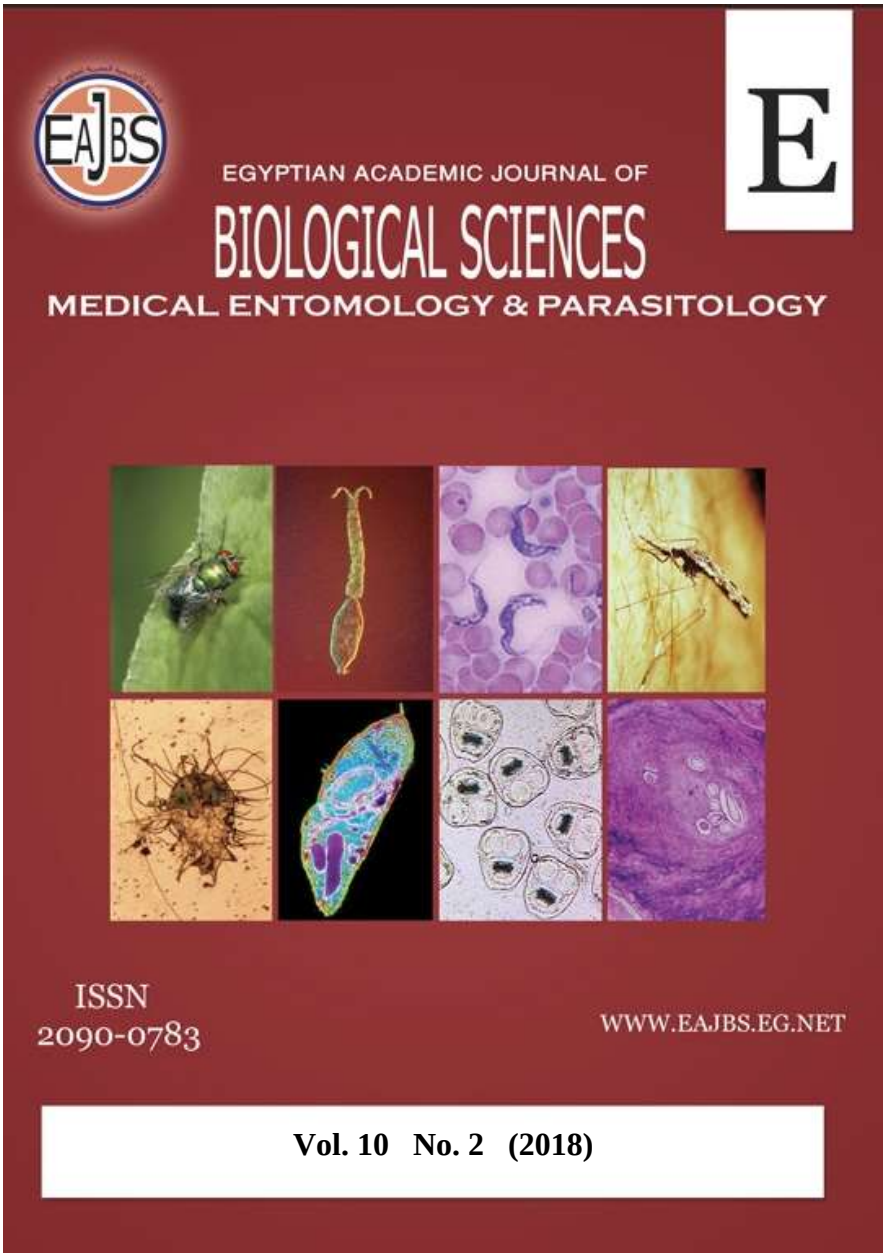

The Journal of Medical Entomology and Parasitology is one of the series issued quarterly by the Egyptian Academic Journal of Biological Sciences. It is an important specialist journal covering the latest advances in that subject.

It publishes original research and review papers on all aspects of basic and applied medical entomology, parasitology and host-parasite relationships, including the latest discoveries in parasite biochemistry, molecular biology, genetics, ecology and epidemiology in the content of the biological, medical entomology and veterinary sciences.

In addition to that, the journal promotes research on the impact of living organisms on their environment with emphasis on subjects such a resource, depletion, pollution, biodiversity, ecosystem…..etc.

www.eajbs.eg.net

Egypt. Acad. J. Biolog. Sci., 10 (2): 31 – 38 (2018)

Egyptian Academic Journal of Biological Sciences
E. Medical Entom. & Parasitology

ISSN: 2090 – 0783
www.eajbs.eg.net

Larvicidal Activity of *Eucalyptus globulus* (Myrtaceae) and *Origanum majorana* (Lamiaceae) Essential Oils against the Sand Fly *Phlebotomus papatasi* (Diptera: Psychodidae)

Maha M. Ahmed[1], Hala A. Kassem[2], Amina M. Faragallah[1], Adel R. Fahmy[3], Janette M. Georgi[1] and Bahira M. El Sawaf[3]

1- Department of Zoology, Women's College for Arts, Science and Education, Ain Shams University, Cairo, Egypt
2- Institute of Environmental Studies and Research, Ain Shams University, Cairo, Egypt
3- Department of Entomology, Faculty of Science, Ain Shams University, Cairo, Egypt.

