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ORIGINAL ARTICLE

Larvicidal activity of camphor and lavender oils against sheep blowfly, *Lucilia sericata* (Diptera: Calliphoridae)

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Abstract In this study, the essential oils of camphor and lavender were tested in vitro against the third instar larvae of *Lucilia sericata* for the first time, following dipping toxicity technique. The toxicity results revealed that *L. sericata* larvae were susceptible to the applied essential oils. Lavender oil was more effective than camphor in killing of *L. sericata* larvae. With 32 % concentration, the mortality percentages of larvae were 100 and 93.3 %, respectively. Light and scanning electron microscopic examinations were done to determine the cuticular changes of *L. sericata* larvae following exposure to the applied essential oils. Larvae showed cuticular swelling and distortion after oil treatment, but its level was greater with lavender oil. The current study suggested that an alternative, effective and natural product can be developed as larvicides against *L. sericata* using camphor and lavender oils.

Keywords *Lucilia sericata* · Larvicide · Camphor · Lavender · Essential oil · In vitro

Introduction

The larva of the blowfly, *Lucilia sericata* (Meigen) (Diptera: Calliphoridae), is a facultative ectoparasite infesting suppurative wounds of humans and animals, and leading to myiasis-infestation with fly larvae (Khater and Khater 2009). Flystrike (cutaneous myiasis) is a serious problem in all the major sheep-producing countries of the world (Phillips 2009). It occurs after gravid female blowflies are attracted to lay their eggs in the wool of sheep by olfactory cues. The resultant damage or 'strike' is mainly due to the mechanical and chemical effects of the feeding of the larvae. The infested animals showed restlessness, irritation at the sites of infestation, dermatitis and purulent infection which retard the growth among lambs, decrease in body gain in adults and loss in meat, milk and wool productions. Severe cases of cutaneous myiasis produce high mortality among infested animals due to pyaemia (El-Khateeb 1999).

Blowfly control is important, and has relied on the use of organophosphate and synthetic pyrethroid dips and sprays (French et al. 1994; Tellam and Bowles 1997) as well as insect growth regulators (French et al. 1994; Levot and Sales 2004). Prior to development and commercial success of synthetic insecticides in mid-1930s to 1950s, botanical insecticides were the foremost weapons against insect pests. The synthetic insecticides are characterized by efficacy, speed of action, ease of use, and low cost. Accordingly, they drove many natural control methods, such as using of botanicals, predators and parasitoids to near obscurity. 20 years after synthetic insecticides were overzealously entrenched in 'modern' agricultural production; they induce widespread environmental contamination, toxicity to non-target organisms, development of resistance against insecticides, and negative effects on animal and human health (Pretty 2009). Thus, naturally alternative means of controlling insects are required. Plant-derived materials are strongly considered the alternative insecticides to synthetic chemicals, and many studies gave good results when evaluated some plant extracts in control of medical and veterinary insects such as *L. sericata* (Morsy et al. 1998; Mazyad et al. 1999; El-Khateeb et al. 2003). Camphor, traditionally obtained through the

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distillation of the wood of the camphor tree, is a major essential oil component of many aromatic plant species, as it is biosynthetically synthesised; it can also be chemically synthesised using mainly turpentine as a starting material. Camphor exhibits a number of biological properties such as insecticidal, antimicrobial, antiviral, anticoccidial, anti-nociceptive, anticancer and antitussive activities, in addition to its use as a skin penetration enhancer (Chen et al. 2013). Lavender oil is useful for use in nervous system stimulants, hypnotics, sedatives, tranquilizers and stress repellents. In addition, it has useful dermatological uses in the treatment sunburn and skin rashes (Cavanagh and Wilkinson 2002), as well as strong antiseptic (disinfectant), and antibiotic (bacteria killing) effects (Lis-Balchin and Hart 1999).

Because the applied essential oils of camphor and lavender are being tested against *L. sericata* for the first time, in vitro assays are useful to prescreen their efficacy on third instar larvae of *L. sericata* following dipping toxicity technique.

Materials and methods

Insect culture

L. sericata larvae were obtained from a laboratory colony maintained at the Department of Entomolgy, Faculty of Science, Cairo University. *L. sericata* was reared under laboratory conditions of 25–30 °C and 60 ± 5 % relative humidity (RH) in the laboratory of the Department of Parasitology and Animal Diseases, Veterinary Research Division, National Research Centre. Adults were fed on sucrose and water but larvae were reared on bovine meat (El-Khateeb 1999).

