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HISTOPATHOLOGICAL EFFECT OF CITRONELLA AND MENTHOL ON INTEGUMENT RED PALM WEEVIL *RHYNCHPHORUS FERRUGINEUS* (OLIVER.)

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Abstract

Plant essential oils and terpenes used as spraying agents for controlling RPW may be implemented as an alternative means of chemical pesticides in integrated pest management (IPM) programs. In this study, We have tested citronella and menthol oils used as novel bio-insecticide to check the histopathological effects on the body wall of the insects. Some pathological changes are represented in the dispersal of the nucleus, the disintegration of muscles fibers compeer the normal untreated fiber, the decomposition of the fat tissues, vacuolization of the fat bodies occurred, detachment of the cuticle from the epidermis in some areas after treatment with LC_{50} and LC_{95} of citronella oil and menthol respectively. The results suggested that the citronella oil and menthol terpenes strongly impact R. *ferrugineus* adult as a spraying agent. A solution may be used in IPM to combat *R. ferrugineus* adult.

Keywords: Citronella oil, Menthol, Histological studies, red palm weevil, *Rhynchophorus ferrugineus*.

Introduction

Rhynchophorus ferrugineus (Olivier.) (Coleoptera: Curculionidae), a severe pest of several palms in the Middle East, South and Southeast Asia, North Africa, and Southern Europe is *Rhynchophorus ferrugineus* (Olivier) (Nirula, 1956). The geographical and host range of the RPW is extensive. It has been found to infest 40 palm species worldwide (Vidyasagar et al. 2000). Females deposit their eggs in palms that have been injured or wounded. The larvae dig into new tissue upon hatching and move to the bud area and heart of the crown, where they feed for two to four months before killing the host plant (Abraham, 1971). The RPW infestation begins with gravid females drawn to palm volatiles to deposit eggs, which hatch into damage-causing larvae.

Agricultural pest management has relied chiefly on synthetic pesticides for field and post-harvest crop protection for the last half-century (Ferry et al., 2004). The emergence of insect resistance to these treatments and high operating costs and pollutants have necessitated the development of alternate techniques to control various insect pests. In this way, essential oils can replace pesticides in various fields and houses (Sarwar et al., 2013).

It also has antifeedant and repellant properties and impacts various biological factors like growth rate, life span, and reproduction (Ebadollahi 2001, Zoubiri et al. 2001). As a result, the identification of novel bio-insecticides may be aided by using plant bioactive (terpenes). In prior investigations, essential oils, monoterpenoids, and IGRS were found to cause histopathological abnormalities in insects' integument, midgut, and ovaries (El-Boki et al. 2010; Salah Eldin, 2016). This study aims to determine the mechanism of action of citronella oil and menthol terpenes on histopathological alterations in body wall adults RPW and to explore these terpenes as bio-insecticide alternatives through an integrated insect program.

Essential oils and terpenes from plants have a wide range of bioactivities against agricultural pests and medically important insect species. They have sublethal effects on ovicidal, larvicidal, pupicidal, and adulticidal actions, including oviposition prevention.

Materials and Methods

Culture of Insects and Toxicity studies

A RPW stock culture was employed in laboratory research at the College of Science and Humanities, Prince Sattam Bin Abdulaziz University, Al Kharj, Saudi Arabia, and was maintained at a temperature of 252°C, 30 percent RH, and an L: D10:14 h. Sugarcane stems are cut lengthwise into 10 cm pieces to provide nourishment and oviposition substrate for adults. The required number of emerging adults was gathered from stock culture-produced pupae. Adults were separated into sexes and kept in 1-liter jars with sugarcane bits as nourishment. The histopathology of integument insects was determined using menthol and citronella oil supplied from Sigma-Aldrich (Germany). Adult insects (males and females) were treated with citronella and menthol diluted with distilled water. The adults were then treated with 0.1 percent Triton X-100 as an emulsifier, and the flies were moved to a natural food palm offset to watch changes and quantify death rates at various time intervals (24, 48, and 72 hours). Only water and 0.1 percent Triton X-100 were used in the control jars. Three repetitions were utilized for each treatment, with ten insects per concentration in each replication.

Histopathological studies

The insects that avoided death are removed and dissected, and each tergum and sternum are separated and placed in Bouin's solution after toxicity testing and measurement of the lethal concentrations. Body walls were fixed individually in alcoholic Bouin's solution for 24 hours, then rinsed in 70% ethanol and dehydrated in a 70-100 percent ethyl alcohol series. Paraffin wax was used to stop the infiltration. A rotary microtome was used to segment the blocks at 6m intervals. Hematoxylin and eosin stains were used to stain the sections, which were subsequently examined

and photographed by compound microscope (Nikon 550S– Japan). The data were analyzed based on bioactive impacts on changes in the histopathology of the insects.

Results and discussion

Impact of Citronella and menthol and Histopathological determinations

Citronella and menthol in various quantities have been used. We observed mortality of different concentrations of citronella and menthol against adults RPW 24, 48, and 72 hours after treatment in a previous study (Al Dawsari and Alam, 2022), and menthol was more toxic than citronella, with LC_{50} values of 1.03, 0.89, and 0.91 mg, and LC_{95} values of 5.09, 2.01, and 1.59 mg, respectively, of menthol and the LC50 values of 2.09, 1.76, Table 1

Compared to untreated treatment (Figure 1), Figure 2 shows the effect of citronella oil with LC_{50} on the body wall after 24 hours, where eating and detachment in the epithelial layer based on the basement membrane and atrophy cells, in addition to finding a lot of vacuoles, and that effect also extends at treatment with the same oil LC_{50} after 48 hours (Figure 3), where record detachment muscles attachment with body wall, found space between fibers, and found vacuoles Also, that oil was treated with LC_{95} concentrate, which caused detachment in the muscles layer that attaches to the body wall, as well as nucleus migration to the outside muscles fiber (figure 4), as well as epithelial layer detachment and numerous vacuoles in both tanned and untanned cuticles (figures 5 and 6).

In the cross sections shown in Figure 7,8, both the LC_{50} and LC_{95} concentrations of menthol had the same histopathological impact on the citronella body wall. After 24-hour treatment with menthol LC_{50}, the epithelium layer and muscles detached, and numerous vacuoles were discovered in fat bodies. On the other hand, after 24 hours of treatment with LC_{95}, the muscle fibers had changed shape. There were many vacuoles in the epithelial layer and cuticle (figure 10) compared to untreated treatment (Figure 1).

