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ALTERATION IN SOME BIOLOGICAL AND BIOCHEMICAL PARAMETERS IN TRIBOLIUM CASTANEUM (COLEOPTERA: TENEBRIONIDAE) DUE TO GARLIC OIL EFFECT

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ABSTRACT

Over the centuries, man has struggled to protect his crops from invasion by insects, microbial pathogens and other pests. Stored grains of almost any kind are subjected to attack by insects, which are highly specialized and in most cases are of small size and have high reproductive potential, which make them easily concealed in grains and carried to many parts of the world. Once established in a commodity, they are usually difficult to control. The extensive and widespread use of synthetic insecticides during the past for the control has caused some concerns regarding the toxicity and environmental impact of these agents. Thus the present study was carried out to evaluate garlic oil as safe alternative to conventional insecticides and fumigants for the protection of wheat, as one of the important, grain products against Trobolium castaneum, as one of those serious stored products pests.

Fourth larval instars of Tribolium castaneum, were reared on wheat treated with different concentration of the garlic oil (0.025, 0.1, 0.3, 0.6, 0.9, 1 ml/ 10gm of wheat) till adult emergence to study changes which could happen in its biological parameters. Also fourth larval instars reared on wheat treated with LC50, which found to be 0.49 ml, of the oil for two days to study some biochemical parameters, accordingly, the potential insecticidal properties of this oil could be assessed.

The results show deleterious effects of garlic oil on the insect development and normal growth; as increasing rates of larval mortalities, extended larval and pupal durations, deformations of larvae shown in shrinking and c-shaped, significant decrease in pupation rate and of emerged adults.

As for the biochemical studies; the protein content found to be increased as well alkaline phosphatase and cholinesterase activities, while glucose and lipid contents were decreased along with acid phosphatase, AST (aspartate aminotransferase) and ALT (alanine aminotransferase) activities, noticing that the decrease in AST was more than in ALT.

Keywords

Tribolium castaneum; garlic oil (Allium sativum L.) and biological and biochemical parameters in insects.

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1-INTRODUCTION

Year after year, insect pests cause heavy losses in food and other crops, particularly in tropical and subtropical regions. In addition, they create serious health problems by transmitting pathogens of man and livestock (Balandrin et al., 1985). Severe insect infections surely cause weight losses and deteriorate kernel quality, which can range from limited to state-wide in scope (Sarwar, 2011 and 2015 and Sarwar and Sattar, 2012).

Many insect pests attack on the stored grains, like Confused flour beetle Tribolium confusum (Duval), Khapra beetle Trogoderma granarium (Everts), Lesser grain borer Rhyzopertha dominica (F.), Angoumois grain moth Sitotroga cerealella (Olivier) and Red flour beetle, Tribolium castaneum (Herbst). Among these, red flour beetle T. castaneum is the main serious pest of wheat (Triticum aestivum) (Sarwar et al., 2004 and Sarwar, 2009 and 2013). This beetle has chewing mouthparts, but do not bite or sting. The red flour beetle may elicit allergic response but is not known to spread disease, and does not feed on or damage the structure of a home or furniture (Alanko et al., 2000). This grain pest is found in temperate areas but can survive the winter in protected places, especially where there is central heat (Tripahti et al., 2001).

T. castaneum is a cosmopolitan pest which primarily attacks milled grain products, such as flour and cereals. Both adults and larvae feed on grain dust and broken grains, but not undamaged whole grains. The beetle spends its entire life cycle outside grain kernels. T. castaneum infests a wide range of commodities and products including barley, beans, biscuits, breakfast cereals, cacao, corn, cornmeal, cottonseed, dried fruits, drugs, flour, legume seeds, milk chocolate, millet, nuts, oats, peas, powdered milk, rice, rye, spices, sunflower seeds, wheat and wheat bran, herbarium and museum collections. T. castaneum successfully feeds on many species of storage fungi, which may also play a significant role in nutrition (Karunakaran et al. 2004).

The widespread use and massive application of synthetic organic pesticides to prevent crop losses and transmission of diseases resulted in the buildup of pest resistance, adverse effects on the environment including pollution, acute and chronic hazards to human and other non- target organisms and upsetting the natural balance. Such environmental problems have focused searching for other means of control using new generation of pesticides occurring naturally in plants. So that many investigators in different parts of the world initiated large screening efforts to find plants which possess medicinal and poisonous effects to use them as pesticides (Balandrin et al., 1985; Lowe et al., 2000 and CERIS, 2004).