Applied essential oils

Two essential oils, Camphor oil, *Cinnamomum camphora*, and Lavender oil, *Lavandula angustifolia* were obtained from El-Captain Co., Al-Obor city, Cairo, Egypt, approved for human use from the Egyptian Ministry of Health.

In vitro treatments

Dipping technique

In vitro larval immersion (dipping) tests were carried out according to Khater et al. (2013), to determine the efficacy of camphor and lavender oils against third instar larvae of *L. sericata*. Five concentrations (2, 4, 8, 16 and 32 %) were freshly prepared in distilled water. Few drops of Tween 80 were added as an emulsifier to the essential oils. The procedures were applied five times for each concentration and ten larvae of *L. sericata* were used per replicate in each test (i.e., 50 larvae were used for each concentration). Each group of larvae was placed in a mesh cloth piece and immersed for 60 s in a 100 ml solution of each material, and then the solution was continuously stirred during the process. The negative control was treated with distilled water and few drops of Tween 80. The immersed larvae were placed in Petri dishes having filter papers (Whatman No. 1) and then dishes were kept at 27 ± 2 °C and 80 ± 5 % RH. The mortality of larvae in all dishes was observed 24 h post treatment. Alive and dead larvae were counted, larvae were considered alive if they exhibited normal behavior when breathed upon or physically stimulated with wooden dowels; larvae which were incapable of movement, maintaining any signs of life, were considered moribund or dead.

Light microscopy

Samples were taken from control larvae as well as those exposing to 32 % concentration of both tested oils, fixed in 10 % buffered formol saline, and processed according to the method of Bancroft et al. (1996). Sections were deparaffinized and stained with hematoxylin and eosin stain for histological examination by light microscopy. The body wall of larvae was studied and photographed using an Olympus CX41 microscope.

Scanning electron microscopy (SEM)

Control larvae and those that exposed to 32 % concentration of both tested oils were fixed intact for 12 h in a 3:1 mixture of 4 % (w/v) glutaraldehyde in 0.12 M Millonig's buffer, pH 7.4 and 1 % aqueous osmium tetroxide. After this, the specimens were processed for SEM following a method previously described by Shalaby et al. (2009).

Results

The present study indicated the in vitro efficacy of the applied oils against the third instar larvae of *L. sericata*. Their efficacy increased as the concentration increased. One hundred percentage of larval mortality was reached 24 h post treatment with 32 % concentration of lavender oil. At the meantime, 93.3 % larval mortality was observed with camphor oil at the same concentration. No mortalities were observed in the negative control group (Fig. 1).

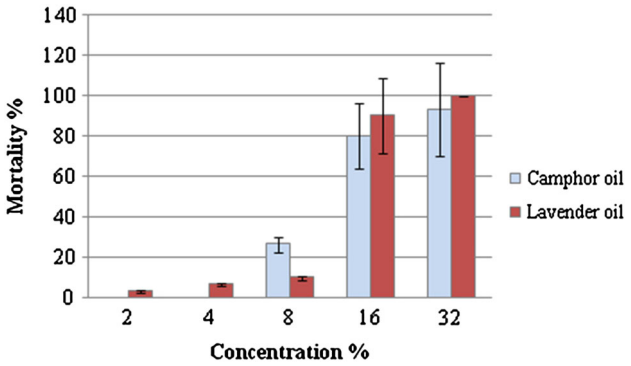

Fig. 1 Mortality percentages of the third instar larvae of *L. sericata* following 24 h post treatment with camphor and lavender oils

Light microscopic observations

Normal control larvae

The normal histological structure of cuticle of *L. sericata* larva was formed of outer electron-dense layer of epicuticle followed by lamellated procuticle consisting of exocuticle and endocuticle and inner cellular layer of epidermal cells (Fig. 2a, b).

Treated larvae

Dipping *L. seicata* larvae in 32 % concentration of camphor oil induced thinning and corrugated cuticular surface with separation of inner cellular layer of epidermal cells in some regions of procuticle (Fig. 2c, d). At a time, some specimens showed severely folded cuticle (Fig. 2c, inset). Also, *L. sericata* larvae dipped in 32 % concentration of lavender oil revealed damage expressed by a swollen cuticle, slight thickness of epicuticle and disruption of inner cellular layer of epidermal cells (Fig. 2e, f).

