# PROTEIN AND ENZYMATIC CHANGES IN *CALLOSOBRUCHUS CHINENSIS* ADULTS (COLEOPTERA : BRUCHIDAE) TREATED WITH STRUCTURALLY DIFFERENT MONOTERPENES

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## ABSTRACT

*Three structurally different monoterpenes; acyclic linalool, monocyclic eugenol and bicyclic α - pinene were tested against C.chinensis adults by using the fumigant toxicity bioassay. Results revealed that the insecticidal activity of the acyclic linalool was much more potent than the monocyclic eugenol and the bicyclic α - pinene. The $LC_{50}$ value of linalool was 10 ppm, versus 41 and 39 ppm for eugenol and α – pinene respectively . Treatment the adult insects with the $LC_{50}$ and $LC_{90}$ of the tested monoterpenes resulted in the appearance of a new peptide band as compared with the control group. The results revealed also that linalool was more effective than eugenol and α - pinene on the physiological processes and consequently the protein synthesis in the adults of C.chinensis.. Concerning the effect of the tested monoterpenes on some enzyme activities of C.chinensis adults, it was noticed that the enzymatic activities of glutamic – oxaloacetic transaminase, glutamic – pyruvic transaminase, acid phosphatase and alkaline phosphatase were significantly increased ($p \leq 0.05$) by treatment with monoterpenes. Among monoterpenes; linalool treatment was significantly the most effective ($p \leq 0.05$) on the activities of all tested enzymes when treatments carried out by both $LC_{50}$ and $LC_{90}$.*


*Keywords: Callosobruchus chinensis, monoterpenes, toxicity, protein patterns polyacrylamide gel electrphoresis, enzymatic activities*

## INTRODUCTION

Synthetic pesticides have been a threat to human health and to the environment, causing among other undesirable effects, phytotoxicity, pollution, development of insecticide resistance, or negative effects on nontarget organisms. One alternative to conventional insecticide is photochemical, which have been used for many years to control insect pests damage on stored products as well as agricultural crops (Lee, *et al.* 2001, Tapondjou, *et al.* 2005). Plants produce a wide range of secondary metabolites (e.g., terpenoids,alkaloids and phenolics) that often possess insecticidal, fungicidal, bactericidal, antiviral, antifeedant or insect growth repellant properties (Singh, *et al.* 1989, Benner, 1993, Wilson, *et al.* 1997). Many of these chemicals are attractive alternatives to synthetic chemicals because none are likely to leach into groundwater or persist in soil or sediments (Isman, 1999) as well as their reduced impact on beneficial insect population, an important component in integrated pest management systems used by many organic farmers (Plimmer ,1993).

Terpenoids (or terpenes) are a group of compounds occur in the plant kingdom. The simpler mono – and sesqui – terpenoids are the chief constituents of the essential oils, the majority of them possessing insecticidal activity (Waliwitiya, *et al.* 2005). Monoterpenoids are divided into three groups: acyclic, monocyclic and bicyclic (Finar, 1986).

Many researchers have reported repellent, antifeedant and toxic properties of monoterpenoids against many stored- product insects. Roger and Hamraoui (1995) reported that monoterpenes had toxic as well as reproductive inhibitory effect on the kidney bean beetle *Acanthocelides obtectus.* Lee, *et al.* (2001) found that the monoterpenes; 1.8 – cineole, limonene and α-pinene had fumigant toxic effects towards the rice weevil, *Sitophilus oryzae.* Huang, *et al.* (2002) reported that eugenol, isoeugenol and methyleugenol showed similar contact toxicity to *S.zeamais* while their toxicities toward *Tribolium castaneum* were in the order isoeugenol > eugenol > methyleugenol. Erler (2005) reported that several monoterpenoids possess fumigant toxic activity against adult's eggs of the confused flour beetle, *Tribolium confusum* and larvae and eggs of the Mediterranean flour moth, *Ephestia kuehniella.* Similar results were achived by Tapondjou, *et al.* (2005) in which the monoterpene cymol showed toxic effect on both *S.zeamais* and *T. confusum.*

In spite of the massive studies on the toxicity of monoterpenes toward insects, little or no researches have been done on the protein and enzymatic changes occurred after treatment with monoterpenes.

In this study, the toxicities of three structurally different groups of monoterpenes (acyclic: linalool ; monocyclic : eugenol ; and bicyclic : α-pinene) on adults of *Callosobruchu chinensis* which considered one of the most major pest to the cow pea, *Vigna sinensis* as well as many legumes. Meanwhile changes in protein electrophoretic patterns and enzymes activities were also investigated.

