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Morphological aberrations induced by Thymol in larvae of *Megaselia scalaris* L. (Diptera: Phoridae)

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ABSTRACT

Megaselia scalaris (Diptera: Phoridae), humpbacked, coffin or scuttle fly, is considered as a cosmopolitan scavenging insect. Despite its profound value in Forensic Entomology and biological control, *M. scalaris* is regarded as a medically notable insect worldwide because it is source of myiasis diseases. Further, it was recorded as a parasitoid infesting some useful insects. On the other hand, it attacks some plants such as corn, food and seed deposits, germinated seeds, cultivated and non-cultivated mushroom and fruits like bananas. Feeding on such infected fruits, can cause intestinal and wound myiasis. Therefore, scuttle fly should be controlled for human welfare and economic reasons. Phytochemical insecticides may be effective, safe and acceptable alternative to traditional chemical insecticides. Thymol, a constituent of oil of thyme (*Thymus vulgaris*), is widely used as food flavorings, mouthwashes, pharmaceutical preparations, besides it has many pesticidal uses; as insecticide, fungicides, rodenticide and antimicrobial effect. Moreover, it degrades rapidly and has no negative effect on seed germination. Accordingly, the present study was conducted to investigate the effects of thymol on the mortality, morphology and surface ultrastructure of the 3rd instar larvae of *M. scalaris*. Results showed that thymol has larvicidal effect, as the estimated LC_{50} averaging 5.22 g/100g media, and the response was a dose-dependent. Morphological observations revealed that thymol treatment resulted in different morphogenic malformations in larval stage. Malformed larvae appeared sluggish and motionless, with symptoms of distorted, darkened and dry bodies. On the other hand, at the ultrastructural level, thymol treatment exhibited many morphological aberrations as compared with the controls. Such aberrations appeared in the malformed structure of short spinous processes and spines of the body wall, bubble membranes, antennae, mouth hooks, maxillary palp complexes and anterior and posterior spiracles. Based on the present results, it is concluded that thymol can prevent adult emergence through killing maggots and protect against re-infestation. Consequently, thymol could be suggested as a safe effective larvicide against the scuttle fly larvae.

Keywords: *Megaselia scalaris*, thymol, toxicity, morphology, ultrastructure, SEM.

INTRODUCTION

Megaselia scalaris (Loew, 1866), (Diptera: Phoridae), humpbacked, coffin or scuttle fly, has a wide geographical distribution and can be found in the tropics and subtropics [1]. The scuttle fly is a common species found amongst indoor and outdoor crime scenes and plays an important role in the decomposition of human remains and is useful in estimating postmortem interval particularly in indoor cases Accordingly, the presence of the larvae in a corpse could be used as entomological evidence in forensic investigations [2].

The larva of *M. scalaris* has scavenging properties. It has been described as parasite, parasitoid, predator, terrestrial detritivore (consumes both animal and plant material), phytophagous, and coprophagous [3]. The parasitoid behavior of the larvae gives this fly the ability to feed on a lot of other insects of agronomic, veterinary and medical importance**.** Such as insects of orders: Orthoptera, Dictyoptera**,** Hemiptera, Diptera, Lepidoptera, Coleoptera, and Hymenoptera, some of which are of agricultural importance [4]. So, it can be a biocontrol agent in biological control methods.

Despite its profound value in forensic entomology and biological control of other harmful insects, *M. scalaris* is considered as a medically important insect worldwide. It affects humans in different ways; it is an aggravating insect for humans and animals, also, a source of myiasis disease. It is well known that flies are transmitters of transmittable and parasitical diseases of both man and animal. When a fly lands on a source of contamination (i.e. a corpse, faeces, open wounds, contaminated food, etc.), it is possible for the fly to then transmit through direct or indirect contact an infectious agent to man or animal. Myiasis refers to the infestation of living tissue from either animals or humans by Diptera larvae. Myiasis caused by Phoridae have been reported as wound myiasis, and non-wound myiasis such as nasopharyngeal, urinogenital myiasis and intestinal myiasis. Besides, it affected non-

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domesticated and domesticated animals [5]. Moreover, *M. scalaris* was found as parasitoid infested some useful insects like *Apis mellifera* [4].

On the other hand, *M. scalaris* was found attacking some plants. It was found infesting corn fields, food and seeds deposits and some germinated seeds [5]**.** The scuttle fly has been reported infesting cultivated and non-cultivated mushroom, which cause damage to mushroom crop via larval feeding directly on mycelia and mush rooms, and/or introducing of disease agents to crops. Moreover, great economic loss in mushroom industry in some countries caused by sciarid flies [6]. Infestation of fruits by the fly larvae is a known occurrence. The first report of *M scalaris* infesting bananas and surviving solely on this fruit for several generations is reported by **Karunaweera**[7]. Further, they stated that a bulky consumption of infected bananas can cause intestinal and wound myiasis in humans make these findings medically important. Unlikely, authors reported that the difficulty to identify the intact and infected bananas from fruiting body increases the bad effects of consumption of affected fruits.

Therefore, scuttle fly should be controlled for human welfare and economic reasons. Elimination of scuttle fly by killing maggots using larvicide is effective. Larvicides that are commonly used in the phorid control program are chemical synthetic insecticides. But, the extensive and widespread use of synthetic insecticides has caused some concerns on the safety and toxicological impacts towards the environment, human and other non-target organisms. The repetitive application of chemical insecticide has affected the natural enemies and increase its resistance to pesticides globally. For the previous reasons, the search for new insect control agents from natural products which are target-specific, biodegradable and of low environmental toxicity is crucial. On consequence, much researches have been focused on the phytochemicals (plant materials and their components) for potential use as alternative pesticides. In the hope such natural materials might provide cheap, biodegradable, low mammalian toxicity, locally produced, environmentally safe and effective control agents, besides their acceptance among users.