This is the first research of its sort on the red palm weevil's body wall, and it uses terpenes to track some histological changes. The effects of citronella oil and menthol on adult RPW were explored in a recent study (Al Dawsari and Alam, 2022), and menthol exhibited a substantial impact compared to citronella oil.

In addition, the impact of histopathology on the body wall RPW was investigated in this study. After treatment with LC_{50} and LC_{95} of citronella oil and menthol, it was discovered that there were some pathological changes in the nucleus dispersal, disintegration of muscle fibers compared to the normal untreated fiber, decomposition of fat tissues, and the presence of vacuoles inside the tissue. Rawi et al (2011) found some histopathological changes on the body wall of *S. littoralis* 4th instar larvae after treatment with LC_{10} crude extracts of *A. indica*. The cytoplasmic contents and cell borders had disintegrated 48 hours after treatment. In addition, compared to the control, the nuclear contents of *Azadiracta indica* are more evenly distributed. The integument of the 4th instar larvae treated with *Spodoptera littoralis* was thick after 10 days, and the body wall thickness was uneven. *Citrullus locynthis* $LC_{10,}$ and LC_{25} extracts resulted in cytoplasm

granulation, fully damaged epidermal cells, fat buildup, and epithelial cell detachment and folding. Muscle fibers are distorted, and the epidermis is separated from the cuticle. Our findings corroborate those of Sharaby and El-Nujiban (2016), who discovered histopathological abnormalities in the body wall of Agrotis ipsilon's third larval instar after treatment with garlic and mint oils. The formation of vacuoles between the cuticle and hypoderm and the evident separation of the cuticle from the epidermis and the undistinguishable cuticular layers are consistent with previous research on *Chrysomyia albiceps* utilizing *Cinnamomum zeylanicum* volatile oil (Sabry, 2004). The vacuolization of fat body cells was seen in the current study's histological modifications. After treatment with *Tagetes minuta* volatile oil, similar outcomes were seen in *L. cuprina* larvae (Chaban et al. 2019; Khater, 2021). According to a search of the literature, there is no research on the histological effects of citronella oil and menthol on the body wall RPW. This is the first research to document some of their histological alterations. The toxicity of volatile oils and terpenes has yet to be fully understood. According to several investigations, the monoterpenoids in these oils may induce insect death by blocking Ache action (Huang et al. 2002; Houghton et al. 2006; Waliwitiya et al. 2008). Lipophilic monoterpenes can enter cell membranes. This mechanism can interfere with the physiological functioning of insects by impairing membrane transport, ion balance, and membrane potential; moreover, mitochondrial malfunction can lead to cell death.

Conclusion

Citronella oil and menthol terpene were the first controls utilized on the RPW. They had a substantial influence on adults, whose histological alterations on the body wall of adults were discovered by light microscopy compared to the untreated control. Terpenes were similar to analogues of juvenile hormones in several aspects. As a result, the findings imply that terpenes in a single spray solution might be employed as a biorational pesticide in an IPM program against *R. ferrugenues*.

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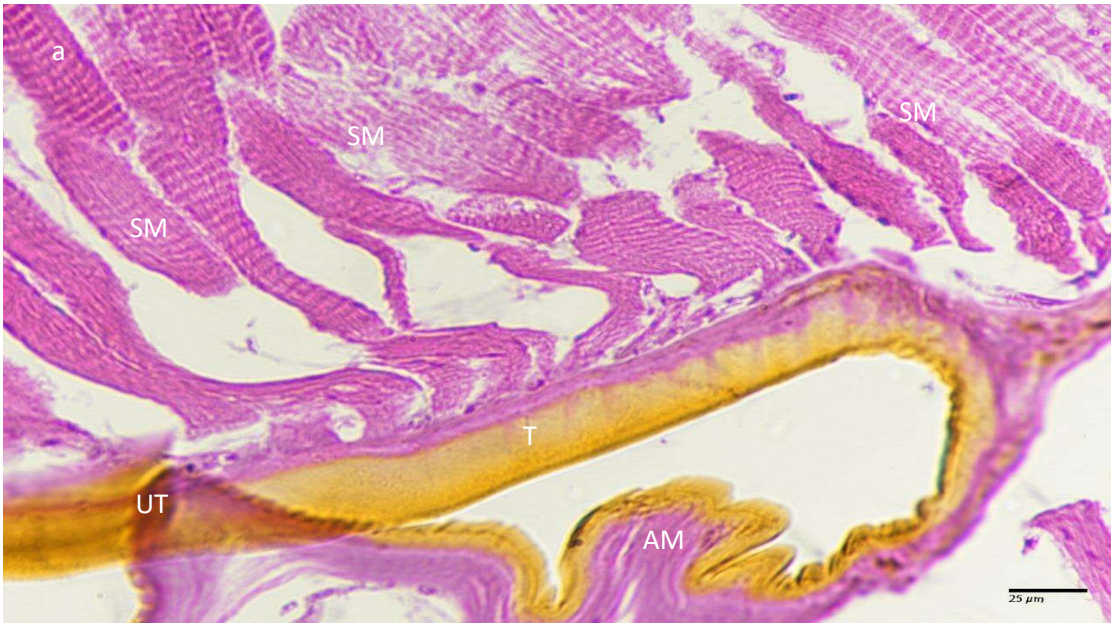


Figure 1a: Cuticle and arthrodial membrane with striped muscle attached of body wall red palm weevil *R. ferrugineus* untreated. Tanned cuticle (T) stains golden- brown, untanned (UT)and partially tanned cuticle red, arthrodial membrane (AM) purple.