## MATERIALS AND METHODS

### 1- Chemical compounds:

The monoterpenes vis: eugenol ($C_{10}$ $H_{12}$ $O_2$), linalool ($C_{10}$ $H_{12}$ O)and α-pinene ($C_{10}$ $H_{16}$) were purchased from Sigma Chemical Co. (St.Louis, Mo, USA). The chemicals were diluted with acetone (analytical reagent grade) to prepare a series of concentrations.

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**2- Insects:**

Culture of *Callosobruchus chinensis* was maintained at the Department of Biological and Geological Sciences, Faculty of Education, Alexandria University, Egypt, The insect was reared on cowpea (*Vigna sinensis*) and maintained at 28 ± 1° C, 60 – 70 % RH, and a photoperiod of 12 : 12 (L:D).

**3- Fumigant toxicity:**

To evaluate the fumigant toxicity of the tested monoterpenes, transparent plastic jars (1 liter) with screwed caps were used as exposure chambers. A Watman No.1 filter paper (2.0 cm diameter) was glued on the underside of the cap to serve as a diffuser for the tested monoterpenes. By means of automatic micropipette concentrations from 20 to 100 ppm for eugenol, 5 to 40 ppm for linalool and 30 to 70 ppm for α-pinene, were dissolved in 0.1ml actone, before being allowed to the filter paper in the inner cap of jars. Control treatments were carried out in the same jars and the filter paper was impregnated with 0.1 ml acetone alone. The solvent was allowed to evaporate for 2 min and the cap containing the treated filter paper was screwed tightly onto the jars containing 50 adult beetles (less than 12 hours old). Three replicates were set up for each treatment and control. The jars containing the tested insects were incubated at (28 ± 1° C), 60 – 70 % RH, and a photoperiod of 12: 12 (L: D). After 24 h, insects were transferred to clean jars covered with a nylon cloth, tied with a rubber band, and returned to the constant room temperature. Mortality was observed at 24-h intervals to determine the endpoint mortality, which was reached after 6 days. The $LC_{50}$ and $LC_{90}$ values were determined according to the FAO method (1974).

**4- Protein determination :**

Protein concentration was measured spectophotometrically, using the Bio-Rad protein Kit (Bradford, 1976). Protein solution 2 µl were pipetted into clean, dry tubes. Five ml of diluted dye reagent (Bio-Rad), was added to each test tube, the contents were mixed by vortex and the absorbance was measured using Pye Uicam SP6-550 spectrophotometer at 595 nm. The protein concentration was determined using a standard curve. The standard curve concentrations ranged from 38 to 525 Ug /protein.

**5- Protein gel electrophoresis:**

Sodium dodecyl sulphate polyacrylamid gel electrophoresis (SDS – PAGE) was carried out using the discontinuous buffer system as described by Laemmli (1970).

**a) Preparation of *C.chinensis* for the analysis:**

Frozen samples of *C.chinensis* adults were ground using liquid nitrogen in a precooled mortar and pestle, then stored at - 20° C. An aliquot of 500 mg were ground in 1ml 0.05 M Tris – HCl buffer, pH 7, then the slurry was incubated on ice for 30 minutes, then centrifuged at 5.000 rpm for 15 minutes ( Shewry, *et al.* 1996).

**b) Preparation of stock solutions :**

- Acrilamide-bis-acrylamide solution was prepared by dissolving 29 g of acrylamide and 1 g bis-acrylamide in a total volume of 100 ml deionized water. The solution was filtered through Whatman filter paper No. 1 and stored at 4°C in a dark bottle.
- N, N, N', N' – Tetramethlethylenediamine (TEMED) was used as supplied and is stable for a long time when stored at 4°C in dark.
- Ammonium persulphate: 10% was freshly prepared.
- Sodium dodecyl sulphate (SDS): 10% (w/v) prepared by dissolving 10 g of SDS in 100 ml distilled water.
- β – mercaptoethanol (ME): used as supplied.