ARTICLE INFO

Article History
Received:16/7/2018
Accepted: 17/10/2018

Key words:
Phlebotomus papatasi
scanning electron microscopy
histopathology
essential oils
Eucalyptus ***globulus***
Origanum majorana

ABSTRACT

The essential oils of *Eucalyptus globulus* and *Origanum majorana* were evaluated for their larvicidal activity against third instar larvae of the sand fly *Phlebotomus papatasi*. *E. globulus* oil showed lethal concentration, LC_{50}, of 677 µg/ml whereas the LC_{50} value for *O. majorana* was 91 µg/ml. Larvae treated with both oils showed surface shrinkage, spinous cells proliferation, bleb formation, abdominal distortion of microtrichia and partial or complete closure of spiracles in scanning electron microscopy (SEM). At 48 h post treatment, both oils induced severe structural changes in the gut cells, brush border and degeneration of the peritrophic matrix. *Origanum majorana* essential oil was more potent than *E. globulus* against third instar larvae of *P. papatasi* based on its lower LC_{50} value. Taking all data into account, essential oils of *E. glolbulus* and *O. majorana* could be considered as a viable option for the development of eco-friendly house hold product against sand flies.

INTRODUCTION

Phlebotomine sand flies are the vectors of medically important viruses, the bacterium *Bartonella bacilliformis*, and most importantly, the protozoan parasites that cause leishmaniasis (WHO, 2008). Sand flies also are often significant biting flies of man; it has been documented that their bites can cause acute dermatitis and delayed-type hypersensitivity reactions (Belkaid *et al*., 2000).

Phlebotomus papatasi (Diptera: Psychodidae) is the vector of *Leishmania major*, the causative agent of zoonotic cutaneous leishmaniasis (ZCL) throughout North Africa, the Middle East, Southwest Asia (WHO, 2008), and in North Sinai, Egypt (Fryauff *et al*., 1993). One of the methods to interrupt disease transmission is to control the vectors. Chemical insecticides play a major role in efforts to manage vector populations. Although chemicals have been used successfully as components of pest management strategies, many of these synthetic insecticides promoted wide spread contamination of the environment, toxicity to non-target organisms, resistance development and destructive effects on human and animal health. An alternative and recent approach for control is the use of natural products of plant origin known as botanical derivatives (George *et al*., 2014).

Plant essential oils (EOs) are lipophilic secondary metabolites of which terpenoids are the main component. Many studies showed neurotoxic action of EOs in insects. The inhibition of acetylcholine esterase (AChE) is the most investigated mechanism of EOs action. Other proposed mechanism of EOs action is a positive allosteric modulation of gamma-aminobuteric acid (GABA) receptors. Essential oils act also via the octopaminergic system. Disruption of octopamine results in the total breakdown of the nervous system in insects. The lack of octopamine receptors in vertebrates provides the mammalian selectivity of EOs as insecticides. A multitude of potential targets in the insect nervous system makes EO components interesting candidates for bio-insecticides (Jankowska *et al*., 2018).

Eucalyptus is one of the most cultivated genera in the world, including more than 700 species belonging to Myrtaceae family. Various biological properties have already been attributed to the genus *Eucalyptus*, among them the repellent action against *Phlebotomus papatasi* (Yaghoobi-Ershadi *et al*., 2006), and insecticidal activity on the different developmental stage of *Lutzomyia longipalpis* in the laboratory (Maciel *et al*., 2010). *Eucalyptus* oil has been placed under generally regarded as safe (GRAS) category by Food and Drug Authority of USA and classified as non-toxic (USEPA, 1993).

The genus *Origanum* is composed of 42 species widely distributed in North Africa (Ietswaart, 1980). These species are used since the ancient times as spices, medicinal, aromatic and ornamental plants (Meyers, 2005). Literature data related to *Origanum majorana* bioactivities are limited. El Idrissi *et al*. (2014) assessed the fumigant action of the EO of *O. majorana* against the weevil *Bruchus lentis* on lentil seeds and recommended the use of oils in integrated management of weevils. Mossa and Nawwar (2010) highlighted that *O. majorana* EO was found to be promising natural sources of bioactive compounds due to its high phenolic and flavonoid contents. Duletić-Laušević *et al*. (2018) found that among tested marjoram samples originated from Serbia, Greece, Egypt and Libya, the most promising were plants from Serbia and Egypt which contained high level of rosmarinic acid. This acid was found to predominant constituent in the extract and presumably had substantial influence on their antioxidant and antineourogenerative activities.