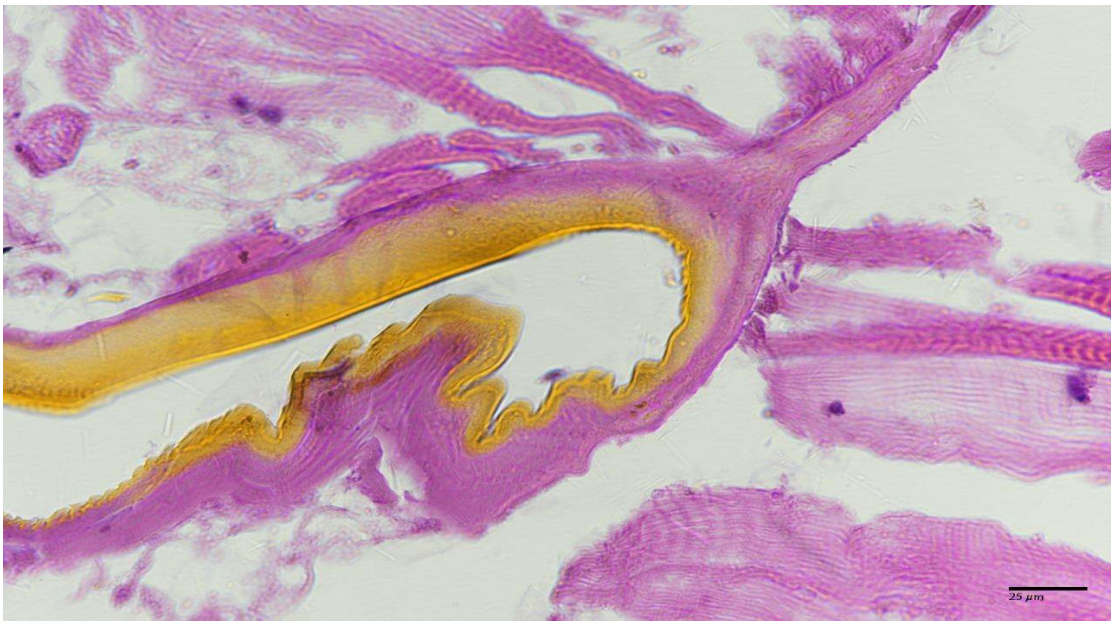


Figure 1b: Cuticle, arthrodial membrane and epithelial cells with striped muscle attached of body wall red palm weevil *R. ferrugineus* untreated. Tanned (T) cuticle stains golden- brown, arthrodial membrane (AM)purple, Epithelial cells (EC), Skeletal muscle (SM) purple.

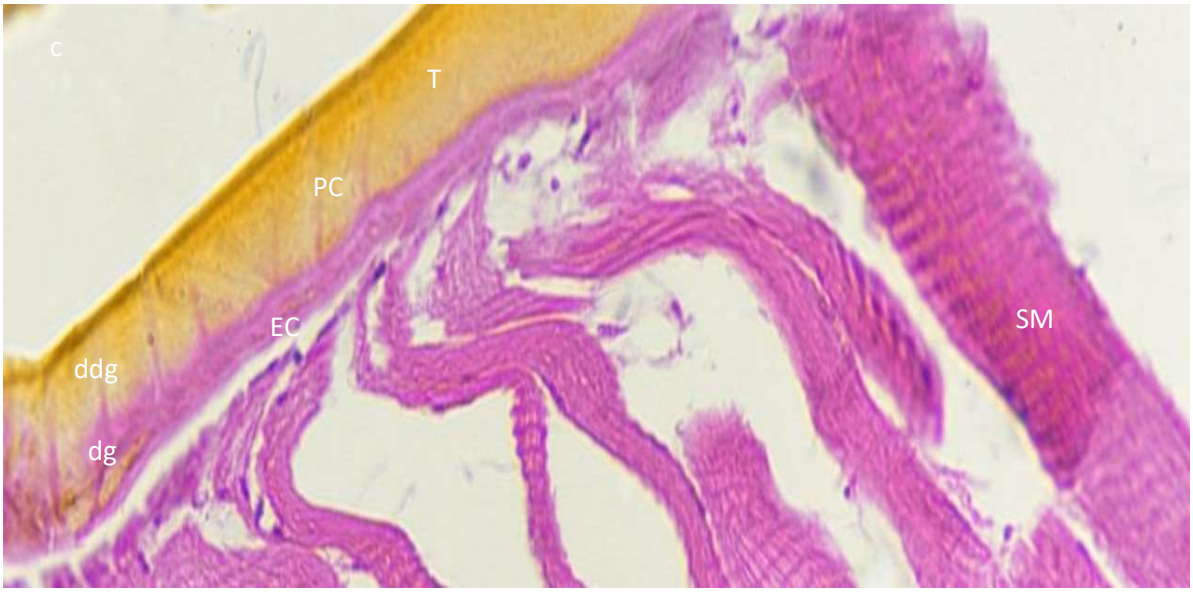


Figure 1c: Pore canal (PC), dermal gland (dg) and duct of dermal gland (ddg) of body wall red palm weevil *R. ferrugineus* untreated. Epithelial cells (EC), Skeletal muscles (SM).

