

## **Biocidal Activity of two botanical volatile oils against the larvae of *Synthesiomyia nudiseta* (Wulp) (Diptera: Muscidae)**

**Abdel Fattah A. Khalaf, Karam T. Hussein and Khater K. Shoukry**

Zoology Department, Faculty of Science, Zagazig University, Zagazig-44519, Egypt

### **ABSTRACT**

The volatile oils of golden pillar, *Cupressus macrocarpa* and galangal, *Alpinia officinarum* were extracted and their constituents were identified. Alpha pinene and trans-caryophyllene were the major isolated constituents of *C. macrocarpa* oil, and 1,8 cineole and 4-terpeniol were the major compounds of *A. officinarum* oil.

The present study revealed that the botanical volatile oils used, had morphogenic effects against *S. nudiseta* stages. These include larval-pupal intermediates, pupal-adult intermediates, deformed adults with crumpled wings and/or deformed thorax and abdomen. Also some adults couldn't emerge and remained in their puparia.

The histological effects induced by oils treatment in the body wall were represented by degeneration and detachment of hypodermis and dissolving of the exocuticle in some regions. The histopathological effects that appeared in the midgut were shrinkage in some epithelial cells and swelling of other cells.

**Key word:** Biocidal, Botanical, *Synthesiomyia nudiseta*

### **INTRODUCTION**

The extensive use of the synthetic insecticides lead to the biological imbalance due to the destruction of beneficial species such as parasites and predators of the pests beside the destruction of pollinating insects such as honey bees. Natural products of plants and plant derivatives are alternative agent to currently use for insect control because they constitute rich sources of bioactive chemicals. They are often active against insect species. They are biodegradable to non-toxic products. Additionally, plant-derived materials are found to be highly effective against insecticide resistant insect pests (Arnason *et al.*, 1989; Kwon *et al.*, 1996 and Ahn *et al.*, 1997), so that many investigators initiated a large screening efforts poisonous effects to use them as insecticides (Khmabay *et al.*, 2002; El-Shazly and Hussien, 2004; Prowse *et al.*, 2006 and Malarvannan and Subashini, 2007).

The main objective of the present study is to assess the toxicity, bioactivity, morphogenic and histological actions of golden pillar, *Cupressus macrocarpa* and galangal, *Alpinia officinarum* plant volatile oils against *Synthesiomyia nudiseta* which has a significant role in transmitting the enteric bacteria to man and causing myiasis to man and many animals.

### **MATERIAL AND METHOD**

#### **Insect culture**

Adults of *Synthesiomyia nudiseta* were collected from some areas in Sharkia province and reared in laboratory for five successive generations before being used in experiments, according to the technique described by Busvine (1962).

### Extraction and analysis technique

Leaves of *C. macrocapra* and rhizomes of *A. officinarum* are used. The plant volatile oils were extracted by steam distillation using 300 g of plant in 300 ml of water for 4-6 h and following the method described by Marcus and Lichtenstein (1979) and Weaver *et al.* (1994). The volatile oils were subjected to further analysis using GC/MS (70 ev. energy) according to the method of Likens and Nickerson (1966) and Bernharde *et al.* (1983). Gas chromatography/mass spectrometry (GC/MS) analysis technique was performed on Hewlett Packard gas chromatogram in National Research Centre, Cairo.

### Test technique

Six different acetone concentrations of the two volatile oils were prepared in 250 ml beaker 10 ml of each concentration were thoroughly mixed with 100 gm of rearing medium by using an electric blender, then acetone was evaporated. Third instar larvae of *Synthesiomyia nudesita* were taken from rearing media and introduced to each beaker (100 larvae). Control larvae were fed in acetone treated diet only. The percent larval mortality was recorded 24 hrs. after treatment, corrected according to Abbott (1925). Log concentration-mortality regression lines (LC.P. lines) were determined and the median lethal concentrations (LC<sub>50s</sub>) were estimated.

### Biological studies

The effect of sublethal concentrations (LC<sub>50</sub>) on some biological aspects of the treated stage and its subsequent developmental stages were determined. Deformed larvae, pupae and adults were also recorded.