Eucalyptus globulus (also called the blue gum) and *Origanum majorana* (also called marjoram) are planted in Egypt. Accordingly, this study investigated for the first time the larvicidal activity of EOs of these two plants on the sand fly *Phlebotomus papatasi*.

MATERIAL AND METHODS

Plant Oils:

Two essential oils were used in this study; *Eucalyptus globulus* and *Origanum majorana*, (Sigma-Aldrich, St. Louis, MO, USA).

Sand Flies:

The sand fly species used in this investigation was a laboratory bred *Phlebotomus papatasi*. Colonization and rearing procedures were those of Modi and Tesh (1983). Sand flies were reared in a walk-in insectary environmentally controlled and regulated to 27±2°C and 60-70 % relative humidity, and 12:12 (L:D) photoperiod. Adults were fed on 30 % sucrose solution and on hamster as a source of blood for females. Larval food consisted of dried rabbit pellets and cow blood; grinded and autoclaved.

Larvicidal Bioassays:

The larvicidal activity of *Eucalyptus globulus* and *O. majorana* EOs were carried out against third instar larvae of *Phlebotomus papatasi*. Ten larvae were added to a 7-dram polystyrene vial with a bottom layer of 2 cm plaster of Paris containing 0.5 mg larval food. Five concentrations (200, 400, 600, 800 and 1000 µg/ml), for *E. globulus* oil and five concentrations (25, 50, 100, 150 and 200

µg/ml) for *O. majorana* oil were used. Each oil concentration was prepared in Tween 80 (3%). Larvae were sprayed with 1 ml of each oil solution. The control group included ten larvae sprayed with 1 ml Tween 80 (3 %). Three replicates for each treatment was carried out. Larval mortalities were recorded after 48 h and data obtained from each oil concentration were subjected to regression analysis by probit to generate values for LC_{50} and LC_{90} using the software package of IBM SPSS Statistics version 20 (SPSS Inc., Chicago, Il, USA).

Scanning Electron Microscopy:

Normal and larvae treated with LC_{50} were washed in saline solution, then fixed in 70% alcohol. They were mounted on aluminum stubs with double-sided clear adhesive tape and sputter-coated on an Eduardo S150 Sputter coater with a thin-layer of gold. Specimens were examined for image analysis with JEOL-JSM-5500LV with high vacuum mode at the Regional Center of Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt.

Histology:

To investigate the changes in midgut cells, larvae were exposed to LC_{50} of EOs of *E. globulus* and *O. majorana* for 48 h. Paraffin sections 6 µm thin, were stained with hematoxylin and eosin (HE) and examined microscopically (X400).

RESULTS AND DISCUSSION

Larval Bioassay:

Larvicidal activity of *E. globulus* and *O. majorana* essential oils was found to be dependent upon oil concentrations tested. Lethal concentration, LC_{50} of *E. globulus* oil was 677 µg/ml; while LC_{90} was 928 µg/ml. The LC_{50} value for *O. majorana* was 91µg/ml; while LC_{90} was 142 µg/ml (Table 1).

Table 1: Mean percent mortality of *Phlebotomus papatasi* third instar larvae treated with *Eucalyptus globulus* and *Origanum majorana* essential oils

	Eucalyptus globulus		*Origanum majorana*	
	Concentration (µg/ml)	Mean mortality percent ± SD	Concentration (µg/ml)	Mean mortality percent ± SD
	200	7± 0.06	25	7± 0.06
	400	17± 0.06	50	17± 0.06
	600	33± 0.06	100	57± 0.06
	800	53± 0.06	150	93± 0.06
	1000	97± 0.06	200	100± 0
Tween 80	3 %	0	3 %	0

From such results, *O. majorana* essential oil was more active against the sand fly larvae based on its lower LC_{50} value. The treated larvae tended to show toxic symptoms including hyperactivity, convulsions and tremors followed by paralysis and death.

Data on the larvicidal bioactivity of EOs especially on the sand fly larvae are scarce. We attempted to compare the lethal dose obtained in the present study with those reported in the literature. Only one study on the New world sand fly *Lutzomyia longipalpis* by Maciel *et al*. (2010) was available. The authors found that *Eucalyptus* EOs were effective on the three phases of the insect (egg, larva and adult) and *E. staigeriana* **was** the most effective followed by *E. citriodora* and *E. globulus*. *Eucalyptus golobulus* showed 100% effectiveness at 40 mg/ ml, while *E. citriodora* and *E. staigeriana* oils showed this result at lower concentrations of 6.5 and 5 mg/ml respectively. Our results for *E. globulus* on *P. papatasi* larvae were much less than that obtained for *Lu. longipalpis* larvae.