Scanning electron microscopic observations

Normal control larvae

The larva of *L. serecata* showed the typical maggot-like body shape. It composed of 12 segments; one cephalic, three thoracic and eight abdominal (Fig. 3a). The anterior end, comprising the cephalic region, was pointed and the posterior end was blunt. The cephalic region was bilobed and had the sensorial structures; a pair of antennae, each equipped with two sensory papillae, and a pair of maxillary palps. It also included two sickle-shaped bucal hooks and oral cristae (Fig. 3b). There was a band of spines between the cephalic region and the first thoracic segment. Two types of spines were observed: one type was flattened with tapered ends and the other was narrower with tapered tips, all of them were projected backward (Fig. 3a, inset). The body cuticle was smooth with the intersegmental line similar to those of the first thoracic segment. The anus had one pair of anal papillae surrounded by numerous tiny spines and was located under the posterior spiracular plate in the last segment, associated with dorsal and ventral papillae which were important to protect the posterior spiracles (Fig. 3c). The anterior spiracle, located in the first thoracic segment, was visible and showed a fan-like structure with 6–7 lobes (Fig. 3d). A pair of posterior spiracles could be seen at the last segment. Their shape was approximately round with a surrounding complete peritremal ring. The button was present on both spiracles, and three slits in each posterior spiracle were straight (Fig. 3e).

Treated larvae

After larval immersion in 32 % concentration of tested oils, cuticular damage had occurred in the majority of examined specimens. Camphor oil induced cuticular swelling at the dorsal surface so that the intercuticular spines appeared to be sunken (Fig. 4a). At the ventral surface, the cuticle had a slightly swollen appearance with loss of spines causing a number of pits (Fig. 4a, inset i). At the anterior end, the sensorial structures; antennae and papillae, were distorted (Fig. 4a, inset ii) and the anterior spiracle was slightly degenerated (Fig. 4a, inset iii). The cuticle appeared deformed with wrinkled surface (Fig. 4b). The posterior end showed broken cuticle with neither papillae nor spines could be observed (Fig. 4c). With lavender oil, the cuticular disruption was more pronounced and both anterior and posterior ends were severely distorted (Fig. 5a–c). The cuticular swelling was similar to that described for camphor oil, except that blebbing of cuticular surface was present in all of the specimens, covering most of the anterior end region (Fig. 5b). Damage to the posterior end was so extreme that little recognizable structure remained (Fig. 5c).

Discussion

There is no doubt that a number of plants possess pesticidal activity and investigations by various research groups in different parts of the world have confirmed this. Aromatic oils obtained through steam distillation of many plant families are highly targeted for anti-insect activities against several insect orders. Approximately 3000 essential oils are known, and 10 % of them have commercial importance in the cosmetic, food, and pharmaceutical industries. They are generally recognized as safe, GRAS, by the US Food and Drug Administration. Complete essential oils are more effective than individual constituents or even a

