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# EFFECT OF GARLIC OIL AND ALLYL DISULFIDE ON PROTEIN AND AMINO ACID COMPOSITION OF TWO STORED-PRODUCT ADULT INSECTS "*CALLOSOBRUCHUS CHINENSIS* (COLEOPTERA: BRUCHIDAE) AND *SITOPHILUS ORYZAE* (COLEOPTERA: CURCULIONIDAE)"

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## ABSTRACT

The effect of treatment of two stored-product adult insects "*Callosobruchus chinensis* (Coleoptera: Bruchidae) and *Sitophilus oryzae* (Coleoptera: curculionidae)" with garlic oil and allyl disulfide was studied. Two different criteria were investigated as changes in protein behaviour on polyacrylamide gel electrophoresis as well as variations in amino acid composition. Native polyacrylamide gel electrophoresis of proteins of control and treated insects showed that allyl disulfide and garlic oil treatments have different distinguished effects on protein synthesis in *C. Chinensis and S. oryzae* at both LC50 and LC90 treatments. The elctrophoretic patterns of *C. chinensis and S. oryzae* were completely different revealing the distinguished variations in protein composition of each insect. Increase the dose of either allyl disulfide or garlic oil to LC90 resulted in more inducing effect for protein synthesis in each type of insects.

SDS-polyacrylamide gel electrophoresis showed that the treatments with allyl disulfide and garlic oil resulted in synthesis of new proteins having low molecular weights and it was pronounced in *S. oryzae* with garlic treatment.

Amino acid composition analysis revealed that phenylalanine is the most abundant amino acid in proteins of both *C. chinensis* and *S. oryzae.* This may reveal the principle role of this amino acid in the physiological behaviour of both types of insects. However, the amino acid composition of protein of each insect is entirely different..

Moreover, some amino acids were more synthesized, especially phenyalanine after treatment with garlic oil and allyl disulfide treatments. It was more remarkable with LC90 allyl disulfide treatment.

# INTRODUCTION

Infestation of grain by various storage-product pests may occur at various stages from time of harvest to consumption by consumers. The susceptibility of stored grain to insect infestation is dependent on a number of factors such as the condition of grain at harvest, environment, cleanliness of bulk storage facilities, and treatment of the grain by protectants, usually fumigants.

In many storage systems, use of fumigants is the most economical tool for managing stored-grain insect pests (Bell, 2000). Fumigants should be biologically active, sufficiently volatile to be removed by aeration, not absorbed by grain, not flammable and non-corrosive. Currently, few chemicals are available for use as fumigants that meet all of these constraints (Lee *et al.,* 2000). Use of methyl bromide, the most effective and widely used fumigant has been phased out in 2005 due to its potential ozone depleting properties (WMO, 1991). Moreover, it is highly toxic to worm-blooded animals including humans (Dansi *et al.,* 1984). Phosphine fumigation, which is widely used, may become increasingly limited in use because pest resistance to phosphine had recorded in more than 45 countries (Bell and Wilson, 1995). In addition, Garry *et al.,* (1989) mentioned that phosphine has genotoxic effect to occupationally exposed fumigators. Because of the increasing drawbacks in continued used of today's conventional fumigants an effort is needed for development of new compounds to replace those currently used.

One potential source for such new compounds is botanical products. Various novel plant-derived compounds have been investigated for their insecticidal properties. These include extracts from the neem tree (Pascual *et al.,* 1990 and Jood *et al.,* 1996), thyme (Mansour *et al.,* 2000), avocado (Rodriguez-Saona and Trumble, 1996) and garlic (Birrenkott *et al.*, 2000).

Garlic, *Allium sativum* L., extracts have shown considerable toxicity to a number of pest species belonging to different insect orders such as Coleoptera, Lepidoptera, Heteroptera and Diptera.

The bulbs of garlic and their extracts have showen to exhibit many promising properties in the control of stored-product pests. Petroleum ether extract of garlic have a repellent action towards *C. chinensis* (Pandey *et al.*,1976). Jood *et al.*, (1993) demonstrated that the powder of garlic bulbs can be used to protect maiz kernels against larvae of *Trogoderma granarium.* Ho *et al.,* (1995) found that the juice of garlic bulbs and ethyl acetate extract of garlic are highly

repellent to *Tribolium castaneum* and *S. zeamais.* Swidan (2005a) found that garlic oil has a fumigant and contact toxicity towards *C. chinensis, C. maculatus, T. Castaneum* and *S. oryzae.* Arannilewa *et al.,* (2006) mentioned that petroleum ether extract has lethal effect against *S. zeamais.*

In spite of the various researches dealt with the effect of garlic juice and its extracts against many insect orders, few studies were concerned with the effect of the active compounds of garlic against stored-product insects. The major volatile compounds of garlic are sulfur-containing compounds such as mono-, di-, and trisulfides. But the most abundant compounds found in garlic juice were allyl di- and trisulfide (Yu *et al.,* 1989). Other compounds were also present at lower concentrations such as allyl methyl trisulfide, diallyl sulfide, diallyl tetra sulfide and allyl methyl tetrasulfide (Prowse *et al.,* 2006).