**c) Preparation of buffers :**

- Resolving gel buffer stock: 1.5 M Tris-HCl (pH 8.8) was prepared by dissolving 18.16 g of Tris in 40 ml distilled water and adjusting to pH 8.8 with 1 N HCl and completing to 100 ml with distilled water, then filtered through Whatman filter paper No. 1 and stored at 4°C.
- Stacking gel buffer stock: 1 M Tris-HCl (pH 6.8) was prepared by dissolving 12.11 g Tris in 40 ml distilled water, adjusting to pH 6.8 with 1 N HCl and completing to 100 ml final volume with water. The solution was filtered and stored at 4°C.
- Running buffer stock: 25 mM Tris, 250 mM glycine and 0.1% SDS (pH 8.3) was prepared as 5x stock solution by dissolving 15.1 g Tris, 94 g glycine in 900 ml deionized water. Then, 50 ml of a 10% (w/v) of SDS was added, and the volume was completed to 1000 ml with deionized water. The solution was then stored at 4°C until used.

**d) Preparation of separating gel:**

A 12% resolving gel was prepared as follows: 12 ml acrylamide-bis-acrylamide, 7.5 ml resolving gel buffer stock, 0.3 ml SDS 10%, 0.3 ml freshly prepared 10% ammonium persulphate, 9.9 ml distilled water, and 0.012 ml TEMED.

**e) Preparation of stacking gel:**

The stacking gel was prepared using the following reagents: 1 ml acrylamide-bis-acrylamide, 0.75 ml stacking gel buffer stock, 0.06 ml SDS 10%, 0.06 ml freshly prepared 10% ammonium persulphate, 4.1 ml distilled water and 0.006 ml TEMED.

**f) Loading of samples:**

After centrifugation as previously mentioned, the supernatant of the sample was transferred into Eppendorf tube containing equal volume of gel loading buffer (50 mM Tris, 10% glycerol, 2% SDS, 0.1% bromophenol blue and 5% β – ME and set pH 6.8), then the samples were denatured by heating at 100°C for 5 min,. followed by immediate cooling on ice and loaded into the gel. Electrophoresis was carried out for one hour at 80 V across the polyacrylamide gel using CONSORT power supply (Belgium) and Mini Protean Cell (Bio-Rad).

**g) Gel staining:**

After the run was completed, the gel was stained as described by Hames and Rickwood. (1990) in 50 ml of staining solution consisting of 0.1% Coomassie blue R-250,dissolved in 40% methanol, and 10% glacial acetic acid. Then gel was distained in distaining solution of 10% glacial acetic acid and 40% methanol.

**h) Protein molecular mass determination:**

Isolated proteins were applied to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) to determine the molecular weight (MW) using standard protein marker according to the method described by Weber and Osborn (1969). Composition of the standard protein marker was as follows:

| Protein | Source | Molecular Mass(KDa) |
|---|---|---|
| Phosphorylase b | *E.coli* | 97 |
| Bovin serum albumin | Bovine plasma | 66 |
| Ovalbumin | Chiken egg white | 45 |
| Carbonic anhydrase | Bovine erythrocytes | 30 |
| Trypsin inhibitor | Soy bean | 20.1 |
| Lysozyme | Chiken egg white | 14.4 |

**Gel scanning:**

Protein bands revealed on gels were *scanned* with Video Copy Processor P65 E (Appligene). Quantitative determination of the resolved protein bands was carried out using the Molecular Dynamic Image Quant V3.3 Program (Appligene).

**6-Spectrophotometric Assay for Enzymatic Activity:**

Enzyme activities of acid phosphatase (ACP), alkaline phosphatase (ALP), glutamic – oxaloacetic – transaminase (GOT) and glutamic – pyruvic transaminase (GPT) were determined in extracts of *C.chinensis* adults (control) as well as those treated with the $LC_{50}$ and $LC_{90}$ of the tested monoterpenes.

Three hundred beetles from each group representing those treated with the $LC_{50}$ and $LC_{90}$ of the different monoterpenes were ground in 5 ml of ice – cold 0.04 M sodium phosphate buffer (pH 7.0) in a tissue grinder. The crude homogenate was centrifuged at 10.000 xg for 5 min at 4°C. The pellet was discarded and the resulting supernatant was immediately used for enzyme assays.

**i) Acid Phosphatase (ACP)**

For assaying the activity of ACP, the instruction of bioMérieux-Kit (France) were followed. The activity was measured spectrophotometrically at 510 nm using phenyl phosphate as a substrate at pH 4.9. The liberated phenol was measured in the presence of amino-4-antipyrine and potassium ferricyanide. To stop the enzymatic reaction, sodium arsenate was used in the reagent (Burtis and Ashwood 1994).