Thymol is a monoterpene phenol derivative of cymene, which is a constituent of oil of thyme, a naturally occurring mixture of compounds in the plant *Thymus vulgaris* and family Lamiaceae. Thymol is widely used in manufacture of perfumes, food flavorings and preservation, mouthwashes, pharmaceutical preparations and cosmetics. Also, thymol has many pesticidal uses, including insecticide, fungicide, rodenticide, antimicrobial [8]. Thymol degrades rapidly in the environment, thus, low risks because of rapid disintegration and low bound residues. Besides, it has no negative effect on seed germination [9]. These characteristics are supporting the use of thymol as a pesticide agent that offers a safe alternative to other chemical pesticides.

M. scalaris is frequently enter houses, either alone or with other phorid species, and may infect man, domestic animals, stored products, fruits and others; as it is considered a cosmopolitan scavenging insect. Phorid larvae are very similar morphologically, but it can be distinguished via SEM observations [10]. Scanning electron microscopy (SEM) gives detailed morphological information that can help in studying the immature forms of flies. Different stages of *M. scalaris* have been studied with light microscopy and with scanning electron microscopy (SEM) [10, 11]. Ultrastructural revelation of *M. scalaris* larvae by means of SEM not only provides details of the normal 3rd larval morphology features, but it can also explain the deleterious effect of thymol on that features.

Unfortunately, there were lack of data regarding the effects of different pesticides on the ultrastructure of the scuttle fly larvae. To our knowledge, this is the first study to examine the effect of botanical pesticide on the ultrastructure of phorid larvae. Thus, the objective of the present study was to determine the effect of thymol on the mortality, morphology and surface ultrastructure of the 3rd instar larvae of *M. scalaris*.

MATERIALS AND METHODS

Insect colony

The scuttle flies, *M. scalaris*, were found infecting house fly breeding cages in the insect laboratory at the Department of Biological and Geological Sciences, Faculty of Education, Ain Shams University, Egypt. Insects were morphologically identified as *M. scalaris* under the guide of Salwa Kamal, Professor at Entomology Department, Faculty of Science, Ain Shams University. A stock culture of *M. scalaris* was established from a single pair (male and female); to keep the genetic homogeneity of the subsequent generations. The adults were allowed free access to sugar and cotton pads soaked in milk powder dissolved in water (10% w/v). Larvae were reared

according to the method described by **Pavela**[12] on a mixture of sterilized bran (38 g), milk powder (2 g) and water (60 ml) and maintained at 26±2°C and 70±5% relative humidity (rh), with a photoperiod of (LD: 16-8).

Thymol

Thymol ($C_{10}H_{14}O$) was purchased from Sigma Aldrich Chemical Co. (St Louis, MO, USA). Thymol (97–99 % purity) (Sigma G8761).

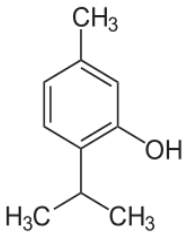

Figure 1. The structure of Thymol

Larvicidal assay:

The larvicidal bioassay was conducted according to **WHO**[13]**.** The susceptibility of 1st instar larvae were assayed towards thymol using the food contamination method. Five concentrations of the tested material (0.3, 1, 3, 6, 9 and 12g/100g media) were prepared by using acetone as a solvent. The treatment was done by mixing the appropriate concentrations with rearing media. Each treatment was divided into 3 equal samples, kept in 250 ml beakers. Control was prepared similarly, without adding tested material. Active 1st instar larvae were collected from the culture. A group of 30 larvae were placed in each beaker. Beakers were labeled, covered with muslin and incubated until pupation at laboratory conditions mentioned before.

All treatments were observed for the number of resulted pupae. The mortality percentages were calculated from the difference between the number of the tested larvae and the number of resulted pupae. The results of the bioassay were corrected for control mortality using formula described by **Abbott**[14], as follows:

$$\text{Corrected mortality (\%)} = \frac{\text{Observed mortality \% - Control mortality \%}}{\text{100 - Control mortality \%}} \times 100$$

Then, results were represented graphically as Probit. Log. Regression Line using Excel (2016) software. The mortality data were subjected to regression analysis, to determine LC_{50} and its lower and higher confidence limits values at 95% probability, according to the method described by **Litchfield and Wilcoxon**[15].

Morphological observations

Healthy 1st instar larvae were exposed to sublethal concentration LC_{50} of thymol. Control treatment was treated similarly without adding thymol. Alive control and treated 3rd instar larvae from treatment were collected for morphological observation. Larvae were preserved in ethanol 70% with glycerin. Morphological changes of the treated larvae were recorded and compared with the control larvae. Photographs were taken with digital camera through the eye lens of the light microscope.

For scanning electron microscope (SEM) study, the control and treated 3rd instar larvae were collected from testing jars, washed with insect saline solution several times, then prefixed with glutaraldehyde (2.5%) at 4°C for 24 hr. After that, Specimens were dehydrated with increased serial concentrations of ethanol (30%, 50%, 70%, 80%, and 90%, for 40 min. each). They were washed three times in 100% for 1 hour each. Then, larvae were transferred to a specimen basket and placed inside a critical point drier. Finally, specimens were attached to double-stick tape on aluminum stub and coated with gold in the sputter-coating apparatus to be viewed and photomicrograph were taken with JEOL JSM 5200 SEM Scanning Electron Microscope, at Applied Center for Ento-Nematode, Cairo University, Egypt.

RESULTS

Larvicidal assay

In the present study, larvicidal assay was conducted with thymol against *M. scalaris* larvae. The obtained data was shown in **Table 1** and **Fig. 2**. The estimated value of LC_{50} was (8.20 > **5.22** > 3.33) g/100 g media at 95% probability. Results showed positive correlation between the concentration of the tested material and its corresponding mortality; as the concentration increased, the corresponding mortality percent was also increased. The response was a dose-dependent, which was also indicated by the positive slope value (**Table 1**).

Table 1. Susceptibility of *M. scalaris* larvae to Thymol

Conc. (g/100g media)	**Dead larvae[(1)]**	**Observed mortality %**	**Corrected mortality%**
0.3	22	24.45	11.70
1	33	36.67	25.98
3	47	52.22	44.16
6	56	62.22	55.84
9	70	77.78	74.03
12	81	90.00	88.31
control	13	14.44	0.00
Slope of the regression line		**6.1025**	
LC_{50} with confidence limits[(2)]		**8.20 > 5.22 > 3.33 g/100g media**	

No. tested larvae: 90 larvae/ concentration, [(1)] sum of 3 replicates,
[(2)] confidence limits at 95% probability.

