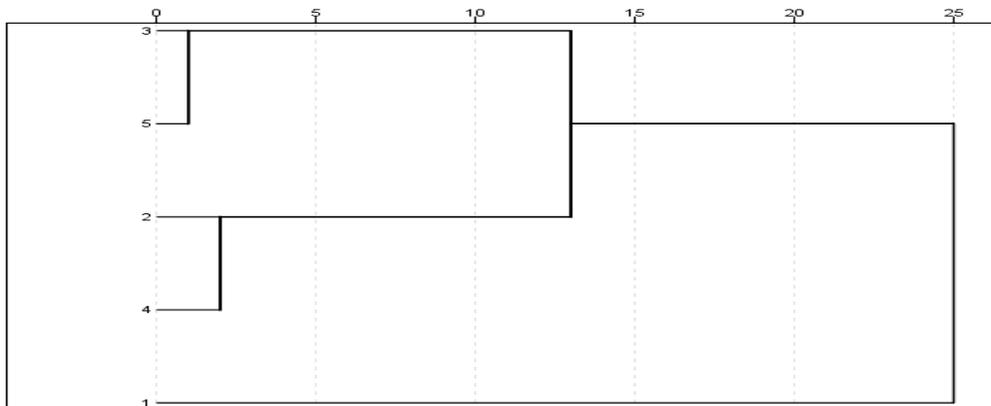


Figure. (4): Dendrogram displaying the results of cluster analysis among *T. castaneum* adults. 1: control adults, 2: adults treated with LC₅₀ of *B. thuringiensis*, 3: adults treated with LC₅₀ of *B. bassiana*, 4: adults treated with LC₅₀ of teflubenzuron, and 5: adults treated with LC₅₀ of spinetoram..

Expression of *CYP 450* in adults *T. castaneum*

The current results demonstrated that *CYP450* was up-regulated at the four tested insecticides. The levels of the up-regulation were ranged from (2.9–25.9) folds when adults were exposed to LC₅₀ of each of these insecticides. The highest level of up-regulation was recorded in adult beetles treated with LC₅₀ of teflubenzuron (25.9-folds) followed by *B. thuringienensis* treated adults (7.6- folds), and finally spinetoram treated adults (6.8-folds). The least level of up-regulation was recorded in adults exposed to LC₅₀ of *B. bassiana* (2.9- folds). In all cases of treatment, the results revealed a significant increase in *CYP450* expression level in *T. castaneum* adults after exposure to each insecticide as compared to control group.

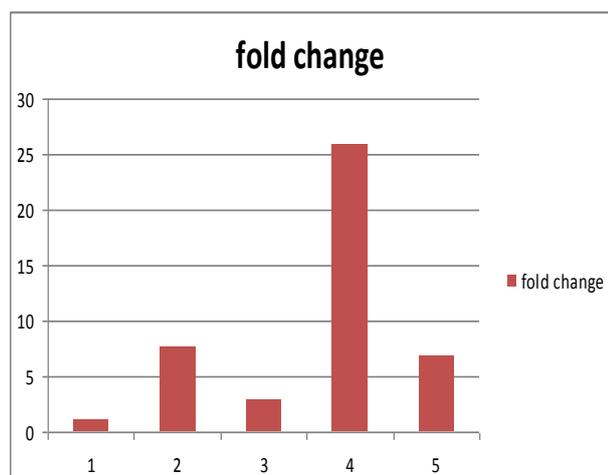


Figure (5): Up-regulation of *CYP450* gene in adults of *T. castaneum* after exposure to different insecticides represented by fold change. 1, control; 2, treated with LC₅₀ of *B. thuringienensis*; 3, treated with LC₅₀ of *B. bassiana*; 4, treated with LC₅₀ of teflubenzuron, and 5, treated with LC₅₀ of spinetoram.