**j) Alkaline phosphatase (ALP)**

ALP activity was measured spectrophotometrically at 510 nm in the presence of phenylphosphate as a substrate at pH 10, using bioMérieux-Kit (France). The liberated phenol was measured in the presence of amino-4-antipyrine and potassium ferricyanide. To stop the enzymatic reaction, sodium arsenate was used in the reagent (Burtis and Ashwood 1994).

**k) Glutamic – Oxaloacetic Transaminase (GOT)**

GOT activity was measured spectrophotometrically at 546 nm in the presense of x-oxoglutarate and L- aspartate using Randox Kit (England). The activity was measured by monitoring the concentration of oxaloacetate hydrazone. The concentration of GOT was measured from the standard curve (Burtis and Ashwood 1994).

**L) Glutamic-Pyruvic Transaminase (GPT)**

GPT activity was measured spectrophotometrically at 546 nm in the presence of α-oxoglutarate and L-alanine using Randox Kit (England). The activity was measured by monitoring the concentration of pyruvate hydrazone formed with 2.4-dinitrophenyl-hydrazine. The concentration of GPT was measured from the standard curve (Burtis and Ashwood 1994).

**7- Statistical analysis:**

Percent mortality as well as the mean values of enzyme activities in the different treatments were transformed to the angular scale for analysis of variance (ANOVA) as given by Steel and Torrie (1980), and the analysis was done using SAS program (SAS, 1985). Means were compared using FLSD 0.5 (Fisher least significant difference). Data obtained from various dose-response bioassays were subjected to probit analysis to estimate the $LC_{50}$ and $LC_{90}$ of the tested monoterpenes (Finny, 1971).

## RESULTS

**I. Bioassay of Fumigant toxicity.**

Results of the fumigant toxicity of the tested monoterpenes towards *C.chinensis* adults revealed that the structural characteristics of the tested monoterpenes influenced the insecticidal activity of such compound as shown in Table 1. The acyclic monoterpene, linalool was more toxic than the monocyclic eugenol and the bicyclic α-pinene against *C.chinensis* adults. Results obtained in Table 1clearly indicated that the $LC_{50}$ of linalool was 10 ppm while

those of eugenol and α - pinene were 41 and 39 ppm, respectively. Also these results showed that the $LC_{90}$ of linalool was 50.1 ppm while those of α - pinene and eugenol were 50.1 and 69 ppm, respectively (Table 1).

**II.Gel Electrophoresis of Proteins.**

Figures (1 and 2) show SDS-PAGE electrophoretic patterns of proteins extracted from *C.chinensis* adults treated with the $LC_{50}$ and $LC_{90}$ of each of linalool, eugenol and α- pinene. The patterns showed the appearance of 11 peptide bands in the control as well as in samples treated with $LC_{50}$ of linalool, eugenol and α- pinene. On the other hand, patterns of treated samples with $LC_{90}$ of linalool, eugenol and α- pinene showed one more additional peptide band, i.e., 12 peptide band. This band was characterized by its low molecular mass (11 KDa). The appearance of such peptide band in those treated samples means that the three different treatments had a positive effective response on the synthesis of such stress protein in *C.chinensis*.adults .This phenomenon was much more pronounced in the insects treated with $LC_{90}$ of eugenol followed by α – pinene and then linalool.

Among the common 11 peptide bands present in all patterns, it was noticed that there were three major bands differ in their concentrations between the control and treated samples. These peptide bands were located on the gel at positions 60, 71 and 103 (Table 2). They have low molecular mass of 38.32 and 24 KDa. For the peptide of 38 KDa, it was noticed that the treatment with eugenol was more effective on the synthesis of such protein by decreasing, since its concentration was declined by 14.8 % with $LC_{50}$ and 31.6 % with $LC_{90}$ as compared with the control. For α -pinene it was more effective at $LC_{50}$, however there is no effect at the $LC_{90}$. Linalool had no effect at both concentrations (Table 2).

Concerning the second stress protein (32KDa) the results showed that its concentration was obviously increased by the three different treatments especially linalool, at both levels of concentrations. Its concentration was increased by 53.3% at $LC_{50}$ and by 40.2% at $LC_{90}$ treatment of linalool. This result reveals that linalool was the most effective treatment on the synthesis of such protein. On the contrary, the results obtained in Table 2 showed that all treatments had a pronounced effect on the synthesis of the third major protein (23 KDa), since its concentration was markedly decreased with both levels of the $LC_{50}$ and $LC_{90}$ values. The decrease in concentration was proportional with increase in levels of treatments. The treatment with linalool was the most effective comparing with other treatments followed by α - pinene then eugenol. It is clear that this protein comprises 36.5% of total protein in the control samples and the decrease in its synthesis by such treatment may affect the physiological processes of *C.chinensis*.

