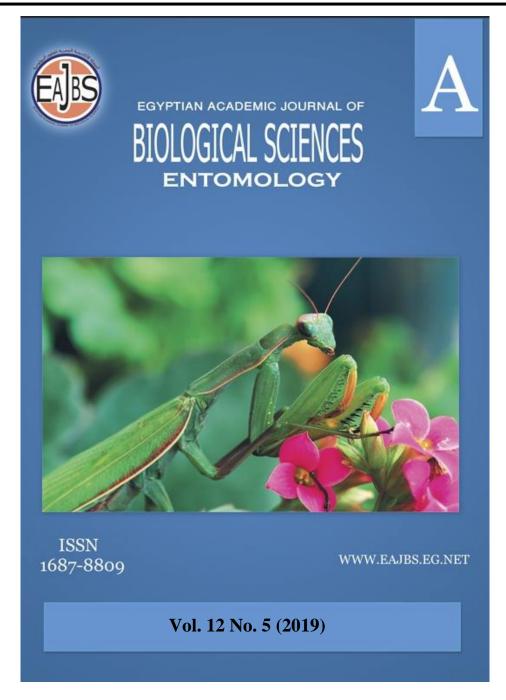
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## Egypt. Acad. J. Biolog. Sci., 12(5):125-140 (2019)



# Egyptian Academic Journal of Biological Sciences A. Entomology

# ISSN 1687- 8809 http://eajbsa.journals.ekb.eg/



Apple Tree Borers at Menoufia, Egypt with Special Reference to the Phytochemical Changes and Their Relation to the Infestation with *Synanthedon myopaeformis* Borkh. (Lepidoptera: Sesiidae).

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#### **ARTICLE INFO**

# **Article History**

Received:2/10/2019 Accepted:26/10/2019

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#### Keywords:

Apple trees, borers, chemical composition, apple clearwing moth

#### **ABSTRACT**

The first part of the work aimed to study the survey of apple trees borers that attack apple orchards in two different geographical regions, at Abo-Mashour and Al-Khatatba locations (Menoufia governorate). Five species were recorded in the study showed, these borers were Synantheden myopaeformis Borkh., Zeuzera pyrina L., Hypothenemus eruditus Westwood., Scolytus amygdali Guer. and Chlorophorus varius Mull (non recorded at Al-Khatatba) . Highest percentages of infestation (26.08 & 21.33 %) were recorded for Sy. myopaeformis followed by Z. pyrina (17.83 &13.01%) at Abo-Mashour and Al-Khatatba, respectively. An annual increase of infested trees with these borers, especially Sy. myopaeformis (10.59 & 9.59) and Z. pyrina (9.22 & 6.62) give serious indicators to quick devastation and the death of infested trees. The weather factors detected a significant correlation with infestation by different borers except Ch. varius. Also significant differences for both Z. pyrina and S. amygdale were detected in the two regions under study, while the infestation showed insignificant differences of both Sy. myopaeformis and H. eruditus The second part of the study aimed to investigate the role of phytochemical components within apple trees and their relation to the infestation with apple clearwing moth Sy myopaeformis. The analysis by GC-MS chromatograph showed differences in both Chemical composition and the percentages of compounds in the tested wood samples from apple trees under study. In the uninfested young trees (resistant trees), the levels of 9-Octadecenoic acid (Oleic acid), 9-Hexadecanoic acid and Ethyl iso-allocholate were found at higher rates than the uninfested old trees, as they were 31.42%, 14.83% and 5.37% respectively. The infested old trees showed high levels of these chemical compounds compared to uninfested ones as the percentages of 9-Octadecenoic acid (Oleic acid), 9-Hexadecanoic acid and Ethyl iso-allocholate increased by 5.8 fold, 3.1 fold and 3.1 fold, respectively, while the percentages in uninfested old trees were 7.42%, 5.39% and 3.34% respectively.

## **INTRODUCTION**

Apple trees (*Malus domestica* Borkh.) are cultivated worldwide and are considered the most widely consumed fruits in the world. Apple trees are deciduous and grow in most

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**Citation**: Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 12(5) pp: 125-140(2019)

countries of the moderate regions and in some tropical areas (Ferree and Warrington 2003). Apple trees, in Egypt and in most world countries are attacked by various insect pests. Aphids, Codling moth, Leopard Moth, Clearwing Moth, dogwood moth, scale insects, leafminers, leafrollers, jewel beetles and bark beetles are the main insect pests attacking fruit trees, including apple orchards (Blommers 1994, Abdel-Azim 1997, Pfammatter & Vuignier, 1998, Anonymous 1999, Brown et al. 2008, Karaca et al. 2010, Simon et al. 2010, Hegazi et al. 2010, Hegazi et al. 2014, Batt & Abd El-Raheem 2017).

In Egypt and several other countries, the xylophagous clearwing moth *Sy. myopaeformis* Borkh. (Lepidoptera: Sesiidae) is a serious pest in the apple orchards (Tertyshny 1995, Al-Antary et al. 2004, Ateyyat 2005, Ateyyat 2006, Aurelian 2011, Batt and Abd El-Raheem 2017). The first and second instar larvae of the apple clearwing moth feed superficially within the bark whereas the older instar larvae feed on the vascular tissues between the bark and cambium. This leads to destructive tunnels inside the trunk of apple trees resulting in economic losses of the crop as a result of the deterioration in the growth of the tree and finally death of the whole tree. (Bergh and Leskey 2003, Kain & straub2001, Aurelian 2011). Thus, several studies were carried out by many authors (Abd El-Kader & Zaklama 1971, Awadallah et al. 1978, Tadros 1993, Tadros 1994 a, 1994 b, Girgis et al. 1995, Abdel-Azim, 1997, Shehata et al.1999, Tadros et al. 2003, Batt & Abd El-Raheem 2017) which aimed to study the Ecology, Biology, monitoring and control of this dangerous pest.