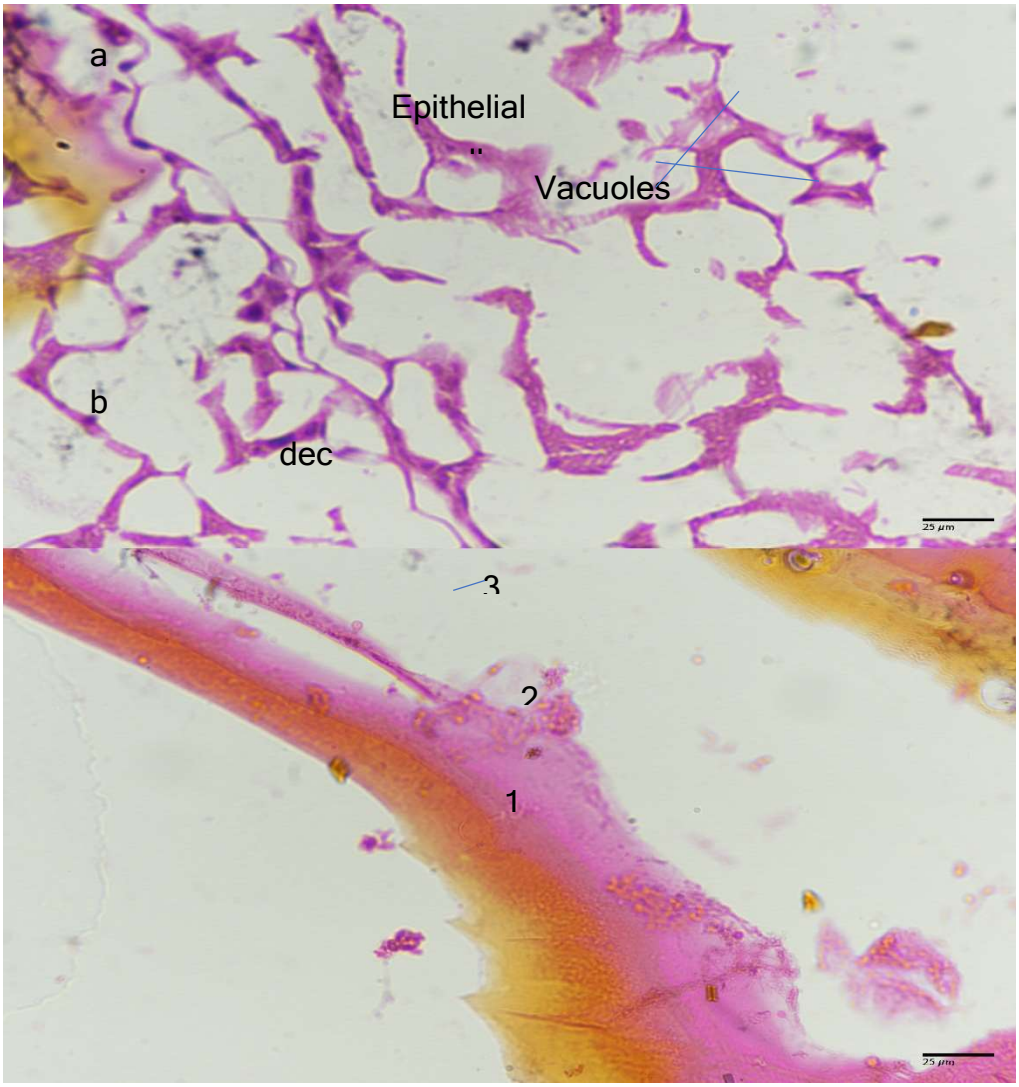


Figure 2: Cross section in the body wall (tergum) of *R.ferrugineus* treated with LC50 of citronella showing vacuolations and atrophy in the epithelial cells layer after 24 h of treatment (a) and detached epithelial cell (dec). (b). 1: tanned cuticle, 2: untanned, 3: basement membrane.

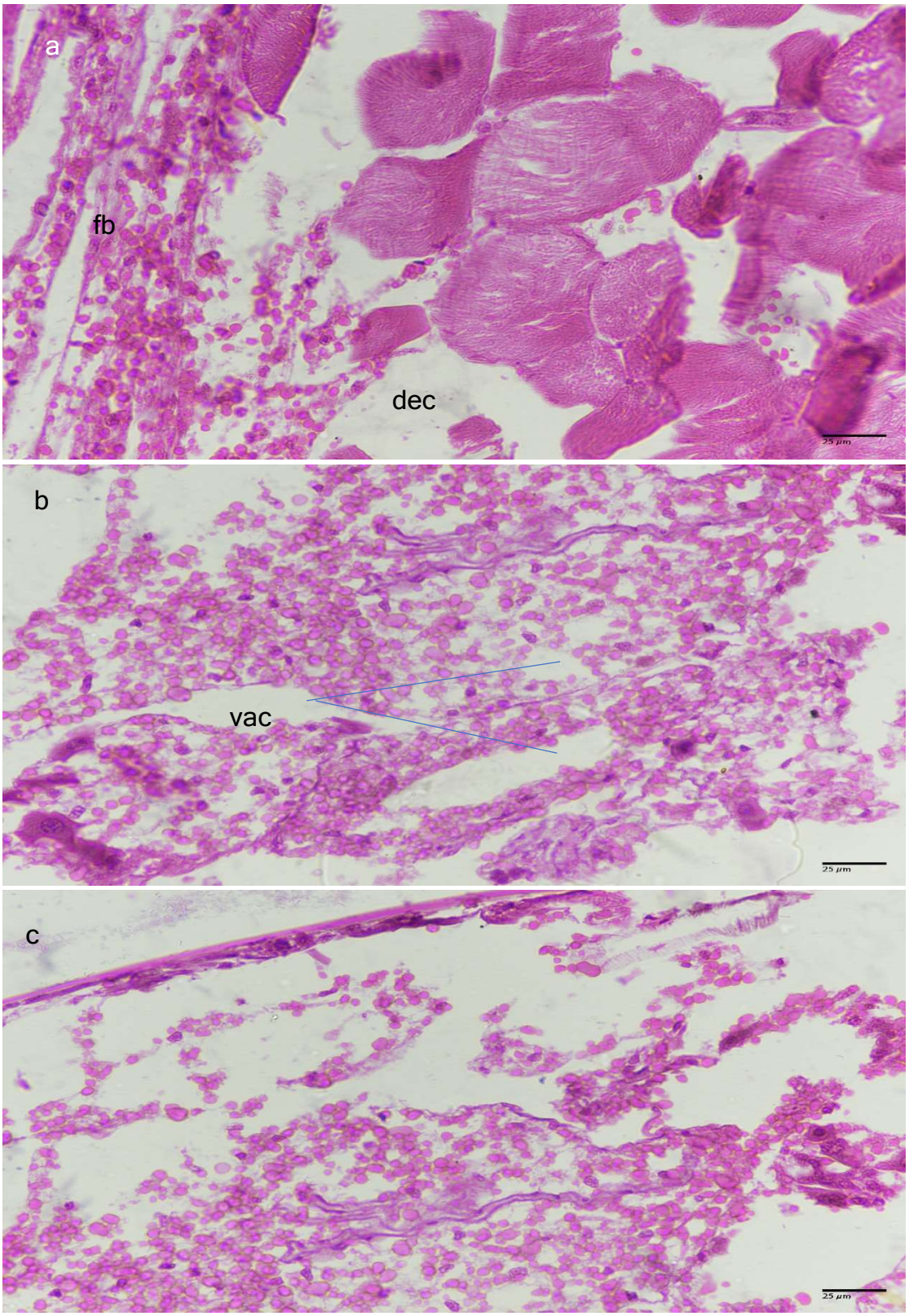


Figure 3: Cross section in the body wall (sternum) of *R.ferrugineus* treated with LC50 of citronella after 48h showing detachment muscles (dec) (a), vacuoles (vac)(b) and eating decaying the fat bodies (fb) (b and c).