2-AIM OF THE WORK

The aim of this work is to evaluate the effect of garlic oil, which already used in our feeding and in medical used without harming, and did not show any adverse effect on seed germination, seedling growth, nodulation, general health and morphology of the host plant, on the 4^{th} larval instar of Tribolium castaneum. Accordingly, the potential insecticidal properties of this oil could be assessed.

3-MATERIALS AND METHODS

Culture of T. castaneum was taken from a laboratory culture which has been reared for many generations throughout 3 years for experiments, to keep it away from any possible pesticide contamination. The method used was according to Hassan et al., (1962).

Cultures and all tested insects were carried out under laboratory conditions, 35^{o}C and RH 60-80% (Shazali and Smith 1986), in Postgraduate laboratory, Department of Entomology, Faculty of Science Alexandria University. The experiment was conducted from November 2014 to February 2015. Larvae were reared in 0.5 liter jars in a diet containing crushed wheat provided with maize and 5% Brewer's yeast. The jars were covered with muslin which fixed tightly by rubber bands, (Khalifa and Badawy, 1961 and Ahmed, 1975). Diet was heated to be sterilized in an oven at 60 $^{\circ}$C for 6 hours before using in the tests. Every five days the diet was replaced by a new one to avoid the accumulation of wastes. It is worth mentioning that crowding was prevented during development which was observed to affect the size of the emerging adults. The newly emerged adults were placed on finely sifted crushed wheat as an ovipositional site. Eggs were obtained by separating them from the diet by using camel's hair brush and incubated in rearing diet.

Four replicates each with 25 fourth instar larvae were reared on wheat treated with the tested oil at 0.025, 0.1, 0.3, 0.6, 0.9 and 1.0 ml/10gm beside the controls. Two controls were made, i.e. grains treated with acetone only (which was used in preparing the different concentrations of the oil) and the other kept without any treatment. The control mortality was not more than five percent, so the percentage mortality did not need to be corrected by Abbott's formula, (Abbott, 1925).

The mean lethal concentration (24-h LC_{50}) of tested oil was determined using Probit analysis, using the computer program, (Stephan 1977).

Some biochemical parameters were done in 100 mg tissue of the all homogenated fourth instar larvae reared for two days on wheat treated with LC50 of the oil and repeated for 30 times. These parameters are: glucose (Singh and Singh 1977), total protein (Gornall et al., 1949), total lipid (Kinght et al., 1972), cholinesterase activity (Weber 1966), acid phosphatase activity (Abbott and Kaplan 1984), alkaline phosphatase catalytic activity (Kind and King 1954), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities (Reitman and Frankel 1957).

All data are represented as mean ± SEM. Statistical analysis was carried out using one-way ANOVA followed by LSD for multiple comparison.

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4-RESULTS

Data obtained indicated that rearing of the larvae on wheat treated with different concentrations of garlic oil induced serious effects in their development and normal growth shown in; c-shaped, shrinking and larvae failed to pupate (fig.1). Numbers of larval mortalities increased and pupae and emergence of adult decreased with increasing oil concentrations (fig2). Larval duration (starting from fourth instar) and pupal duration increased (fig.3).

Rearing 4th instar larvae on wheat treated with LC50 (0.49 ml) for two days resulted in decreasing glucose (as an indicator of carbohydrate metabolism) and lipid content and increasing protein content (fig. 4). Alkaline phosphatase activity was found to be higher while acid phosphatase activity was found to be decreased, while cholinesterase was found to be increased, (fig. 5). Both ALT and AST activities were found to be less than in control (fig. 6) and the decrease in AST was found to be more than in ALT (fig. 7).