From these results one can concluded that the treatment with linalool was more effective on the insect physiology and consequently the protein synthesis of *C.chinensis* adults,than eugenol and α - pinene. Meanwhile increasing the doses of linalool, eugenol and α - pinene to the $LC_{50}$ levels resulted in synthesis of a new protein of low molecular mass and it was pronounced with eugenol than the other treatment. Such stress protein is formed in *C.chinensis* adults as a protective biological factor against toxic substances (Table 2).

**III.Enzyme activities.**

The effect of treatment by monoterpenes on enzyme activities of *C.chinensis* adults was shown in Table 3. It was clear that the activities of GOT, GPT, ALP and ACP were significantly increased ($p \leq 0.05$) with treatment by monoterpenes comparing with control. Among the three different treatments, linalool treatment was significantly the most effective on the enzyme activities of GOT, GPT and ACP at both $LC_{50}$ and $LC_{90}$ values. However, eugenol treatment with the $LC_{90}$ was significantly the most effective on the ALP activity. Results showed also that with increasing levels of treatment from $LC_{50}$ to $LC_{90}$, the activities of all enzymes were significantly increased comparing with control.

From these results it can be concluded that:

a. The three different treatments by monoterpenes had variable effects on the activities of the above mentioned enzymes.

b. The treatment by linalool had the highest significant increase in the enzyme activities comparing with eugenol and α -pinene.

c. Increasing the level of treatments from $LC_{50}$ to $LC_{90}$ resulted in a significant increase in the enzymatic activities.

**Table 1:** Fumigant toxicity of eugenol, linalool and α - pinene against *C.chinensis* adult.

| | $LC_{90}$(ppm) | $LC_{50}$(ppm) | Slope ± SE | $r^2$ |
|---|---|---|---|---|
| **Eugenol** | 69 | 41 | 5.67 ± 2.63 | 0.6084 ns |
| **Linalool** | 16 | 10 | 6.68 ± 2.81 | 0.9216** |
| **α – pinene** | 50.1 | 39 | 11.94 ± 3.25 | 0.8190* |

$LC_{50}$ and $LC_{90}$ data were determined by Probit analysis; concentration (ppm) in acetone.
ns: Not significant, *, **, significant at 0.05 and 0.01 level of probability.

**Table 2: Scanning analysis of polyacrylamid gel electrophoretic patterns presented in figures 1 and 2.**

| Band No. | Position | Control | Band % | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | L50 | E50 | P50 | L90 | E90 | P90 |
| 1 | 6 | 4.7 | 4.4 | 3.7 | 8.3 | 9.8 | 4.6 | 6.0 |
| 2 | 15 | 3.6 | 4.2 | 2.9 | 7.6 | 8.2 | 3.8 | 5.3 |
| 3 | 30 | 3.2 | 3.9 | 3.5 | 2.8 | 4.1 | 2.6 | 4.0 |
| 4 | 38 | 6.5 | 8.0 | 6.3 | 5.3 | 6.6 | 7.2 | 6.3 |
| 5 | 46 | 3.6 | 2.8 | 2.0 | 1.7 | 2.4 | 1.9 | 2.7 |
| 6 | 60 | 15.5 | 15.8 | 13.2 | 12.6 | 15.3 | 10.6 | 15.4 |
| 7 | 71 | 12.2 | 18.7 | 17.7 | 12.8 | 17.1 | 12.5 | 10.2 |
| 8 | 80 | 2.9 | 4.4 | 5.3 | 4.4 | 4.3 | 2.4 | 3.3 |
| 9 | 103 | 36.5 | 26.9 | 33.1 | 30.9 | 26.4 | 29.4 | 27.1 |
| 10 | 113 | 9.2 | 7.1 | 7.9 | 9.4 | 6.3 | 10.9 | 8.0 |
| 11 | 149 | 2.7 | 3.3 | 3.1 | 4.4 | 2.9 | 3.8 | 4.3 |
| 12 | 164 | - | - | - | - | 4.8 | 10.6 | 7.4 |

