Disturbance of the Main Body Metabolites in Larvae and Pupae of *Spodoptera littoralis* (Lepidoptera: Noctuidae) by Certain Sesquiterpene Compounds

Hamadah, Kh.¹; Ghoneim, K.¹*; Selim, Sh.²; Waheeb, H.¹
1-Department of Zoology and Entomology, Faculty of Science, Al-Azhar University, Cairo, Egypt
2-Department of Pesticide Chemistry and Technology, Faculty of Desert and Environmental Agriculture, Matrouh University, Matrouh, Egypt
*Corresponding author: Email: karemghoneim@gmail.com

**ABSTRACT**

The Egyptian cotton leafworm *Spodoptera littoralis* (Boisduval) is a dangerous pest of many field crops and vegetables in the world. The present study was conducted to investigate the disturbing effects of the sesquiterpene compounds, Farnesol, Nerolidol, and Bisabolol, on the main body metabolites in larvae and pupae of this insect. The newly moulted last (6th) instar larvae were treated with LC₅₀ values of these compounds (33.67, 42.24 and 59.31 ppm, respectively) and the determination of the main metabolites was achieved in haemolymph and fat bodies of larvae and the homogenate of pupae. The most important results could be summarized as follows. The present study recorded predominant reducing effects of these compounds on the protein content in larvae and pupae with an exceptional enhancing effect of some compounds at certain ages of last instar larvae. Also, the lipid content in haemolymph and fat bodies of larvae as well as in the pupae was remarkably reduced. In addition, all compounds exhibited predominant reducing effects on the carbohydrate content in haemolymph and fat bodies of larvae and in the pupae, with two exceptions of increasing carbohydrate content in larval haemolymph (at 24 hr post-treatment) and in larval fat bodies by Nerolidol (at 72 hr post-treatment).

**INTRODUCTION**

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), is a serious insect pest damaging more than 90 host plants belonging to 44 plant families of important field crops and vegetables, including cotton (Kandil et al., 2003). This pest is native in Africa (Shonouda and Osman, 2000) but distributed throughout the world, in Southern Europe, Africa, Asia Minor and the Middle East (Sadek, 2003; Pineda et al., 2007; Lanzoni et al., 2012; El-Sabrou, 2013; Azzouz et al., 2014; EPPO, 2019). The economic losses are attributed to the high fecundity of adult moths and voracious feeding of caterpillars on leaves, flower buds, fruiting buds, and bolls (El-Khawas and Abd El-Gawad, 2002; Korrat et al., 2012; Mokbel et al., 2019).

Pest management programs for controlling *S. littoralis* up till the year 2000 were directed mainly for handpicking of egg-masses early in the season by children and to implement the application of synthetic insecticides purposely for their larvicidal activities later. However, due to the recent universal announcement of childhood rights, using children in collecting egg-masses is forbidden and restricted by law (Abd-El-Aziz and Sayed, 2014).
In Egypt, several types of insecticides have been used for controlling S. littoralis, including synthetic pyrethroids, organophosphates, and non-steroidal compounds (Casida and Quistad, 1998). Although some insecticides are toxic to humans and the environment, they have already been recommended for controlling this insect pest (Pluschkell et al., 1998, Abd El-Mageed and Shalaby, 2011, Ghoneim et al., 2012).

The continuous and intensive uses of synthetic insecticides to control agricultural pests usually lead to adverse effects on beneficial insects, fish, and wildlife, hazards to man and animals by environmental pollution, residues in foods (Abdel-Rahim and Azab, 2008; Osman and Mahmoud, 2009; Ehab, 2012). Over the past 40 years, the intensive use of broad-spectrum insecticides against S. littoralis had led to the development of resistance to many of them (Aydin and Gurkan, 2006 and Rizket et al., 2010). Thus, the crop protection by many insecticides becomes insufficient (Abo Elghar et al., 2005).

Therefore, there is a need to search for alternative strategies based on environmentally safe products and with low risks for human health. In this context, plant-derived products offer new and promising alternatives causing no damages to the environment and non-target organisms (Isman, 2008, 2015; Pavela and Benelli, 2016 a,b; Benelli et al., 2017). The insecticidal potential of various plants against S. littoralis has been demonstrated by many researchers in Egypt (Mansour et al., 2012; Mendez et al., 2011; Pavela, 2014). It is important to point out that the plants produce a wide diversity of compounds involved in their chemical defense. Among these natural products, terpene compounds have been shown to have a significant potential for insect control (Luigards-Moura et al., 2002; Copping and Duke, 2007; Alecio et al., 2014; Dambolena et al., 2016). In insects, terpenes play important roles in communication and defense, especially the C15sesquiterpenes, which often act as sex, alarm, or aggregation pheromones or protection against enemies (Gershenzon and Dudareva, 2007; Blomquist et al., 2010; Vandermeuten et al., 2012).

Farnesol is an acyclic representative of Sesquiterpenes. Chemically, Farnesol (3, 7, 11-trimethyl-2, 6, 10-dodeca-triene-1-ol, Molecular Formula: C15H26O) is a naturally occurring aliphatic sesquiterpenoid alcohol (Jung et al., 2018) and isolated from essential oils of various plants in nature, such as citronella, lemongrass, tuberose, cyclamen, rose, neroli, balsam, and musk (Ishizaka et al., 2002; Schulz, 2013; Azanchiet al., 2014; Krupciket al., 2015). As reported by Kumar and Gupta (2017), Farnesolcan disrupt the normal metabolic function and therefore, affects various life processes of the insects. Recently, Ghoneim et al. (2020) recorded different toxic and disruptive effects of Farnesol on growth, development and metamorphosis of S. littoralis. Nerolidol (3,7,11-trimethyl-1,6,10-dodecaatrien-3-ol, Molecular Formula: C15H26O), also known as peruviol and penetrol, is one of the most important acyclic Sesquiterpenes. It is aliphatic sesquiterpene alcohol isolated from essential oils of various sources (Pacifico et al., 2008). Nerolidol isomers function as insect attractants (Aldrich et al., 1993; Binder et al., 1995), antifeedants (Dokstech et al., 1980; Wheeler et al., 2002, 2003), and larvicidal agents (Chantaraine et al., 1998). Recently, Hamadah et al. (2020) studied the drastic effects of Nerolidol on adult performance and reproduction of S. littoralis. α-Bisabolol is a plant-derived monocyclic sesquiterpene alcohol(6-methyl-2-(4-methylcyclohex-3-en-1-yl)hept-5-en-2-ol) with the molecular formula: C15H26O. It is more formally α-(-)-bisabolol and also known as levomenol (Rohstoff-Lexikon, 2008). The present study was conducted aiming at the investigating of the disturbing effects of the sesquiterpene compounds, Farnesol, Nerolidol, and Bisabolol, on the main body metabolites (proteins, lipids and carbohydrates) in larvae and pupae of S. littoralis.
MATERIALS AND METHODS