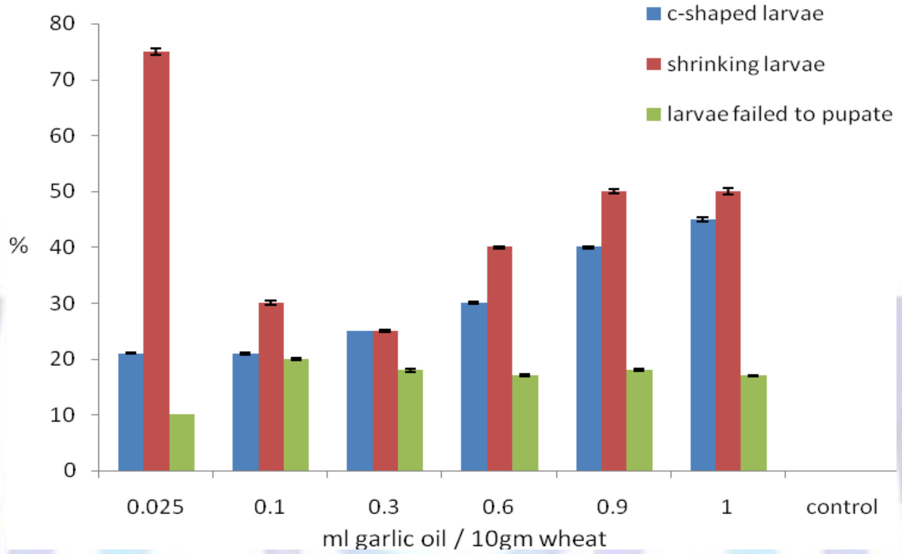

Figure 1: Effect of different concentrations (ml) of garlic oil on 4th instar larvae of T. castaneum; showing percentages of c-shaped larvae, shrinking larvae and larvae failed to pupate

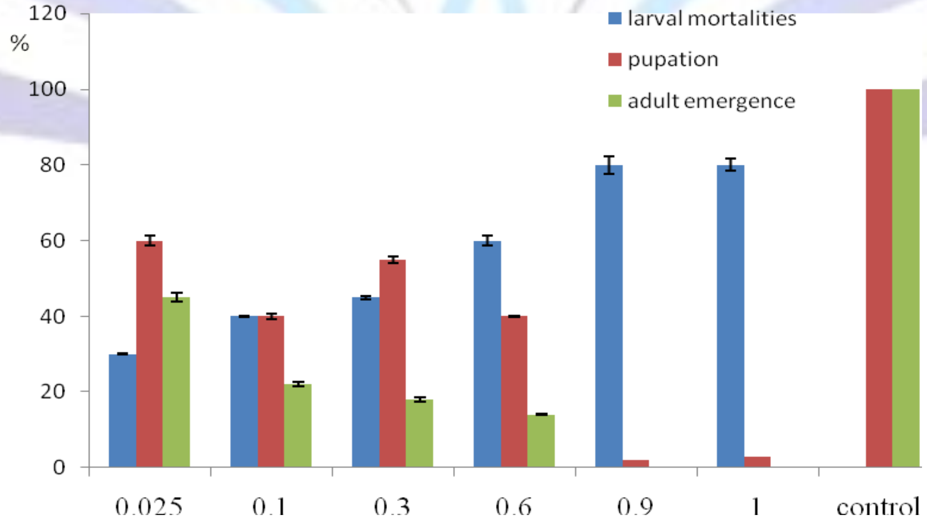

Figure 2: Effect of different concentrations (ml) of garlic oil on 4th instar larvae of T. castaneum; showing percentages of larval mortality, pupation, and adult emergence.

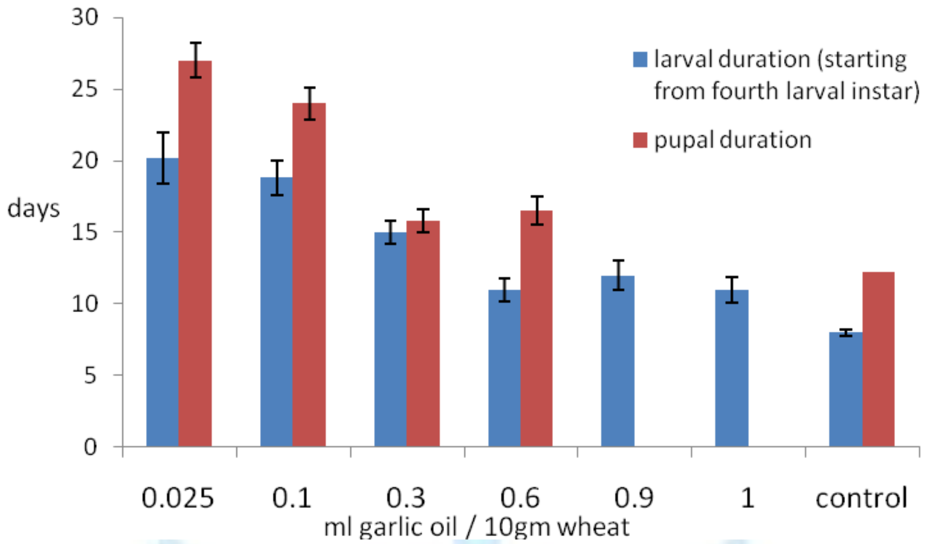

Figure 3: Effect of different concentrations (ml) of garlic oil on 4th larval instar of T. castaneum; showing relations between these concentrations and both larval and pupal durations (days) (starting from fourth instar larvae).