- : Not present. L : Lianool P :α - pinene E : Eugenol

**Table 3:** Effect of treatments by monoterpenes on some enzyme activity of *C.chinensis* adults.

| Monoterpenes | Mean values of enzyme activities (Unit/litre) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | GOT | | GPT | | ALP | | ACP | |
| | $LC_{50}$ | $LC_{90}$ | $LC_{50}$ | $LC_{90}$ | $LC_{50}$ | $LC_{90}$ | $LC_{50}$ | $LC_{90}$ |
| **Eugenol** | $1020.0^{d}$ | $2040.0^{b}$ | $590.0^{g}$ | $880.3^{c}$ | $69.2^{c}$ | $85.4^{a}$ | $520.0^{d}$ | $839.0^{b}$ |
| **α - Pinene** | 956.7e | 2020.0b | 681.3e | 931.3b | 64.6c | 80.0b | 508.3e | 759.0c |
| **Linalool** | $1729.0^{c}$ | $3720.0^{a}$ | $710.3^{d}$ | $1198.7^{a}$ | $78.1^{b}$ | $78.2^{b}$ | $1174.0^{a}$ | $1178^{a}$ |
| **Control** | $240.7^{f}$ | | $640.0^{f}$ | | $56.1^{d}$ | | $211.0^{f}$ | |

*Within columns: means with different superscript letters differ significantly ($p \leq 0.05$)

## DISCUSSION

In the present study, the fumigant toxicity of three structurally different monoterpenes was investigated towords *C.chinensis* adults. The study revealed that the acyclic or aliphatic monoterpene (linalool) was much more toxic than the monocyclic and the bicyclic (or aromatic) monoterpenes eugenol and α–pinene, respectively. Hummelbrunner and Isman (2001) reported that thymol, which is an acyclic monoterpenes, had toxic and deterrent effect towards *spodoptera litura* larvae than other monoterpenes. Similar result was achieved by Waliwitiya, *et al.* (2005) in their work on *Agriotes obscurus* larvae. Harwood *et al* (1990) found that several monoterpenes altered growth, feeding and pupation in *Peridroma saucia* larvae. Guillet, *et al* (1998) reported that monoterpenes had a toxic as well as synergistic action towards third instar larvae of *Osrtinia nubilalis.*

Roger and Hamraoui (1995) tested the fumigant toxic activity of several monoterpenes towards the kidney bean beetles, *Acanthoscelides obtectus.* They found that oxygenated monoterpenes such as linalool and eugenol were the most toxic monoterpenes than the unoxygenated ones. Lee, *et al* (1999) tested acyclic, monocyclic and bicyclic monoterpenes towords *Ostrinia nubilalis* larva. They found that the monocyclic monoterpene pulegone was the most active one towards the tested insect. Lee, *et al* (2001) mentioned that there is no correlation between monoterpenes structure and their toxicity towards *Sitophilus oryza.* Similar result was achived by Erler (2005) in his work on the toxicity of monoterpenes towards *Tribolium confusum* and *Ephestia kuehniella.*

It could be concluded from the aforementioned results that toxicity of monoterpenes is species specific. Further investigation must be done to elucidate the most active group(s) of these chemical to be used commercially.

Concerning the effect of monoterpenes on protein analysis of *C.chinensis* adults, polyacrylamide gel electrophoretic techniques are used successfully for monitoring or analysis any changes in cellular composition of microorganisms. One of these techniques is sodium dodecyle sulphate polyacrylamide gel electrophoresis (SDS-PAGE). By this technique, any change in protein composition can be detected as well as the molecular mass of polypeptides under analysis can be estimated.

Although several investigations dealt with the effect of different chemicals on protein composition of different insect species, no data were available on the effect of monoterpenes on such protein analysis.

Yi and Adams (2000) tested the effect of the juvenile hormone analogue (JHA), pyriproxfen, on the Colorado potato beetle, *Leptinotarsa decemlineata*. They found that JHA treatment causes qualitative changes in hemolymph protein as well as the synthesis of new diapause proteins

Sankhon, *et al.* (1999) found that treatment of *Dermacentor variabilis* females with the JHA methoprene resulted in the production of new oocyte protein with a low molecular weight. Similar result was achieved by Delisle and Cusson (1999) in their work on *Choristoneura fumiferana* and *C. rosaceana* moths .

Mendoza, *et al.* (2000) found that in a malathion – resistant strain of *Habrobracon hebetor* , two extra protein bands with high mobilities were detected . These bands were absent in the susceptible strain of the same insect.

It could be concluded from the aformentioned results that treatment of *C.chinenises* adults with different monoterpenes had led to the formation of new stress proteins which are a biologically defense mechanisms towards toxicant matters.