Experimental Insect:
A sample of the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) pupae was kindly obtained from the culture of susceptible strain maintained for several generations in Plant Protection Research Institute, Agricultural Research Center, Doqqi, Giza, Egypt. In the laboratory of Insect Physiology, Faculty of Science, Al-Azhar University, Cairo, a culture was established under laboratory-controlled conditions (27±2°C, 65±5% R.H., photoperiod 14 h L, and 10 h D). Rearing procedure was carried out according to Ghoneim (1985) and improved by Bakr et al. (2010). Egg patches were kept in Petri dishes until hatching. The hatched larvae were transferred into glass containers containing a layer of dry sawdust and tightly covered with muslin cloth secured with rubber bands. Larvae were provided daily with fresh castor bean leaves *Ricinus communis*. The resulting pupae were then collected and placed in clean jars provided with a layer of moistened sawdust. All jars had been kept in suitable cages provided with branches of fresh Tafla plant, *Nerium oleander*, as oviposition sites. The emerged adults were provided with 10% honey solution on a cotton wick as a food source. Moths were allowed to lay eggs on branches, then the egg patches were collected daily, and transferred into Petri dishes for another generation.

Selected Compounds:
The tested Sesquiterpene compounds, Farnesol, Nerolidol and Bisabolol, in the present study were purchased from ABCR GmbH, Karlsruhe, Germany. Farnesol 96% (mixture isomers) has the chemical name: [(2E,6E)-3,7,11-trimethylodocaa-2,6,10-trien-1-ol] and Formula: C_{15}H_{26}O. Nerolidol 98% has the chemical name: *(cis + trans)* [3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol] and Formula: C_{15}H_{26}O. α-Bisabolol 95% has the chemical name: [6-methyl-2-(4-methyl cyclohex-3-en-1-yl)hept-5-en-2-ol] and Formula: C_{15}H_{26}O.

Larval Treatment:
A series of concentrations of each compound (400.0, 200.0, 100.0, 50.0, 25.0, 12.5 & 6.25 ppm) was prepared for calculating the LC_{50} of each compound against the 6th (last) larvae of *S. littoralis*. Discs of fresh castor bean leaves were dipped in each concentration for 5 minutes and air-dried before introduction to larvae as food for 24 h under the aforementioned laboratory conditions. Control larvae received leaf discs after dipping in Tween 60 and alcohol (95 %) solution for 5 minutes. LC_{50} values of Farnesol, Nerolidol and Bisabolol, were found 33.67, 42.24 and 59.31 ppm, respectively. After treatment of the newly moult last instar larvae with these LC_{50} values, samples of larval haemolymph and fat bodies had been obtained.

Tissue Preparation:
Larval Haemolymph:
For the determination of the main metabolites, haemolymph was collected from treated and control 6th (last) instar larvae (at 24 and 72 hr post-treatment). The haemolymph was obtained by amputation of one or two prothoracic legs of the larva with fine scissors. Gentle pressure was done on the thorax until a drop of haemolymph appeared at the point of amputation. Haemolymph was drawn into Eppendorf Pipetman containing few milligrams of phenoloxidase inhibitor (Phenylthioure) to prevent tanning or darkening and then diluted 5x with saline solution 0.7%. The diluted haemolymph was frozen for 20 s to rupture the haemocytes. Collected haemolymph samples were then centrifuged at 2000 r.p.m. for 5 min, and only the supernatant fractions were used for assay directly or frozen until use. Three replicates were used and the haemolymphs of two individuals were never mixed.

Larval Fat Body:
For the determination of the main metabolites and enzyme activities, fat bodies (parietal and visceral) were carefully collected (by dissection) from the treated and control last instar larvae (24 and 72 hr post-treatment). Collected samples of fat bodies were weighed and then homogenized in a saline solution (the fat body of one insect / 1 ml saline solution 0.7 %) using a fine electric
homogenizer, tissue grinder for 2 min. Homogenates were centrifuged at 4000 r.p.m. for 15 min. The supernatant was used directly or frozen until use. Three replicates were used and the fat bodies from two individuals were avoided to be mixed.

**Pupal Homogenate:**

For the determination of the main metabolites, healthy treated and control pupae (of different ages: early-aged, mid-aged, and late-aged pupae, or 1-day, 4-day, and 7-day old pupae, respectively) were weighed and then homogenized in a saline solution (one pupa / 1 ml saline solution 0.7 %) using a fine electric homogenizer, tissue grinder for 2 min. Homogenates were centrifuged at 4000 r.p.m. for 15 min. The supernatant was used directly or frozen until use. Three replicates were used and homogenates of two individuals were avoided to be mixed.

**Determination of the Main Body Metabolites:**

**Total protein Content:**

Quantitative determination of the total protein content was conducted in the larval tissues and pupal homogenate according to the method of Weichselbaum (1946) and using the kit of Biodiagnostics. The method depends on the protein forms a violet complex with cupric ions in analkaline medium, and then measured the absorbance at 550 nm using a spectrophotometer.

**Total Lipid Content:**

Quantitative determination of the total lipid content was conducted in the larval tissues and pupal homogenate according to the technique of Folch et al. (1957) and lipid estimation was taken place by phosphovanillin reagent depending on Knight et al. (1972) and using the Spectrophotometer at 520 nm.

**Total Carbohydrate Content:**

Quantitative determination of the total carbohydrate (as glycogen) content was conducted in the larval tissues and pupal homogenate using the anthrone reagent according to Singh and Sinha (1977) and utilizing the Spectrophotometer at 620 nm.

**Statistical Analysis Of Data:**

Data obtained were analyzed by the Student’s t-distribution, and refined by Bessel correction (Moroney,1956) for the test significance of the difference between means using GraphPad InStat® v. 3.01 (1998).