Chiam *et al.,* (1999) investigated the effect of allyl disulfide towards adults and larvae of *T. castaneum* and adults of *S. zeamais.* They found that *T. castaneum* adults were more susceptible to the fumigant toxicity of allyl disulfide than those of *S. zeamais.* Huang *et al.,* (2000) tested the fumigant and contact toxicity of methyl allyl disulfide and dially trisulfide towards *S. zeamais* and *T. castaneum.* They found that the fumigant and contact toxicity of diallyl trisulfide were greater than that of methyl allyl disulfide to the adults of these two species of insects. These two compounds were also more toxic to *T. castaneum* adults than *S. zeamais* adults. Swidan (2005b) tested the fumigant and contact toxicity of allyl disulfide against adults of *C. chinensis, C. maculatus, T. castaneum* and *S. oryzae.* He found that allyl disulfide was more toxic to *C. chinensis* than the other insects in both fumigant and contact toxicity.

The aim of the current study was to determine the effects of garlic oil and allyl disulfide on the changes in the amino acid composition and the protein electrophortic patterns of *Callosobruchus chinensis* and *Sitophilus oryzae* in order to evaluate the physiological effects of these compounds against the above insects.

## MATERIAL AND METHODS

### 1-Chemicals

The chemicals used in this investigation were allyl disulfide ($C_6$ $H_{10}$ $S_2$) (80% purity, MW 146.28, density 1.008 g/ml) was purchased from Sigma-Aldrich, St. Louis, USA..Garlic oil was extracted from garlic cloves by water

steam distillation. The chemicals were diluted with acetone (analytical reagent grade) to prepare the different concentrations.

**2- Insects**

Adults of *S. oryzae* and *C. chinensis* were obtained from laboratory cultures at the Department of Biology, Faculty of Education, Alexandria University and maintained in a controlled environmental chamber at 28±2°C, 60-70 ± 5% R.H. and a photoperiod of 12:12h (L:D). Insecticide-free whole wheat grains were used as a culture media for *S. oryzae;* while insecticide-free cowpea grains were used for *C. chinensis.*

**3- Bioassay**

To evaluated the effect of allyl disulfide and garlic oil on amino acid composition and the protein electrophoretic patterns of *C. chinensis* and *S. oryzae,* fumigant bioassay was used. This bioassay was performed in exposure chambers composed of transparent plastic jars (1 liter) with screwed caps. A Watman No. 1 filter paper (2.0 cm diameter) was glued on the underside of each cap and served as a diffuser for garlic oil and allyl disulfide. By means of automatic micropipette the $LC_{50}$ and $LC_{90}$ values of both compounds (Table 1) were dissolved in 0.1 ml acetone, before being allowed to the filter paper in the inner cap of the jars. Control treatments were carried out using the same jars and the filter papers were impregnated wit 0.1ml 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 each containing 50 adult insect (less than 12 hours old for *C. chinensis* and one week old for *S. orzae*). The jars containing the tested insects were incubated at 28 ± 2°C, 70 ± 5% R-H and a photoperiod of 12:12 h (L : D). After 12, hours insects were transferred to clean jars covered with a nylon cloth, tied with a rubber band and left for another 12 hours in the controlled environmental chamber. After this period, insects were kept at -20°C until analysis for amino acid and protein patterns.

Table 1: $LC_{50}$ and $LC_{90}$ values of fumigant toxicity of garlic oil and allyl disulfide for *C. chinensis* and *S. oryzae* (Swidan, 2005 a & b).

| Tested insect | Garlic oil | | Allyl disulfide | |
|---|---|---|---|---|
| | $LC_{50}$ (ppm) | $LC_{90}$ (ppm) | $LC_{50}$ (ppm) | $LC_{90}$ (ppm) |
| *C. chinensis* | 0.081 | 0.14 | 0.46 | 1.31 |
| *S. oryzae* | 1.77 | 7.66 | 3.75 | 12.65 |

**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), were 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 µg /protein as bovine serum albumin.

**5-Native-polyacrylamide gel electrophoresis (Native-PAGE)**

Native-PAGE at pH 8.6 was carried out using vertical electrophoresis apparatus (PROTEAN. II cell, Bio-Rad, Richmond, CA, USA) at 120V for 2h. Gels used were 4.5% T for stacking and 10% T for separating (resolving gel). TEMED and ammonium persulphate (final concentrations 0.04% (v/v) and 0.07% (w/v), respectively) were added to the stacking gel containing 7.5% glycerol in 0.5M tris-Hcl buffer (pH 68). The separation gel contained TEMED and ammonium persulphate (final concentration 0.03% (v/v) and 0.07% (w/v), respectively) in 1.5M tris-Hcl buffer (pH 8.8). The electrode and running buffer consisted of 0.19 M glycine and 0.24M tris (pH 8.6) (Hames and Rickwood, 1990).