In all, Variation in phytochemicals or metabolic diversity in plants possibly explains variation in response to a diversity of natural enemies, including specialist and generalist insect herbivores (Becerra 1997, Dyer et al. 2004, Lankau 2007, Kursar et al. 2009). These studies have provided the information needed to find novel technologies and resources for assessing and understanding pest management issues according to the relation between the insects and the host plants.

According to the aforementioned views, the main objectives of our study were to (1) survey wood borers attacking apple orchards in Menoufia governorate, (2) annual percentages and progress of infestation for different apple tree borers, (3) changes of chemical components and their relation of infestation with apple clearwing moth.

### **MATERIALS AND METHODS**

# Survey of Apple Tree Borers in Two Geographical Regions of Menoufia Governorate:

The present study was carried out on apple orchards during 2017–2018 with 15-20 years-old apple trees. Infested apple orchard at two geographical regions at Menoufia governorate (South of the Nile Delta in northern Egypt), the first, at Abo-Mashour village (Berkat Al-Saba district, 30.68390° N, 31.06222° E) and the second Al-Khatatba location (Al-Sadat district, 30.33938° N, 30.70440° E), were chosen to survey the stem tree borers species infesting apple trees. The borer species were recognized by different infestation symptoms of each species.

In the last week of December 2017, the infested apple trees with each borer species were counted and marked by colored paint. The percentage of infestation for each borer species was calculated. A monthly examination was done to determine the new numbers of infested apple trees and then marked. The progress of infestation was estimated by a monthly cumulative number of infested trees. The annual percentage of infestation by borer species at the end of December 2018 was determined as follows:-

Infestation % = No. of infested trees / borer species ×100 Total number of examined trees Increase (progress) of infestation % =

Infested number secondly of trees – infested number firstly of trees  $\times$  100

The examined number of trees

## Analysis of Chemical Components of Infested and Uninfested Apple Trees:

The study was carried out in apple orchard, Abo-Mashour (30.68390° N, 31.06222° E), with 15-20-year-old apple trees but has several areas with trees less than four years old of the same species instead of the old damaged ones. The orchard was neglected, heavily attacked by insect borer *Sy. myopaeformis* and no pesticides were applied for more than 3 years.

## **Apple Trees Were Divided Into 3 Groups:**

A- non-infested young trees (resistant trees), b- non-infested old trees and c- infested old trees.

The trunk samples including bark, phloem, cambium and xylem were taken from 30 trees (10 from each of the previous groups) 10-50 cm above the ground. The samples of each group were mixed well together and 30g were taken from each mixture to be analyzed.

# **Sample Preparation:**

#### 1. Extraction:

The dried samples were grounded to a fine powder using a pestle and mortar. The sample powder was put into a methanol solution for two days before filtering the solution. The solution was filtered to separate the solids away using a filter paper. The methanol solution that remains after the filtration process was evaporated using rotary evaporator till dried. The solution in methanol was 3:1 and injected  $1\mu L$  in GC-MS.

## 2. Gas Chromatography–Mass Spectrometry (GC-MS) Analysis:

The chemical composition of the collected samples was performed using Trace GC 1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m  $\times$  0.25 mm  $\times$  0.25 µm film thickness). The column oven temperature was initially held at 60°C and then increased by 5 °C /min to 150 °C withhold 2 min then increased to 300 with 10 C/min and hold for 5 min. The injector temperature was kept at 250 °C. Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 2 min and diluted samples of 1 µl were injected automatically using Autosampler AS3000 coupled with GC in the split mode. All mass spectra were recorded in the electron impact ionization (EI), mass spectra were collected at 70 electron volts ionization voltages over the range of m/z 50 – 650 in full scan mode. The ion source and transfer line temperatures were set at 200 and 250 °C respectively. The components were identified by comparison of their retention times and mass spectra with those of (NIST and WILEY) mass spectral database, Davies (1990) and Wiley NIST (2008).

#### **Statistical Analysis:**

The data obtained from apple trees attacked with different borer species in infested orchards at Abo-Mashour (Berkat Al-Saba district) and Al-Khatatba (Al-Sadat district) locations Menoufia governorate, during 2018 were estimated by (SAS program, 2001).

### RESULTS AND DISCUSSION

# Survey of Apple Tree Borers in Two Geographical Regions of Menoufia Governorate:

Cultivated apple trees in infested orchards with fruit tree borers at Abo-Mashour (Berkat Al-Saba district) and Al-Khatatba location (Al-Sadat district) Menoufia governorate, indicated that five borers species infested the apple trees in the Abo-Mashour region. These borers are *Synanthedon myopaeformis* Borkh. (Lepidoptera: Sesiidae), *Zeuzera pyrina* L., (Lepidoptera: Cossidae), *Hypothenemus eruditus* Westwood, *Scolytus amygdali* Guer. (Coleoptera: Curculionidae: Scolytina) and *Chlorophorus vairus* Mull. (Coleoptera: Cerambycidae), while four species only (the same of previous species except for

Chlorophorus vairus) recorded at Al-Khataba location.