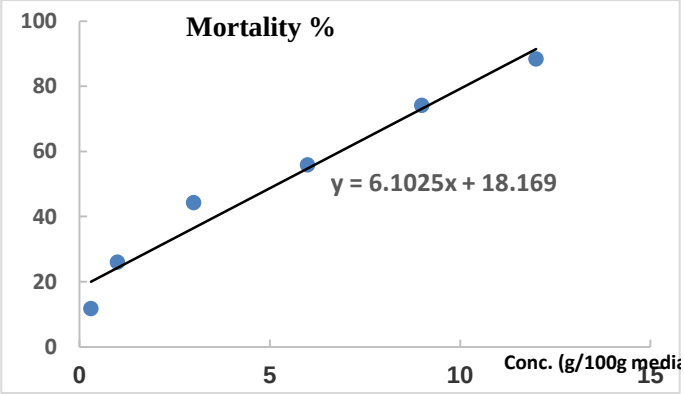

Figure 2. Susceptibility line of *M. scalaris* 3rd instar larvae to thymol treatment.

Morphological observations

Light microscopic observations revealed that the normal third-instar larva of *M. scalaris* is relatively small, averaging 4.54 mm in length (range 3.83-5.21, n=10). It is creamy-white in color and legless. The body is vermiform-shaped with pointed head and blunt end and body segments bear short fleshy processes (**Fig. 3-a**). Larvae have 12 abdominal segments in which bilateral spines are present from the third thoracic to the last abdominal segment. Larval body is somewhat transparent, so, it can easily distinguish internal organs under light microscope, like intestine, gonads, buccopharyngeal skeleton.

Treatment of larvae with LC_{50} of thymol resulted in different morphogenic malformations in larval stage (**Fig. 3b-i**). Malformed larvae appeared sluggish and motionless. Larval malformations showed symptoms of distorted body, dwarfism and shrinkage. Treated larvae averaging 3.98 mm in length (range 3.50-4.97, n=10). Some larvae were appeared with irregular, compressed or curved bodies and with thorax or abdomen constriction. Others appeared with darkened and dry bodies. In few cases, part of exuvial cuticle remained attached to the larval body at the front end.

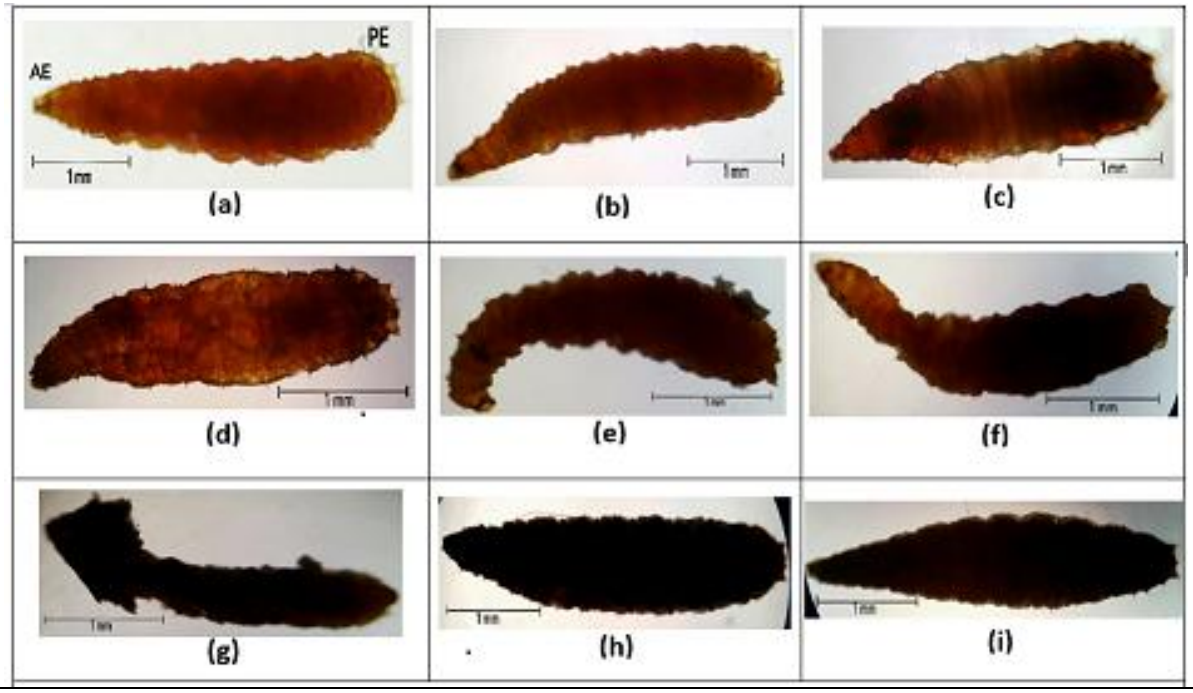

Figure 3: Normal and malformed larvae of *M. scalaris* after treatment with thymol (scale bar= 1 mm). (a) Normal 3rd instar larva. (b-i) Malformed larvae; (b) compressed thorax, (c) flattened and sluggish, (d) distorted and shrinkage body, (e) curved and shrinkage, (f) curved with thorax constriction, (g) distorted, dry and darkened with head exuvial remnants, (h,i) dark and motionless larvae.

Scanning Electron Microscope (SEM) examination showed that the body of normal 3rd instar larva of *M. scalaris* is cylindrical, pointed at the head while blunt at the end (**Fig. 4a-c**).

The body integument contains short spinous processes located dorsally and laterally. Dense, tooth-like spines, each bearing a single, sharp pointed tip, are arranged in several rows on the thoracic segment, interwind with rows of hairy spines (**Fig. 5a-d**). The bubble membranes, a group of globular structures on the dorsolateral border of the 5th segment, is seen (**Fig. 5a,b**).