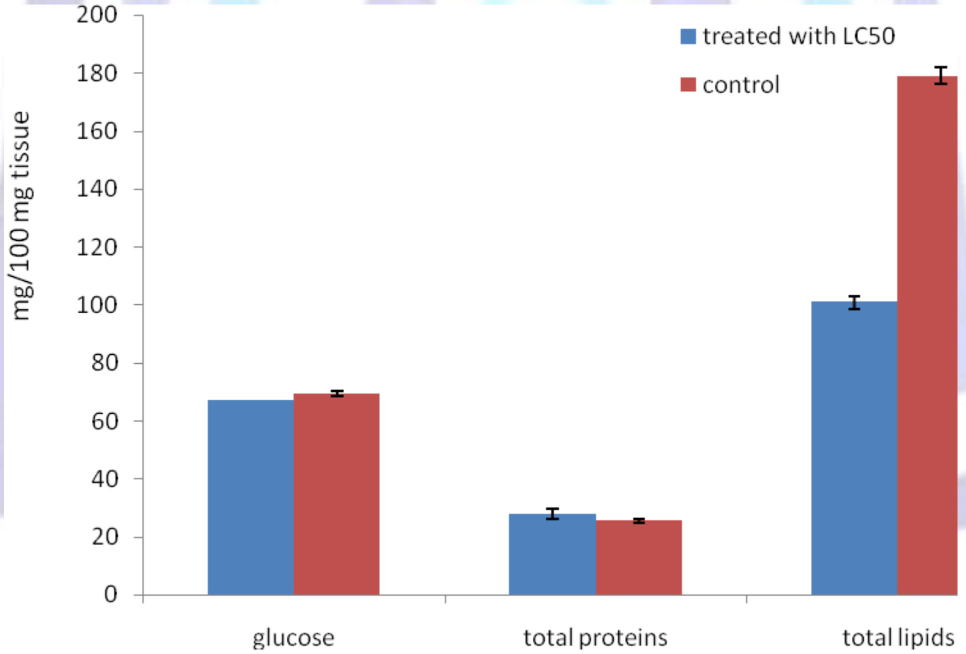

Figure 4: Shows glucose, total protein and total lipids levels in 4th instar larvae of T. castaneum after two days of rearing them on wheat treated with garlic oil at LC50 concentration (0.49ml).

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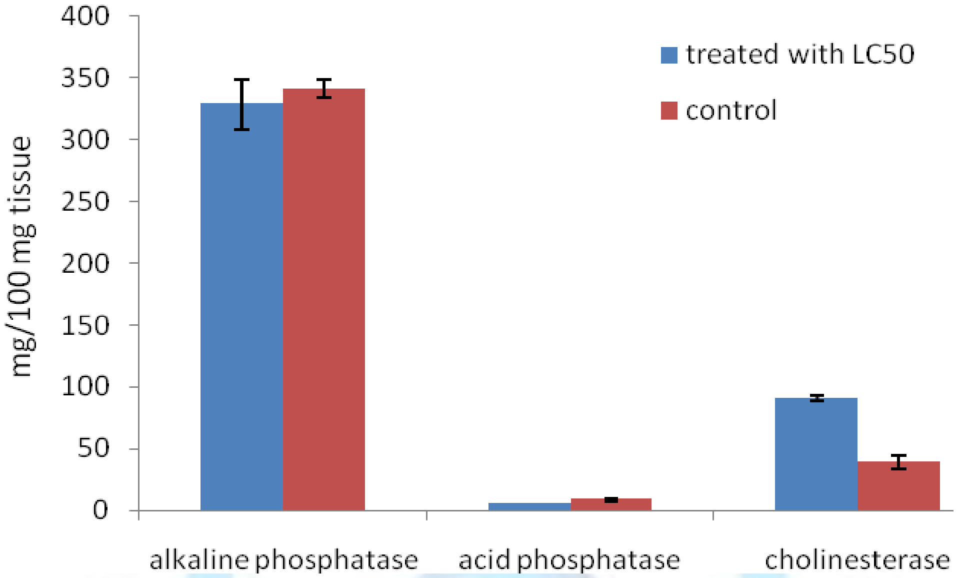

Figure 5: Effects of garlic oil on alkaline phosphatase, acid phosphatase and cholinesterase after two days of rearing 4th instar larvae of T. castaneum on wheat treated with LC50 concentration (0.49ml).