The previous studies point that all borers that have been found in the study have been established as pests on fruit trees in Egypt, for instance, Willcocks 1924, Batt 2002, 2008, Hegazi et al. 2010, 2014, Tadros 1994 a, 1994 b, Girgis 198, Abd El-Moaty et al. 2013.

### **Monthly Changes of Infestation:**

At the two geographical regions in our study, under the different effects for each of weather factors, the new infestations of various borers attacking the apple trees were recorded in the Table (1) and Table (2).

### 1. At Abo-Mashour Location:

As shown in Table (1), the number of new infestations during the year of study showed that the highest monthly infestations were recorded during August (8 infestations) with both *Z. pyrina & Sy. myopaeformis*; during July (5 infestations) with both *H. eruditus & S. amygdali* and during August (3infestations) with *Ch.vairus*.

Table (1). Monthly numbers of new infestations for borers of attacking apple trees in infested orchards at Abo-Mashour (Berkat Al-Saba district) location Menoufia governorate, during 2018.

|          | 5,              | Species of apple tree borers |                     |     |                |     |                |     |               | 1   | Veather factors     |                    |      |
|----------|-----------------|------------------------------|---------------------|-----|----------------|-----|----------------|-----|---------------|-----|---------------------|--------------------|------|
| Months   | Z.pyrina        |                              | Sy.<br>myopaeformis |     | H.<br>eruditus |     | S.<br>amygdali |     | Ch.<br>varius |     | Minimum<br>Temp .°C | Maximum<br>Temp.°C | Rh%  |
|          | No.             | Cu.                          | No.                 | Cu. | No.            | Cu. | No.            | Cu. | No.           | Cu. | 1                   |                    |      |
| Dec.2017 | 44              | 44                           | 79                  | 79  | 45             | 45  | 8              | 8   | 14            | 14  |                     |                    |      |
| Jan.     | 1               | 45                           | 3                   | 82  | 1              | 46  | 1              | 9   | 0             | 14  | 10.3                | 17.96              | 51.1 |
| Feb.     | 3               | 48                           | 3                   | 85  | 2              | 48  | 2              | 11  | 0             | 14  | 10.7                | 19.4               | 55.6 |
| Mar.     | 3               | 51                           | 4                   | 89  | 2              | 50  | 0              | 11  | 2             | 16  | 13.5                | 23.7               | 57.2 |
| Apr.     | 6               | 57                           | 5                   | 94  | 2              | 52  | 2              | 13  | 1             | 17  | 14.6                | 26.6               | 49.3 |
| May      | 4               | 61                           | 5                   | 99  | 4              | 56  | 3              | 16  | 2             | 19  | 19.06               | 32.15              | 50.2 |
| Jun.     | 5               | 66                           | 6                   | 105 | 3              | 59  | 4              | 20  | 0             | 19  | 21.33               | 32.53              | 52   |
| Jul.     | 7               | 73                           | 7                   | 112 | 5              | 64  | 5              | 25  | 2             | 21  | 23.4                | 34.43              | 55.4 |
| Aug.     | 8               | 81                           | 8                   | 120 | 4              | 68  | 4              | 29  | 3             | 24  | 26                  | 36.6               | 54.7 |
| Sep.     | 5               | 86                           | 5                   | 125 | 2              | 70  | 3              | 32  | 0             | 24  | 24.95               | 35.7               | 50.2 |
| Oct.     | 3               | 89                           | 4                   | 129 | 1              | 71  | 1              | 33  | 1             | 25  | 21.3                | 30.95              | 59.8 |
| Nov.     | 1               | 90                           | 2                   | 131 | 0              | 71  | 0              | 33  | 0             | 25  | 15.95               | 25.23              | 71.6 |
| Dec.2018 | 1               | 91                           | 2                   | 133 | 1              | 72  | 1              | 34  | 0             | 25  | 11.4                | 20.4               | 64.9 |
| A        | 91 133 72 34 25 |                              |                     |     |                |     |                |     |               |     |                     |                    |      |
| В        | 510             |                              |                     |     |                |     |                |     |               |     |                     |                    |      |

A = Total number of infested trees

B = Number of examined trees

N = number of infested trees

Cu. =Cumulative number of infested trees

#### 2. At Al-Khataba Location:

The highest numbers of new infestations for apple tree borers (Table2) were recorded during August with *Z. pyrina* (5 infestations) & *H. eruditus* (4 infestations) and during July with *Sy. myopaeformis* (7 infestations) & *S. amygdale* (3 infestations).