**6- 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* and *S. oryzae* for the analysis:**

Frozen samples of *C.chinensis* and *S. oryzae* 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 |

**i) 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).

**7-Amino acid analysis**

Determination of the amino acids presents in the protein component of *C.chinensis* and *S. oryzae* proteins was performed according to the method described by Ozols (1990). Protein samples were first oxidized with performic acid to protect methionine and cysteine from distraction during acid hydrolysis. Acid hydrolysis was carried out in closed conical flask for determining all amino acids. Samples equal to 10 mg of protein was weighed in the conical flask and 5 ml of performic acid was added. The flask was closed and placed in ice bath for 16 hours followed by the addition of 0.84 mg sodium disulfide and 25 ml of 6 N HCL. The flasks were placed in an oven for 24 h at 110°C. The flasks were then opened and by the mean of rotary evaporator the volume of each flask was reduced to 5-10 ml under vacuum at 40°C. The pH of each sample was adjusted to 2.20 by sodium hydroxide solution. Suitable volume of sodium citrate buffer (pH 2.20) was added to each hydrolyzed sample. After all soluble materials were completely dissolved; the samples are ready for the analysis. The samples were analyzed by the high performance Beckman 7500 amino acid analyzer at the Central Laboratory for Food and Feed, Agricultural Research Centre, Cairo, Egypt.

## RESULTS

**I. Gel electrophoresis of proteins**

**A. Native - polyacrylamide gel electrophoresis (Native PAGE).**

To study the effect of allyl disulfide as well as garlic oil treatments on the physiological behaviour of *C. chinensis* and *S. oryzae* as considered stored – product insects; soluble proteins were extracted from each type of insect after treatments and subjected to the molecular technique analysis, e.g. polyacrylamide gel electrophoresis, without any changes in the nature of the proteins, i.e., to be native protein.

Figure 1 shows the electrophoretic patterns of proteins extracted from *C. chinensis* after treatment with $LC_{50}$ and $LC_{90}$ of allyl disulfide (1 & 2) and $LC_{50}$ and $LC_{90}$ of garlic oil (3 & 4). The electrophoretic patterns showed the appearance of several peptide bands in control sample as well as in treated samples. These bands differed in number, migration positions and band's intensity. In control samples, only 6 peptide bands were observed separated at different migration positions, starting from Rf 17 to Rf 102 (Table 2). Three bands of those are majors, and separated at positions of Rf 17, Rf 24 and Rf 102. They are representing 18.8, 22.8 and 21.9% of the total bands, respectively.

The $LC_{50}$ of allyl disulfide pattern showed the appearance of one more band, which separated at Rf 118. Meanwhile, there was a marked decrease in the intensity (concentration) of a peptide band, separated at position (Rf 102). The presence of such peptide indicated that the treatment with $LC_{50}$of allyl disulfide resulted in the synthesis of a new protein (Rf 118) at the same time suppressed to some extent the synthesis of two proteins (Rf 39 & Rf 102). When the dose of allyl disulfide was increased to $LC_{90}$, more pronounced changes were recorded. Since 9 peptide bands were appeared on the gel. This means that two more polypeptides (Rf 146 & Rf 173) were synthesized comparing with $LC_{50}$. meanwhile, this dose resulted in suppression of not only two proteins but also three proteins (Rfs 39 & 83 & 102). From this result it is clear that increase the dose of allyl disulfide was more effective on the physiological behaviour of *C. chinensis* specifically for protein synthesis. It is expected that, the decrease in protein synthesis of 3 polypeptides will affect the behaviour of *C. chinensis* towards allyl disulfide treatment.

When garlic oil was used instead of allyl disulfide; the protein patterns showed that at the $LC_{50}$ of garlic oil different effect was noticed. Since two polypeptides (Rfs 39 & 102) were completely disappeared comparing to the control (Table 2). At the same time a new polypeptited (Rf 118) was appeared. This result indicated that the $LC_{50}$ garlic oil treatment resulted in blocking the synthesis of two polypeptides but enhanced or induced the synthesis of other new polypeptides. On the other side the treatment led to increase in the concentration of the common polypeptide (Rf 83) with the control one.

When garlic oil concentration was increased to the $LC_{90}$, the effect was more remarked. Since 8 polypeptides were observed on the gel. Three of them (Rfs 39, 146 & 173) were new proteins comparing with the treatment of $LC_{50}$ garlic oil.

From these results, it can be concluded that the treatments with allyl disulfide and garlic oil have different effects on the protein synthesis of *C. chinensis* and increasing the dose to $LC_{90}$ in both treatments results in synthesis of new proteins not present in the control .

Concerning the effect of allyl disulfide and garlic oil on *S. oryzae,* the electrophoretic patterns (Figure 2) showed that the control sample was containing 7 peptide bands. These bands differed also in migration position and band's intensity. It was clear that no major bands were remarked among these bands. This pattern is completely different than that of *C. chinensis.* Another evidence for the

difference is the number of bands and their intensities also the migration positions of bands separated in pattern of each type of insects.