Alkaline phosphatase (ALP) is a ubiquitous enzyme in all organisms. ALP is abundant, high-PH metal protein known to play roles in phosphate uptake and in secretary process in epithelia in mammals. However, the precise physiological role of ALP remains unknown (Cabrero, *et al.* 2004). ALP is found in the midgut microvillar membrane of insects (Fitches and Gatehouse, 1998). It could have a role in epithelial transport in *Drosophila melanogaster*. Fitches and Gatehouse (1998) found an increase in the ALP activity after treating larvae of *Lacanobia oleracea* with the phytochemical proteins, lectins. They found that these lectins cause disruption in the midgut epithelial cell morphology, which led to a disturbance in enzyme regulatory mechanisms. The same result was achived by Pusztai, *et al.* (1996) in which lectins cause disruption in the rat gut epithelium. Eisemann. *et al.* (1994) found that different kinds of lectin are bound and reduced the permeability of the peritrophic membrane matrix of the blow fly *Lucilia cuprina*. They added that this change in the membrane environment led to consequent disruption of the enzyme recycling mechanism which could explain the increase in the enzyme activity. Acid phosphatase (ACP) is found in several tissues of insects including fat body, the midgut epithelium, the epidermal cells and the salivary glands (Dean, 1978). ACP involves in the oocyte maturation of *Musca domestica* (Ribolla, *et al.*, 2001). Van Pelt – Verkuil (1979) found that ACP activity largely increased at the beginning of metamorphosis, and this increase was associated with an increase in the lysosomal system in the cell. Van Pelt – Verkuil (1979) found that injection of *Calliphora erythrocephala* larvae with ecdysteroid led to a large increase in the activity of ACP, which is probably brought about via a transcriptional and translational pathway. Swidan (2006) found that thymol, which is an ayclic monoterpenes, causes a complete distruction in the ultra structure of the brush border of the midgut epithelium of the cotton leafworm, *Spodoptera littoralis*. In addition to that thymol affects oocyte maturation of this insect.

It seems from the previous results that the increase in activities of ALP and ACP of *C.chinensis* adults treated with monoterpenes is a result of a disruption in the enzyme secreting centres, which led to an increase in the enzymatic concentration.

It is well known that transaminases enzymes (GOT & GPT) are widely distributed in animal tissues.and catalyze the interconversion of amino acid and α-oxo- acids by transfer of amino groups. Tabassum, *et al.* (1998) tested the effect of neem-based insecticides on GOT and GPT activity in *Alphitobius diaperinus*. They found that the enzymatic activity decreased after such treatment. On the other hand transaminases activities were found to increase significantly in *Tribolium castaneum* adults after treatment with pyrethroids (Tabassum, *et al.* 1998). This difference in enzymatic activity in stored grain insects could be due to the difference in insect strain. In addition to that, different materials could exhibit different behaviour against insect species.

From the above mentioned results one can conclude that the monoterpenes tested in the present investigation affected *C.chinensis* adults in different ways. They exhibited a lethal effect towards adult insect, they led to synthesis a new stress protein as a result of its treatment with monoterpenes and finally they increase some enzymatic activities in this insect.

Further studies must be done to elucidate the exact role of different kind of monoterpenes towards stored grain insects.