### Histological studies

Histological examinations were carried out on normal third instar larvae and those treated with sublethal concentration (LC<sub>50</sub>) of plant oil (24 hrs. post treatment). Routine histological technique was used for comparisons.

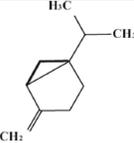
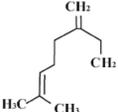
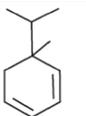
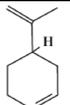
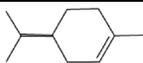
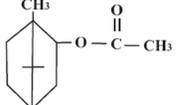
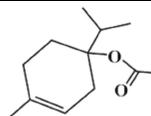
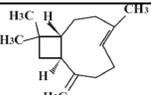
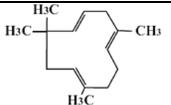
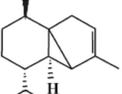
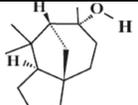
## RESULTS AND DISCUSSION

### 1. Extractions and identification of volatile oils components

Only 9 and 15 gm volatile oils were extracted from one kg of leaves from *Cupressus macrocapra* and rhizomes from *Alpinia officinarum* by steam distillation. High resolution GC/ MS resulted in thirty volatile components in both *Cupressus macrocapra* and *Alpinia officinarum*; only thirteen and sixteen major components are identified from *C. macrocarpa* and *A. officinarum*.

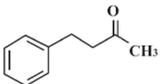
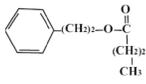
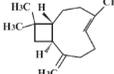
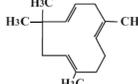
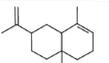
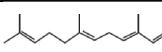
Analysis of *Cupressus macrocarpa* essential oil showed it to be rich in alpha pinene (67.94%), trans-caryophyllene (5.10%), alpha cedrol (5.00%), alpha Humulene (3.79%), Limonene (3.69%), and alpha Myrcene (2.98%), (Table 1).

Table (1): Common name and chemical structure of isolated volatile oil components of *Cupressus macrocarpa*

Common name	Empirical formula	Structural formula	Component %
Pinene (alpha)	C <sub>10</sub> H <sub>16</sub>		67.94
Sabinene	C <sub>10</sub> H <sub>16</sub>		2.94
Myrcene (Beta)	C <sub>10</sub> H <sub>16</sub>		2.98
Phellandrene (alpha)	C <sub>10</sub> H <sub>16</sub>		0.76
Limonene	C <sub>10</sub> H <sub>16</sub>		3.69
Terpinolene (alpha)	C <sub>10</sub> H <sub>16</sub>		2.05
1-Bornyl acetate	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>		0.89
Terpinenyl acetate	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>		2.29
Trans-caryophyllene	C <sub>15</sub> H <sub>24</sub>		5.10
Humulene (alpha)	C <sub>15</sub> H <sub>24</sub>		3.79
Cubebene (alpha)	C <sub>15</sub> H <sub>24</sub>		2.07
(+)-chamigrene	C <sub>15</sub> H <sub>24</sub>		0.44
Cedrol (alpha)	C <sub>15</sub> H <sub>26</sub> O		5.00

Analysis of *Alpinia officinarum* showed it to be rich in 1,8-cineole (72.33%), 4-terpeniol (3.61%), linalyl propionate (3.29%), alpha pinene (3.11%), camphor (2.36%) and gamma Muurlene (2.20%), (Table 2).

Table (2): Common name and chemical structure of isolated volatile oil components of *Alpinia officinarum*