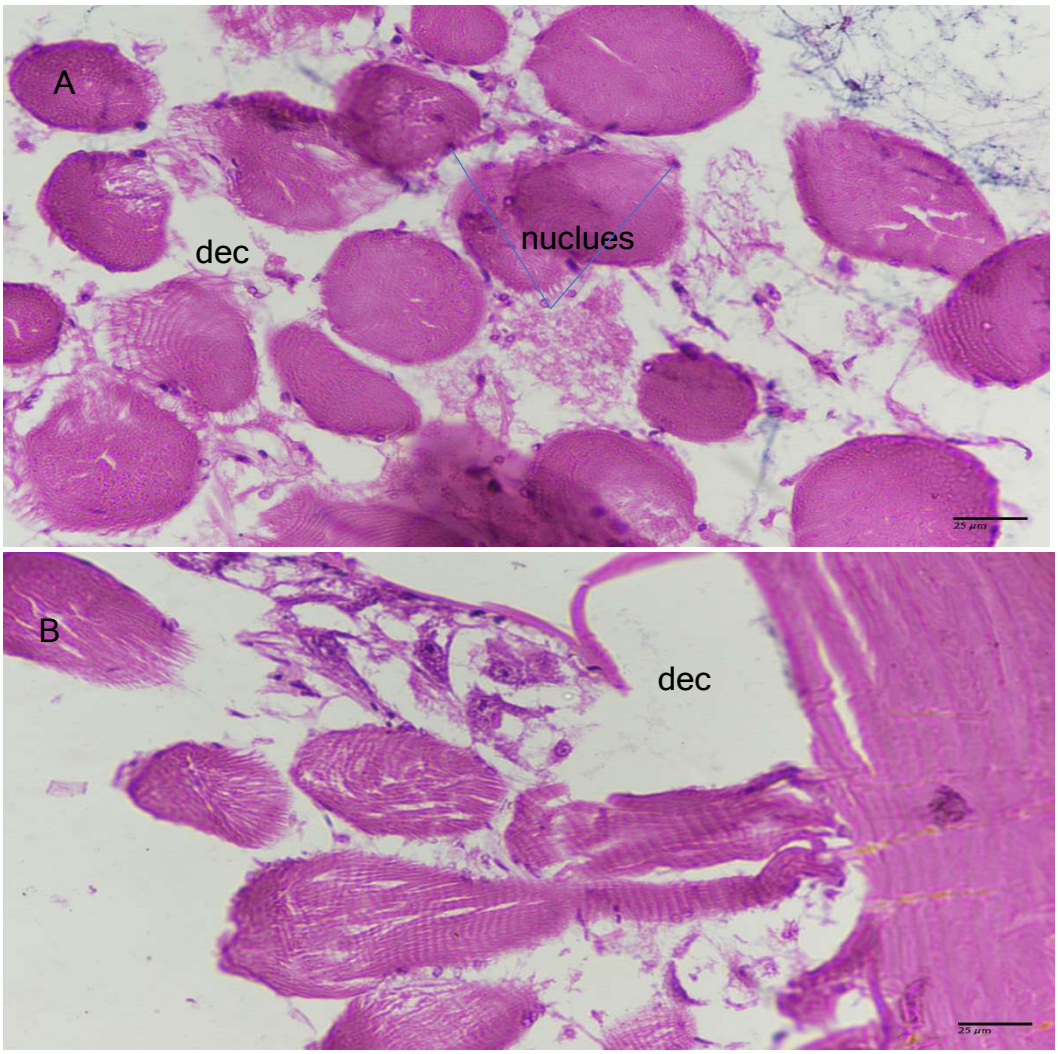


Figure 4: Cross section in the body wall (sternum) of *R.ferrugineus* after 48h treatment with LC95 of citronella showing detachment muscles and migration the nucleus out the muscle tissue (a, b).

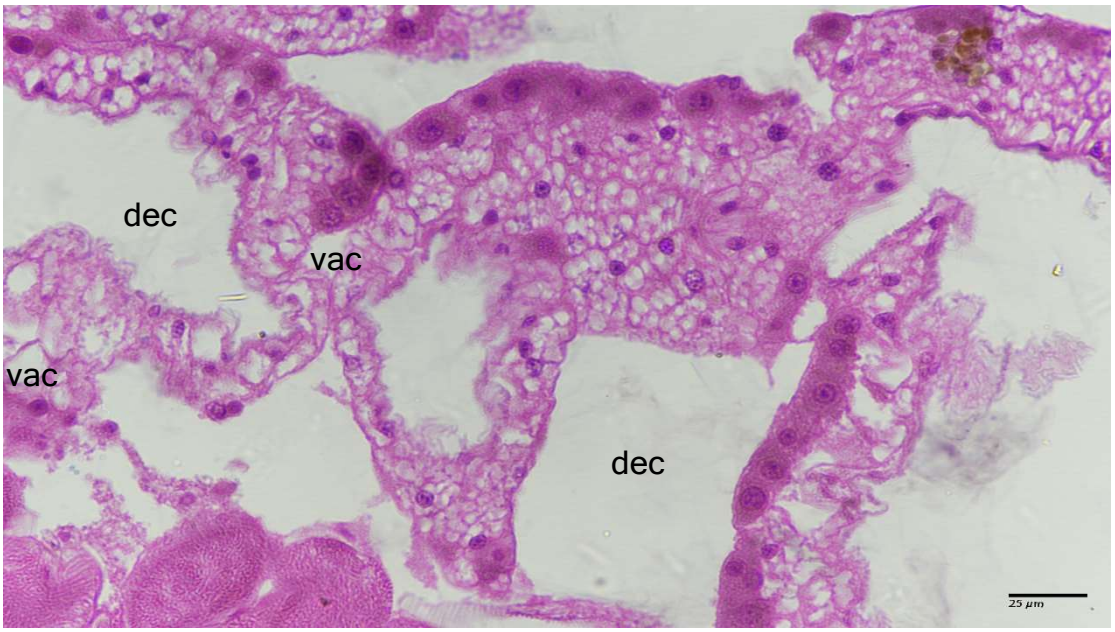
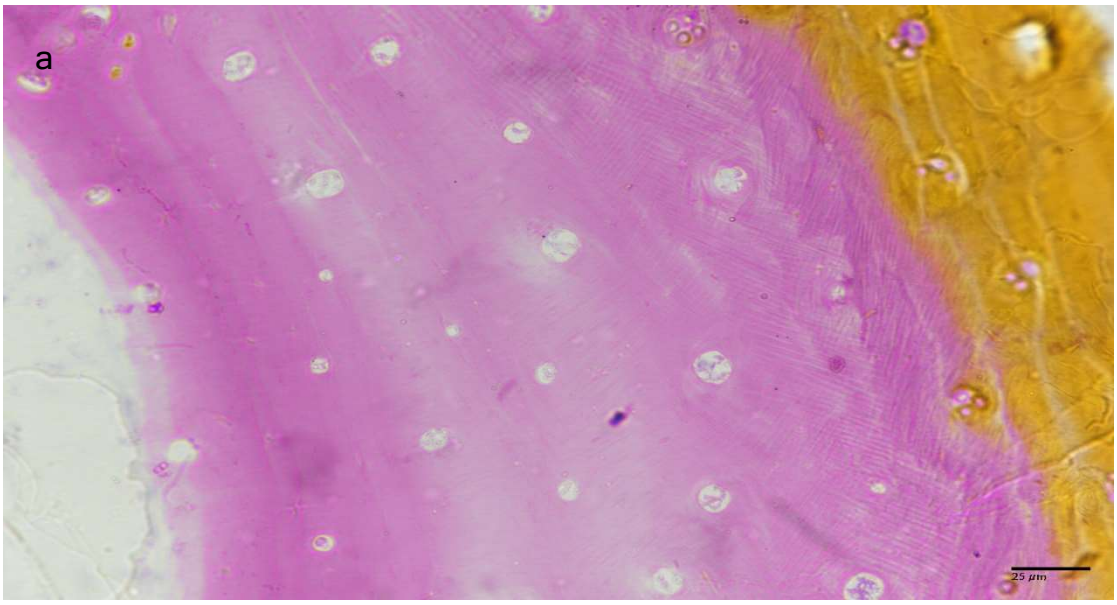