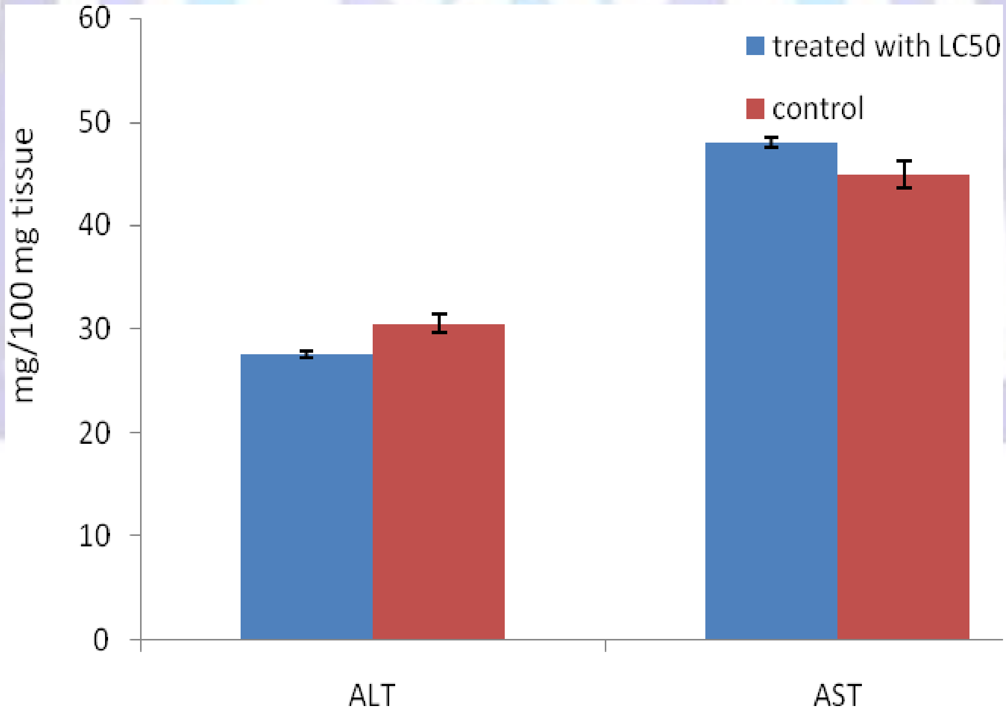

Figure 6: Shows ALT and AST activities in 4th instar larvae of T. castaneum after two days of rearing 4th instar larvae of T. castaneum on wheat treated with garlic oil at LC50 concentration (0.49ml).

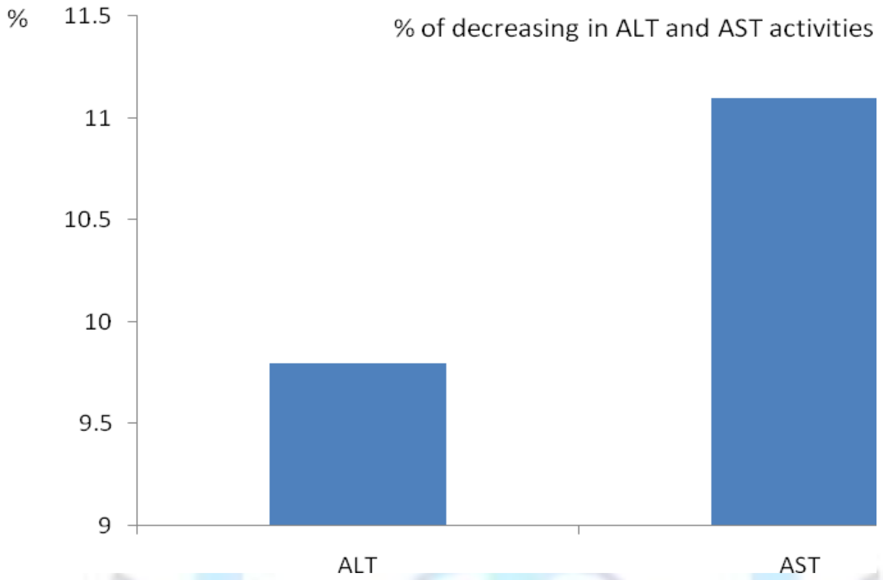

Figure 7: Comparisons between the decreasing percentages of ALT and AST activities in 4th instar larvae of T. castaneum after two days of rearing them on wheat treated with garlic oil at LC50 concentration (0.49ml).