The electrophoretic patterns of $LC_{50}$ of allyl disulfide showed the appearance of 6 peptide bands. Of these 5 common bands with the control sample; however, a new major band (Rf 26) was appeared. Meanwhile, two peptides were disappeared (Rfs 13 & 37) from the pattern of $LC_{50}$. This result indicated that this treatment had a remarkable effect on the synthesis of a new protein (stress protein, which can be considered as a protective effect) and prevention of synthesis of another two peptides. When the concentration of allyl disulfide was increased to the $LC_{90}$, 8 peptide bands were observed. Two of them were new (Rfs 26 & 96). It is clear that one of them (Rf 26) was common with $LC_{50}$ pattern but the other (Rf 96) was unique. This finding indicates that increase the concentration of allyl disulfide resulted in synthesis of more peptides. Although, this effect of allyl disulfide was similar in *C. chinensis,* the new peptides in each type of insects were completely different in their properties due to the difference in migration positions in each case, which reflects the differences in the density of net charges on each type of protein, consequently, its primary structure.

Figure 2 showed also the changes in electrophoretic patterns of *S. oryzae* due to the treatment with $LC_{50}$ and $LC_{90}$ of garlic oil. The patterns showed the appearance of only 5 peptide bands treated with $LC_{50}$ garlic oil. Four of them were common with control; however, the fifth peptide was unique and separated at Rf 26 (Table 3). This means that $LC_{50}$ of garlic oil was more effective on the synthesis of *S. oryzae* proteins. When garlic oil was used at the concentration of $LC_{90}$, the pattern showed the appearance of one more peptide band (Rf 115) compare with $LC_{50}$.

From these results it can be concluded that a) allyl disulfide and garlic oil treatments have different distinguished effects on both *C. chinensis* and *S. oryzae* at both levels of treatments. b) the electrophoretic patterns of *C. chinensis* and *S. oryzae* were completely differents. c) increase the dose of either allyl disulfide or garlic oil results in more inducing effect for protein synthesis in each type of insects.

**B. SDS-polyacrylamide gel electrophoresis.**

In order to verify the characteristics of the separated polypeptides, i.e. molecular masses (KDa) of each type of insects, another molecular technique was applied. This is known as polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE). Figure 3 shows SDS-PAGE

electrophoretic patterns of *C.chinensis* treated with $LC_{50}$ and $LC_{90}$ of allyl disulfide and garlic oil. The total lab analysis of each pattern showed that in control sample 10 polypeptides having molecular masses ranged from 45.7 to 164 KDa were present (Table 4). In pattern of $LC_{50}$ of allyl disulfide two polypeptides (52 & 57 KDa) were disappeared but one (43.2 (KDa) polypeptide was appeared. In pattern of $LC_{90}$ of allyl disulfide two polypeptides (38.7 & 43.2 KDa) were appeared and were not present in control. In contrast to that the treatment with garlic oil at $LC_{50}$ and $LC_{90}$ resulted in appearance of higher number of polypeptides especially those of 22.5 to 43.2 KDa. From these results, it is clear that treatment of *C.chinensis* with allyl disulfide or garlic oil resulted in synthesis of new peptides having low molewlar weights ranged from 22.5 to 43.2. This phenomenon was more pronounced with garlic oil treatment than allyl disulfide treatment. Figure 4 shows SDS-PAGE electrophoretic patterns of *S. oryzae* treated with $LC_{50}$ and $LC_{90}$ of allyl disulfide and garlic oil. The behaviour was completely different in case of *S.oryzae,* since 14 polypeptides having molecular masses ranged from 21.4 to 179 KDa were recorded in control sample (Table 5). The same peptides were appeared in $LC_{50}$ of allyl disulfide. But in pattern $LC_{90}$ of allyl disulfide, two low molecular mass peptides (15.2 & 18.7 KDa) were appeared.

The treatment with garlic oil at $LC_{50}$ and $LC_{90}$ showed the appearance of the same number of peptides having a wide range of molecular masses (8.7 to 179 KDa).

It should be taken into account that in native-PAGE each peptide is separated in one band; but in SDS-PAGE the same peptide may be splited into several bands i.e., subunits depending on the number of disulfide bands present in the structure of that protein.

From these results it can be concluded that the treatments with allyl disulfide or garlic oil result in synthesis of new polypeptides having low molecular masses, and it was pronounced in *S. oryzae* with garlic treatment.

**II. Amino acid composition**

Table 6 shows amino acid composition of proteins extracted from *C. chinensis* which treated with allyl disulfide and garlic oil. It is clear that glutamic acid and phenylalanine are the major amino acids in the protein of control sample, wherease, methionine and cystein were the minor ones. This means that the content of sulpher-containing amino acid in control sample was very limited while the most abundant amino acid was phenyl alanine. When $LC_{50}$ of garlic oil was used, the amino acid pattern of *C. chinensis* protein was not affected comparing to

the control one except a decline in valine content in $LC_{50}$ sample was occurred. With $LC_{90}$ garlic oil it was noticed that the contents of all amino acids were increased comparing to the control. This means that this treatment had a pronounced effect on the synthesis rate of all amino acids, which form the whole protein of the insects.

When allyl disulfide $LC_{50}$ was applied, its effect was similar to that of garlic $LC_{90}$, except there was a decrease in the content of the major amino acid (phenylalanine) in protein of $LC_{50}$ allyl disulfide treatment. The results showed also that $LC_{90}$ allyl disulfide treatment resulted in more decrease in phenylalanine content comparing to the control or $LC_{50}$ allyl disulfide. But there was a marked increase in methionine content in $LC_{90}$ allyl disulfide treatment. The increase rate was about 4.3 much more times as that of control.