Available literature on the activity of *O. majorana* oil and its components showed fumigant action, ovicidal and adulticidal activity against pests of stored products (e.g. El Idrissi *et al*., 2014).

Recently, mosquito control programs target the elimination of mosquito larval stages at their breeding sites with larvicides since adulticides may reduce the adult population only temporarily (Chung *et al*., 2009; Conti *et al*., 2010). To apply this approach to control sand flies, more conclusive studies to locate their breeding sites are required.

Scanning Electron Microscopy (SEM):

To visualize the effect of oil treatment on the larval surface, scanning electron microscopy was employed. Figures 1, 2 and 3 compare the structural changes in larvae treated with LC_{50} of each EOs and untreated ones. *E. globulus* oil-treated larvae (Fig. 2) showed significant shrinkage of intersegmental region, distortion of microtrichia (m) (Fig. 2A and B), malformation or degeneration of anterior spiracle (Fig. 2C) as compared to the untreated ones (Fig. 1); in addition to partial closure of posterior spiracle (Fig. 2D).

Origanum majorana oil caused severe shrinkage of larval segments (Fig. 3A). wrinkled cuticular surface of the head and thorax (Fig. 3B). The abdominal segments showed distortion of microtrichia and squamiform sensilla (Fig. 3C). Figure 3D, highlights deformed and complete closure of the abdominal spiracles.

The above observations indicated that *E. globulus* and *O. majorana* oils caused substantial damages on the surface of sand fly larvae. A clear damage was also observed on the spiracles which suffered degeneration, deformation and partial or complete closure. It is hypothesized that the EO causes intoxification to the larvae, and in response the larvae close the spiracles on the abdominal segments as a detoxification process. *Eucalyptus globulus* oil-treated larvae of *Musca domestica* showed shrinkage, spinous cells proliferation, deformation in the integument intersegmental spines and bleb formation in SEM observation analysis (Sukontason *et al*., 2004; Kumar *et al*., 2012).

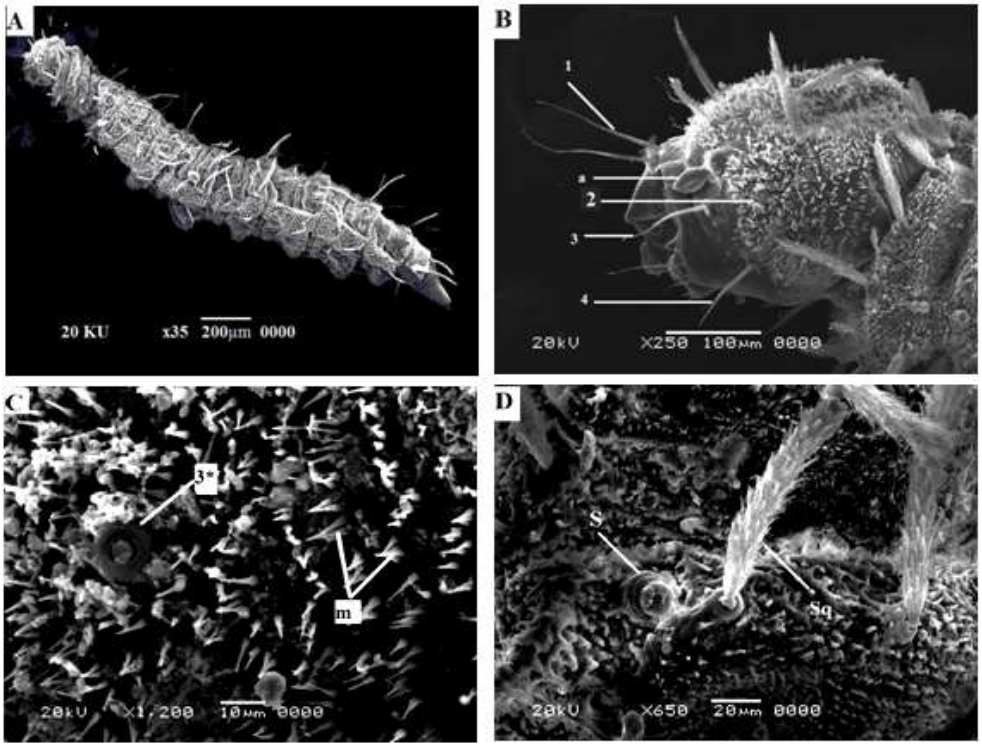

Fig. 1: Scanning electron microscopy of the third instar larva of *Phlebotomus papatasi*. (A) lateral view of untreated larvae. (B) head in lateral view showing, 1: anterior frontal seta, a: antenna, 2: campaniform sensillum, 3: dorsal genal seta, 4: lateral genal seta. (C) higher magnification of microtrichia (m) and campaniform sensillum (3*). (D) higher magnification of squamiform sensilla (Sq) and spiracle (S).