5-DISCUSSION

It is generally accepted, that toxic materials of plant origin exert an inhibitory growth effect, leading to prolonged larval and pupal durations. Similar effects of some phytochemicals on some insect species have been documented by many authors such as Al-Sharoock et al. (1991) in their work studying the effect of crude seed extracts on mosquito larvae and El-Shazly et al. (1996) in their work on 2nd instar larvae of Muscina stabulans with ethanolic extract of Nerium oleander.

Increasing mortalities found herein come in agreement with Nammour et al. (1989) who evidenced that the disulphides and trisulphides (components of garlic) lead to mortality of the Coleopteran Bruchidus atrolineatus. And with Flint et al. (1995) and Gurusubramanian and Krishna (1996) who found a repellent and toxic effects of garlic extracts upon Hemiptera. It also come in agreement with Ansari et al. (2000) found that peppermint oil had a larvicidal activity against different mosquito species, since they found that it causes larval mortality, inhibits adult emergence and the few adults which emerged did not oviposit. Also with Al-Moajel (2004) who tested the effectiveness of powders from parts of eleven different plant species including Allium cepa against T. granarium adults and larvae and found a significant mortality of the larvae at all concentrations.

Increasing larval and pupal abnormality, mortality and duration come in agreement with Abdel Halim and Morsy (2005) who evaluated the activity of Eucalyptus globulus oil (camphor oil) against the larval maturation and adult emergency of the house-fly, Musca domestica 3rd stage and found that the developed pupae did not emerge to adults. They also concur with Mohamed (2006) using neem seed oil for controlling T. granarium as he found that the larval mortalities increased than control with all neem oil concentrations. The results also come in agreement with the finding of Singh et al. (2006) in their work with Melia azedarach L. against Plutella xylostella. They also agreed with Rachid and Fouad (2007) who explain the effects of methanol extract of Peganum harmala L. seeds against Tribolium castaneum (Herbst). They also come in agreement with Sadeghi et al. (2008) who found that lectins from garlic retarded the development of the cotton leaf-worm larvae, Spodoptera littoralis Boisd and their metamorphosis and was detrimental to the pupal stage resulting in lethal abnormalities. They also agreed with Omar (2010) who found that oils of seven different plant species including garlic (Allium cepa) oil at different concentrations leads to increase larval and pupal duration, mortalities and abnormalities. Also with Kumar et al. (2011) who found that the larvicidal and pupicidal effect of the essential oils of six plant species against the house fly, Musca domestica L. (Diptera: Muscidae) increased larval mortality, and the crude oils of two of them suppressed the emergence of adult flies by 100% and that come in agreement with the herein findings.

Larval and pupal abnormality and mortality could be due to the fact that garlic oil may possess anti-ecdysone activity, which could influence insect development (Harmatha and Dinan, 2003). The larval mortalities could be also due to the antifeeding or growth regulating effects of tested oil on larvae and that might have resulted in arrest of further development and survival. It is therefore assumed that the mortality is likely a consequence of starvation (Yilin et al., 2005).

The present results also showed that treatment with plant oils affected biochemical activities which lead to the disturbance in total lipid and other biochemical activities and that was also the opinion of Hill and Izatt (1974) and Khalaf (1998).

Glucose content was found to be less than control which come in agreement with Fell et al. (1982); Rajendra (1990) and Shakoori and Salem (1991) who suggested that carbohydrates converted to proteins in detoxification mechanism against toxicants that enter the animal body. Also with Abo el- Ghar et al. (1995) who found that the petroleum ether extract of Ammimajus and Apium graveolens fed to 6th instar larvae of Agrotis ipsilon greatly reduced the haemolymph carbohydrates. The same results were obtained when the acetone and ethanol extracts of Melia azedarach were used. Also Shoukry and Hussein (1998) had obtained the same findings with the larvae of Galleria mellonella when they were treated with volatile oils of Lantana camara and Vitexaganus custus plants. The results also agreed with Shoukry et al. (2002a and 2003) who studied the biochemical changes in the haemolymph of Plodia interpunctella larvae treated with sublethal concentrations of two volatile oils and three fixed oils and found that all oil treatments decreased the levels of haemolymph carbohydrates contents. That is also agreed with Shoukry et al. (2002b) who noted that all oil treatments decrease haemolymph carbohydrates content. It also agreed with the finding of Gareth et al. (2006) and Omar (2010) how noted that all oil treatment decrease carbohydrates content in insects.