**RESULTS**

**Effects of Sesquiterpene Compounds on The Protein Content in Larvae and Pupae:**

Depending on the data assorted in Table (1), the total protein content in haemolymph of control last instar larvae of *S. littoralis* increased with the age (4.45±0.03 & 5.20±0.06 g/dL, in haemolymph at 24 hr post-treatment & 72 hr post-treatment, respectively). In contrast, total protein content in fat bodies of the same larvae decreased with the age (49.87±1.07 & 48.67±0.33 mg/g in fat bodies at 24 hr post-treatment & 72 hr post-treatment, respectively).

As seen in the same table, Farnesol prohibited the treated larvae to attain normal protein content in haemolymph (17.66 & 20.12% reductions at 24 hr post-treatment & 72 hr post-treatment, respectively). On the contrary, each of Nerolidol and Bisabolol exhibited a diverse effect on this metabolite, since Nerolidol insignificantly induced the proteins in haemolymph, at 24 hr post-treatment (1.49% increment) but slightly reduced it at 72 hr post-treatment (3.13% decrement). Also, Bisabolol slightly reduced the protein content at 24 hr post-treatment (7.23% decrement) but slightly enhanced it at 72 hr post-treatment (5.85% increment).

With regard to the total protein content in fat bodies of larvae, data of the previously mentioned table exiguously revealed contradictory effects of the tested compounds, since Farnesol prohibited larvae to attain normal protein level in fat bodies (1.12 & 5.82% reductions, at 24 hr post-treatment & 72 hr post-treatment, respectively) while Bisabolol enhanced similar larvae to gain more proteins in fat bodies (2.76 & 12.91% increments, at 24 hr post-treatment & 72 hr post-treatment, respectively) while Bisabolol enhanced similar larvae to gain more proteins in fat bodies (2.76 & 12.91% increments, at 24 hr post-treatment & 72 hr post-treatment, respectively). In addition, Nerolidol exhibited a diverse effect because it prohibited the treated larvae (at 24 hr post-treatment) to attain normal protein content (6.42% reduction) but induced these larvae to gain more proteins in fat bodies (0.14%...
increment, at 72 hr post-treatment. After treatment of newly moulted last instar larvae with LC<sub>50</sub> values of the Sesquiterpene compounds, data of disturbed protein content in the successfully developed pupae were arranged in Table (2). According to these data, all compounds prevented the pupae to attain normal protein content. For some detail, the most potent reducing action on total proteins was exerted by Farnesol on the mid-aged pupae (22.15±1.02, vs. 35.23±0.19 mg/g in control pupae, with 38.80% decrease) while the least reducing action was exerted by Nerolidol on the early-aged pupae (23.00±1.13, vs. 25.56±1.08 mg/g in control pupae, with 11.44% decrease). In respect of the control pupae, data of the same table demonstrated aconceivable curve of protein content with a peak in the mid-aged pupae, i.e., the total proteins increased in these pupae (25.56±1.08, 35.23±0.19 & 20.67±0.52 mg/g, in early-aged, mid-aged and late-aged pupae, respectively).

**Table 1:** Total protein content in last instar larvae of *S. littoralis* as influenced by treatment of the same larvae with LC<sub>50</sub> values of Sesquiterpene compounds.

<table>
<thead>
<tr>
<th>Sesquiterpene compound</th>
<th>Tissue</th>
<th>Sampling time</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 hrs post-treatment</td>
<td>72 hrs post-treatment</td>
<td></td>
</tr>
<tr>
<td><strong>Farnesol</strong></td>
<td><strong>Haemolymph</strong></td>
<td>mean±SD</td>
<td>3.87±0.12 c</td>
<td>4.09±0.05 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Change (%)</td>
<td>-17.66</td>
<td>-20.12</td>
</tr>
<tr>
<td></td>
<td><strong>Fat body</strong></td>
<td>mean±SD</td>
<td>51.17±1.33 a</td>
<td>46.25±1.76 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Change (%)</td>
<td>-1.12</td>
<td>-5.82</td>
</tr>
<tr>
<td><strong>Nerolidol</strong></td>
<td><strong>Haemolymph</strong></td>
<td>mean±SD</td>
<td>4.63±0.21 a</td>
<td>4.96±0.05 c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Change (%)</td>
<td>+1.49</td>
<td>-3.13</td>
</tr>
<tr>
<td></td>
<td><strong>Fat body</strong></td>
<td>mean±SD</td>
<td>48.43±0.53 a</td>
<td>49.18±1.17 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Change (%)</td>
<td>-6.42</td>
<td>0.14</td>
</tr>
<tr>
<td><strong>Bisabolol</strong></td>
<td><strong>Haemolymph</strong></td>
<td>mean±SD</td>
<td>4.36±0.11 a</td>
<td>5.42±0.25 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Change (%)</td>
<td>-7.23</td>
<td>+5.86</td>
</tr>
<tr>
<td></td>
<td><strong>Fat body</strong></td>
<td>mean±SD</td>
<td>53.18±1.36 b</td>
<td>55.45±0.67 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Change (%)</td>
<td>+2.76</td>
<td>+12.91</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td><strong>Haemolymph</strong></td>
<td>mean±SD</td>
<td>4.45±0.03</td>
<td>5.20±0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Change (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Fat body</strong></td>
<td>mean±SD</td>
<td>49.87±1.07</td>
<td>48.67±0.33</td>
</tr>
</tbody>
</table>

*Farnesol LC<sub>50</sub> = 33.67 ppm, Nerolidol LC<sub>50</sub> =42.24 ppm, Bisabolol LC<sub>50</sub> =59.31 ppm. Mean±SD followed with letter a: insignificant (P >0.05), b: significant (P<0.05), c: highly significant (P<0.01), d: extremely significant (P<0.001).
Table 2: Total protein content (mg/g) in pupae of *S. littoralis* as influenced by the treatment of the newly moulted last instar larvae with LC50 values of Sesquiterpene compounds.