| ut       | during 2016. |                              |     |                     |     |                |     |             |                 |         |       |  |
|----------|--------------|------------------------------|-----|---------------------|-----|----------------|-----|-------------|-----------------|---------|-------|--|
|          |              | Species of apple tree borers |     |                     |     |                |     |             | Weather factors |         |       |  |
| Months   | Z.pj         | Z.pyrina                     |     | Sy.<br>myopaeformis |     | H.<br>erudītus |     | S.<br>gdali | Min.            | Max.    | Rh%   |  |
|          | No.          | Cu.                          | No. | Cu.                 | No. | Cu.            | No. | Cu.         | Temp.°C         | Temp.°C |       |  |
| Dec.2017 | 28           | 28                           | 51  | 51                  | 39  | 39             | 3   | 3           |                 |         |       |  |
| Jan.     | 1            | 29                           | 3   | 54                  | 1   | 40             | 0   | 3           | 8.65            | 18.8    | 62    |  |
| Feb.     | 1            | 30                           | 2   | 56                  | 0   | 40             | 0   | 3           | 9.23            | 20.8    | 61.3  |  |
| Mar.     | 3            | 33                           | 3   | 59                  | 3   | 43             | 0   | 3           | 11.13           | 24      | 60.98 |  |
| Apr.     | 4            | 37                           | 4   | 63                  | 2   | 45             | 1   | 4           | 14              | 28.5    | 55.98 |  |
| May      | 3            | 40                           | 3   | 66                  | 3   | 48             | 2   | 6           | 17.13           | 32.5    | 51.12 |  |
| Jun.     | 4            | 44                           | 5   | 71                  | 3   | 51             | 2   | 8           | 19.8            | 34.7    | 50.5  |  |
| Jul.     | 4            | 48                           | 7   | 78                  | 2   | 53             | 3   | 11          | 21              | 35.3    | 50.3  |  |
| Aug.     | 5            | 53                           | 6   | 84                  | 4   | 57             | 2   | 13          | 21.5            | 35.9    | 62.8  |  |
| Sep.     | 0            | 53                           | 3   | 87                  | 1   | 58             | 1   | 14          | 19.83           | 34.15   | 57.9  |  |
| Oct.     | 2            | 55                           | 2   | 89                  | 0   | 58             | 2   | 16          | 17.15           | 31.43   | 65.1  |  |

Table (2). Monthly numbers of new infestations for borers of attacking apple trees in infested orchards at Al-Khatatba ((Al-Sadat district)) location Menoufia governorate, during 2018.

A = Total number of infested trees

56

57

57

2

93

93

B = Number of examined trees

58

58

N = number of infested trees

Nov.

Dec.2018

A

В

Cu. =Cumulative number of infested trees

0

17

17

13.48

10.4

25.9

20.6

68.88

65.34

### Annual percentages and progress of infestation for different apple tree borers:

438

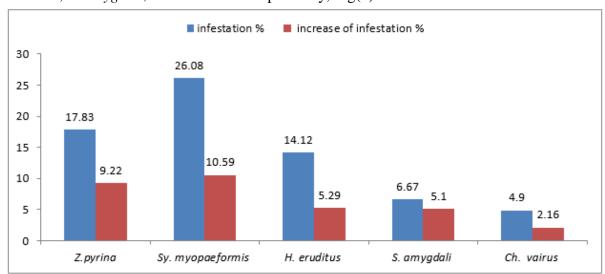
0

The annual cumulative numbers of infestation during the study period for each apple tree borer at Abo-Mashour and Al-Khataba locations indicated that the infestation percentages of different borers were represented in Fig. (1) and Fig. (2) respectively.

### 1. At Abo - Mashour Location:

The percentages of infestation were 17.83, 26.08, 14.12, 6.67 and 4.90% for *Z. pyrina*, *Sy. mopeaformis*, *H. eruditus*, *S. amygdali and Ch. varies* respectively.

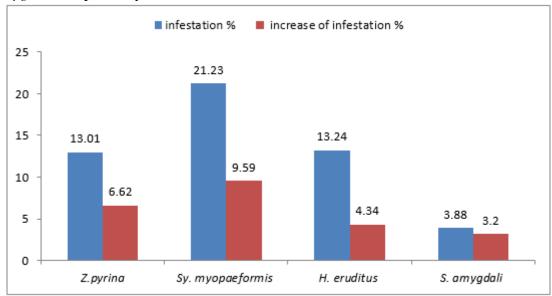
The data also detected that the increase of infestation recorded during the one year study was as follows 9.22, 10.59, 5.29, 5.10 and 2.16 % for each of *Z.pyrina*, *Sy. mopeaformis*, *H. eruditus*, *S. amygdali*, and *Ch. varies* respectively, Fig(1).



**Fig** (1) Annual percentages and progress of infestation for different borers of apple trees at Abo-Mashour location during 2018

#### 2. At Al-Khatataba Location:

As shown in Fig(2) the annual percentages of infestation recorded 13.01, 21.23, 13.24 and 3.88 for each of *Z. pyrina, Sy. mopeaformis, H. eruditus*, *S. amygdali* respectively, in this respect the progress of infestation indicated that the annual increase of infestation recorded 6.62, 9.59, 4.34 and 3.20% for each of *Z. pyrina, Sy. mopeaformis, H. eruditus* and *S. amygdali* respectively.



**Fig (2)** Annual percentages and progress of infestation for different borers of apple trees at Al-Khatatba location during 2018

# Comparative Study between the Infestation by Apple Tree Borers at Abo-Mashour and Al-Khatatba Locations:

The obtained data in Table (3) indicated that the infestation by *Z. pyrina* and *S. amygdali* was significant while the differences between the infestation of both *Sy. Myopaeformis* and *H. eruditus* were insignificant. (T= 1.37and 1.12, respectively).