Common name	Empirical formula	Structural formula	Component %
Thujene (alpha)	C <sub>10</sub> H <sub>16</sub>		1.84
Camphene	C <sub>10</sub> H <sub>16</sub>		2.06
Pinene (alpha)	C <sub>10</sub> H <sub>16</sub>		3.11
Cineole (1.8)	C <sub>10</sub> H <sub>18</sub> O		72.33
Camphor	C <sub>10</sub> H <sub>16</sub>		2.36
4 Terpinol	C <sub>10</sub> H <sub>18</sub> O		3.61
Linalyl propionate	C <sub>10</sub> H <sub>18</sub> O		3.29
4-phenyl -2- butanon	C <sub>10</sub> H <sub>12</sub> O		1.30
2-phenyl ethyl butanoate	C <sub>12</sub> H <sub>16</sub> O <sub>2</sub>		0.77
(Trans)caryophyllene	C <sub>15</sub> H <sub>24</sub>		1.82
Bergamotene (alpha)	C <sub>15</sub> H <sub>24</sub>		2.00
Humulene (alpha)	C <sub>15</sub> H <sub>24</sub>		0.74
Selinene	C <sub>15</sub> H <sub>24</sub>		0.74
Farnesene	C <sub>15</sub> H <sub>24</sub>		1.08
Gamma Muurolene	C <sub>15</sub> H <sub>24</sub>		2.20
8-methoxy-2-tetralone	C <sub>12</sub> H <sub>12</sub> O <sub>3</sub>		1.04

## 2. Biological studies

The insecticidal activity of the volatile oils of *C. macrocarpa* and *A. officinarum* was evaluated. On the basis of mortality percentages, it was found that they showed a promising insecticidal activity.

The toxicity of the volatile oils of *C. macrocarpa* and *A. officinarum* against the third instar larvae of *S. nudiseta* exposed for 24 hours showed that the LC<sub>50</sub>s are 1.11% and 2.37%, respectively (Table 3 and Fig.1).

The effect of oil treatment on treated larval stage and its subsequent developmental stages were recorded.

Table (3): Toxicity and the relative potency of *Cupressus macrocarpa* and *Alpinia officinarum* volatile oils against *Synthesiomyia nudiseta*

Tested plants	Conc. %	Mortality %	LC <sub>50</sub> (confidence limits)	Slop functions	Relative potency based on	
					<i>C. macrocarpa</i>	<i>A. officinarum</i>
<i>Cupressus macrocarpa</i>	0.39	25	1.11 (0.891 – 1.374)	1.307	1	0.478
	0.78	40				
	1.56	50				
	3.12	75				
	6.25	85				
	12.5	100				
<i>Alpinia officinarum</i>	0.39	15	2.37 (2.05-2.72)	1.662	2.09	1
	0.78	25				
	1.56	25				
	3.12	45				
	6.25	90				
	12.5	100				

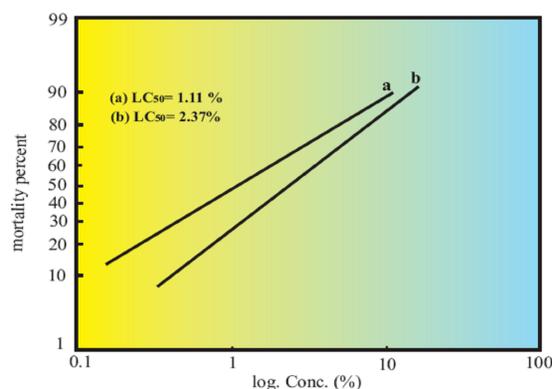


Fig. (1):Log. Conc.-Mortality regression lines of the plant volatile oils against *Synthesiomyia nudiseta* larvae. (a) *Cupressus macrocarpa* (b) *Alpinia officinarum*.

### A. Delayed effect of oils treatment on some biological activities

The results in Table (4) illustrate that both oils treatment of the third instar larvae induced a decrease in the percentage of pupation by 91.57 % and 91.34 % in treatment with *C. macrpocarpa* and *A. officinarum* respectively.

The obtained Results in Table (4) clearly demonstrate the treated third instar larvae of *S. nudiseta* with LC<sub>50</sub>s, sublethal concentrations of the two volatile oils reduced percentage of adult emergence by -91.57% and -91.34% with *C. macrpocarpa* and *A. officinarum* respectively. In treatment with the two volatile oils no oviposition observed at all.

Table (4): Effect of larval treatment with plant volatile oils on pupation and percentage adult emergence of *Synthesiomyia nudiseta*.

Treatments	Pupal stage		Adult stage	
	Pupation% (Mean±SE)	Change %	Adult emergence% (Mean±SE)	Change%
<i>Cupressus macrocarpa</i>	11.80±0.80*	-86.8	7.4±0.87*	-91.57%
<i>Alpinia officinarum</i>	13.20±1.31*	-85.27	7.6±1.12*	-91.34%
Control	89.6±3.26	-	87.80±2.65	-

(-) reduction from control.