DISCUSSION

The RAPD-PCR technique has been used for analysis and detection of DNA alterations produced by environmental genotoxic chemicals. Studies have reported that RAPD-PCR can be used for examination and estimation of genomic variation in genotoxic studies (Rocco *et al.*, 2014). This technique is highly sensitive to nucleotide differences between template DNA and the primer, allowing detection of single nucleotide changes (Williams *et al.*, 1990). This feature makes RAPD markers ideal for detecting variation in closely related individuals and in less polymorphism species (Williams *et al.*, 1991). RAPD analysis is reproducible, sensitive and capable of detecting temporary DNA changes at lower concentrations of pollutants that may not finally manifest themselves as mutations (Liu *et al.*, 2005; Atienzar and Jha 2006). The results of our study support the use of RAPD-PCR analysis as a preliminary tool to monitor DNA changes occurred after exposure to insecticides. As suggested by Liu *et al.*, (2005), RAPD analysis in conjunction with other biomarkers proved to be a tool to study genetic- toxicological effects. The change in the number of bands and the variation in their intensity in RAPD-PCR profile might be associated with alterations of genetic material. Analysis of RAPD band

gain or loss and variation between exposed and non-exposed individuals may be related to DNA damage, mutations or structural rearrangements induced by genotoxins, affecting the primer sites or interpriming distances (Atienzar *et al.*, 2002). RAPD assays have been shown to detect DNA damage caused by plant extracts against mosquitoes at different doses by Lalrothuanga *et al.*, 2011. They attributed the changes occurring in RAPD profiles due to formation of covalently bound adducts between the insecticide, microorganism or its metabolites and the DNA. Faulty repair of these adducts often results in mutations and, sometimes, cytogenetic changes (Lalrothuanga *et al.*, 2011). Similar results were obtained by Mahmoud (2018) on *Spodoptera littoralis* using two different bioinsecticides. The genomic template stability (GTS, %) is directly related to the extent of DNA damage and also to the efficiency of DNA repair and replication (Rocco *et al.*, 2011).

RAPD marker in the present study was used to evaluate genetic diversity. Cluster analysis is a hierarchical method that will place isolates in groups with some level of implicit relatedness. The dendrogram from RAPD data clearly gave comparable results and showed that there were homogeneity between adults treated with LC₅₀ of *B. thuringienensis* and teflubenzuron in a subcluster and adult beetles treated with LC₅₀ of *B. bassiana* and spinetoram in another subcluster indicating that there was genetic diversity between the treated adult beetles. Similar results were observed by Golshan *et al.*, 2014 using different isolates of *B. bassiana* against *T. castaneum* adults by RAPD-PCR.

Up-regulation of *CYP* genes was reported to be rapid in response to insecticides even at low concentrations and depended on the type of insecticides. Liang *et al.*, (2015) indicated that there were no specific patterns for predicting the up-regulation of specific *CYP* gene families based on the insecticide classification. Moreover, up-regulation of *CYP* genes mediated by insecticides and other xenobiotic compounds have been reported in many insect species. (Feyereisen, 2011).

Up-regulated *CYP* P450 super-family gene expression has been proved to be closely associated with resistance to pyrethroid insecticides in many insects over expression of *CYP6D123* and *CYP6A3624* in resistant strains of houseflies (Zhu *et al.*, 2008) and *CYP6F1* in *Drosophila melanogaster* (Daborn *et al.*, 2001). Assie *et al.*, (2007) showed that *CYP4Q4* and *CYP4Q7* genes were related to pyrethroid resistance in *T. castaneum*. Up-regulation of *CYP* genes could potentially have a significant impact on insect's ability to metabolize xenobiotics, which may lead to detoxification of insecticides and even the development of insecticide resistance in the insect populations. To date, however, limited information is available with respect to the relationship between *CYP* genes and the type of insecticides. In addition, little is known about the level of the up-regulation of *CYP* genes in relation to the insecticide concentration and the exposure duration in insects. Recently, Zhu *et al.* (2010) identified six *CYP* up-regulated genes in deltamethrin