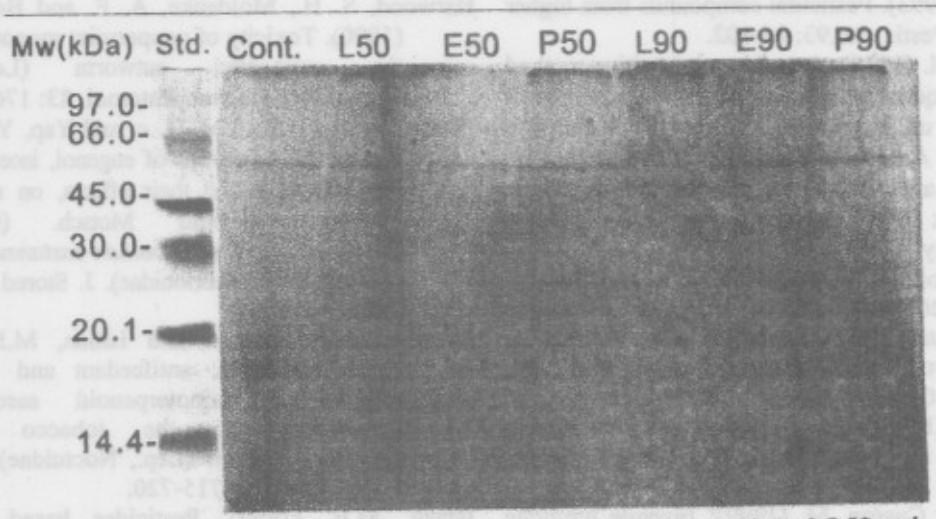


**Figure 1 : SDS - PAGE of *C.chinensis* proteins treated with the LC 50 and LC 90 of the tested monoterpeins. Anode (+) is toward bottom of photo. Each lane had 20 µg protein for each sample.**
**L, E, and P: Linalool, Eugenol and α-Pinene with LC 50 and LC 90, respectively.**

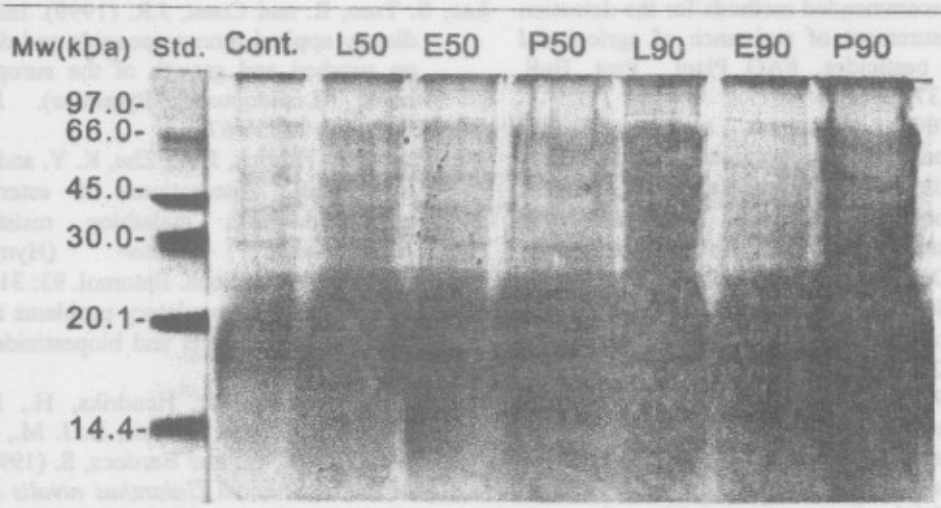


**Figure 2 : SDS - PAGE of *C.chinensis* proteins treated with the LC 50 and LC 90 of the tested monoterpeins. Anode (+) is toward bottom of photo. Each lane had 40 µg protein for each sample.**
**L, E, and P: Linalool, Eugenol and α-Pinene with LC 50 and LC 90, respectively.**

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## الملخص العربى

## التغير في المحتوى البروتيني و الأنزيمي في حشرة خنفساء اللوبيا بعد معاملتها ببعض التربينات الأحادية مختلفة التركيب

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تم في هذا البحث اختبار فاعلية ثلاث أنواع من التربينات الأحادية ذات تراكيب مختلفة على خنفساء اللوبيا تضمنت تلك الأنواع: اللينالول (مركب لاحلقي)، اليوجينول (مركب أحادي الحلقة) و الألفابينين(مركب ثنائي الحلقة)، و قد أظهرت النتائج المتحصل عليها أن سمية المركب اللاحلقي أكبر من المركبين الآخرين حيث كانت الجرعة نصف المميتة($LC_{50}$) للمركب الحلقي هي ١٠ أجزاء في المليون بينما كانت الجرعات المماثلة لمركبي اليوجينول و الألفابينين هي ٣٩، ٤١ جزء في المليون على الترتيب. و عند معاملة حشرة خنفساء اللوبيا بكل من الجرعة نصف المميتة($LC_{50}$) و الجرعة المميتة($LC_{90}$) تبين ظهور بروتينات جديدة غير متواجدة في الحشرات غير المعاملة. كما أظهرت النتائج أيضاً أن لمركب اللينالول تأثيراً أكبر على فسيولوجية الحشرة مقارنة بالمركبين الآخرين.

كما أوضحت النتائج أن معاملة الحشرات بالجرعة نصف المميتة و الجرعة المميتة بالمركبات السابقة أدت إلى زيادة ملحوظة في نشاط أنزيمات: GOT, GPT, ACP,ALP و قد كان مركب اللينالول هو أكثر تلك المركبات تأثيراً في زيادة نشاط تلك الأنزيمات.