<table>
<thead>
<tr>
<th>Sesquiterpene compound</th>
<th>Pupal homogenate</th>
<th>Pupal age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early-pupae**</td>
<td>Mid-pupae***</td>
</tr>
<tr>
<td>Farnesol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean±SD</td>
<td>17.39±0.54 d</td>
<td>22.15±1.02 d</td>
</tr>
<tr>
<td>Change (%)</td>
<td>-33.04</td>
<td>-38.80</td>
</tr>
<tr>
<td>Nerolidol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean±SD</td>
<td>23.00±1.13 b</td>
<td>29.82±1.53 c</td>
</tr>
<tr>
<td>Change (%)</td>
<td>-11.44</td>
<td>-17.60</td>
</tr>
<tr>
<td>Bisabolol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean±SD</td>
<td>20.35±0.36 c</td>
<td>26.12±0.79 d</td>
</tr>
<tr>
<td>Change (%)</td>
<td>-21.64</td>
<td>-27.83</td>
</tr>
<tr>
<td>Control</td>
<td>mean±SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25.56±1.08</td>
<td>35.23±0.19</td>
</tr>
</tbody>
</table>

*, c, d: See footnote of Table (1). **: 1-day old, ***: 4-day old, ****: 7-day old.

Effects of Sesquiterpene Compounds on the Lipid Content in Larvae and Pupae:

After treatment of newly moulted last instar larvae with LC50 values of the Sesquiterpene compounds, data of disturbed lipid content in the larval haemolymph and fat bodies were arranged in Table (3). Depending on these data, the total lipid content in haemolymph of control larvae decreased with the age (2.11±0.05 & 1.76±0.02 g/dL, at 24 hr post-treatment & 72 hr post-treatment, respectively). The reverse trend was recorded for the total lipids in larval fat bodies since this metabolite increased with age (24.35±0.67 & 25.27±0.43 mg/g, at 24 hr post-treatment & 72 hr post-treatment, respectively).

In respect of the disturbed lipid content in haemolymph of treated larvae, data of the previously mentioned table obviously revealed that all Sesquiterpene compounds prohibited the larvae to attain normal lipid content since remarkably reduced lipid content had been determined. For some detail, Farnesol showed a strong reducing effect on lipids in larval haemolymph, regardless of the age (34.85 & 33.54% reductions, at 24 hr post-treatment & 72 hr post-treatment, respectively). A lesser potent reducing effect was exhibited by Bisabolol (22.22 & 14.02% reductions, at 24 hr post-treatment & 72 hr post-treatment, respectively). In contrast, Nerolidol enhanced larvae to gain more lipids than control larvae (1.75 & 1.27% increments, at 24 hr post-treatment & 72 hr post-treatment, respectively).

With regard to the disturbance of lipid content in larval fat bodies after treatment of larvae with the tested Sesquiterpene compounds, data of Table (3) clearly displayed considerably reducing effects of all compounds on lipids. For some detail, Farnesol exhibited the strongest inhibitory effect on lipid content (19.58 & 24.42% lipid decrements, at 24 hr post-treatment & 72 hr post-treatment, respectively). On the other hand, Bisabolol exerted the least reducing action on lipids in fat bodies (15.76 & 10.17% decrements, at 24 hr post-treatment & 72 hr post-treatment, respectively).

In addition, the lipid content perturbation by the tested Sesquiterpene compounds had been recorded in the successfully developed pupae of *S. littoralis*, as shown in Table (4). According to data of this table, the lipid content was detrimentally declined, regardless of the compound. For some detail, the prevalent reducing potency of each compound gradually increased with the pupal age. The strongest reducing potency on lipids was recorded for Farnesol in the late-aged pupae (15.63±0.45, compared to 24.18±0.27 mg/g in control pupae, with 41.06% reduction). However, the lipid content gradually decreased with the age in control pupae (129.17±2.36, 59.56±0.54 & 24.18±0.27 mg/g lipids in early-aged, mid-aged & late-aged pupae, respectively).
Table 3: Total lipid content in last instar larvae of *S. littoralis* as influenced by the treatment of the same larvae with LC_{50} values of Sesquiterpene compounds*.

<table>
<thead>
<tr>
<th>Sesquiterpene compound</th>
<th>Tissue</th>
<th>Sampling time</th>
<th>24 hrs post treatment</th>
<th>72 hrs post treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Farnesol</strong></td>
<td><strong>Haemolymph</strong></td>
<td>mean±SD</td>
<td>1.29±0.06 d</td>
<td>1.09±0.03 d</td>
</tr>
<tr>
<td></td>
<td>(g/dL)</td>
<td>Change (%)</td>
<td>-34.85</td>
<td>-33.54</td>
</tr>
<tr>
<td></td>
<td><strong>Fat body</strong></td>
<td>mean±SD</td>
<td>21.28±1.16 b</td>
<td>19.47±1.04 d</td>
</tr>
<tr>
<td></td>
<td>(mg/g)</td>
<td>Change (%)</td>
<td>-19.58</td>
<td>-24.42</td>
</tr>
<tr>
<td><strong>Nerolidol</strong></td>
<td><strong>Haemolymph</strong></td>
<td>mean±SD</td>
<td>1.75±0.16 b</td>
<td>1.27±0.09 d</td>
</tr>
<tr>
<td></td>
<td>(g/dL)</td>
<td>Change (%)</td>
<td>-11.62</td>
<td>-22.56</td>
</tr>
<tr>
<td></td>
<td><strong>Fat body</strong></td>
<td>mean±SD</td>
<td>23.39±1.10 a</td>
<td>21.33±0.67 c</td>
</tr>
<tr>
<td></td>
<td>(mg/g)</td>
<td>Change (%)</td>
<td>-11.60</td>
<td>-17.20</td>
</tr>
<tr>
<td><strong>Bisabolol</strong></td>
<td><strong>Haemolymph</strong></td>
<td>mean±SD</td>
<td>1.54±0.06 d</td>
<td>1.41±0.12 c</td>
</tr>
<tr>
<td></td>
<td>(g/dL)</td>
<td>Change (%)</td>
<td>-22.22</td>
<td>-14.02</td>
</tr>
<tr>
<td></td>
<td><strong>Fat body</strong></td>
<td>mean±SD</td>
<td>22.29±1.08 b</td>
<td>23.14±1.36 a</td>
</tr>
<tr>
<td></td>
<td>(mg/g)</td>
<td>Change (%)</td>
<td>-15.76</td>
<td>-10.17</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td><strong>Haemolymph</strong></td>
<td>mean±SD</td>
<td>2.11±0.05</td>
<td>1.76±0.02</td>
</tr>
<tr>
<td></td>
<td>(g/dL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Fat body</strong></td>
<td>mean±SD</td>
<td>24.35±0.67</td>
<td>25.27±0.43</td>
</tr>
<tr>
<td></td>
<td>(mg/g)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*, a, b, c, d: See footnote of Table (1).