\* very high significant.

It is generally accepted that toxic materials of plant origin exert an inhibitory growth effect. Similar effects of some phytochemicals on some insect species have been documented by many authors. Shoukry (1996) attributed the toxicity of volatile oils of *C. inermis* leaves and *M. chamomilla* flowers on house fly, due to the presence of monoterpene, hydrocarbons, and sesquiterpene. Khalaf and Hussein (1997) declared that the toxicity of *C. citratus* and *R. officinalis* volatile oil attributed to the presence of terpenoid compounds which have different methyl and hydroxyl groups in different position. Similar results were recorded by Shoukry *et al.* (2002) on *P. interpunctella*, El-Domiaty *et al.* (2003) on *M. domestica*, El-Shazly and Hussein (2004), on *M. domestica*, Traboulsi *et al.* (2005) on *C. pipiens* and Wang-Jian *et al.* (2005) on *M. domestica*.

The percentage pupation was decreased due to larval treatment with volatile oils, similar effects of some botanical plant extracts have been reported by Abou El-Ela *et al.* (1995) and Youssef (1997) on the house fly *M. domestica*, Nassar *et al.* (1997) on *P. argyrostoma* and Sabry (2004) on *C. albicipes*.

High reduction in adult emergence was achieved by larval treatment with *C. macrocarpa* and *A. officinarum* volatile oils. These results are in agreement with the findings of Hussein and Aioub (2000) on *M. stabulans*, El-Shazly and Hussein (2004) on *C. pipiens*, *M. domestica* and *C. capitata* larvae and Traboulsi *et al.* (2005) on *C. pipiens* larvae.

### B. Morphogenic abnormalities

Treatment of the third larval instar of *S. nudiseta* with the tested two volatile oils decrease the survival of the larvae and gave rise to noticeable larval, pupal and adult abnormalities (Figs. 2, 3 & 4).

#### I. Deformed larvae

Some deformed larvae were pigmented and larval-pupal intermediate which had parts of pupal cuticle with persisting last larval skin in their anterior end (Fig. 2 b & c).

#### II. Deformed pupae

Some of treated larvae were able to pupate, however, the resultant puparia of some individuals showed C-shaped pupae, elongated pupae and balloon shaped pupae (Fig. 3b, c & d).

#### III. Deformed adult

From Fig (4b & c), most of the pupae failed to reach adults however some emerged adult have various degrees of morphological abnormalities. Some individuals showed a dominance of incomplete adult eclosion varying from complete eclosion of adults with only their legs or wings were partially attached to the puparium. However in most cases, only the head and thorax were emerged from the puparium. Mean while

some adult were completely free but possessed abnormal appearance such as: severely crumpled wings, deformation in the thorax and abdomen.

These results are in agreement with the data reported by Hussein (1995) on *P. aegyptiaca* after treatment with some plant extract. Abahussain (1999) studied the morphological effects induced by *C. procera* in *C. pipiens* and *A. multicolor*, those morphological effects were larval-pupal intermediate and pupal-adult intermediate. Hussein (2000b) on *S. littoralis* treated with volatile oil. El-Domiaty *et al.* (2003) found shrinkage of the pupae and folding of the wings of adults as a result of treatment of third instar larvae of *M. domestica* with *P. nigra* volatile oil. Sabry (2004) stated that, treatment of third instar of *C. albiceps* with *T. vulgaris* and *Z. officinale* produce larval pupal intermediate and adults with crumpled wings. Ibrahim (2006) found larval pupal intermediate as a result of treatment of *S. littoralis* larvae with seeds extracts of *E. cyclocarpum*.

This indicates that volatile oil of *C. macrocarpa* and *A. officinarum* might act as an insect growth inhibitor, where Wright (1970) indicate that such malformation is a true juvenile hormone effect. Cruichashank and Plamere (1971) mentioned that terpenoid amides act as insect juvenile hormone, however the two volatile oils used in this study belong to terpen group in their structure and act as juvenoids or IGRs. Our results are in accordance with David *et al.* (1988), Singh *et al.* (1989) and Schearer (1984).