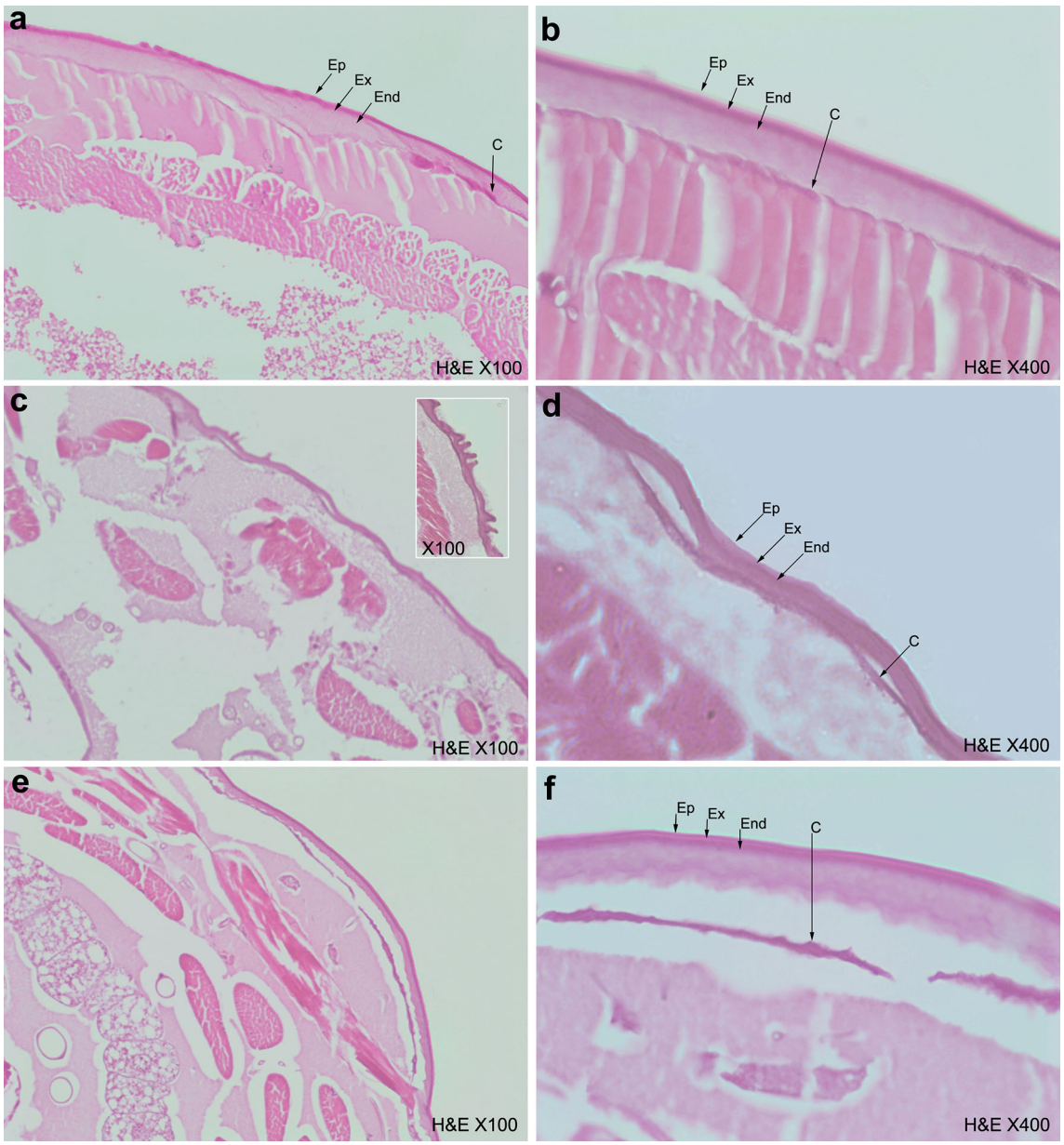

Fig. 2 Light micrographs of the cuticle cross section of the third instar larvae of *L. sericata*. **a**, **b** Normal control larvae. **c**, **d** Following 24 h post treatment with 32 % concentration of camphor oil. Note thinning and corrugated cuticular surface. Some specimens showed severely folded cuticle (*inset*). **e**, **f** Following 24 h post treatment with 32 % concentration of lavender oil. Note disruption of inner cellular layer of epidermal cells. *Ep* epicuticle, *Ex* exocuticle, *End* endocuticle, *C* cellular layer of epidermal cells

combination of constituents (Tripathi et al. 2009). In this study, the larvicidal effect of camphor and lavender oils against *L. sericata* was revealed. Besides, light and scanning electron microscopic observations could be used to determine the cuticular changes, as the cuticle of larvae was essential for protective and sensorial functions. Light microscopy was used to observe the changes of cuticular structure, while cuticular surface changes could be observed by scanning electron microscopy. The Notes for Guidance published by the Working Party on the Efficacy of Veterinary Medicines or Products (European Commission III/3682/92-EN) indicated that the overall efficacy of ectoparasiticides for the treatment of infestations by diptera species should be between 80 and 100 %, preferably more than 90 %. The efficacy of the applied oils met these criteria for the applied dipping assays. Similarly, larvae of *L. sericata* had been controlled efficiently by some essential oils, for example, fenugreek (*Trigonella foenum-graecum*), celery (*Apium graveolens*), radish (*Raphanus sativus*), and mustard (*Brassica campestris*) (Khater and Khater 2009); lettuce (*Lactuca sativa*), chamomile (*Matricaria chamomilla*), anise (*Pimpinella*