Table 4: Total lipid content (mg/g) in pupae of *S. littoralis* as influenced by the treatment of the newly moulted last instar larvae with LC_{50} values of Sesquiterpene compounds*.

<table>
<thead>
<tr>
<th>Sesquiterpene compounds</th>
<th>Pupal homogenate</th>
<th>Pupal age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Early-pupae**</td>
</tr>
<tr>
<td><strong>Farnesol</strong></td>
<td>mean±SD</td>
<td>111.29±3.71 c</td>
</tr>
<tr>
<td></td>
<td>Change (%)</td>
<td>-11.94</td>
</tr>
<tr>
<td><strong>Nerolidol</strong></td>
<td>mean±SD</td>
<td>119.81±1.28 c</td>
</tr>
<tr>
<td></td>
<td>Change (%)</td>
<td>-5.20</td>
</tr>
<tr>
<td><strong>Bisabolol</strong></td>
<td>mean±SD</td>
<td>112.37±2.11 d</td>
</tr>
<tr>
<td></td>
<td>Change (%)</td>
<td>-11.09</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>mean±SD</td>
<td>129.17±2.36</td>
</tr>
</tbody>
</table>

*, a, c, d: See footnote of Table (1). **, ***: See footnote of Table (2).

Effects of Sesquiterpene Compounds on the Carbohydrate Content in Larvae and Pupae:

After treatment of newly moulted last instar of *S. littoralis* with LC_{50} values of the Sesquiterpene compounds, data of the disturbed carbohydrate content in haemolymph and fat bodies of larvae were arranged in Table (5). As clearly shown in this table, all compounds exerted slight reducing actions on this metabolite in haemolymph of larvae, at both 24 hr and 72 hr post-treatment with an exception of Nerolidol which slightly enhanced increasing carbohydrate content in larval haemolymph only at 24 hr post-treatment (0.25±0.04, vs., 0.24±0.01 g/dL in control congeners, with 4.17% increment). The most potent reducing action on carbohydrate content was exerted by Farnesol, at 72 hr post-treatment (26.92% reduction) while the least reducing action was exerted by Nerolidol, at 72 hr post-treatment (3.85% reduction).

With regard to the perturbed
carbohydrate content in larval fat bodies, data listed in the aforementioned table exiguously revealed a diverse effect of Nerolidol which prohibited the treated larvae to attain an abnormal level of carbohydrates, at 24 hr post-treatment but induced it at 72 hr post-treatment. Both Farnesol and Bisabolol exerted strongly reducing actions on carbohydrate content. For some detail, Farnesol exhibited the strongest reducing effect on this metabolite in fat bodies of larvae at 72 hr post-treatment (2.27±0.19, vs., 2.96±0.10 mg/g in fat bodies of control larvae, with 24.58% decrement). On the other hand, the least reducing effect was displayed by Nerolidol on carbohydrates in fat bodies of larvae, at 24 hr post-treatment (5.04±0.36, vs., 5.21±0.20 mg/g in fat bodies of control larvae, with 6.15% reduction).

Table (6) contains the data of disturbing carbohydrate content in the successfully developed pupae after larval treatment with LC50 values of Farnesol, Nerolidol, and Bisabolol. In respect of the control pupae, carbohydrate content could be conceived as a curve the bottom of which was represented by the mid-aged pupae (3.37±0.06, 3.18±0.17 & 3.96±0.15 mg/g, in early-aged, mid-aged and late-aged pupae, respectively). As clearly seen in this table, the disturbance of carbohydrate content was found in various reductions. The strongest reducing action was exerted by Bisabolol (30.34% reduction, in late-aged pupae) while the least reducing action was exerted by Farnesol (1.29% reduction, in mid-aged pupae).

Table 5: Total carbohydrate content in last instar larvae of S. littoralis as influenced by the treatment of the same larvae with LC50 values of Sesquiterpene compounds*.

<table>
<thead>
<tr>
<th>Sesquiterpene compound</th>
<th>Tissue</th>
<th>Sampling time</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 hrs post-treatment</td>
<td>72 hrs post-treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Change (%)</td>
<td>Change (%)</td>
<td></td>
</tr>
<tr>
<td>Farnesol</td>
<td>Haemolymph (g/dL) mean±SD</td>
<td>0.23±0.02 a</td>
<td>0.19±0.04 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fat body (mg/g) mean±SD</td>
<td>4.35±0.21 c</td>
<td>2.27±0.19 c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Change (%)</td>
<td>-4.17</td>
<td>-26.92</td>
<td></td>
</tr>
<tr>
<td>Nerolidol</td>
<td>Haemolymph (g/dL) mean±SD</td>
<td>0.25±0.04 a</td>
<td>0.25±0.05 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fat body (mg/g) mean±SD</td>
<td>5.04±0.36 a</td>
<td>3.04±0.27 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Change (%)</td>
<td>-4.17</td>
<td>-8.35</td>
<td></td>
</tr>
<tr>
<td>Bisabolol</td>
<td>Haemolymph (g/dL) mean±SD</td>
<td>0.22±0.05 a</td>
<td>0.20±0.03 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fat body (mg/g) mean±SD</td>
<td>4.76±0.12 b</td>
<td>2.51±0.07 c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Change (%)</td>
<td>-8.33</td>
<td>-23.08</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Haemolymph (g/dL) mean±SD</td>
<td>0.24±0.01</td>
<td>0.25±0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fat body (mg/g) mean±SD</td>
<td>5.21±0.20</td>
<td>2.96±0.10</td>
<td></td>
</tr>
</tbody>
</table>

*, a, c: See footnote of Table (1).
Disturbance of the Main Body Metabolites in Larvae and Pupae of *Spodoptera littoralis*

### Table 6: Total carbohydrate content (mg/g) in pupae of *S. littoralis* as influenced by the treatment of the newly moulted last instar larvae with LC50 values of Sesquiterpene compounds*.