On the other hand, our results disagree with Sabry (2004) who showed that, the treatment with plant fixed and volatile oil affect biochemical activities of myiasis producing fly C. alliceps, and the content of homogenate carbohydrate significantly increased in treated larvae.

Total protein in larvae treated with LC50 of garlic oil was found to be higher than in control. This finding may be due to the conversion of carbohydrates and lipids to proteins as stated by Kinnear (1968) who suggested that the increased of protein level was due to increased synthesis of new proteins by fat body, haemolymph and other tissues of the larvae. The results come in agreement with Wilkinson (1976); Fell et al. (1982); Rajendra (1990); Shakoori and Saleem (1991) and Omar (2010) who stated that increase in protein content could be due to increase in protein biosynthesis by its tool (amino acids) for the detoxification mechanism, since protein helps to synthesize microsomal detoxifying enzyme which assists to detoxify the toxicants that entered into the insect body.

Total lipid found to be significantly less than in control. This finding comes in agreement with Fell et al. (1982); Rajendra (1990) and Shakoori and Salem (1991) who suggested that lipids converted to proteins in detoxification mechanism against toxicants that enter the animal body. The result herein come in agreement with Shoukry and Hussein (1998) who showed that treatment of third instar larvae Galleria mellonella with sublethal concentrations of Lantana camara and Vitex agnus castus reduced the total lipids in last larval instar. Also with Abou El- Ela et al. (1998) who found significant reduction in lipid contents of Spodoptera littoralis as a result of treatment with three different insecticides and this decrease was in favor of adults than in 5^{th} instar larvae, the results also come in agreement with Shoukry et al. (2004) who found that S. officinalis oil produced a highly significant decreased in total lipid. The results also come in agreement with Sabry (2004) who showed that, the treatement with plant fixed and volatile oil affect biochemical activities of myiasis producing fly C. alliceps. He also stated that the content of haemogenate lipid significantly decreased with volatile oils treatment and significantly increased with fixed oil treatment. The results also agreed with the finding of Rachid and Fouad (2007) using Peganum harmala extracts on Tribolium castaneum; And also with the finding of Omar (2010) using seven different plant species oil including Allium cepa against T. granarium.

Since lipid accumulation is more likely to be related to a lake of Juvenile hormone, so inhere decrease of total lipid may be due to increase in secretion of Juvenile hormone and that could be due to disturbance in Corpora allata or that the plant oils act as juvenile hormone. This conclusion is strengthening by the herein finding of longer larval duration since treatment.

The results disagree with Mostafa (1993) who found that the total lipid content was significantly increased in T. granarium as a result of treatment with plant extracts. Also with Abou El-Ela et al. (1995) who found the same result on Musca domestica after treatment with water extracts of some plants, and with Shoukry et al. (2003) who studied the biochemical changes in the haemolymph of Plodia interpunctella larvae treated with sublethal concentrations of two volatile oils and three fixed oils and found that all oil treatments increased the levels of haemolymph lipids and proteins but decreased their carbohydrates contents, and also with Shoukry et al. (2004) on their work with T. foenum graceum, A. nilotica and R. dentatus which leads to highly significant increase in total lipid content and all these experiments was in larvae of Indian meal moth Plodia interpunctella.

Alkaline phosphatase found to be lower in treated larvae than in untreated ones. That comes in agreement with Dua et al., 2010; Werdin et al., 2010 and Omar (2010) who stated that the plant oils may have juvenile or insect growth regulator effect. So the increase in the alkaline phosphatase could be due to juvenile hormone effect of the garlic since juvenile hormone leads to increase alkaline phosphatase.

Acid phosphatase content in treated larvae with LC50 of the oil was found to be less than in the control. The results come in agreement with Mostafa (1993) who recorded significant reduction in the acid phosphatase activity at all time intervals when treatments carries out to study the effect of the formulation of plant extract (Margason- o) on 4^{th} and 6^{th} instar larvae of Spodoptera littoralis. It also agreed with Abdel- Hafez et al. (1993) who found significant reduction in acid phosphatase when using two OP insecticides on laboratory strain of Spodoptera littoralis. And that indicate that the tested oils have insecticidal effects. The results also come in agreement with Liu et al. (2005) who found indirect evidence that Arabidopsis (Arabidopsis thaliana) vegetative storage protein (VSP) could play a role in defense against herbivorous insects. AtVSP2 (an anti-insect vegetable storage protein) when incorporated into the diets of insects having acidic mid-gut as coleopteran it decrease acid phosphatase activity in the insects and significantly delayed their development and increase their mortality (which suggest the presence of anti insect storage protein in the oil), and it has been shown previously that treatment with the tested oils at any concentration delayed the development and increased the mortality.