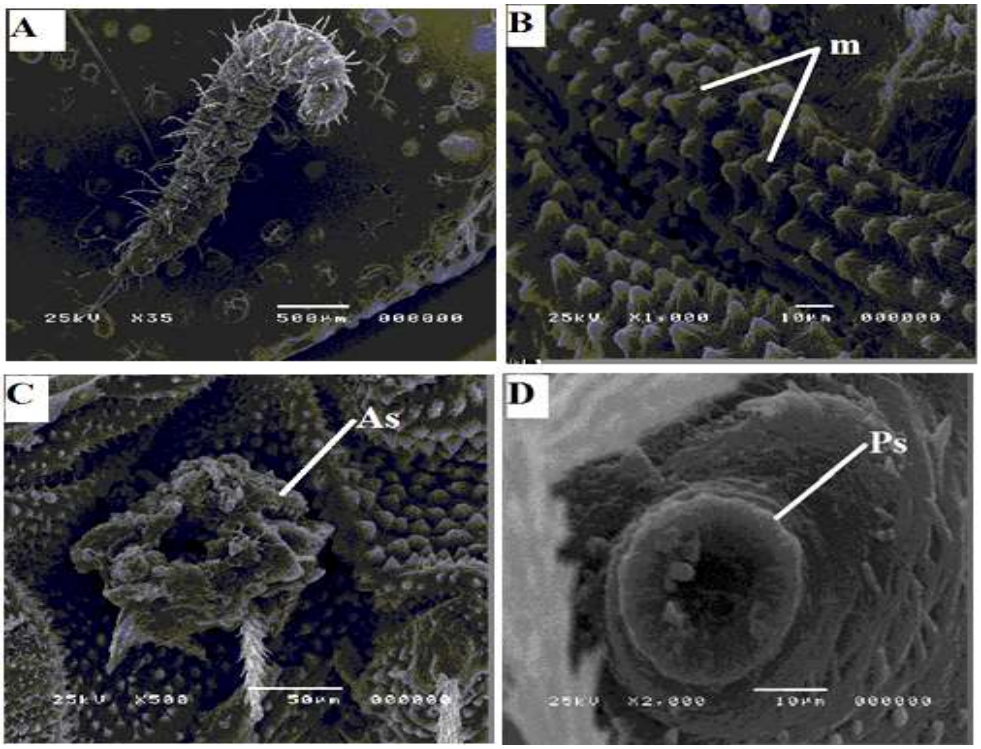

Fig. 2: Scanning electron microscopy of the third instar larva of *Phlebotomus papatasi* treated with *Eucalyptus globulus* oil**. (A)** top view of treated larva showing wrinkled cuticular surface. **(B)** abdominal segment showing distortion of microtrichia (m). **(C)** degenerated anterior spiracle (As). **(D)** partially closed posterior spiracle (Ps).