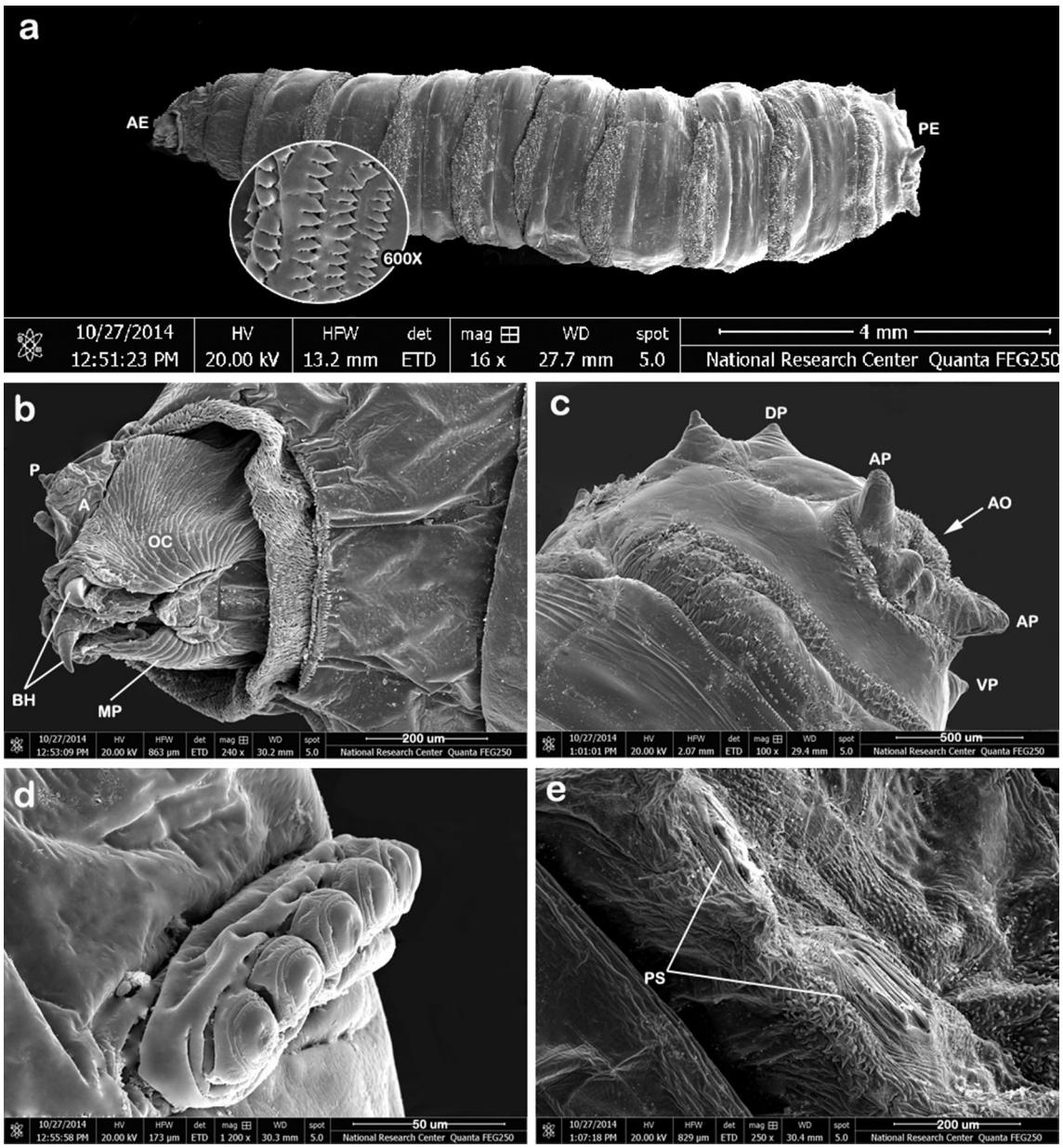

Fig. 3 Scanning electron micrographs (SEMs) of the normal third instar larvae of *L. sericata*. **a** SEM of whole larva shows the typical maggot-like body shape. The body cuticle is smooth with intersegmental spines projected backward (*inset*). **b** SEM of the anterior end of larva. **c** SEM of the posterior end of larva. **d** SEM of the anterior spiracle shows a fan-like structure with 6-7 lobes. **e** SEM of posterior spiracles shows the button and three straight slits in each posterior spiracle. *AE* anterior end, *PE* posterior end, *A* antenna, *P* papilla, *OC* oral cristae, *MP* maxillary palp, *BH* bucal hook, *AP* anal papilla, *DP* dorsal papilla, *VP* venteral papilla, *AO* anal opening, *PS* posterior spiracle