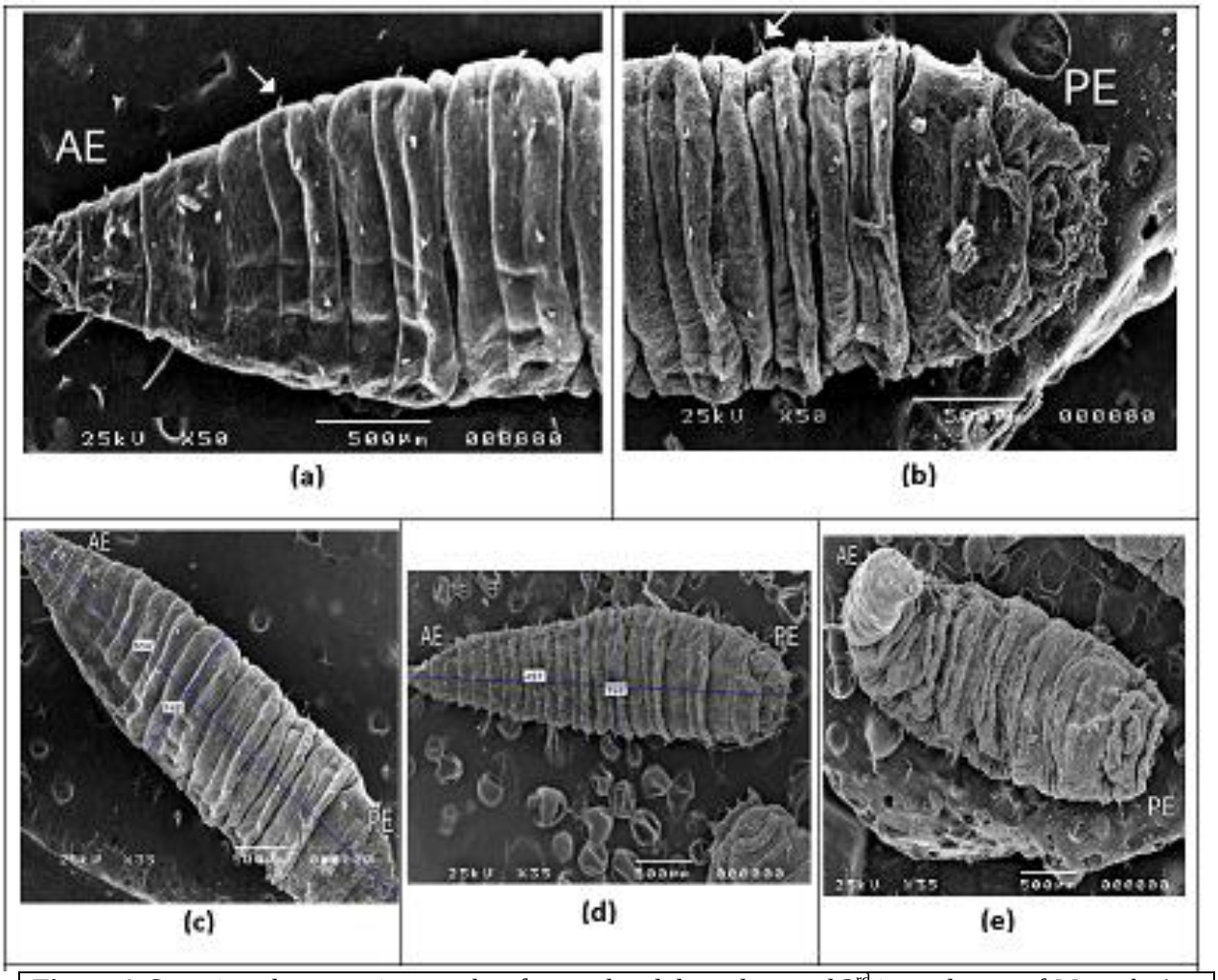

Figure 4. Scanning electron micrographs of normal and thymol-treated 3rd instar larvae of *M. scalaris.* (a,b) Ventral view of the normal larval body, anterior end (AE) to the left, posterior end (PE) to the right. White arrow indicated short spinous process. (c) Normal larva (d) Treated larva (shrinkaging and darker), (e) Another treated larva (curved and shrinkaging).

The cephalic region is composed of two cephalic lobes which separated by a deep groove. The cephalic segment is composed of a pair of antennae, a pair of maxillary palp complexes and mouthparts (**Fig. 6a**). A pair of dome-shaped antennae (or dorsal organs) are located dorsally on each side of the cephalic region. The prominent features of the mouthpart are the labrum, labium, a pair of mouth hooks and an oral groove (**Fig. 6b,c**). A tuberculate labrum is located between the deeply serrated mouth hooks. Each mouth hook appears as a semicircular lobe that has its peripheral margin deeply serrated. While, the labium appears as a large triangular-shaped structure that curves ventrally.