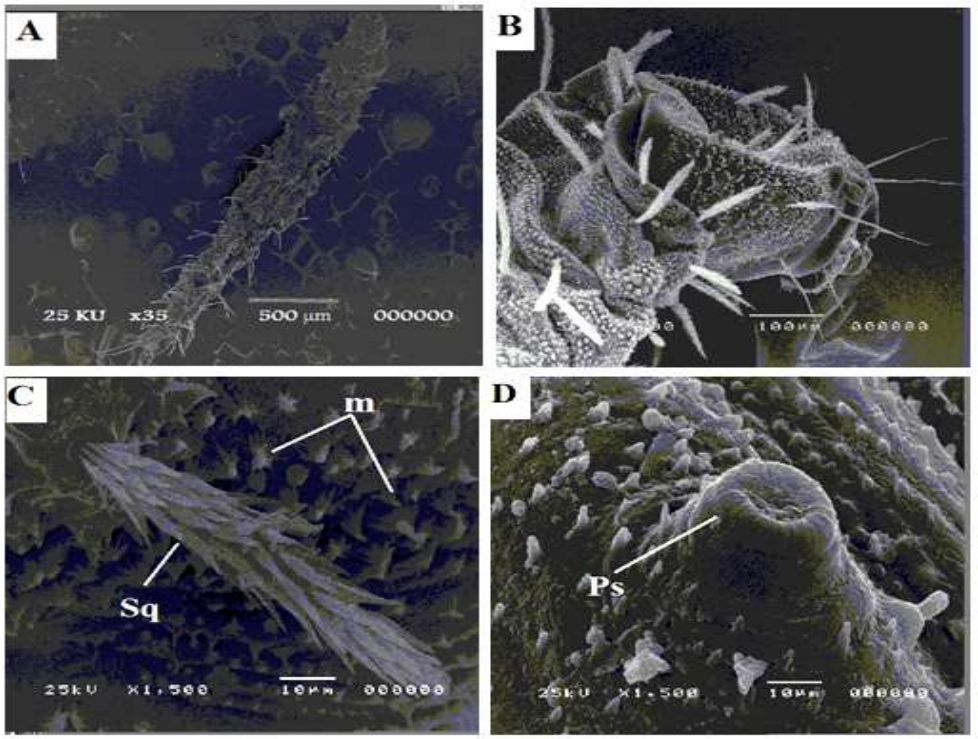

Fig. 3: Scanning electron microscopy of the third instar larva of *P. papatasi* treated with *Origanum majorana* oil**. (A)** deformed and wrinkled cuticular surface. **(B)** wrinkled thoracic segments. **(C)** abdominal segment showing distortion of the microtrichia (m) and squamiform sensilla (Sq). **(D)** complete closure of the posterior spiracle (Ps).

Histological Analysis:

Histology data of larvae treated with LC_{50} of *E. globulus* was performed 48 h post exposure to confirm histological changes. In the control group, the midgut tissue is formed of columnar epithelial cells arranged in a single layer and a well-developed brush border. The epithelium along the midgut rested on non-cellular basal lamina, under which there is a thin musculature consisting of inner circular and outer longitudinal muscles. The peritrophic matrix is present as a thin thread like membrane within the midgut lumen (Fig. 4A).