Table (3): Comparative study for infestation by apple tree borers at Abo-Mashour and Al-Khatatba locations

| Species of apple tree | Abo-Mashour      | Al-Khatatba | T- Test |  |
|-----------------------|------------------|-------------|---------|--|
| borers                | Mean <u>+</u> SE | Mean ± SE   | 1- 1651 |  |
| Z. pyrina             | 3.92 + 0.65      | 2.42 + 0.45 | 1.82    |  |
| Sy. myopaeformis      | 4.5 + 0.52       | 3.5 + 0.46  | 1.37    |  |
| H. eruditus           | 2.25 + 0.41      | 1.58 + 0.40 | 1.12    |  |
| S.amygdali            | 2.17 +0.45       | 1.17 +0.28  | 1.79    |  |

#### **Probable Effect of Weather Factors:**

Many factors may play an important role in affecting the state of trees and the monthly abundance of different borer species for apple trees. The temperature and relative humidity are some of the weather factors affecting the infestation. The correlations between these factors and infestation by apple tree borers showed significant differences in two geographical regions (Abo-Mashour and Al-Khatatba), Table (4).

| locations             |          |         |          |         |                      |        |  |  |
|-----------------------|----------|---------|----------|---------|----------------------|--------|--|--|
| Abo-Mashour           |          |         |          |         |                      |        |  |  |
| Species of apple tree | Min. Te  | emp. °C | Max. T   | emp. °C | RH%                  |        |  |  |
| borers                | r        | b       | r        | b       | r                    | b      |  |  |
| Z. pyrina             | 0.740**  | 0.306   | 0.784**  | 0.279   | -0.540*              | -0.190 |  |  |
| Sy. myopaeformis      | 0.794**  | 0.263   | 0.823**  | 0.234   | -0.555*              | -0.156 |  |  |
| H. eruditus           | 0.577    | 0.151   | 0.644*   | 0.144   | -0.539*              | -0.120 |  |  |
| S.amygdali            | 0.697*   | 0.201   | 0.722**  | 0.179   | -0.541*              | -0.133 |  |  |
| Ch. varius            | 0.457 Ns | 0.087   | 0.510*   | 0.084   | -0.216 <sup>N₅</sup> | -0.035 |  |  |
|                       |          | Al-     | Khatatba |         | •                    | •      |  |  |
| Species of apple tree | Min. Te  | emp. °C | Max. T   | emp. °C | RH%                  |        |  |  |
| borers                | r        | b       | r        | b       | r                    | b      |  |  |
| Z. pyrina             | 0.529*   | 0.181   | 0.569*   | 0.122   | -0.504*              | -0.131 |  |  |
| Sy. myopaeformis      | 0.689*   | 0.245   | 0.658*   | 0.175   | -0.610*              | -0.164 |  |  |
| H. eruditus           | 0.512*   | 0.156   | 0.552*   | 0.126   | -0.568*              | -0.132 |  |  |
| S.amygdali            | 0.889**  | 0.194   | 0.892**  | 0.132   | -0.542*              | -0.10  |  |  |

Table (4). The correlations between weather factors (Min. Temp. °C, Max. Temp. °C and RH %) and the infestations by apple tree borers at Abo-Mashour and Al-Khatatba locations

## **Chemical Components Of Apple Trees:**

Analysis of methanol extract of trunk wood of infested and uninfested apple trees detected the chemical components of each young, old infested and uninfested apple trees, as in the following results:

# 1. GC-MS Chromatogram of the Methanol Extract of Resistant Young Apple Trunk Wood:

GC-MS chromatogram of the methanol extract of apple trunk wood depicted 20 peaks indicating the presence of 20 compounds. The chemical components identified in the methanol wood extract from resistant young apple trees with the retention time ranging from 2.17 to 33.33 minutes were presented in (Table 5). Twenty compounds were detected as results of the GC-MS analysis of the wood composition. These are 1-Gala-1-ido-octose (0.03%), 2,2-Dideutero octadecanal (0.10%), 2-pentanone,4-hydroxy-4-methyl- (2.43%), 5-Cyclopropylcarbonyloxypentadecane (0.03%),N-2,4-Dnp-L-arginine Benzenemethanol, à-(1-aminoethyl)- (0.03%),[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester (0.01%), Dodecanoic acid, 3-hydroxy- (0.10%), 2-Aminoethanethiol hydrogen sulfate (ester) (0.24%), Z-8-Methyl-9-tetradecenoic acid (0.05%), 9-Hexadecenoic acid (14.83%),9-Octadecenoic acid (Z)- (Oleic acid) (31.42%), Oxiranecarboxamide,2-ethyl-3propyl-(6.58%), Z-(13,14-Epoxy)tetradec-11-en-1-o l acetate (9.96%), 7-Methyl-Ztetradecen-1-ol acetate (2.35%),1-Heptatriacotanol (11.87%), Ethyl iso-allocholate 2-Hydroxy-3-[(9E)-9-octad ecenoyloxy]propyl(9E)-9-octadecenoate Tetraneurin - A- diol (5.31%) and Alanine, 3-(benzyloxy)-, L- (3.23%).