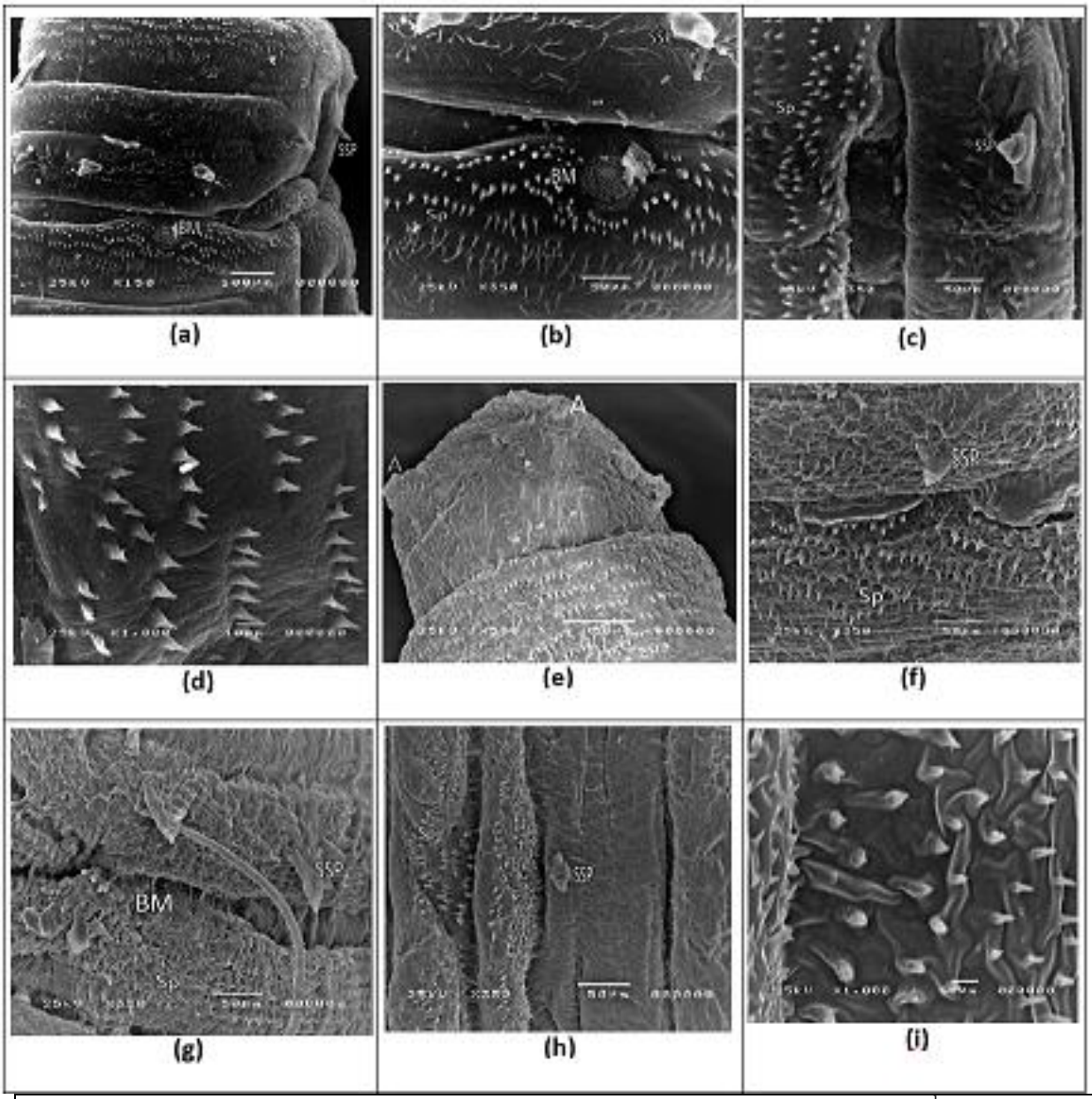

Figure 5. Scanning electron micrographs of body wall of normal and thymol-treated 3rd instar larvae of *M. scalaris*. (a-c) postero-lateral view of normal larva, showing rows of spines (Sp), short spinous processes (SSP) and bubble membranes (BM). (d) magnified spines. (e) dorso-lateral view of treated larva showing malformed antenna (A) and spines (Sp). (f-h) treated larvae with distorted spines, short spinous processes and bubble membrane. (i) Rows of spines, lost their pointed tips, of treated larval body.

A pair of protruding maxillary palp complex (or terminal organ), located on each cephalic lobe, contains several types of papillae arranged in a row (**Fig. 6d**). There are also well-developed oral grooves which is located as cuticular ridges associated with the mouth.