### 3. Histopathological studies.

#### A. Integument:

The structure of the normal integument of larvae of *Synthesiomyia nudiseta* consists of the inner basement membrane, a single cell layer and outer cuticle which is differentiated into an outer epicuticle and an inner endocuticle (Fig. 5a).

Severe damage was occurred on the integument of *S. nudiseta* larvae when they were treated by volatile oils. Complete disintegration in the hypodermis of larval cuticle was observed. They elicited a lack of differentiation between exocuticle and endocuticle, destruction of the basement membrane and appearance of vacuoles between cuticle and hypodermis (Fig.5b).

Similar observations were reported with azadirachtin against the larvae of *E. varviests*. They showed degeneration in the hypodermal cells in addition to cuticular abnormalities (Schluter & Schulz, 1983 and Schuter, 1986). Also the integument of *S. littoralis* larvae was affected, as degeneration of hypodermal cells, and irregular thickness of the cuticle when the larvae were fed on castor bean leaves treated with *A. monosperma*, *Z. coccineum* and *L. termis* extracts. Also, treatment of *S. littoralis* larvae with plant extracts of both *B. tournefortii* and *Z. coccineum* elicited degeneration of the cuticle and detachment of the epidermal cell from each other (Younes *et al.*, 1999). Also, Shoukry *et al.* (2002b) found detachment of cuticle from hypodermis, disintegration in the hypodermis and destruction of the basement membrane when *P. interpunctella* larvae treated with certain volatile oils. Sabry (2004) estimate the similar results on *C. albiceps*.

#### B. Muscles:

The muscles are composed of striated fibers. Each fiber consists of a number of parallel fibrillate or sacrostyles, occupying the whole of the cross section of the fiber and are laid down in plasma or sarcoplasm. The nuclei of the sarcoplasm are disposed immediately beneath the sarcolemma (Wigglesworth, 1947).

The histopathological effect of volatile oils on muscles was ranged between slight degeneration by the occurrence of fissures, to complete destruction of the whole

tissue. The appearance of fissure and the breaking down of muscles into small parts are attributed to the destruction of the sarcolemma (Fig.5b).

These results are well agreed with those obtained by Hussein (1995); Shoukry (1996); Hussein (2000b); Shoukry *et al.* (2002b), Sabry (2004) and Ibrahim (2006).

#### **C. Fat bodies:**

Histological structure of the normal fat bodies indicated that they are composed of two layers. An outer or partial layer which is formed of ribbons beneath the body wall and an inner or visceral layer surrounding the various organs. The ribbon consist of many irregular cells. Their cells surrounded by sheath (Fig.5a).

The histological changes were caused by the two volatile oils used in this study showed a noticeable destruction on the fat body cells, as vacuolization of the fat cells, destruction of the membranous sheath (Fig.5b).

#### **D. Midgut:**

Cross section in the normal midgut shows one layer of epithelial cells resting on the basement membrane, muscular layer consisting of inner circular muscle, and outer scattered groups of longitudinal muscles (Fig.6a).

Volatile oil treatment induced severe effect on the mid-gut, where they are regarded shrinkage in some epithelial cells and swelling of other cells presumably due secretory activities. The boundaries of epithelial cells were extensively damaged, showed a great destruction of muscular cells, necrotic epithelium and detachment of the basement membrane (Fig.6b).

These results are in agreement with findings of Emar and Assar (2001), Shoukry *et al.* (2002), Sabry (2004) and Ibrahim (2006).

#### **E. Salivary gland:**

The wall of normal salivary gland consists of a small number of large cells, which have large nuclei (Fig.7a). Signs of necrosis and degeneration in the nuclei and cytoplasm of the salivary gland cells are regarded as a rupture of the cells membrane of the salivary gland with volatile oil treatment (Fig.7b).

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(a)

(b)

(c)

Fig. (2): Normal and deformed larvae of *Synthesiomyia nudiseta* treated with volatile oils.  
 a) Normal larvae. b) Larval pupal intermediate. c) Larvae with darkened cuticle.