<table>
<thead>
<tr>
<th>Sesquiterpene compounds</th>
<th>Pupal homogenate</th>
<th>Pupal age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early-pupa</td>
<td>Mid-pupa</td>
</tr>
<tr>
<td>Farnesol</td>
<td>mean±SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.25±0.13 a</td>
<td>3.05±0.05 a</td>
</tr>
<tr>
<td>Change (%)</td>
<td>-5.52</td>
<td>-1.29</td>
</tr>
<tr>
<td>Nerolidol</td>
<td>mean±SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.00±0.36 a</td>
<td>2.75±0.26 a</td>
</tr>
<tr>
<td>Change (%)</td>
<td>-12.79</td>
<td>-11.00</td>
</tr>
<tr>
<td>Bisabolol</td>
<td>mean±SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.08±0.13 b</td>
<td>2.69±0.10 b</td>
</tr>
<tr>
<td>Change (%)</td>
<td>-10.47</td>
<td>-12.95</td>
</tr>
<tr>
<td>Control</td>
<td>mean±SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.37±0.06</td>
<td>3.18±0.17</td>
</tr>
</tbody>
</table>

*a, b, c, d: See footnote of Table (1). **, ***: See footnote of Table (2).

**DISCUSSION**

In insects, the main body metabolites (proteins, lipids and Carbohydrates) have an important role in biological and physiological activities, such as body size, growth rate, fecundity, and at higher levels of organization has been linked to population dynamics and life histories (Fagan *et al.*, 2002). Therefore, the content of macromolecules is a good indicator of the level of metabolism in insects after treatment with chemicals (Zhu *et al.*, 2012). On the other hand, the potential effects of botanicals on the biochemical milieu of insect pests are of great interest in biological control applications (Medhini *et al.*, 2012). The plant-derived compounds or phytochemicals have been reported to have the ability to drastically influence various metabolic components (carbohydrates, lipids, proteins, etc.) in the body of insects leading to the impairment of internal metabolism, which may explain its mortality.

On the other hand, haemolymph is the only extracellular fluid in the insect body that is usually kept in circulation by an open heart within the body cavity. It transports food materials to the cells and metabolic waste products away from those same cells. Hormones that regulate larval moult, growth, metamorphosis, metabolism and other physiological processes of insects are secreted and circulated in the haemolymph (Hietakangas and Cohen, 2009). One of the most characteristic features of insect haemolymph plasma is the high level of free amino acids ranging from 25 to 75 mM, and functioning as a buffer in osmoregulation and as substrates for protein synthesis and energy production (Chapman, 2013). As reported by Rodriguez-Ortega *et al.* (2003), exposure of an organism to xenobiotic products can modify the synthesis of certain metabolites and disturb the functionality of the organism. In insects, the use of haemolymph as a medium for controlling insect pests has been made because the changes occurring in the haemolymph are quickly transferred to other portions of the insect’s body (Pugazhvendan and Soundararajan, 2009).

In addition, the fat body of insects carries out a variety of different metabolic activities comparable to mammalian liver. It is the main site for protein synthesis as well as the intermediating metabolism of amino acids, which are utilized for the production of hormones and enzymes and the composition of protein in the body as a whole may be greatly modified (Arrese and Soulages, 2010). Thus, the fat body is the important organ that synthesizes and stores energy reserve, in addition, to regulate metabolic activities and reproduction (Park *et al.*, 2006; Vivekananthan *et al.*, 2010). To perform multiple metabolic functions to fulfill the changing physiological needs of the insect during development, the fat body must be able to integrate signals from other organs. Thus the fat body is the target organ of several hormones (Gade, 2004).
The Disturbed Protein Content of S. littoralis by Sesquiterpene Compounds:

Proteins are the most important organic constituents of animal tissues including insects and play an important role in energy production (Taşkı̇n and Aksoẏlar, 2011). As reported by many authors (Hassan, 2002; Cohen, 2010; Chapman, 2012), proteins perform a vast array of functions within living organisms, including catalyzing metabolic reactions, replicating DNA, synthesis of ATP, responding to stimuli, and transporting molecules from one location to another. In addition, proteins in all viable cells, as nucleoproteins, are essential to cell division, enzymes and hormones controlling many chemical reactions in the cell metabolism. In insects, protein metabolism plays a key role in rebuilding adult structures during the transformation of larvae/pupae into adults (Resmitha et al., 2014). It is very important to point out that protein synthesis is necessary for insect reproduction (Taşkı̇n and Aksoẏlar, 2011).

Depending on the currently available literature, some studies have examined the disturbing effects of certain plant compounds on protein content in haemolymph or fat bodies of a number of insects. For example, protein content significantly increased in haemolymph and fat body of the silkworm Bombyx mori larvae after topical application with Benzyladenine and 3-indoleacetic acid (IAA) (plant growth regulators, PGRs) (Hugar and Kaliwal, 1997). Similar results of increasing proteins were recorded in B. mori after treatment with Para-Aminobenzoic Acid, 2,4-Dichlorophenoxy acetic acid, and betanaphthoxyacetic acid (Goudar and Kaliwal, 2001) or indole-3-butryc acid (IBA) and indole-3-pyruvic acid (IPA) (Bhattacharya et al., 2011). The plant product BiostopMoustiques® (derived from coconut oil) was applied onto 4th instar larvae of susceptible and resistant strains of the mosquito Anopheles gambiae. Protein content significantly increased in larvae of both strains (Ahadji-Dabla et al., 2015). Rearing larvae of the greater wax moth Galleria mellonella on a diet supplemented with Gibberellic acid (GA3) led to increased protein in the larval haemolymph (Uçkan et al., 2011b).

Contradictory to those reported results of increasing protein content, Farnesol prohibited the last (6th) instar larvae of S. littoralis to attain normal protein content in haemolymph, in the current investigation. On the contrary, each of Nerolidol and Bisabolol exhibited a diverse effect (reducing or inducing) on protein content, depending on the larval age. In the fat bodies of larvae, Farnesol treatment caused a reduction of protein content, while Bisabolol treatment enhanced it. In addition, Nerolidol exhibited a diverse effect on protein content in larval fat bodies because the protein was declined at 24 hr post-treatment but induced at 72 hr post-treatment. All Sesquiterpene compounds prevented the successfully developed pupae to attain normal protein content. The most potent reducing action on proteins was exerted by Farnesol on the mid-aged pupae while the least reducing action was exerted by Nerolidol on the early-aged pupae. In general, the present study recorded predominant reducing effects of Sesquiterpene compounds on protein content in larvae and pupae of S. littoralis, with an exception of increased protein content at a certain age of larvae.

These results were, to a great extent, in agreement with some reported results of the protein reduction in haemolymph and/or fat bodies of a number of insects after treatment with certain plant compounds, such as the 5th (last) instar nymphs of the migratory locust Locusta migratoria after topical application with GA3 (Abdellaouiet et al., 2013), last instar larvae of the wax moth Galleria mellonella after treatment with Ethephon (ETP) (Altuntas (2015b) and last instar larvae of the Mexican bean beetle Epilachnavarivestis by high doses of azadirachtin (Schloter, 1985). Coumarin (isolated from of Chicory flower) and Neemix (Azadirachtin formulation) caused as significant decrease in the total protein content in the 4th instar larvae of S. littoralis (Gaaboubet et al., 2012).