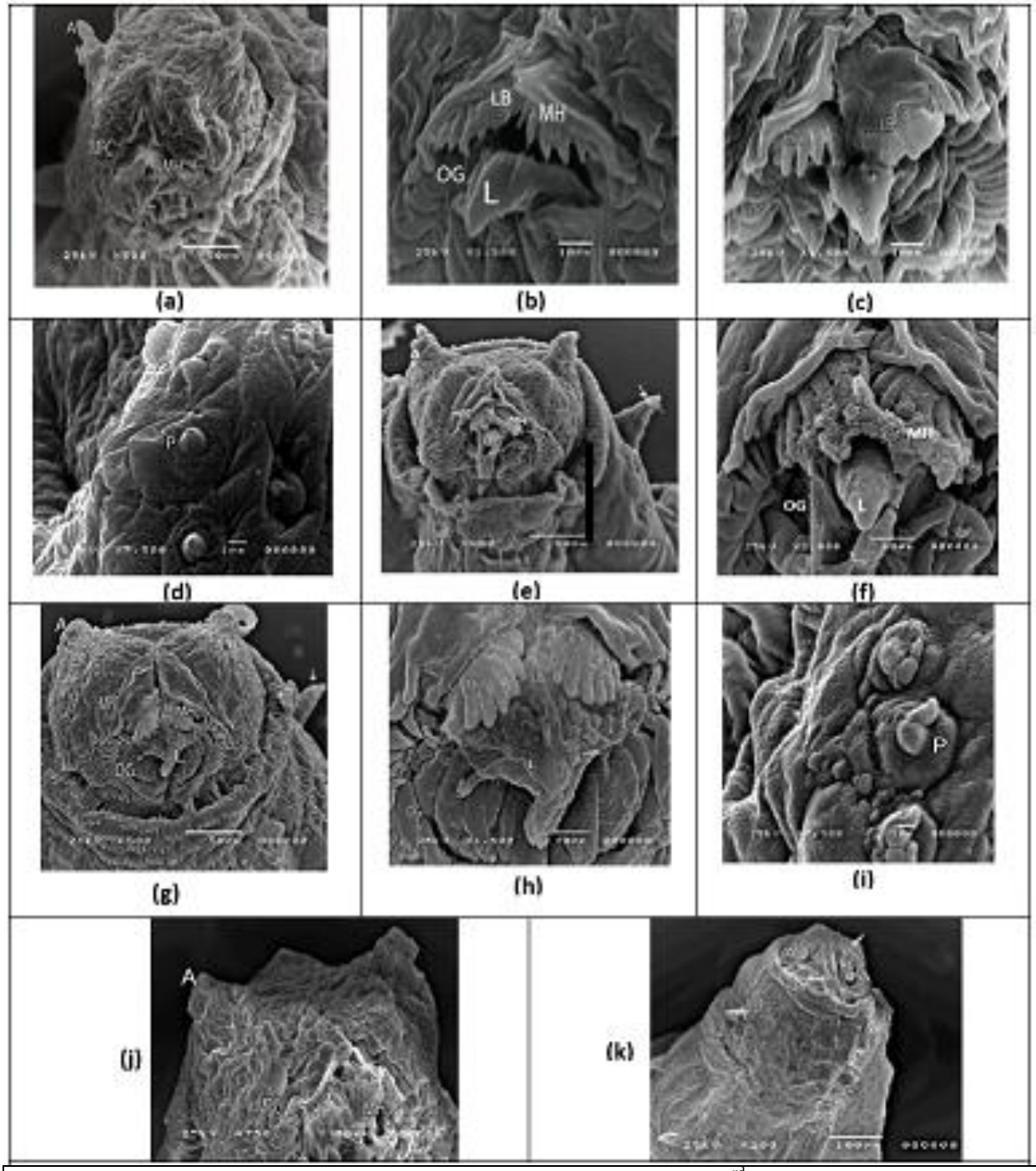

Figure 6. Scanning electron micrographs of normal and thymol-treated 3rd instar larvae of *M. scalaris*. (a) fronto-lateral view of normal cephalic segment showing antenna (A), maxillary palp complex (MPC), mouth hooks (MH) and labium (L). (b,c) magnified mouthparts of normal larva showing labrum (LB), L, MH and oral groove (OG). (d) papillae (P) on the normal MPC. (e,g) cephalic segment of treated larvae, short spinous process (arrow). (f,h) mouthparts of treated larvae. (i) cleavage papillae on the maxillary palp complex of treated larvae. (j) frontal view of cephalic head of treated larva showing malformed A and MH. (k) dorso-apical view of cephalic segment of treated larva showing antennae, SSP and anterior spiracle (AS).

The anterior spiracles are located on each latero-posterior edge of the prothorax (**Fig. 6k**). Each prothoracic spiracle appeared as a circular papilla-like structure that contains two straight spiracular openings (slits), with one end closed together, while the other is far apart (**Fig**. **7a,b**).

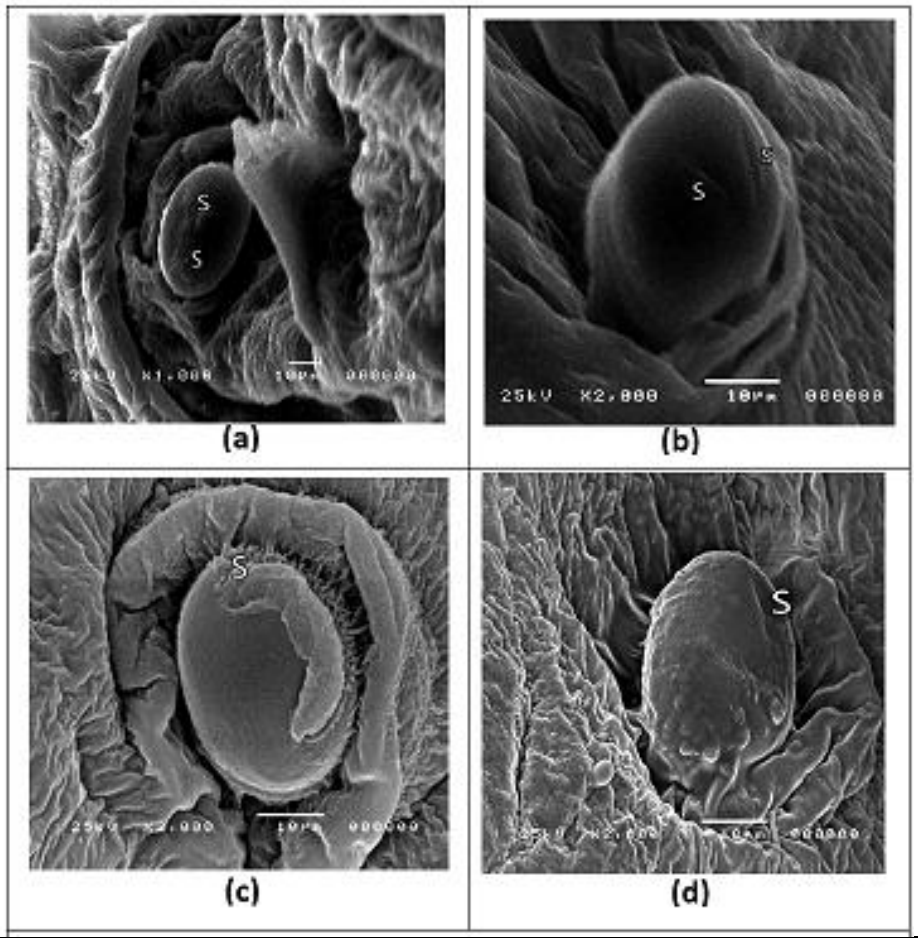

Figure 7. Scanning electron micrographs of the anterior spiracle (AS) of normal and thymol-treated 3rd instar larvae of *M. scalaris*. (a,b) AS of normal larva with circular structure, normal two straight slits (S) coalesced at one end and smooth integument. (c,d) AS of treated larva showing distorted slits with rough integument.

On the other hand, A pair of posterior spiracular discs protrudes dorsally on the 12th segment (**Fig. 8a,b**). Each appears as a large cone which is constricted centrally, with smooth cuticle and intact posterior spiracle. Each posterior spiracle has four intact straight spiracular slits arranged as two opposite groups. The posterior spiracular hairs (sun ray structures) appear centrally, at the area of constriction (**Fig.8c**).