resistant strain of *T. castaneum*. *CYP6BQ9*, a brain-specific gene, showed over 200-fold constitutive over expression, and can be up-regulated when the insects were exposed to deltamethrin (Zhu et al, 2013). Scott (1999) concluded that *CYP*-mediated detoxification is an essential resistance mechanism that might cause a significantly higher level of resistance to many insecticides in insect populations. Feyereisen (2005) reported that the diverse functions of cytochrome P450s are primarily due to the diversity of *CYP* genes. Thousands of *CYP* genes in general have been identified in insects (Urlacher and Girhard, 2012) and the number is still growing rapidly as more insect genomes are sequenced. Moreover, *CYP* genes effect on insect growth, development and nutrition, besides detoxification (Bautista, et al., 2007). Several microarray-based studies on *Drosophila melanogaster* have identified xenobiotic-inducible *CYP* genes (Giraud et al., 2010 and Le Goff et al., 2006). The use of microarrays on insecticide-resistant mosquitoes, including *Anopheles gambiae* (David, et al., 2005), *Aedes aegypti* and *Culex quinquefasciatus* (Komagata, et al., 2010) have collectively identified a relatively small number of up-regulated *CYP* genes after exposure of mosquitoes to different concentrations of insecticides.

CONCLUSION

The results revealed that teflubenzuron and *B. thuringiensis* were the most effective followed by *B. bassiana* and *spinetoram*. The results were confirmed by qRT-PCR, which revealed up-regulation in *CYP450* by all tested insecticides.

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تحليل تفاعل البلمرة المتسلسل (RAPD-PCR) والتعبير الجيني لجين سيتوكروم بي 450 في خنفساء الدقيق الصدفية استجابة لمبيدات حشرية مختلفة

الملخص العربي

شيماء حسين محمود^{1*}، عايدة سعيد كامل²

كلية العلوم جامعة المنوفية - قسم علم الحيوان¹

كلية العلوم جامعة بنها - قسم علم الحشرات²

تم استخدام تفاعل البلمرة المتسلسل (RAPD) كأداة لفحص الاختلاف الجيني في طور البالغ لخنفساء الدقيق الصدفية المعاملة بالتركيزات المميّنة من كل من بكتيريا *الباسيليس ثيورينجينسس* وفطر *البوفاريا باسينيا* ومنظم النمو تيفلوبنزرون والمبيد الحيوي اسبينوترام باستخدام عشرة من البادئات العشوائية. وأظهرت نتائج التحليل العنقودي تقارب بين الخنافس البالغة المعالجة بالتركيز المميّنت من منظم النمو تيفلوبنزرون والخننافس البالغة المعالجة بـ *الباسيليس ثيورينجينسس* (مجموعة)، وبين الخنافس البالغة المعالجة بالاسبينوترام والخننافس البالغة المعالجة بالتركيز المميّنت من فطر *البوفاريا باسينيا* (مجموعة أخرى). وعلاوة على ذلك تم دراسة التعبير الجيني لجين سيتوكروم بي 450 لكل من المبيدات الحشرية في خنفساء الدقيق الصدفية باستخدام تفاعل البلمرة المتسلسل الكمي اللحظي. أوضحت النتائج حدوث زيادة في التعبير الجيني في طور البالغ لخنفساء الدقيق الصدفية مع جميع المبيدات الحشرية التي تم اختبارها وكان أعلى مستوى تم تسجيله في الخنافس البالغة المعالجة بالتركيز المميّنت من منظم النمو تيفلوبنزرون (بمقدار 25.9 ضعفا) تلاه الخنافس البالغة المعالجة بـ *الباسيليس ثيورينجينسس* (بمقدار 7.6 أضعاف) يليه الخنافس البالغة المعالجة بالاسبينوترام (بمقدار 6.8 أضعاف). تم تسجيل أقل مستوى للتعبير الجيني في الخنافس البالغة المعالجة بالتركيز المميّنت من فطر *البوفاريا باسينيا* (بمقدار 2.9 - أضعاف) بالمقارنة مع المجموعة الضابطة مما يعكس كفاءة بكتيريا *الباسيليس ثيورينجينسس* ومنظم النمو تيفلوبنزرون في مكافحة هذه الحشرة .