Regarding the amino acid composition of *S. oryzae* protein as affected by the same above mentioned treatments, results (Table 7) showed that phenylalanine was the most abundant amino acid in control sample followed by glutamic, glycine and alanine. This pattern is similar to that of *C. chinensis* in the dominance of phenylalanine but different in other amino acid contents. With $LC_{50}$ garlic oil treatment, the amino acid composition was slightly different than that of control, i.e, a slight increase in most amino acids content was observed. But there was a decrease in phenylalanine content. By $LC_{90}$ garlic oil treatment, there was an obvious increase in the contents of glutamic, glycine, valine and histidine.

On the other side the treatment with $LC_{50}$ of allyl disulfide resulted in marked increase in all amino acids except methionine and phenylalanine. On the contrary, $LC_{90}$ treatment led to marked increase in the content of phenylalanine.

On the bases of the present results it can be concluded that a) phenylalanine is the most abundant amino acid in protein of both *C. chinensis* and *S. oryzae.* b) the amino acid composition of protein of each insect is completely different in amino acid contents. c) garlic oil and allyl disulfide treatments of $LC_{50}$ and $LC_{90}$ had significant effects on the amino acid composition of protein of each insect by increasing the synthesis of amino acids specially phenyl alanine by $LC_{90}$ allyl disulfide treatment.

## DISCUSSION

This study is probably the first investigation to examine the influence of allyl disulfide and garlic oil on protein and amino acid compositon of adults of *C. chinensis* and *S. oryzae.*

Formation of new proteins were observed in different insect species often insecticide or juvenile hormone treatments. Delisle and Cusson (1999) reported the formation of new, low molecular weight protein, after juvenile hormone treatment in females of *Choristoneura fumiferana.* This low molecular weight protein was the vitellogenin protein found in the oocytes of the tested insect. Dekort and Koopmanschap (1994) found that photoperiodic conditions affects protein composition of the haemolymph of *leptiotarsa decemlineata.* They added that after juvenile hormone treatment, a new polypeptite was detected in *L. decemlineata.* This result was also confirmed by De Kort *et al.,* (1997). This new polypeptide was considered as a diapausing protein and appears only after juvenile hormone treatment and induce the insect to enter the diapausing period.

In the present study allyl disulfide and garlic oil could prevent the expression of the gene responsible for protein synthesis in the control and accelerates their mRNA in the treated insects while led to the formation of new proteins. Additional biochemical and molecular studies are necessary to elucidate the exact role of the allyl disulfide and garlic oil towards different insect species.

Lefevere *et al.*, (1989) mentioned that amino acid concentration increased as a result of different physiological factors in different insect orders. Yi and Adams (2000) found an increase in amino acid concentration after Juvenile hormone treatment in *L. decemlineata.* Similar results were also obtained by De Kort and Kramer (1976). The increase in amino acid concentration in *C. chinensis* and *S. oryzae* as a result of allyl disulfide and garlic oil treatments could be related to the synthesis, uptake and degeneration of major body proteins, vitellogenins, lipophorins, enzymes, peptides and nitrogenous products.

On the bases of these findings, the study suggests that the physiological behaviours of *C. chinensis* and *S. orysae* are different due to the complete difference in protein behaviour and structure. Treatments with garlic oil and allyl disulfide led to suppression of synthesis of some proteins in both types of insects, which may play an important physiological role. On the other side, new proteins were synthesized as stress proteins to overcome the effect of treatments.

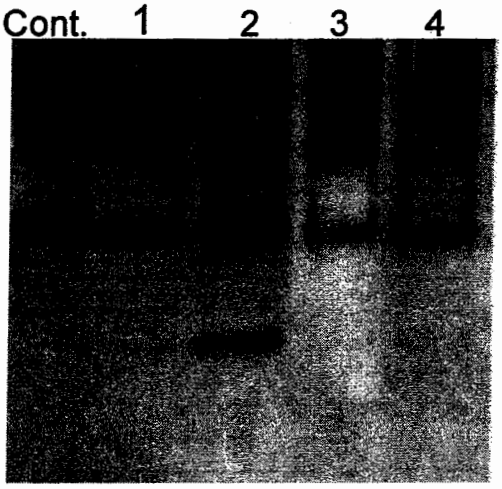


Figure 1: Alkaline native-PAGE of *C. chinensis* proteins treated with allyl disulfide and garlic oil. Cont: control. 1 & 2: beetles treated with $LC_{50}$ and $LC_{90}$ of allyl disulfide respectively. 3 & 4: beetles treated with $LC_{50}$ and $LC_{90}$ of garlic oil, respectively. Anode (+) is toward bottom of photo.