Figure 5: Cross section in the body wall (sternum) of *R.ferrugineus* after 48h treatment with LC95 of citronella showing detachment and scatter epithelial cells layer (dec) and, vacuoles (vac) and distortion of muscle.



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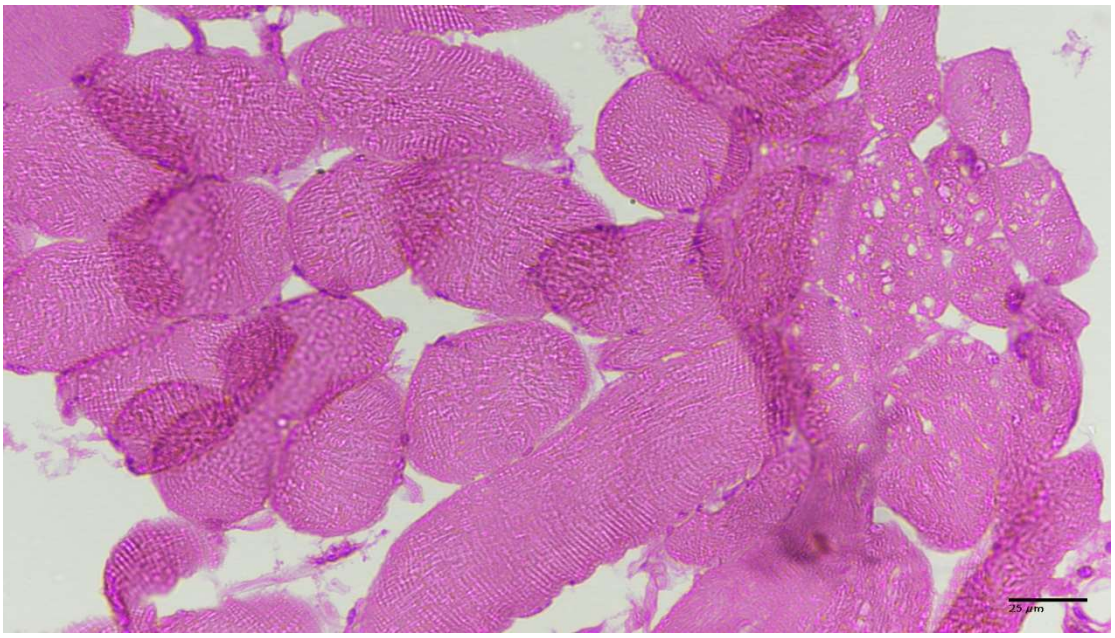
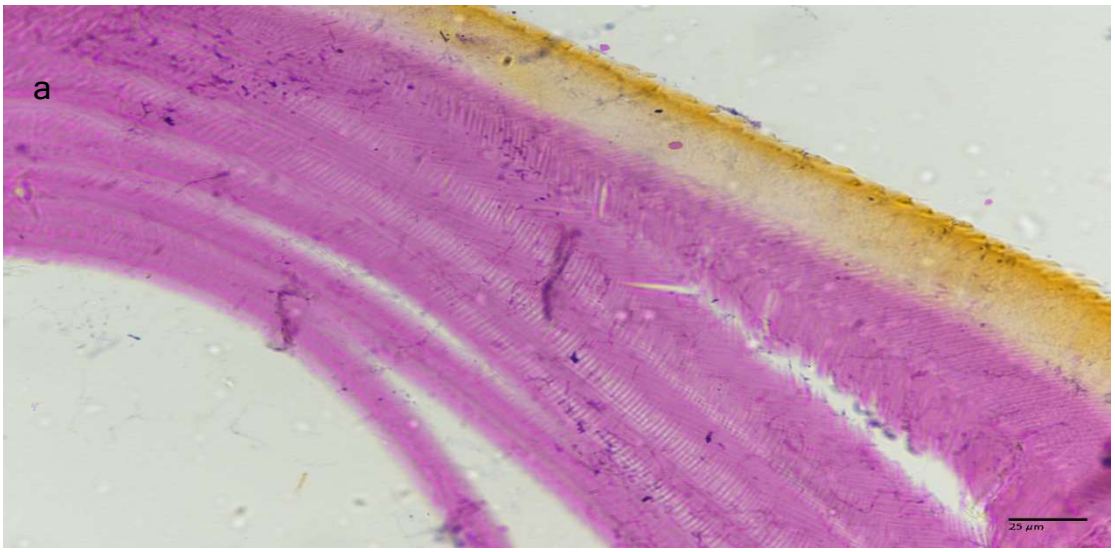


Figure 6: Cross section in the body wall (tergum) of *R.ferrugineus* after 48h treatment with LC95 of citronella showing vacuoles in the tanned and untanned cuticle (a), vacuoles and disingration muscle tissue (b).