anisum), and rosemary (*Rosmarinus officinalis*) (Khater et al. 2011); the American wormseed (*Chenopodium ambrosioides*) and thyme (*Thymus vulgaris*) (Morsy et al. 1998); and dill (*Anthem graveolens*) and burnoof (*Conyza dioscoridis*) (Mazyad et al. 1999). Concerning camphor oil, it was highly effective in controlling *Oestrus ovis* (Mazyad and Soliman 2001), one of obligate-myiasis-producing flies. Besides, it induced pronounced in vitro and in vivo pediculicidal activity against the buffalo louse, *Haematopinus tuberculatus* (Khater et al. 2009). Lavender oil had been demonstrated to have a larvicidal effect against camel nasal botfly *Cephalopina titillator* (Khater et al. 2013). It induced 100 % larval mortality after treatment for 24 h with 50 % concentration. In the present study, lavender oil was more effective than camphor in killing of *L. sericata* larvae. With 32 % concentration, the mortality percentages of larvae were 100 and 93.3 %, respectively. Similarly, the relative efficacy of tested oils,

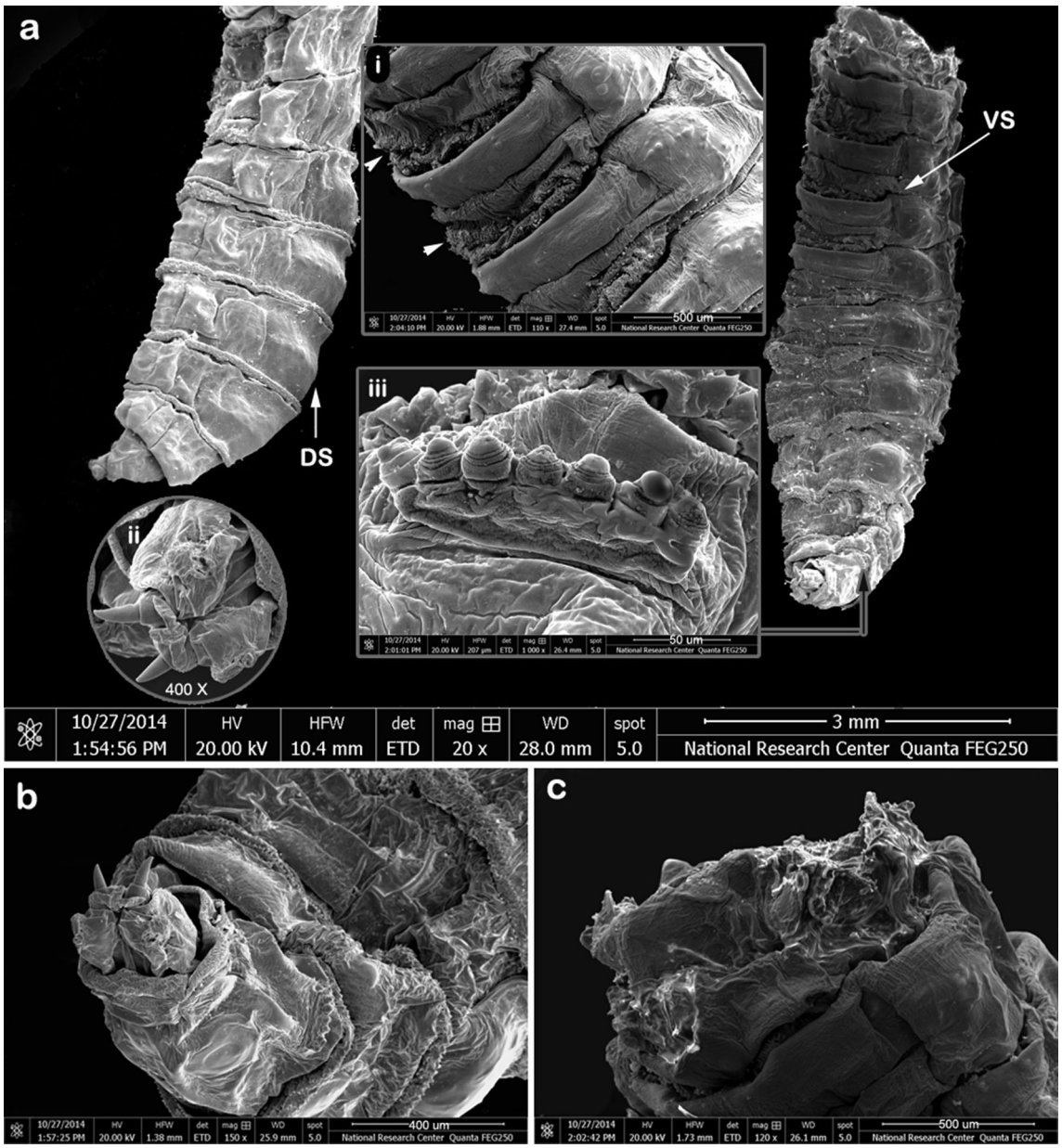

Fig. 4 SEMs of the third instar larvae of *L. sericata* following 24 h post treatment with 32 % concentration of camphor oil. **a** SEM of whole larva shows cuticular damage with loss of spines at the ventral surface (*inset i, head arrow*), distortion of the sensorial structures (*inset ii*) and slight degeneration of the anterior spiracle (*inset iii*). **(b)** SEM of the anterior end of larva shows deformed and wrinkled cuticular surface. **c** SEM of the posterior end of larva shows broken cuticle. *DS* dorsal surface, *VS* ventral surface

24 h post treatment with 50 % concentration, against *C. titillator* larvae indicated that lavender oil was two times effective than camphor oil (Khater et al. 2013).

On the other hand, histological observations were only recorded on the cuticle which appeared to be more swollen than normal. This swelling became pronounced, with disruption of epidermal cell layer in case of lavender oil treatment. At the meantime, the gut remained unaffected by oil treatment. Light microscopic observations could be used to determine the ability of tested oils to penetrate through the larval cuticle (Abdel-Shafy et al. 2009). Hence, in the dipping assay, it might be suggested that the transcuticular uptake of applied oils could be the main route of their entry into *L. sericata* larvae. The internal changes in the cuticle observed in this study were compatible with surface changes seen in the scanning electron microscopic observations. Larvae showed cuticular swelling and distortion after oil treatment, but its level was greater with lavender oil. This might be linked to disruption of epidermal cell layer of the cuticle and explain the observed