# 2. GC-MS Chromatogram of the Methanol Extract of Uninfested Old Apple Trees Trunk Wood:

The methanol extract of trunk wood from uninfested old apple trees with the retention time ranging from 3.37 to 31.00 minutes revealed the presence of 12 compounds summarized in (Table 6). The major identified chemical components were, 2-Pentanone,4-hydroxy-4-

<sup>\* =</sup> Significant \*\* = highly Significant Ns = non-significant

methyl- (9.46%), Benzene, 1,3-bis(1,1-dimethylethyl) (0.37%), 2,4-Bis(1,1-Dimethylethyl)Phenol(1.81%), 13,16-Octadecadiynoic acid, methyl ester (1.33%), Tetradecanoic acid (14.99%), 9-Hexadecenoic acid (5.39%), 9-Octadecenoic acid (Z)- (Oleic acid) (7.42%),7-Methyl-Z-tetradecen-1-ol acetate (3.44%), Ethyl iso-allocholate (3.34%), 1-Heptatriacotanol (5.20%), 1,2-benzenedicarboxylic acid, diisooctyl ester (46.73%) and Alanine, 3-(benzyloxy)-, L-(0.52%).

# 3. GC-MS Chromatogram of the Methanol Extract of Infested Old Apple Trees Trunk Wood:

The chemical compounds present in the methanol extracts of trunk wood from infested old apple trees were identified by GC-MS analysis resulting in the presence of 16 chemical compounds with the retention time ranging from 2.08 to 33.28 minutes as shown in (Table 7). The identified compounds were, 12-Methyl-E,E-2,13-octadecadien-1-ol (0.85%),12,15-Octadecadiynoic acid, methyl ester (0.22%), 9,12-Octadecadienoyl chloride,(Z,Z)- (0.08%), Dodecanoic acid, 3-hydroxy (1.95%), 2-Aminoethanethiol hydrogen sulfate (ester) (0.24%), Dodecanoic acid, 3-hydroxy- (1.21%), Phenol, 2,4-bis(1,1-dimethylethyl) (5.97%), 9-Hexadecenoic acid (16.93%), 9-Octadecenoic acid (Z)- (Oleic acid) (42.87%), Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate (2.59%), 7-Methyl-Z-tetradecen-1-ol acetate (0.24%),1-Heptatriacotanol (7.33%), Hexadecadienoic acid, methyl ester (4.80%), Ethyl iso-allocholate (10.19%), Tetraneurin - A- diol (4.01%) and Alanine, 3-(benzyloxy)-, L- (0.50%).

Table (5). Major chemical components identified from trunk wood of uninfested young apple trees

| RT 1  | Compound Name  | Area<br>% | Molecular<br>Formula | Molecular<br>Weight |
|-------|--|-----------|----------------------|---------------------|
| 2.17  | 1-Gala-1-ido-octose  | 0.03      | C8H16O8              | 240                 |
| 2.84  | 2,2-Dideutero octadecanal  | 0.10      | C18H34D2O            | 270                 |
| 3.37  | 2-pentanone,4-hydroxy-4-methyl-                                      | 2.43      | C6H12O2              | 116                 |
| 5.50  | 5-Cyclopropylcarbonyloxypentadecane                                  | 0.03      | C19H36O2             | 296                 |
| 5.61  | N-2,4-Dnp-L-arginine   | 0.02      | C12H16N6O6           | 340                 |
| 5.82  | Benzenemethanol, à-(1-aminoethyl)-                                   | 0.03      | C9H13NO              | 151                 |
| 11.92 | [1,1'-Bicyclopropy1]-2-octanoic acid, 2'-hexyl-,<br>methyl ester     | 0.01      | C21H38O2             | 322                 |
| 12.16 | Dodecanoic acid, 3-hydroxy-  | 0.10      | C12H24O3             | 216                 |
| 14.33 | 2-Aminoethanethiol hydrogen sulfate (ester)                          | 0.24      | C2H7NO3S2            | 157                 |
| 16.59 | Z-8-Methyl-9-tetradecenoic acid                                      | 0.05      | C15H28O2             | 240                 |
| 26.31 | 9-Hexadecenoic acid  | 14.83     | C16H30O2             | 254                 |
| 26.64 | 9-Octadecenoic acid (Z)- (Oleic acid)                                | 31.42     | C18H34O2             | 282                 |
| 27.06 | Oxiranecarboxamide,2-ethyl-3-propyl-                                 | 6.58      | C8H15NO2             | 157                 |
| 27.55 | Z-(13,14-Epoxy)tetradec-11-en-1-o 1 acetate                          | 9.96      | C16H28O3             | 268                 |
| 28.33 | 7-Methyl-Z-tetradecen-1-ol acetate                                   | 2.35      | C17H32O2             | 268                 |
| 30.21 | 1-Heptatriacotanol   | 11.87     | C37H76O              | 536                 |
| 30.68 | Ethyl iso-allocholate  | 5.37      | C26H44O5             | 436                 |
| 31.15 | 2-Hydroxy-3-[(9E)-9-octad<br>ecenoyloxy]propyl(9E)-9-octadecenoate # | 6.04      | C39H72O5             | 620                 |
| 32.16 | Tetraneurin - A- diol  | 5.31      | C15H20O5             | 280                 |
| 33.33 | Alanine, 3-(benzyloxy)-, L-  | 3.23      | C10H13NO3            | 195                 |