Cholinesterase was found to be greater than in control. Sakata and Miyata (1994); Yuan and Chambers (1998) Guerrero, et al. (1999) and Riely, et al. (1999) stated that garlic compounds may have significant effects on numerous essential physiological enzymes containing thiol groups (e.g. glutathione S-transferase). Thiol containing enzymes such as the esterase family have been shown to be involved in insecticide resistance. Falak, et al. (2004) on their investigation of the level of resistance in various populations of T. granarium by using various esterases, found that when exposing 4th instar larvae to phosphine at LC_{20} for different hours duration that cholinesterase activity increased after prolonged administration.

The results showed that ALT and AST in 4th larval instar reared on the tested oil at concentration of LC50 was less than in control. That comes in agreement with the results of Omar (2010) in his work testing the effectiveness of seven plant oils including Allium cepa against T. granarium.

From the results it was obvious that the inhibition of AST was more than in ALT. These results were found to agree with Tabassum, et al. (1998) which reported that as far as the activity of transaminase is concerned, pyrethroil and neem formulation compounds were found to have inhibitory effects and the decrease of AST was more than in ALT. Hassan (2002) denoted that the activity of tissue specific enzymes has been used to diagnose damage to specific organs and tissues resulting from chemical toxicity. The two enzymes showing the greatest diagnostic potential are aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The results agreed also with the finding of Omar (2010) on T. granarium using seven tested oils.

Since transaminase enzymes help in the production of energy, ALT and AST help in the transfer of amino groups and play an important role in the Kreb's cycle or the high energy producing cycle. In other words; the transaminases form a link between the metabolism of amino acids, lipids and carbohydrates, (Sharma and Singh, 1977 and Azmi, et al., 1998). So using plant oils which found to inhibit these enzymes will help in controlling insects' pests.

6-SUMMARY

This study was done to evaluate some of the biological and biochemical effects of garlic (Allium sativum L.) on T. castaneum treated as 4th instar larvae. According to these evaluations, the potential insecticidal properties of this oil could be assessed. Data obtained indicated that exposure of the 4th instar larvae of T. castaneum to concentrations ranged from 0.025 to 1.0 ml per ten gm of wheat induced deleterious effects on their mortality, development and normal growth. The effects appeared in increased larval mortality, extended larval and pupal durations, significant decreased in pupation rate, deformations of larvae shown in shrinking and c-shaped. Larval abnormalities and pupal mortality were generally increased with increasing oil concentrations. While the adult emergence decrease. The results showed that the oil may possess anti-ecdysone activity, which could influence insect development. Another mechanism might be minded that the tested oil act principally as insect growth inhibitors (IGIS) leading to the disruption of the insect development, which usually ends by death. Another explanation of the obtained results could be due to the antifeeding effect of tested oil, thus this oil has growth regulating effects on T. castaneum larvae and that might have resulted in arrest of further development and survival. It is therefore assumed that the mortality is likely a consequence of starvation. LC 50 which found to be 0.49 ml was used to treat fourth larval instar for two days before applying biochemical tests and comparing them with control. The results showed that glucose (as an indicator of carbohydrate metabolism) and lipid content in treated larvae was less than in control while protein content was higher. This could be due conversion of glucose and fat to protein in detoxification mechanism considering plant oil has insecticidal probarities; since protein helps to synthesize microsomal detoxifying enzyme which assists to detoxify the toxicants that entered into the animal body. As for decreasing in lipid content another explanation could be the disturbance in corpora allata or that plant oils act as juvenile hormone. The results show that alkaline phosphatase was increased which assist the previous suggestion that garlic oil has a juvenile hormone effect. Acid phosphatase content in treated larvae was found to be less than in the control and that could be due to the presence of anti-insect protein in the tested oil that its defense function is correlated with acid phosphatase activity and cause loss of it. Cholinesterase was found to be increased and that could be due to its involvement in insecticide resistance (considering plant has insecticidal effect). The results show that both ALT and AST contents in 4th instar larvae were found to be decreased and the decrease of AST was found to be more than ALT.

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