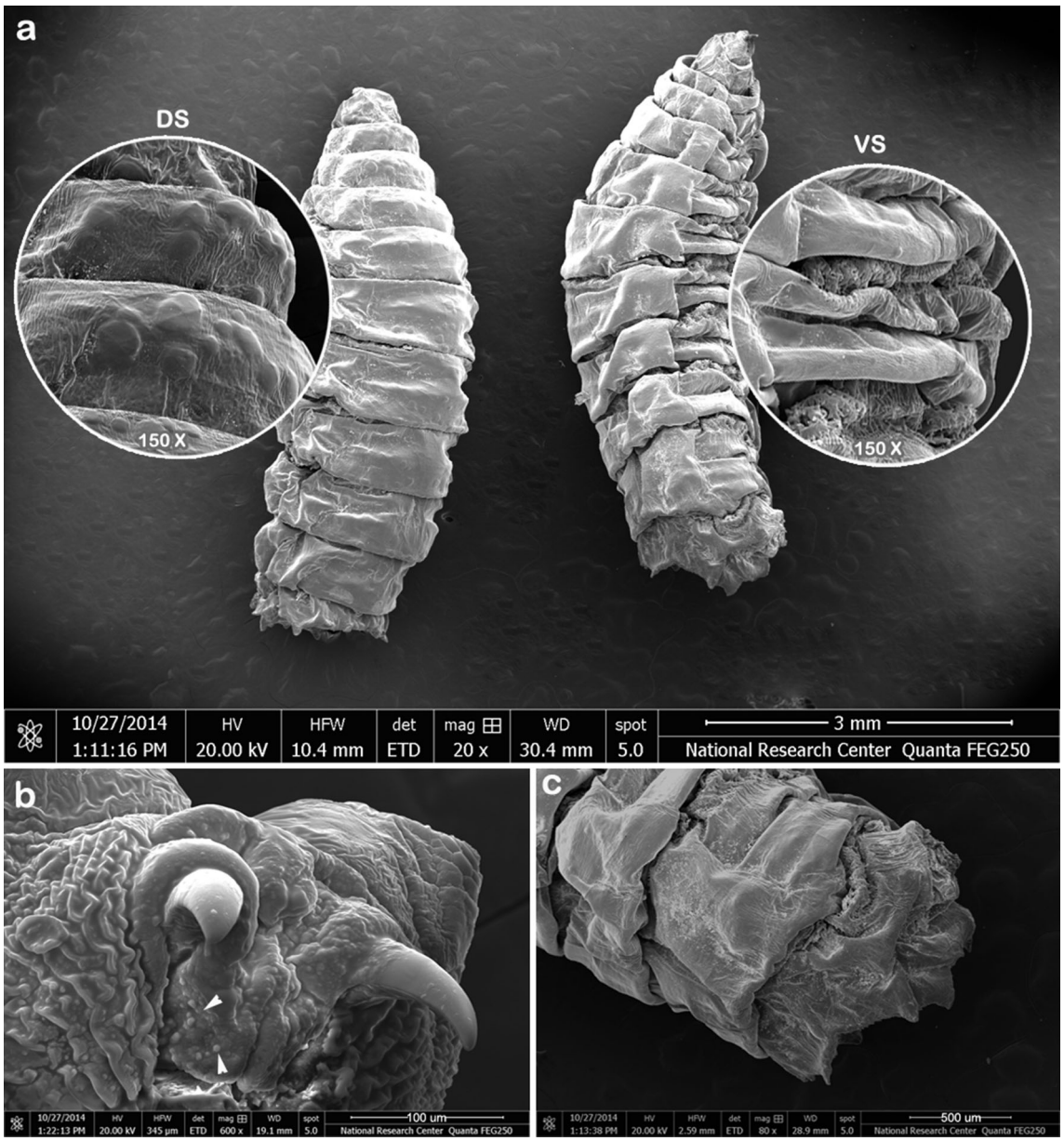

Fig. 5 SEMs of the third instar larvae of *L. sericata* following 24 h post treatment with 32 % concentration of lavender oil. **a** SEM of whole larva shows cuticular distortion at both dorsal and ventral surfaces. **b** SEM of the anterior end of larva shows blebbing of the cuticular surface (*head arrow*). **c** SEM of the posterior end of larva reveals extreme cuticular damage so that little recognizable structure remained. *DS* dorsal surface, *VS* ventral surface

larval mortality with low concentration of lavender oil. Using light and scanning electron microscopic observations to determine the cuticular changes of *L. sericata* larvae following exposure to essential oils has not been done before. Therefore, the results were discussed with those for other insects. Similar cuticular changes were observed in *Chrysomyia albiceps* larvae treated with spinosad; a metabolite of actinomycete *Saccharopolyspora spinosa*, ginger roots of *Zingiber officinale* and garlic fruits of *Allium sativum* (Shams El-Din 2010) as well as *C. megacephlala* treated with volatile oils of *Eucalytal eucalyptal* (Sukontason et al. 2004) and neem extract (Siriwattanarungsee et al. 2008). Comparable swelling, blebbing and distortion of the body cover in intestinal and liver parasites were observed following treatment with essential oils of myrrh (Massoud et al. 2012), *Nigella sativa* (Shalaby et al. 2012; Shalaby and El-Moghazy 2013) and *Allium sativum* (Shalaby and Farag 2014).

Lavender essential oil has more than 100 components with linalool, linalyl acetate, 1,8-cineol and camphor as the major constituents (Kara and Baydar 2013). It was noted that camphor was a major component of the essential oil of

aromatic plants and synergy between the essential oil components might lead to an increased insecticide response (Gillij et al. 2008). Therefore, the neat oil might be more effective compared to the individual components. The current study and those cited above indicate that an alternative, effective and natural product can be developed as larvicides against *L. sericata* using camphor and lavender oils that could offer a suitable and cheaper alternative for the more expensive insecticides. Further studies with in vivo effect of camphor and lavender oils on *L. sericata* are clearly warranted.

Compliance with ethical standards

Conflict of interest None.

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