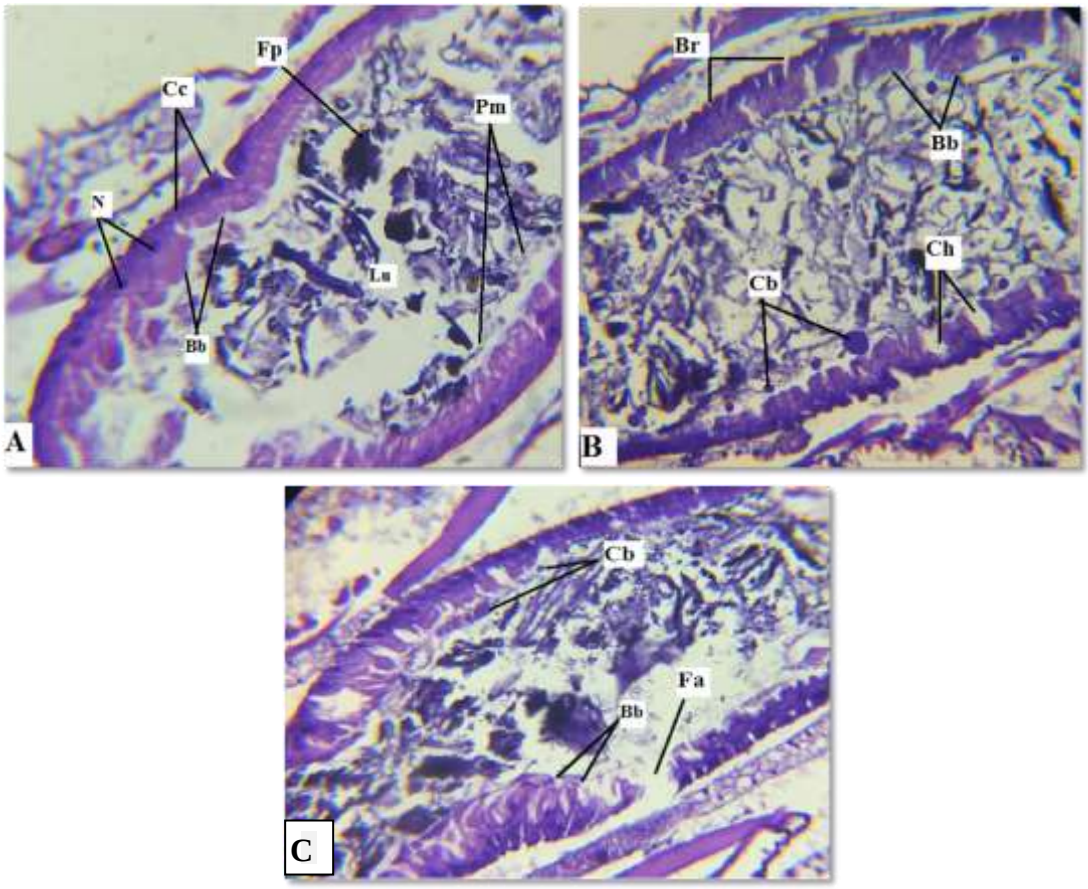

Fig. 4: Histological sections of the midgut of *Phlebotomus papatasi* larvae X400. **(A)** untreated larval midgut showing columnar cells (Cc), dense basophilic spherical nuclei (N), lumen (Lu), food particles (Fp), peritrophic matrix (Pm), well developed brush border (Bb). **(B)** midgut of larvae exposed to *Eucalyptus globulus*, showing over growth of brush border (Bb), cell blebbing (Cb), cell hypertrophy (Ch), separation of intestinal cells from the basal region (Br). **(C)** midgut of larvae exposed to *Origanum majorana*, showing over growth of brush border (Bb), focal areas (Fa), cell blebbing (Cb).

In the larvae exposed to *E. globulus* (Fig. 4B), the gut cells were detached from the basal lamina and were separated from each other leaving clear gaps. Marked blebbing and release of cell content in the lumen was also observed. The brush border increased in size reaching almost the length of the epithelial cells. Disintegration of the peritrophic matrix and release of the food particles in the ectoperitrophic space was noticed.

When the third instar larvae of *P. papatasi* were exposed to *O. majorana* (Fig. 4C), the columnar cells exhibited marked elongation and development of protrusions growing out towards the gut lumen. Outgrowth of the brush border, degeneration of the peritrophic matrix was observed.

The mechanism of action of EOs on larvae has not been clearly elucidated. Although its larvicidal activity was reportedly mediated by their comparative

absorption to larvae midgut (Rey *et al*., 1999) and neurotoxicity potential (Coats *et al*., 1991). Midgut cells have been described as a possible target for plant-derived compounds, resulting in various midgut disorders and destruction of brush border (Ribeiro-Neto *et al*., 2017). Treatment of *P. papatasi* larvae with both EOs resulted in the degeneration of the peritrophic matrices thus releasing midgut content into the lumen and the ectoperitrophic space. The peritrophic matrices are considerable physical barriers between the contents of the insect-gut lumen and the underlying digestive epithelial cells of the insect midgut (Tellam, 1996). The observed histopathological effect on the midgut of *P. papatasi* larvae due to *E. globulus* and *O. majorana* oils **were** similar to the effect of other xenobiotics which triggered a detoxification process (Alves *et al*., 2018). Regardless of the type of substance used, these changes were common response to cellular intoxication.

Results obtained by *E. globulus* and *O. majorana* EOs shed light on their possible role in developing eco-friendly product for sand fly control. The EOs mechanism of action is not well established, however, toxicity by contact might be reasonable way of action.

REFERENCES

Alves, K. F.; Caetano, F. H.; Garcia, I. J. P.; Santos, H. L.; Silva, D. B.; Siqueira, J. M.; Tanaka, A. S. and Alves, S. N. (2018). *Baccharis dracunculifolia* (Asteraceae) essential oil toxicity to *Culex quinquefasciatus* (Culicidae). Environ. Sci. Pollut. Res. Int., https://doi.org/10.1007/s11356-018-3149-x.

Belkaid, Y.; Mendez, S. and Lira, R. (2000). A natural model of *Leishmania major* infection reveals a prolonged ″silent″ phase of parasite amplification in the skin before the onset of lesion formation and immunity. J. Immunol., 165: 969-977.

Chung, I. M.; Seo, S. H.; Kang, E. Y.; Park, S. D.; Park, W. H. and Moon H. I. (2009). Chemical composition and larvicidal effects of essential oil of *Dendropanax morbifora* against *Aedes aegypti* L., Biochem. Syst. Ecol., 37: 470-473.

Coats, R.; Karr, L. L. and Drewes, C. D. (1991). Toxicity and neurotoxic effects of monoterpenoids in insects and earthworms In: Hedin, P. (Ed.). Natural occurring pest Bioregulators. Am. Chem. Soc. Symp. Ser., 449: 305-316.

Conti, B.; Canale, A.; Bertoli, A; Gozzini, F. and Pistelli, L. (2010). Essential oil composition and larvicidal activity of six Mediterranean aromatic plants against the mosquito *Aedes albopictus* (Diptera: Culicidae). Parasitol. Res., 107: 1455-1461.

Duletić-Laušević, S.; Aradski, A. A.; Kolarević, S.; Vuković-Gačić, B.; Oalđe, M.; Živković, J.; Šavikin, K. and Marin, P. D. (2018). Antineurodegenerative, antioxidant and antibacterial activities and phenolic components of *Origanum majorana* L. (Lamiaceae) extracts. J. Applied Botany and Food Quality, 91:126-134. https://doi.org/10.5073/JABFQ.2018.091.018.