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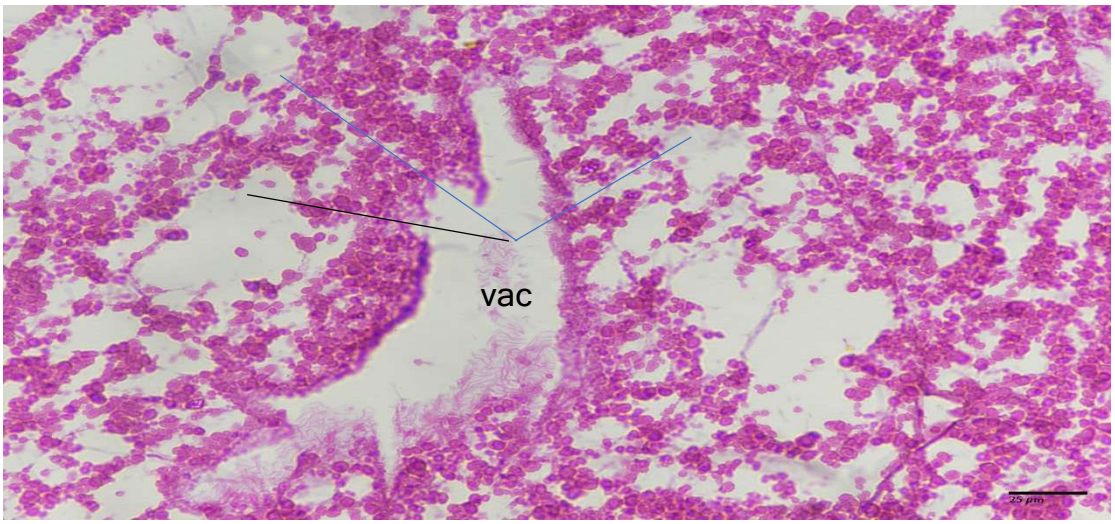

Figure 7: Cross section in the body wall (tergum) of *R.ferrugineus* after 24 h treatment with LC50 of menthol showing detachment epithelial cells layer (dec) and, muscle (a), vacuoles (vac) in the fat bodies (b).

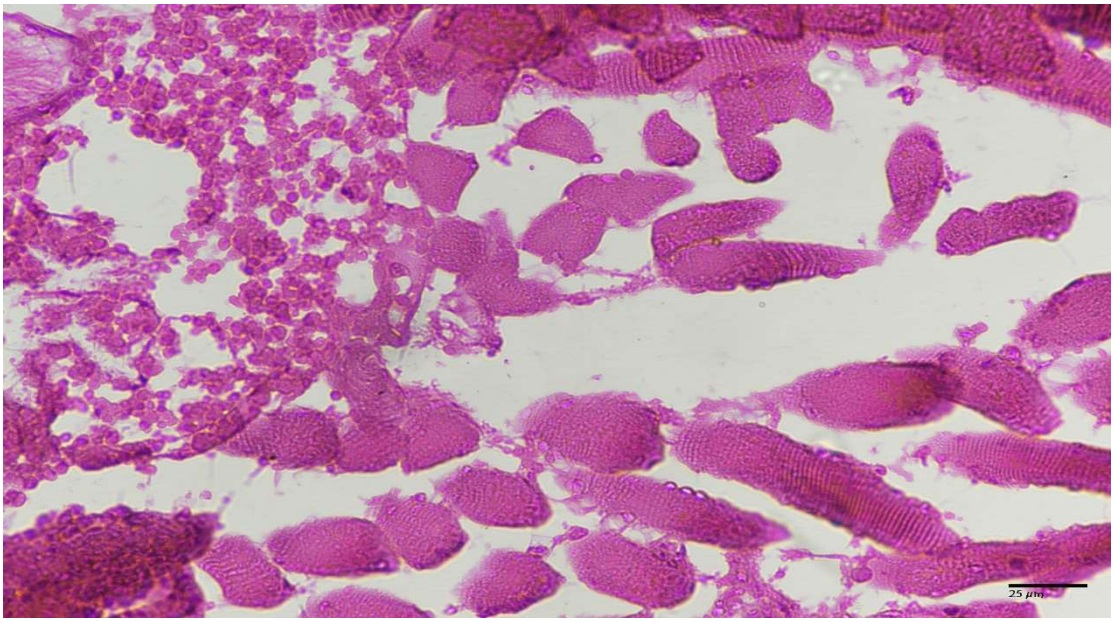

Figure 8: Cross section in the body wall (sternum) of *R.ferrugineus* after 24 h treatment with LC50 of menthol showing vacuoles and disinegration of muscle tissue.