(a)

(b)

(c)

(d)

Fig. (3): Normal and deformed pupae of *Synthesiomyia nudiseta* treated with volatile oils.  
 a) Normal pupae. b) C-Shaped pupae.  
 c) Elongated pupae. d) Balloon Shaped pupae.



(a)

(b)

(c)

Fig. (4): Normal & deformed adults of *Synthesiomyia nudiseta* treated with volatile oils.  
 a) Normal adult ( ). b) Incomplete adult eclosion. c) Adult with crumpled wings.

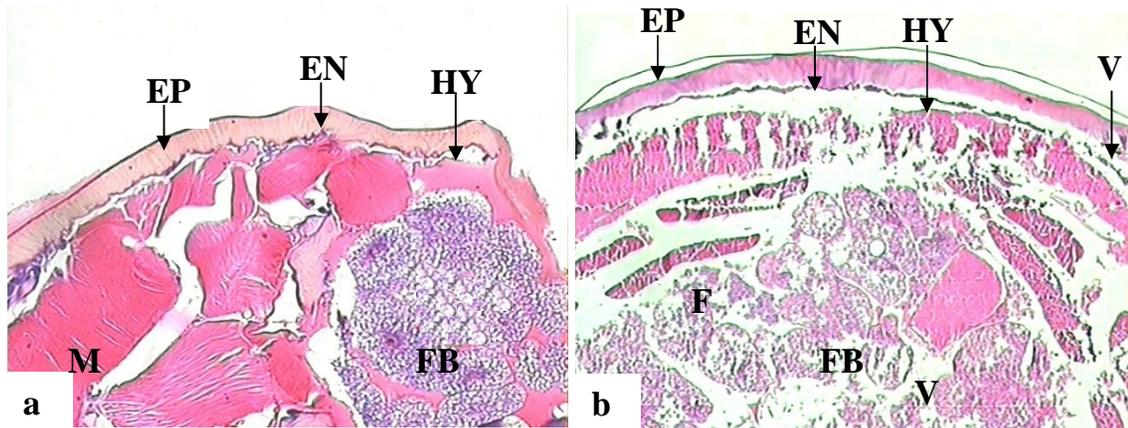


Fig. (5): Photomicrographs of T-S in the cuticle, muscles and fat bodies of untreated and plant volatile oil treated *Synthesiomyia nudiseta* larvae, (X= 200).  
 a- Untreated                      b- Treated

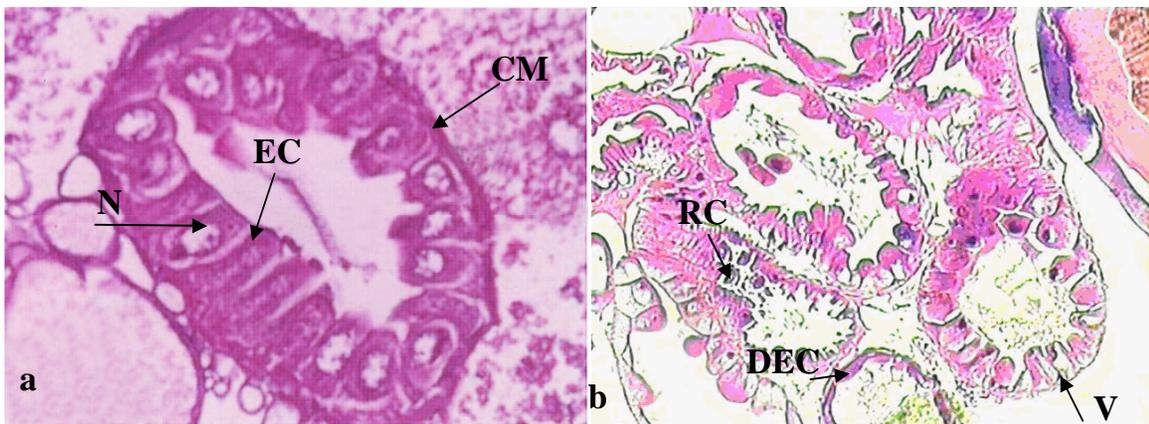


Fig. (6): Photomicrographs of T-S in the mid-gut of untreated and plant volatile oil treated *Synthesiomyia nudiseta* larvae, (X= 200).  
 a- Untreated                      b-Treated