The reduction of protein content in haemolymph and fat bodies of S.
littoralis larvae and pupae, in the present study, after larval treatment with sesquiterpene compounds, could be interpreted by some conceivable suggestions, as provided herein. (1) After the treatment of insects with exogenous substances, the reduction in protein content may reflect the decrease in the activity of those enzymes engaged in the protein synthesis (Kyung and Kim, 1990). (2) The protein content in the insect body is related to the rate of biosynthesis, and the rate of breakdown of proteins into amino acids (Nath et al., 1997; Medhini et al., 2012). Different stresses on an insect can inhibit the total protein in certain body tissues which could be attributed to the breakdown of proteins into amino acids for involving in the TCA cycle, they will be exhausted to supply energy for the insect needs (Etebari and Matindoost, 2004 a,b; Ghoneim et al., 2014a). Therefore, the reduction in protein content in haemolymph and fat bodies of S. littoralis larvae and pupae, in the present study, might be due to the increase in breakdown of proteins into amino acids for detoxifying the tested sesquiterpene compounds and to aid the insect to recover from their insecticidal stress (Vijayaraghavan and Chitra, 2002; Ali et al., 2014). (3) In this context, it was suggested that the protein plays a major role in synthesis of the microsomal detoxifying enzymes against toxicants (foreign compounds) entering into the insect body (Kyung and Kim, 1990). Proteins can bind with the tested sesquiterpene compounds and therefore the reduction of proteins, in the present study, might reflect the depressed activity of the detoxifying enzymes. (5) The protein reduction in the current study might, also, be due to the interference of tested sesquiterpene compounds with the insect endocrine system causing a hormonal imbalance (Hajjar and Casida, 1979) and affecting the general metabolism (De Mark and Bennett, 1989) or protein synthesis, in particular (Padmaja and Rao, 2000). (6) The tested compounds may either act on the hormonal regulation of the protein synthesis, degradation and inhibition or act on the neurosecretory cells which control endocrine organs (Bouazizet et al., 2011; Djeghader et al., 2014). (7) The suppression of the ATP synthesis and inhibition of RNA synthesis are also the main causes of decreased protein content (Nabi et al., 1990). Moreover, Ali et al. (2011) reported that the deficiency in protein synthesis could also be related to the reduction in levels of DNA and RNA. (8) Another point of interest is the protein depletion in haemolymph and fat body in some developmental stages may be understood in the light of decreasing enzyme constituents, especially transaminases (El-Sheikh, 2002). In the foreseeable future, further investigation should be conducted for good understanding the modes of metabolic action of the tested sesquiterpene compounds, Farnesol, Nerolidol and Bisabolol.

**Disturbed Lipid Content of S. littoralis by Sesquiterpene Compounds:**

In insects, lipids represent an important source of energy, hormone precursors and structural members. They are transported from the synthesis site of storage through the haemolymph towards the user organs, such as the cuticular synthesis (Dapporto et al., 2008) and vitellogenesis (Zhou and Miesfeld, 2009). In addition to the sites of lipid storage in the body, lipids located in the egg play a very important role inachievinthenergy needed for the developing embryo (Boz and Gülel, 2012). Lipid turnover in insects is regulated by neuroendocrine-controlled feed-back loops (Downer, 1985). As reported by Canavoso et al. (2001), the quantity of lipids available for the reserves seems to be the result of a balance between the catch of food and the requests for reserves by processes, such as maintenance, growth and reproduction, and this balance is disturbed by any toxic product.

As reported in the available literature, few studies examined the effects of plant compounds on the lipid content in insects. For example, treatment of larvae of B. mori, with the plant growth regulators, IBA and IPA, led to increased lipid content in the fat body (Bhattacharya et al., 2011). After feeding of 3rd and 4th instar larvae of B. mori on fresh
mulberry leaves treated with IAA, Bharathi and Lakshmikantham (2012) determined increases in total lipid content in the midgut of 5th instar larvae. Topical application or forced ingestion of GA3 into the newly hatched nymphs of L. migratoria was carried out by Abdellaoui et al. (2013). They estimated a significant increase in the total lipid content in haemolymph of 5th (last) instar nymphs.

Results of the present study disagreed with those reported results of increasing protein content because the treatment of last instar larvae of S. littoralis with the Sesquiterpene compounds (Farnesol, Nerolidol and Bisabolol) led to a remarkable reduction of lipids in haemolymph. Farnesol showed a strong reducing effect on lipids in larval haemolymph, regardless the age. The least potent reducing effect was exhibited by Bisabolol. Also, lipid content in the fat bodies of larvae was considerably reduced after treatment with these Sesquiterpene compounds. Farnesol exhibited the strongest reducing effect on lipid content. In the successfully developed pupae, lipid content was detrimentally declined, regardless of the compound. The strongest reducing potency on lipids was recorded for Farnesol in the late-aged pupae. In general, lipid content in haemolymph and fat bodies of larvae, as well as lipid content in pupae, had been remarkably reduced, with an exception of Nerolidol which enhanced the treated larvae to gain more lipids in haemolymph than control larvae.

The present results were, to some extent, in accordance with some reported results of decreased lipid content in haemolymph of larvae after treatment with certain plant growth regulators, such as G. mellonella after treatment with GA3 (Uğkan et al., 2011b) or ETP (Altuntaş, 2015b). A similar reducing effect on lipid content was recorded in the early- and late-aged 3rd instar larvae of the house fly Musca domestica by Neemazal® (azadirachtin formulation) (Kassem et al., 2011).