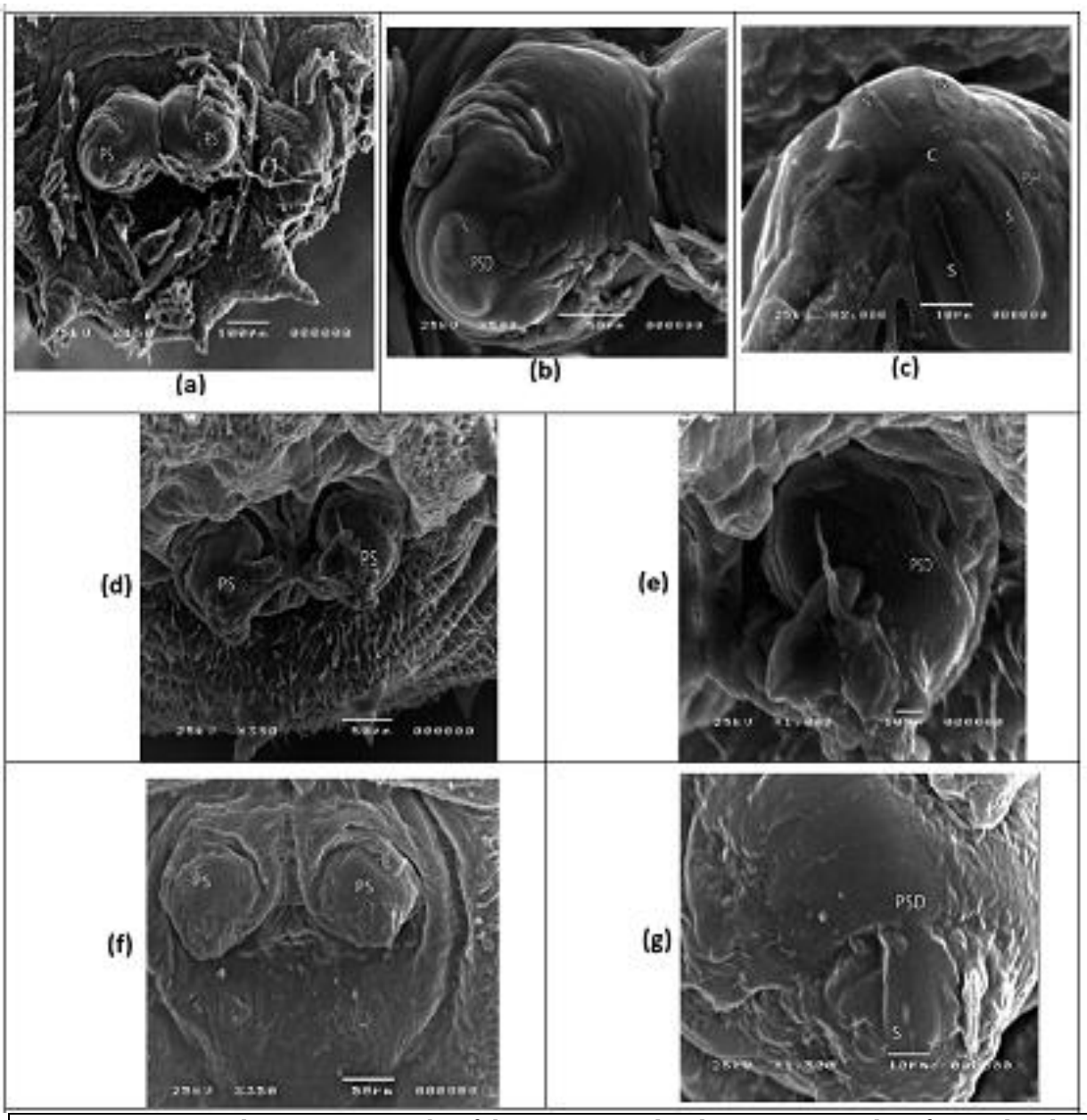

Figure 8. Scanning electron micrographs of the posterior end and posterior spiracles of normal and thymol-treated 3rd instar larvae of *M. scalaris*. (a) posterior end of normal larva showing the pair of intact posterior spiracles (PS). (b,c) Posterior spiracular discs (PSD) with constriction (C) in the middle, each bearing two straight slits (S), and surrounding by posterior spiracular hairs (PSH). (d,e) Posterior end of treated larva showing malformed PSD with semi-closed slits. (f,g) Another treated larva with distorted PSD .

SEM observations of *M. scalaris* 3rd instar larvae treated with sublethal concentration LC_{50} of thymol revealed obvious signs of toxicity. Thymol-treated larvae exhibited many morphological aberrations as compared with that of the control larvae **(Fig. 4-d,e)**. The treated larvae appeared with distorted, darken and shrunken body (**Fig. 5-e-g**). Short spinous processes and spines appeared malformed and lose their pointed tips (**Fig. 5-f-i**). Bubble membranes could not be clearly seen under SEM observation but are quite apparent as circular structures containing remaining traces indicate it (**Fig. 5-g**).

Ultrastructure examination of the cephalic segment showed that antennae of treated larvae appeared compressed and shorter than that of control ones (**Fig. 5-e, 6-g,j,k).** Besides, mouthparts showed somewhat less aberrations; in some treated larvae, mouth hooks appeared adhered (**Fig. 6-f**), while in few ones appeared distorted (**Fig. 6-j**). In some cases, mouth opening closed with particles which may be came from the alimentary canal or from outside medium (**Fig. 6-h**). Also, thymol treatment led to an obvious deformity where each papilla on the maxillary palp complex manifested cleavage into a few projections (**Fig. 6-i**).

Furthermore, the prothoracic spiracles appeared malformed with blocked spiracle openings (slits) with distorted bubbly cuticle (**Fig. 7c,d**). As well as, thymol-treatment led to damage in the posterior spiracular discs, which appeared slightly shrunken with billow shaped cuticle**.** Posterior spiracles appeared damaged with distorted closed or semi-closed spiracular slits **(Fig. 8 d-g)**.