El Idrissi, M.; Marmouch, M. and Amechrouq, A. (2014). Chemical composition and biological activity of essential oils of *Origanum majorana* L. (Lamiaceae) and *Salvia officinalis* (L.) (Lamiaceae) against *Bruchus lentis* (Coleoptera, chrysomelidae). International Journal of latest Research in Science and Technology (IJLRST), 3: 78-84.

Fryauff, D. J.; Modi, G. B.; Mansour, N. S.; Kreutzer, R. D.; Soliman, S. and Youssef, F. G. (1993). Epidemiology of cutaneous leishmaniasis at a focus monitored by the multinational force and observers in the northeastern Sinai desert of Egypt. Am. J. Trop. Med. Hyg., 49: 598-607.

George, D.R..; Fin, R.D.; Graham, K.M. and Sparagano, O.A. (2014). Present and future potential of plant-derived products to control arthropods of veterinary and medical significance. Parasite Vectors, 7: 1-12.

Ietswaart, J. H. (1980). A taxonomic revision of the genus *Origanum* (Labiatae). Leiden Botanical series 4. The Hague, Leiden: Leinden University Press.

Jankowska, M.; Rogalska, J.; Wyszkowska, J.; Stankiewicz, M. (2018). Molecular targets for components of essential oils in the insect nervous system- a review. Molecules, 23:34. https://doi.org/10.3390/molecules23010034

Kumar, P.; Mishra, S.; Malik, A. and Satya, S. (2012). Compositional analysis and insecticidal activity of *Eucalyptus globulus* (Family: Myrtaceae) essential oil against house fly (*Musca domestica*). Acta Trop., 122: 212-218.

Maciel, M. V.; Morais, S. M.; Bevilaqua, C. M. L.; Silva, R. A.; Barros, R. S.; Sousa, R. N.; Sousa, L. C.; Buto, E. S. and Sousa-Neto, M. A. (2010). Chemical composition of *Eucalyptus* spp. Essential oils and their insecticidal effects on *Lutzomyia longipalpis*. Vet. Parasitol., 167: 1-7.

Meyers, M. (2005). Oregano and Marjoram: The Herb Society of America. Guide to the genus *Origanum*. Kintland, Ohio: The Herb Society of America.

Modi, G. B. and Tesh, R. B. (1983). A simple technique for mass rearing *Lutzomyia longipalpis* and *Phlebotomus papatasi* (Diptera: Psychodidae) in the laboratory. J. Med. Entomol., 20: 568-569.

Mossa, A. T. H. and Nawwar, G. A. M. (2010). Free radical scavenging and anticholinesterase activities of *Origanum majorana* L. essential oil. Hum. Exp. Toxicol. 30: 1501-1513.

Rey, D.; Pautou, M. P. and Meyran, J. C. (1999). Histopathological effect of tannic acid on the midgut epithelium of some aquatic dipteran larvae. J. Invertebr. Pathol., 73:173-181.

Ribeiro- Neto, J. A.; Pinto, M. E. A.; Ferreira, V. V.; Tibúrcio, J. D.; Varotti, F. P.; Azevedo, D. O.; Siqueira-Filho, E. P.; Serrão, J. E.; Lima, L. A. S. and Alves, S. N. (2017). Larvicidal activity of vegetable oils and esterified compounds against *Culex quinquefasciatus* (Diptera: Culicidae). Ecotoxicol. Environ. Safety, 143:57-61. https://doi.org/10.1016/j.ecoenv.2017.05.004

Sukontason, K. L.; Boonchu, N.; Sukontason, K. and Choochote, W. (2004). Effect of eucalyptol on house fly (Diptera: Muscidae) and blow fly (Diptera: Calliphoridae). Rev. Histol. Med. Trop. S. Paulo, 46: 97-101.

Tellam, R. L. (1996). The peritrophic matrix. In Biology of the Insect Midgut (ed. M. J. Lehane and P. F. Billingsley), pp. 86-114. Cambridge: Chapman and Hall.

USEPA (United States Environmental Protection Agency) R. E. D. Facts. Flower and vegetable oils. (1993). Available online at http:// www.epa.gov. oppsrrd 1/ REDs/ fact sheets/ 4097 fact pdf.

WHO, (2008). Leishmaniasis. (http:// www.who.int/leishmaniasis/en/).

Yaghoobi- Ershadi, M. R.; Akhavan, A. A.; Jahanifard, E.; Vantandoost, H.; Amin, G. H.; Moosari L.; Ramazani, A. R. Z.; Abdoli, H. and Arandian, M. H. (2006). Repellency effect of Myrtle essential oil and DEET against *Phlebotomus papatasi*, under laboratory conditions. Iran. J. Publ. Hlth., 35: 7-13.