| RT 1   | Compound Name                                  | Area<br>% | Molecular<br>Formula | Molecular<br>Weight |
|--------|--|-----------|----------------------|---------------------|
| 3.37   | 2-Pentanone,4-hydroxy-4-methyl-                | 9.46      | C6H12O2              | 116                 |
| 10.18  | Benzene, 1,3-bis(1,1-dimethylethyl)            | 0.37      | C14H22               | 190                 |
| 17.98  | 2,4-Bis(1,1-Dimethylethyl)Phenol               | 1.81      | C14H22O              | 206                 |
| 21.0 8 | 13,16-Octadecadiynoic acid, methyl ester       | 1.33      | C19H36O2             | 296                 |
| 22.04  | Tetradecanoic acid                             | 14.99     | C14H28O2             | 228                 |
| 26.29  | 9-Hexadecenoic acid                            | 5.39      | C16H30O2             | 254                 |
| 26.74  | 9-Octadecenoic acid (Z)- (Oleic acid)          | 7.42      | C18H34O2             | 282                 |
| 28.42  | 7-Methyl-Z-tetradecen-1-ol acetate             | 3.44      | C17H32O2             | 268                 |
| 29.94  | Ethyl iso-allocholate                          | 3.34      | C26H44O5             | 436                 |
| 30.21  | 1-Heptatriacotanol                             | 5.20      | C37H76O              | 536                 |
| 30.81  | 1,2-benzenedicarboxylic acid, diisooctyl ester | 46.73     | C24H38O4             | 390                 |
| 31.00  | Alanine, 3-(benzyloxy)-, L-                    | 0.52      | C26H44O5             | 195                 |

Table (6). Major chemical components identified from trunk wood of uninfested old apple trees

Table (7). Major chemical components identified from trunk wood of infested old apple trees

| RT 1  | Compound Name                               | Area<br>% | Molecular<br>Formula | Molecular<br>Weight |
|-------|---|-----------|----------------------|---------------------|
| 2.08  | 12-Methyl-E,E-2,13-octadecadien-1 -o1       | 0.85      | C19H36O              | 280                 |
| 3.43  | 12,15-Octadecadiynoic acid, methyl ester    | 0.22      | C19H30O2             | 290                 |
| 7.48  | 9,12-Octadecadienoyl chloride,(Z,Z)-        | 0.08      | C18H31C1O            | 298                 |
| 12.98 | Dodecanoic acid, 3-hydroxy                  | 1.95      | C12H24O3             | 216                 |
| 14.87 | 2-Aminoethanethiol hydrogen sulfate (ester) | 0.24      | C2H7NO3S2            | 157                 |
| 15.44 | Dodecanoic acid, 3-hydroxy-                 | 1.21      | C12H24O3             | 216                 |
| 17.98 | Phenol, 2,4-bis(1,1-dimethylethyl)          | 5.97      | C14H22O              | 206                 |
| 26.24 | 9-Hexadecenoic acid                         | 16.93     | C16H30O2             | 254                 |
| 26.53 | 9-Octadecenoic acid (Z)- (Oleic acid)       | 42.87     | C18H34O2             | 282                 |
| 27.51 | Z-(13,14-Epoxy)tetradec-11-en-1-o1 acetate  | 2.59      | C16H28O3             | 268                 |
| 28.46 | 7-Methyl-Z-tetradecen-1-ol acetate          | 0.24      | C17H32O2             | 268                 |
| 30.47 | 1-Heptatriacotanol                          | 7.33      | C37H76O              | 536                 |
| 30.53 | Hexadecadienoic acid, methyl ester          | 4.80      | C19H36O              | 280                 |
| 30.74 | Ethyl iso-allocholate                       | 10.19     | C26H44O5             | 436                 |
| 32.19 | Tetraneurin - A- diol                       | 4.01      | C15H20O5             | 280                 |
| 3328  | Alanine, 3-(benzyloxy)-, L-                 | 0.50      | C26H44O5             | 195                 |

According to the available Knowledge, this study is the first in Egypt interested in the interaction between *Sy. myopaeformis* and apple trees. Previous ecological studies on *Sy. myopaeformis* Borkh (Batt & Abd El-Raheem, 2017), detected that the young apple trees (less than 4 years old) were not infested by the apple clearwing moth, however, the old trees (15-20 years old) were heavily attacked. These observations are consistent with (Brunner 1915, Kinawy 1981, Johnson 1993).

The differences in the principle chemical compositions of infested and uninfested apple trees wood considering, the tree age, led us to predict that these variations can be the key reason for being attacked by the apple clearwing moth or not. This prediction is supported by the view of (Schultz 1988) who mentioned that Plant chemistry changes with plants maturity, Nykänen & Koricheva (2004) who pointed out that changes in woody plants chemicals occur as a result of insects injury, also the damage in the early season caused more changes in plant chemicals than late-season damage, thus, the time of injury is an important factor in determining the response of the plant to the feeding by insects. Similar observation on fig

trees was obtained by Kinawy (1981) who reported that the chemical composition varied according to the age of fig trees as the total phenols were found to be less in old fig trees than the young ones.

The analysis results showed differences in both Chemical composition and in the percentages of compounds in the different wood samples for the infested old trees, uninfested old trees and the resistant young trees. We confined our emphasis to the chemical components presented at high concentration and which have previous references as a bioactive components against insects (Table 8). 9-Octadecenoic acid (Oleic acid), 9-Hexadecanic acid, Ethyl iso-allocholate, 1,2-Benzenedicarboxylic acid, diisooctyl ester and Tetradecanoic acid were the most important chemical components identified from infested and uninfested old trees, and uninfested young trees.