DISCUSSION

Larvicidal assay

Larvicidal assay of thymol against *M. scalaris* larvae revealed that there was a positive correlation between the concentration of the tested material and its corresponding mortality; as the concentration increased, the corresponding mortality percent was also increased. The present results agree with those of some authors who studied the toxic effects of different botanical pesticides against various insect species and found that adult mortality increased gradually with increasing concentration of the tested materials**. Waliwitiya**[9] reported that thymol had great contact toxicity against larvae of *Agriotes obscurus* and had no negative impact on corn seed germination. Also, thymol had a significant toxic effect against early third-stage larvae of *Culex tritaeniorhynchus, Aedes albopictus,* and *Anopheles subpictus*, as stated by **Govindarajan**[16]**.** Moreover, it showed profound toxic effect in both laboratory and greenhouses against the maize stem borer, *Chilo partellus*[17]**.** Likely, it was found that thymol has low risks to environment because it degrades rapidly as reported by **Hu and Coats**[8], (DT_{50} 16 days in water, 5 days in soil). So, thymol can be used as botanical pesticide offering safe alternative to chemicals.

Morphological observations

M. scalaris has six stages in its life cycle, the adult, the egg, first instar larva, second instar larva, third instar larva and pupa. Their life cycle is approximately 18 days for males and 20 days for females under 28°C (seven days in three larval instars) [3]. In the present study, morphological observations of normal third-instar larva of *M. scalaris* indicated that the same external features were also reported by several authors [2, 11, 18]**.**

While, light Microscope (LM) has been the most used technique in observing morphology, but, Scanning Electron Microscope (SEM) is more efficient for observing the fine details of the specimen's morphology. So, SEM is helpful tool for entomologist to give a precise morphological information about larval body.

SEM examination of normal 3rd instar larva of *M. scalaris* resembles that of other authors who studied the same scuttle fly larvae [2, 11] and *M. spiracularis* [18]. Additionally, the present morphological investigation was in accordance with that of *Chrysomya megacephala*, *C. rufifacies* and *C. hominivorax* [19]**.**

The bubble membranes, which found on the dorsolateral border of the 5th segment, is considered as one of taxonomic differentiation between forensically important flies [10, 18], and the number of globules is different between such insects. Also, a pair of dome-shaped antennae (or dorsal organs) are located dorsally on each side of the cephalic region. **Akent'eva**[20] reported that the dome-shaped antenna acted as an olfactory receptor when he studied *M. domestica* larvae using transmission electron microscopy and SEM.

Concerning mouthparts, the labium appears as a large triangular-shaped structure that curves ventrally. As an adaptation, the ventral curvature of the labium may help the larva in feeding; where it is used to anchor the larva to the tissue, so it can successfully feed on the host either animal or plant. Besides, the presence of antennae, papillae of maxillary palp complexes and several sensory hairs on the mouth parts could enable the larvae to have various receptors and thus, perceive considerable information on their environs [2].

Morphological examination of the 3rd larval instar of *M. scalaris* clearly demonstrated presence of the cephalic sensory organs and well-developed mouthparts. The ultrastructure of sensory organs (antenna and maxillary palp) and mouthparts shares many morphological features those described in several other dipterous insects [5, 18, 19].

SEM investigation have shown well-developed mouth parts in *M. scalaris* larvae, which were reported only in cyclarrhaphous Diptera, which in turn make that larvae capable of feeding on a wide variety of host material [21]. Mouth hooks found also in *C. megacephala*, *A rufifacies* and *H. ligurriens*, which serves their feeding behavior to a great extent [19].

At the larval posterior end, a pair of posterior spiracular discs protrudes dorsally, each appears as a large cone which is constricted centrally. The posterior spiracular hairs appear centrally, at the area of constriction. According to **Dama**[22], the posterior spiracular hairs help the larvae to raise their posterior spiracles above the medium and increase the angle of contact with air. This enables the larvae to feed longer than the ones without the sun rays.

Ultrastructure examination of thymol-treated larvae showed that thymol has a deleterious effect on both anterior and posterior spiracles which in turn interrupts the ion regulation of larvae and further causes the imbalance of homeostasis. Additionally, that spiracular distortions may lead to spiracles malfunction. So, the entry of surrounding medium particles could be easy inside spiracle atrium through distorted slits which harms the respiratory system of the larvae and may lead eventually to death. Similar action has been described by **Wigglesworth**[23] whereby kerosene entered the tracheae from terminal spiracles in mosquito larvae and caused the finest capillaries to disappear from the tracheal system. Consequently, the assumed interruption of the osmo-regulatory system and the malfunctioned spiracles of respiratory system are suggested to contribute to the death of larvae which in turn stops re-infection.

In general, the present investigation clearly shows that thymol treatment affects many morphological features of the body of the treated 3rd larval instar of *M. scalaris*. Similar aberrations were reported by **Bianco**[24] when they treated *Ae. aegypti* larvae with extracts of natural products such as red seaweed *Laurencia dendroidea* and dried fruits of peppercorns. Comparable abnormalities have been recorded for *M. domestica* after treatment of the third larval instars with sesame, nigella, onion, diflubenzuron, and pyriproxfen [25]. The abnormalities could be attributed to the probable metamorphosis-inhibiting effect of thymol suggesting a type of insect growth regulating activity.

It is worthy to note that the scuttle fly larvae tend to embed themselves in the food medium while feeding even for a long period of time until pupation. So, its body is full of bacteria could not totally be cleaned when observed by SEM which in turn affects the quality of some micrographs in the present study.

CONCLUSION

The control of scuttle flies is important in terms of both human welfare and national economy but presents challenges concerning the identification of new, safe, and environmentally acceptable insecticides. The development of natural pesticides would help to decrease the negative impact of synthetic agents, such as residues, mammalian toxicity, resistance and environmental pollution. In this respect, phytochemical insecticides may be effective, selective, biodegradable, and less toxic to environment. In the present study, thymol not only killed larvae when used at high concentrations, but also, at sublethal concentration LC_{50}, induced morphological abnormalities that caused physiological disturbances which eventually lead to death. The present investigation showed that thymol can prevent adult emergence through killing maggots and protect against re-infestation.

Based on the present results, thymol could be suggested as alternative safe insecticides. However, further studies are required to determine the cost, applicability and safety to the non-target organisms and environment. Further investigations concerning the efficacies of such botanicals may lead to the future development of effective natural phorid fly control agents that could be integrated into integrated pest control programs (IPM). Therefore, the present study recommend thymol for clinical and field evaluations.

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