To interpret the reduction of lipids in larvae and pupae of S. littoralis, after treatment with sesquiterpene compounds in the present study, some suggestions could be provided herein. (1) It is important to point out that the lipid turnover in insects is regulated by neuroendocrine-controlled feedback loops (Downer, 1985). Moreover, many biochemical and physiological changes in insects have been reported to occur in different metabolism pathways under the control of hormones (Leonardi et al., 2001; Kim et al., 2002; Etebari et al., 2007). Therefore, the decreased lipid content, in the current investigation, might be due to the inhibitory effects and stress of the tested sesquiterpene compounds on neurosecretion or other hormones in larvae and pupae of S. littoralis (Gade et al., 1997; Bouaziz et al., 2011). (2) The fat body is hypothesized to be a dynamic organ playing roles as reserves and signaling molecules, affecting many insect physiological processes (Arrese and Soulages, 2010) and their decrease in the fat body may indicate both energy conversion and signal for detoxification cascade (Martins et al., 2011a). In the light of this information, decreasing lipids in larvae and pupae of S. littoralis, as an effect of sesquiterpene compounds in the present study, suggested the use of these energy molecules for some detoxification of these compounds (Cossolin et al., 2019). (3) The declined lipid content might be due to a shift in energy metabolism towards lipid catabolism in S. littoralis larvae and pupae as a result of physiological stress induced by the tested sesquiterpene compounds (El-Sherif, 1995). (4) It may be important to highlight the mode of the sesquiterpene compounds action on lipids in S. littoralis last instar larvae. These compounds induce stress on larvae to use lipids and glucose for cell repair and increasing protein catabolism which may be stimulated due to high energy demand under such stress conditions (Sancho et al., 1998).

The Disturbed Carbohydrate Content of S. littoralis by Sesquiterpene Compounds:

Carbohydrates play an important role in the structure and function of all tissues during metamorphosis as well as for the normal functioning of the male and female
reproductive organs and embryonic development (cf. Chippendale, 1978). They increase during the rest periods, like metamorphosis, and decrease during the growth periods, like the stages of maturation of the gonads in insects (Bouaziz et al., 2011). On the other hand, the carbohydrate content in the haemolymph is an important indicator of the level of metabolism in insects, and a dynamic balance of the absorption, metabolism, and utilization by different tissues (Zhu et al., 2012). It is important to point out that the production and/or utilization of the main body metabolites in insects, such as carbohydrates, are suggested to be regulated by the endocrine products, such as juvenile hormone (Gade, 2004; Sugumaran, 2010) or neurosecretion (Gade et al., 1997). It is interesting to mention that the carbohydrates, as energy elements, play a crucial role in the physiology of those insects, are disturbed by exogenous toxic materials (Kaufmann and Brown, 2008).

Some reported research works in the literature obviously indicate inconsistent effects of botanicals on the carbohydrate content, depending on the insect species and its developmental stage, efficiency of the plant extract and its concentration. The available literature contains few reported studies investigating the diverse effects of isolated plant compounds on carbohydrate content in tissues of insects. For example, topical application of BAP and IAA onto B. mori last instar larvae led to a remarkable increase of the fat body glycogen content but a significant decrease of haemolymph trehalose content (Hugar and Kaliwal, 1997). By feeding Z. paravittiger larvae on a diet containing GA3, carbohydrate content was significantly decreased at 1000 and 2000 ppm but significantly increased at 4000 ppm (Rup et al., 1998a). The disturbing effects of GA3, Alar-B9, IBA, CGA, Cytokinin, and MH on the carbohydrate content in the aphid L. erysimi were investigated. The carbohydrate content was reduced after treatment with GA3, Alar-B9, IBA, or CGA; MH enhanced the carbohydrate content and CK failed to affect the carbohydrate content (Rup et al., 2000a).

Treatment of B. acucurbitae larvae with GA3, IAA, kinetin, and Coumarin induced the quantitative changes in haemolymph carbohydrate content (Kaur and Rup, 2003 a,b). Topical application of IBA onto B. mori last instar larvae resulted in increase of both glycogen of fat body and trehalose of haemolymph (Bhattacharya et al., 2011). Uçkan et al. (2011b) reared G. mellonella larvae on a diet supplemented with GA3 and determined a reduction of carbohydrate content in the haemolymph of larvae. Topical application or forced ingestion of various concentrations of Gibberellic acid into the newly hatched nymphs of L. migratoria was carried out. A significant decrease in the total carbohydrate content was determined in haemolymph of the 5th (last) instar nymphs (Abellaoui et al., 2013). Ghoneim et al. (2006) recorded enhancing or inhibitory action of Margosan-O (an azadirachtin formulation) on the carbohydrate content throughout the pupal stage of M. domestica, depending on the day of life and concentration. A potent reducing action on carbohydrate content was exerted in the early- and late-aged 3rd instar larvae of M. domestica by Neemazal® (azadirachtin formulation) (Kassem et al., 2011).

In the present study, treatment of newly moulted last instar of S. littoralis with LC50 values of the Sesquiterpene compounds, Farnesol, Nerolidol and Bisabolol, resulted in a reduction of carbohydrate content in haemolymph of larvae. The most potent reducing action on carbohydrate content was exerted by Farnesol (at 72 hr post-treatment) while least reducing action was exerted by Nerolidol (at 72 hr post-treatment). An exceptional case of increased carbohydrate content in larval haemolymph was recorded for Nerolidol, only at 24 hr post-treatment. In the larval fat bodies, both Farnesol and Bisabolol exerted strongly reducing actions on carbohydrate content in the fat bodies of larvae. Farnesol exhibited the strongest reducing effect (at 72 hr post-treatment) and the least reducing effect was displayed by Nerolidol (at 24 hr post-treatment). A diverse
The main body metabolites have an important role in biological and physiological activities in the body of insects. Depending on the results of the present study, sesquiterpene compounds generally disturbed the contents of proteins, lipids and carbohydrates in larvae and pupae of S. littoralis, with few exceptions. Therefore, the tested sesquiterpene compounds may be recommended to take a part in the Integrated Pest Management program of this dangerous pest.

REFERENCES


Azanchi, T.; Shafaroodi, H. and Asgarpanah,


EPPO, (2019): *Spodoptera littoralis* distribution. EPPO Global Database.
Available: https://gd.eppo.int/taxon/SPODLI/distribution [5 February 2019]


Disturbance of the Main Body Metabolites in Larvae and Pupae of *Spodoptera littoralis* 151


GraphPad InStat© v. 3.01 (1998): GraphPad Software, Inc. 7825 Fay Avenue, Suite 230 La Jolla, CA 92037 USA. Available online at: http://www.graphpad.com/scientific.software/instat/


Disturbance of the Main Body Metabolites in Larvae and Pupae of *Spodoptera littoralis*


Shonouda, M.L. and Osman, S.I. (2000): New botanical derivatives, used in medicinal preparations, showing bioactive action on insect pests. I-