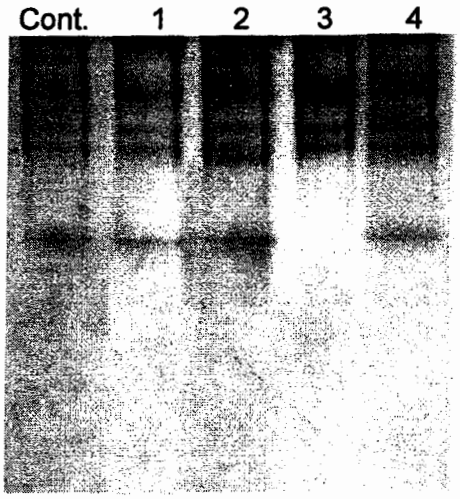


Figure 2: Alkaline-native PAGE of S. oryzae proteins treated with allyl disulfide and garlic oil. Cont: control. 1 & 2: weevils treated with LC50 and LC90 of allyl disulfide respectively. 3 & 4: weevils treated with LC50 and LC90 of garlic oilrespectively. Anode (+) is towards bottom of photo.

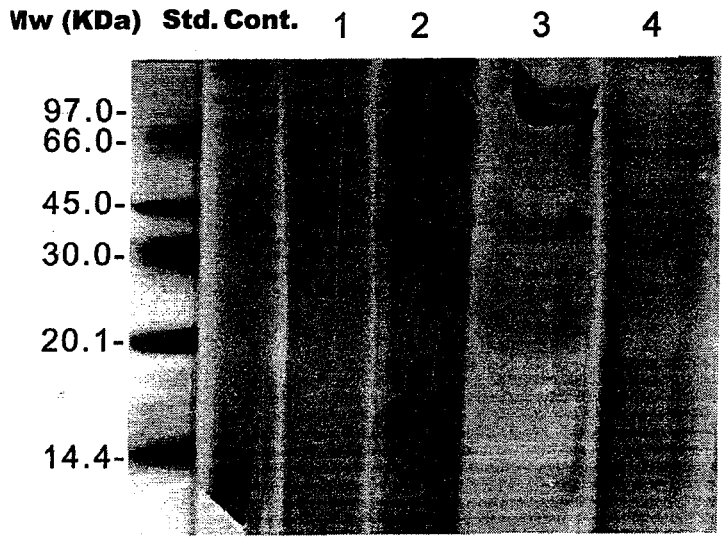


Figure 3: SDS-PAGE of *C. chinensis* proteins treated with allyl disulfide and garlic oil Cont: control. 1 & 2: beetles treated with $LC_{50}$ and $LC_{90}$ of allyl disulfide respectively. 3 & 4: beetles treated with $LC_{50}$ and $LC_{90}$ of garlic oil respectively. Anode (+) is towards bottom of photo.

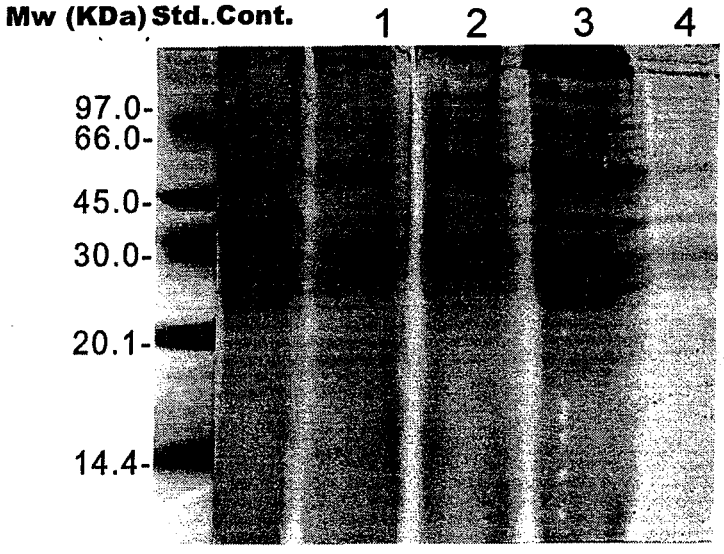


Figure 4: SDS-PAGE of *S. oryzae* proteins treated with allyl disulfide and garlic oil.. Cont: control. 1 & 2: weevils treated with $LC_{50}$ and $LC_{90}$ of allyl disulfide respectively. 3 & 4: weevils treated with $LC_{50}$ and $LC_{90}$ of garlic oil respectivelyl. Anode (+) is towards bottom of photo.

**Table 2: Scanning analysis of alkaline native-PAGE protein patterns of *C. chinensis* treated with $LC_{50}$ and $LC_{90}$ of allyl disulfide and garlic oil presented in Figure 1.**

| Band No. | Rf | Band % | | | | |
|---|---|---|---|---|---|---|
| | | Control | 1 | 2 | 3 | 4 |
| 1 | 17 | 18.8 | 19.7 | 18.2 | 16.9 | 20.3 |
| 2 | 24 | 22.8 | 20.5 | 23.4 | 21.2 | 23.2 |
| 3 | 39 | 10.6 | 5.1 | 6.1 | - | 3.6 |
| 4 | 56 | 15.0 | 14.2 | 11.2 | 16.0 | 11.9 |
| 5 | 83 | 9.7 | 10.0 | 5.3 | 18.3 | 13.1 |
| 6 | 102 | 21.9 | 9.4 | 7.4 | - | - |
| 7 | 118 | - | 21.1 | 20.8 | 27.6 | 25.4 |
| 8 | 146 | - | - | 2.2 | - | 1.4 |
| 9 | 173 | - | - | 5.4 | - | 1.1 |