The infested old trees showed high levels of these chemical compounds compared to uninfested ones as the percentages of 9-Octadecenoic acid (Oleic acid), 9-Hexadecanoic acid and Ethyl iso-allocholate increased by 5.8 fold, 3.1 fold and 3.1 fold respectively, (Table 8). In comparison with the infested old trees, the levels of these chemical compounds in the uninfested old trees were found in lower concentrations (maybe natural levels) as they were 7.42% for 9-Octadecenoic acid (Oleic acid), 5.39% for9-Hexadecanoic acid and 3.34% for Ethyl iso-allocholate. The presence of these chemical components at their natural rates in uninfested old trees may not be sufficient to protect trees from injury and this is what drives the trees to produce two new and exclusive chemical components in high level, Tetradecanoic acid (14.99%) and 1,2-Benzenedicarboxylic acid, diisooctyl ester (46.73%) to make the apple trees unfavorable for the apple clearwing moth feeding and thus the apple trees are not attacked. These changes in apple trees phytochemicals may explain the role of these chemical compounds in the self-defence mechanism against the apple clearwing moth attack, as these bioactive plant defense compounds may cause toxicity or repel the attacking insects, Wink (2006) and Fürstenberg-Hägg *et al.* (2013).

Our interpretation is that the increased production of such chemical components may be due to the feeding of the apple clearwing moth larvae, the results indicated by Suckling et al. (2012) prove our interpretation as he found that the apple seedlings infested with the herbivore *Epiphyas postvittana* larvae produced larger amounts of chemical components than uninfested ones. Additionally, the obtained results are consistent with the view of Lämke & Unsicker (2018) who stated that feeding by insects represents stress for the attacked trees, and therefore induce the trees to increase the concentration of phytochemicals, which has a role in self-defence. On the same approach, Inbar *et al.* (1999), Bernays & chapman (1994) and Traw & Dawson (2002) mentioned that herbivore feeding can induce the plant to increase its chemical defences or decrease its nutritional quality which leads to reduced performance of herbivores. Moreover, Marquis et al. (2001) and Poorter *et al.* (2004) assumed that morphological and phytochemical plant traits are the main reason for the intensity of herbivore damage to its host plants.

Regarding the chemical composition content of the uninfested young trees (resistant), the levels of 9-Octadecenoic acid (Oleic acid), 9-Hexadecanoic acid and Ethyl iso-allocholate were found at higher rates than the uninfested old trees (Table 8), as they were 31.42%, 14.83% and 5.37% respectively. This is evidence that the young trees naturally contain a high level of such chemical components that protect them from the apple clearwing moth attacking.

The results also showed that young apple trees, containing many chemical compounds at relatively high portion such as 2-Pentanone,4-hydroxy-4-methyl-(2.43%), Oxiranecarboxamide,2-ethyl-3-propyl- (6.58%),Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate (9.96%), 7-Methyl-Z-tetradecen-1-ol acetate (2.35%), 1-Heptatriacotanol (11.87%), 2-Hydroxy-3-[(9e)-9-octadecenoyloxy]propyl(9e)-9-octadecenoate (6.04%), Tetraneurin - a-

diol (5.31%) and Alanine, 3-(benzyloxy) - , l- (3.23%). We expect that such compounds may have a role in preventing apple trees from infestation by the apple clearwing moth, (Table 5).

In accordance with the previous findings, this part of the research has helped to illustrate some of the principal characteristics of constitutive defenses of apple trees against the apple clearwing moth. Yet, the full information on the mechanisms behind the evolution of apple trees defense is still limited, and further studies are needed to reach new facts in the development of apple trees defense against the apple clearwing moth, which could be used to develop new approaches in the future for apple orchards protection and improvement of apple crop production.

Table (8). The proportions and biological activity of the most important chemical components identified in methanol extract from wood of old trees (infested and uninfested) and resistant young trees.

|   |          | Old tree   |                  | Young tree | Biological activity  |  |
|---|----------|------------|------------------|------------|--|--|
| Compound name                                     | Infested | Uninfested | Increasing ratio | resistance | (previous studies)   |  |
| 9-Hexadecenoic acid                               | 16.93%   | 5.39%      | 3.1              | 14.83%     | Pesticide and larvicide<br>[Abdul Rahuman et al.(2000),<br>Rajalakshmi & Mohan<br>(2016), Abubakar & Majinda<br>(2016), Kumar et al.(2010),<br>Kala & Ammani (2017),<br>Sharma et al.(2018) and<br>Ahmed et al.(2018)] |  |
| 9-Octadecenoic acid (Z)-                          | 42.87%   | 7.42%      | 5.8              | 31.42%     | Pesticide and insectfuge<br>[Rajalakshmi & Mohan<br>(2016), Chandrasekaran et<br>al.(2011), Salem et al.(2016),<br>Sharma et al.(2018)]  |  |
| Ethyl iso-allocholate                             | 10.19%   | 3.34%      | 3.1              | 5.37%      | Pesticide<br>[Saravanan et al.(2014)]  |  |
| Tetradecanoic acid                                | 0.00%    | 14.99%     | -                | 0.00%      | larvicidal and repellent<br>activity<br>[Gomathy & Rathinam<br>(2017), Sharma et al.(2018)<br>and Duke & Duke's, (1992)]   |  |
| 1,2-benzenedicarboxylic<br>acid, diisooctyl ester | 0.00%    | 46.73%     | -                | 0.00%      | Pesticide<br>[Khalil et al.(2014)]   |  |

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