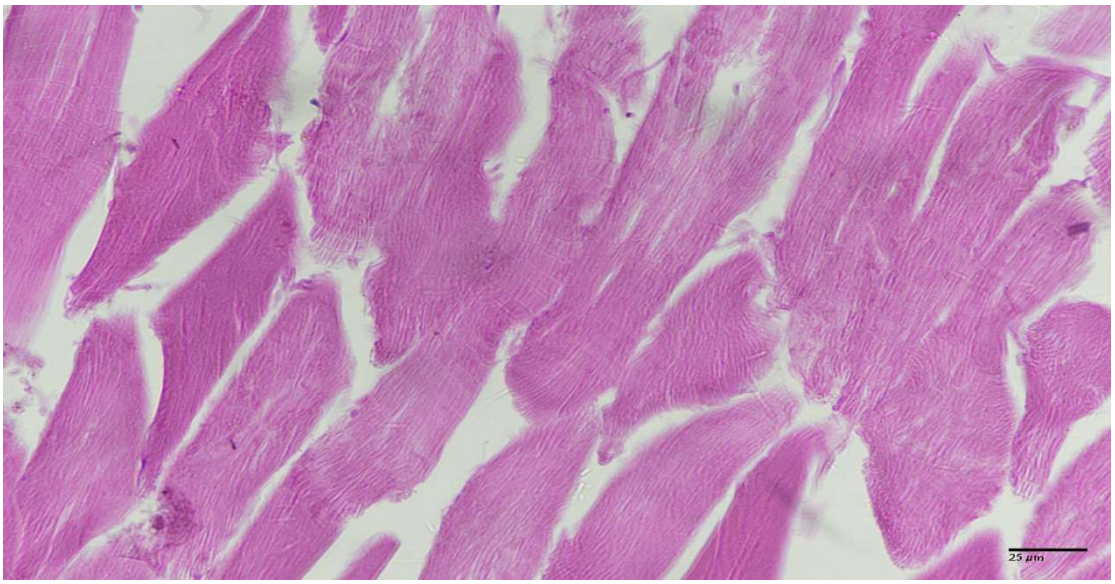

Figure 9: Cross section in the body wall (sternum) of *R.ferrugineus* after 24 h treatment with LC95 of menthol showing disintegration of muscle fibers

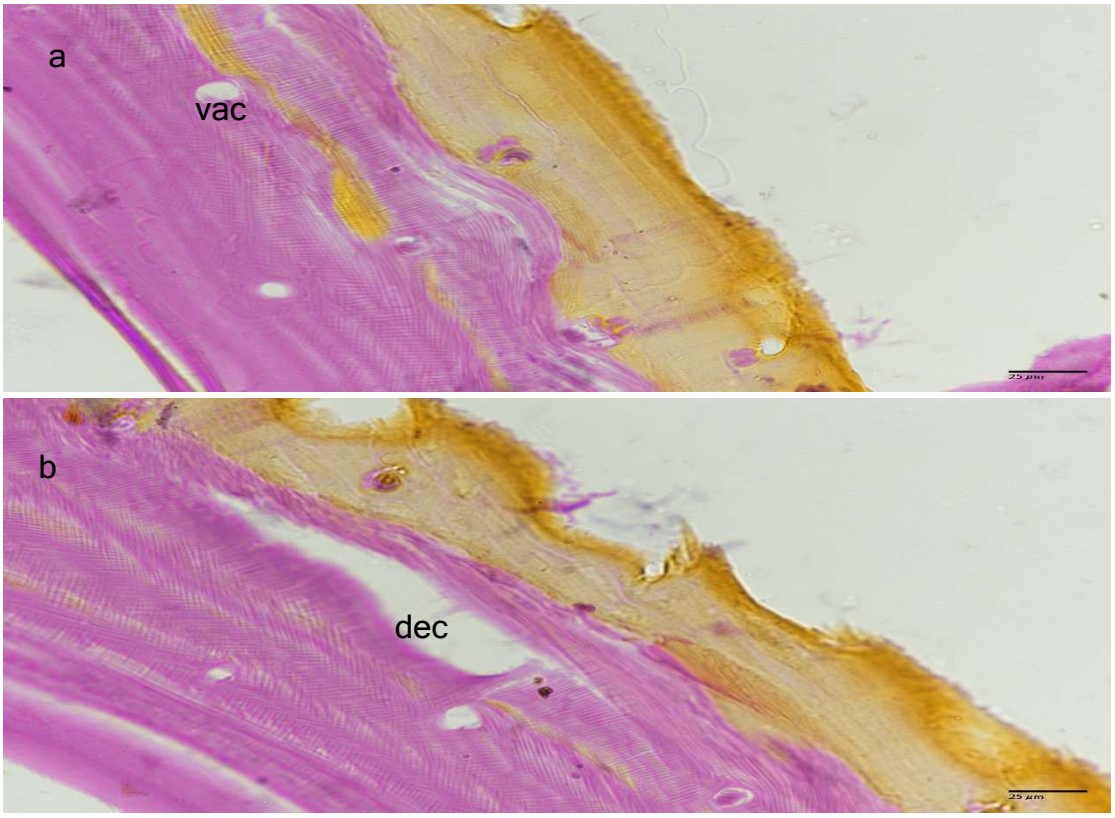

Figure 10: Cross section in the body wall (tergum) of *R.ferrugineus* after 24 h treatment with LC95 of menthol showing detachment (dec) and vacuoles (vac) in epithelial layer (a,b).

Table 1: Estimated LC_{50} and LC_{95} values of menthol against RPW at different time intervals after treatment.

Time	LC_{50} (mg) (95% fiducial limits)	LC_{95} (mg) (95% fiducial limits)	Histopathological changes at LC_{50} (mg)	Histopathological changes at LC_{95} (mg)
24	1.03 (0.59-1.39)	5.09 (3.68 - 9.55)	Detachment epithelial cells layer (dec) and, muscle, vacuoles in the fat bodies,vacuoles and disinegration of muscle tissue	Disintegration of muscle fibers detachment (dec) and vacuoles (vac) in epithelial layer
48	0.891 (0.57-1.00)	2.01 (1.61 - 3.36)	-	-
72	0.91 (0.60-1.06)	1.59 (1.304-3.959)	-	-

Table2: Estimated LC_{50} and LC_{95} values of citronella against RPW at different time intervals after treatment.

Time	LC_{50} (mg) (95% fiducial limits)	LC_{95} (mg) (95% fiducial limits)	Histopathological changes at LC_{50} (mg)	Histopathological changes at LC_{95} (mg)

24	1.03 (0.59-1.39)	5.09 (3.68-9.55)	Vacuolations and atrophy in the epithelial cells layer after 24 h of treatment and detached epithelial cell (dec)	-
48	0.891 (0.57-1.00)	2.01 (1.61-3.36)	Detachment muscles (dec), vacuoles (vac) and eating decaying the fat bodies (fb)	Detachment muscles and migration the nucleus out the muscle tissue. detachment and scatter epithelial cells layer (dec) and, vacuoles (vac) and distortion of muscle, vacuoles in the tanned and untanned cuticle, vacuoles and disingration muscle tissue
72	0.91 (0.60-1.06)	1.59 (1.304-3.959)	-	-