**(-): Not present.**
**1 & 2: Beetles treated with $LC_{50}$ and $LC_{90}$ of allyl disulfide.**
**3 & 4: Beetles treated with $LC_{50}$ and $LC_{90}$ of garlic oil.**

**Table 3: Scanning analysis of alkaline native-PAGE protein patterns of *S.oryzae* treated with $LC_{50}$ and $LC_{90}$ of allyl disulfide and garlic oil presented in Figure 2.**

| Band No. | Rf | Band % | | | | |
|---|---|---|---|---|---|---|
| | | Control | 1 | 2 | 3 | 4 |
| 1 | 13 | 19.2 | - | - | - | - |
| 2 | 26 | - | 23.4 | 13.4 | 11.9 | 20.4 |
| 3 | 37 | 16.7 | - | 3.0 | - | - |
| 4 | 49 | 14.9 | 13.2 | 6.1 | 20.0 | 11.7 |
| 5 | 60 | 13.4 | 13.1 | 12.2 | 23.1 | 13.8 |
| 6 | 71 | 12.9 | 11.8 | 14.3 | 24.6 | 15.9 |
| 7 | 82 | 12.7 | 15.3 | 4.0 | 20.2 | 14.5 |
| 8 | 96 | - | - | 24.1 | - | - |
| 9 | 115 | 10.2 | 23.2 | 22.9 | - | 23.8 |

**(-): Not present.**
**1 & 2: Weevils treated with $LC_{50}$ and $LC_{90}$ of allyl disulfide.**
**3 & 4: Weevils treated with $LC_{50}$ and $LC_{90}$ of garlic oil.**

**Table 4: Scanning analysis of SDS-PAGE of protein patterns of *C. chinensis* treated with $LC_{50}$ and $LC_{90}$ of allyl disulfide and garlic oil presented in Figure 3.**

| Band No. | Rf | MW (KDa) | Band % | | | | |
|---|---|---|---|---|---|---|---|
| | | | Control | 1 | 2 | 3 | 4 |
| 1 | 19 | 164 | 14.2 | 17.4 | 16.9 | 10.4 | 4.1 |
| 2 | 24 | 149 | 9.7 | 7.3 | 15.7 | - | 5.0 |
| 3 | 33 | 122 | 17.2 | 18.9 | 16.4 | - | 14.1 |
| 4 | 47 | 91.5 | 23.2 | 28.9 | 24.8 | 26.3 | 15.1 |
| 5 | 56 | 74 | 18.4 | 20.9 | 11.4 | 29.4 | 33.5 |
| 6 | 59 | 70 | 4.4 | 2.3 | 3.1 | 8.4 | 20.9 |
| 7 | 70 | 57 | 2.5 | - | 1.3 | 7.95 | 1.0 |
| 8 | 83 | 52 | 3.9 | - | 1.7 | 8.88 | 0.73 |
| 9 | 94 | 49 | 3.4 | 2. | 3.3 | 1.2 | 1.05 |
| 10 | 105 | 45.7 | 3.0 | 1.5 | 2.1 | 1.4 | 0.95 |
| 11 | 119 | 43.2 | - | 1.02 | 2.3 | 1.0 | 0.73 |
| 12 | 124 | 38.7 | - | - | 0.95 | 0.83 | 0.55 |
| 13 | 137 | 27.4 | - | - | - | 2.0 | 1.2 |
| 14 | 144 | 22.5 | - | - | - | 2.3 | 1.0 |

**(-): Not present. 1 & 2: Beetles treated with $LC_{50}$ and $LC_{90}$ of allyl disulfide. 3 &4: Beetles treated with $LC_{50}$ and $LC_{90}$ of garlic oil.**

**Table 5: Scanning analysis of SDS-PAGE of protein patterns of *S.oryzae* treated with $LC_{50}$ and $LC_{90}$ of allyl disulfide and garlic oil presented in Figure 4.**