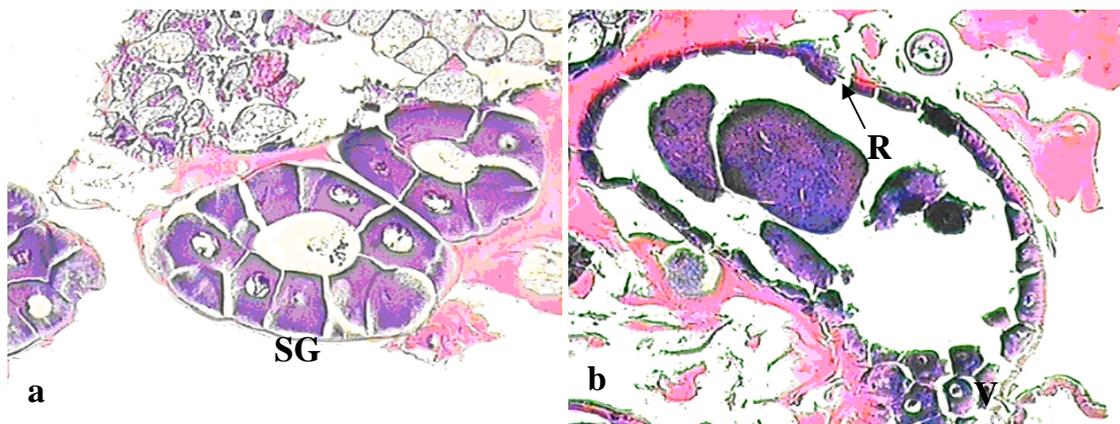


Fig. (7): Photomicrographs of T-S in the salivary gland of untreated and plant volatile oil treated *Synthesiomyia nudiseta* larvae, (X= 200)  
 a- Untreated                      b- Treated

EN: Endocuticle    EP: Epicuticle    F: Fissures    FB: Fat Bodies    HY: Hypodermis    M: Muscles            V: Vacuoles  
 R: Rapture        SG: Salivary gland    CM: Circular muscles    DEC: Degenerated epithelial cells    EC: Epithelial cells  
 N: Nucleus        RC: Ruptured cells

## ARABIC SUMMARY

الإبادي الحيوي لزيوت نباتية طيارة ضد يرقات ذبابة السينزسوميا نيوديسييتا ( مسكيدي- ذات الجناحين)

عبد الفتاح عبد المجيد أحمد خلف- كرم طلب حسين- كريمة شكرى خاطر  
قسم علم الحيوان – كلية العلوم – جامعة الزقازيق

استخدم التقطير البخاري لاستخلاص الزيوت النباتية الطيارة من نباتي السرو الليموني والجانجال وجمعت هذه النباتات من محافظة الشرقية، كما استخدم جهاز التحليل الكروماتوجراف الغازي ومطياف الكتلة للتعرف على مكونات هذين الزيتين. وجد أن مركب الألفابينين ومركب الترانس كاربوفلين يشكلان النسبة العالية لزيوت السرو الليموني، بينما شكل مركب (8.1) سينيول و 4- تربنيول النسبة العالية لزيوت نبات الخنجان. أدت معاملة الطور البرقي الثالث لذبابة السينزيسوميا نيوديسييتا بالتركيزات النصف مميته إلى التأثير الواضح على النواحي البيولوجية والمورفولوجية على نفس الطور المعامل وعلى الأطوار الأخرى التالية. أدت المعاملة إلى ظهور تشوهات في كل من اليرقات والعدارى وكذلك الطور الكامل لهذه الذبابة. أظهرت الدراسة الهستولوجية أن المعاملة بالزيوت الطيارة أثرت على تركيب كل من الجليد والعضلات والأجسام الدهنية والمعى المتوسط والغدة اللعابية. وفي النهاية نوصي باستخدام هذين الزيتين كوسيلة آمنة في مقاومة هذه الحشرة الضارة.