| Band No. | Rf | MW (KDa) | Band % | | | | |
|---|---|---|---|---|---|---|---|
| | | | Control | 1 | 2 | 3 | 4 |
| 1 | 14 | 179 | 13.2 | 11.4 | 12.9 | 17.6 | 19.3 |
| 2 | 18 | 165 | 10.1 | 9.4 | 8.9 | 13.2 | 17.1 |
| 3 | 22 | 151 | 4.8 | 2.1 | 2.7 | 1.9 | 2.1 |
| 4 | 37 | 119 | 5.1 | 4.2 | 3.5 | 2.9 | 3.0 |
| 5 | 45 | 92 | 3.2 | 2.7 | 3.4 | 3.5 | 3.2 |
| 6 | 59 | 76 | 2.9 | 3.3 | 2.9 | 2.7 | 2.6 |
| 7 | 72 | 55 | 10.7 | 7.1 | 11.4 | 13.9 | 7.5 |
| 8 | 83 | 52 | 12.0 | 16.2 | 11.9 | 10.4 | 6.3 |
| 9 | 98 | 44.5 | 2.4 | 1.7 | 11.03 | 2.5 | 2.4 |
| 10 | 109 | 40.3 | 11.9 | 18.4 | 15.6 | 11.2 | 9.2 |
| 11 | 114 | 39.9 | 10.3 | 17.5 | 4.8 | 8.1 | 8.8 |
| 12 | 130 | 32.2 | 5.9 | 1.0 | 0.75 | 0.95 | 1.2 |
| 13 | 142 | 28.7 | 5.4 | 2.2 | 2.9 | 1.4 | 1.6 |
| 14 | 157 | 21.4 | 2.1 | 2.8 | 1.4 | 0.88 | 0.95 |
| 15 | 161 | 18.7 | - | - | 3.5 | 2.3 | 1.2 |
| 16 | 170 | 15.2 | - | - | 2.4 | 1.9 | 3.5 |
| 17 | 188 | 14.1 | - | - | - | 2.2 | 1.2 |
| 18 | 193 | 11.9 | - | - | - | 1.7 | 0.82 |
| 19 | 207 | 10.2 | - | - | - | 0.50 | 5.03 |
| 20 | 219 | 8.7 | - | - | - | 0.27 | 3.0 |

**(-): Not present.**
**1 & 2: Weevils treated with $LC_{50}$ and $LC_{90}$ of allyl disulfide.**
**3 & 4: Weevils treated with $LC_{50}$ and $LC_{90}$ of garlic oil .**

**Table 6: Percentage of amino acid composition of the protein of *Callosobruchus chinensis* treated with the $LC_{50}$ and $LC_{90}$ of garlic oil and allyl disulfide.**

| Amino acid | Control | Garlic oil | | Allyl disulfide | |
|---|---|---|---|---|---|
| | | $LC_{50}$ | $LC_{90}$ | $LC_{50}$ | $LC_{90}$ |
| Aspartic | 1.26 | 1.49 | 1.76 | 1.90 | 1.61 |
| Methionine | 0.32 | 0.31 | 0.42 | 0.42 | 1.36 |
| Thionine | 0.63 | 0.64 | 0.83 | 0.84 | 0.79 |
| Serine | 0.65 | 0.63 | 0.78 | 0.85 | 0.73 |
| Glutamic | 2.05 | 2.17 | 2.52 | 2.83 | 2.28 |
| Glycine | 1.42 | 1.64 | 1.90 | 1.91 | 1.69 |
| Alanine | 1.25 | 1.20 | 1.52 | 1.57 | 1.40 |
| Gystein | 0.12 | 0.22 | 0.20 | 0.17 | 0.22 |
| Valine | 1.03 | 0.76 | 1.41 | 1.45 | 1.27 |
| Isoleucine | 0.70 | 0.76 | 0.95 | 1.15 | 0.89 |
| Lencine | 1.27 | 1.32 | 1.59 | 1.62 | 1.50 |
| Phenylalanine | 2.85 | 2.58 | 2.89 | 2.76 | 2.40 |
| Histidne | 0.88 | 1.04 | 1.13 | 1.32 | 1.07 |
| Lysine | 1.12 | 1.06 | 1.39 | 1.44 | 1.33 |
| Arginine | 0.91 | 1.11 | 1.12 | 1.17 | 1.12 |

Data are expressed as means of two replicates (Steel and Torrie, 1980).

**Table 7: Percentage of amino acid composition of the protein of *Sitophilus oryzae* treated with the $LC_{50}$ and $LC_{90}$ of garlic oil and allyl disulfide.**

| Amino acid | Control | Garlic oil | | Allyl disulfide | |
|---|---|---|---|---|---|
| | | $LC_{50}$ | $IC_{90}$ | $LC_{50}$ | $IC_{90}$ |
| Aspartic | 1.78 | 1.87 | 1.91 | 2.02 | 2.22 |
| Methionine | 0.28 | 0.13 | 0.13 | 0.14 | 0.17 |
| Thionine | 1.00 | 1.03 | 1.02 | 1.12 | 1.20 |
| Serine | 0.98 | 0.96 | 0.95 | 1.03 | 1.18 |
| Glutamic | 2.78 | 2.80 | 2.92 | 3.07 | 3.28 |
| Glycine | 2.52 | 3.00 | 2.85 | 3.24 | 3.17 |
| Alanine | 2.03 | 2.19 | 2.10 | 2.37 | 2.39 |
| Gystein | 0.25 | 0.35 | 0.31 | 0.39 | 0.21 |
| Valine | 1.67 | 1.65 | 1.91 | 4.56 | 2.05 |
| Isoleucine | 1.22 | 1.32 | 1.34 | 1.45 | 1.40 |
| Lencine | 1.77 | 1.86 | 1.81 | 2.07 | 2.00 |
| Phenylalanine | 4.00 | 3.17 | 3.88 | 3.16 | 5.54 |
| Histidine | 0.60 | 0.65 | 1.01 | 0.74 | 0.71 |
| Lysine | 1.55 | 1.25 | 1.30 | 1.35 | 1.44 |
| Arginine | 1.37 | 1.34 | 1.66 | 1.60 | 1.64 |

Data are expressed as means of two replicates (Steel and Torrie, 1980).

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