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Larvicidal potential and ultra-structural changes induced after treatment of *Culex pipiens* L. (Diptera: Culicidae) larvae with some botanical extracted oils

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Abstract
Searching for new alternatives for the limitation of the vector of diseases, *Culex pipiens*, the larvicidal actions of three plant oil extracts were assessed. On the base of 24hr. LC_{50} values, the supreme toxic extract against *Culex pipiens* larvae was *Piper nigrum* (black pepper), followed by *Eucalyptus regnans* and *Azadirachta indica* (neem) oil (6.99×10^{-3}, 2.24×10^{-2}, and 0.44 ppm, respectively). The histopathological results assured the toxicological data. The most characteristic disorder signs visualized in the mid gut region of treated larvae were epithelial cells elongation and vacuolization, rupturing of the peritrophic membrane, detachment of basement membrane, disorganization of microvilli, and furthermore, muscle layer degeneration. The most dramatic changes were observed in the midgut of mosquito larvae treated with *Piper nigrum;* that could serve as a potential larvicidal agent. Further studies should be considered to discover the stability and residual activity of the tested oils under field conditions.

Keywords: *Azadirachta indica, Culex pipiens, Eucalyptus regnans, histopathology, Piper nigrum*, vector control

1. Introduction

Mosquitoes stand for a chief public health dilemma than any other arthropod vector of diseases. They were registered to affect millions of people worldwide as they lead to stark human diseases (malaria, filariasis, encephalitis, dengue, hemorrhagic fever, yellow fever, and arbovirosis) plus intolerable biting nuisance [1, 2].

Larval stages of mosquitoes are attractive goals for insecticides because of mosquito breeding in aquatic media, and thus, in this habitat, it is simple to regulate them. Synthetic organic chemical insecticides have worked for disease vector control for many years. However, they have resulted in a problem of insect resistance [3]. Moreover, the continuous use of chemical insecticides has often directed to environmental pollution and severe harm to health and ecosystem, like poisoning, genetic damage, cancer and mortality [4, 5]. These restrictions create a significant market opportunity for alternative biocontrol agents. Efforts are being made to segregate, screen and improve phytochemicals (botanical insecticides) which possess insecticidal activity. These categories of pesticides are known as biopesticides [6], which are very promising in this respect. Biopesticides have the advantages of selectivity towards the target insect; they don't distress predators or beneficial organisms, safe to human and environment. Biopesticides are biodegradable, non-toxic and don't trigger contamination of food, soil or water, and readily available worldwide [7, 8]. A vast number of studies have underlined the research and expansion of plant-extracts for mosquito control [9, 10]. Essential oils isolated from plants are volatile elements and discovered among a number of species [11].

An example of the botanical extract is *Azadirachta indica* A. Juss (*A. indica*) (F. Meliaceae) (or commonly: the neem) which have revealed a high potential insecticidal activity [12]. Family Meliaceae is native to India and Burma but was hosted to other countries in the late 19^{th} Century. *Azadirachta indica* is an economically feasible bioinsecticide, less contaminating and low remaining toxic to birds and mammals [13].

Indeed, *A. indica* contains several biologically active components such as azadiractin, salani, meliantrol, nimbin, and nimidin [14]. Many authors have stated that A. indicahinders the growth of the insect, affects insect survival and causes repellence and nourishing prevention, lessens the fertility of female insects and triggers anatomical deformities in multiple species of insects. It has a direct cytotoxic upshot on glands and affects protein metabolism and enzyme synthesis [12, 15-18].

Another botanical extract which had shown a strong repellence and insecticidal activity is Eucalyptus sp. extract (F. Myrtaceae); while being very eco-friendly at the same time [19-21]. The plant family Myrtaceae is a capable supplier of mosquito repellent composites [20]. Eucalyptus sp is also known to possess strong pesticide activity against other insect species [22, 23].

Among the plant families explored to date and showing immense potential is the pepper family (Piperaceae) [24]. Piperaceae contains approx. 2,000 species which are extensively developed and usually used in tropical regions as medicine, spice, and condiments in local cuisine [25]. The photochemical examination of Piper nigrum (P. nigrum) (black pepper, a member of Piperaceae) fruit illustrates that it holds 4% alkaloids in the berry [26]. *Piper nigrum* is famous as the species emperor caused by its pungent quality. Some pepper spp were described to have remarkably insecticidal possessions against many mosquito species like *Culex pipiens* (*Cx. pipiens*), *Aedes aegypti* (*A. Aegypti*), *Aedes togoi,(A. togoi*) and Anophele*s sp* [5, 27-30]. Different studies have estimated the toxicity of *P. nigrum* extract against other different insects, in addition, it was stated as having also ovicidal and repellents effects [29, 31-39].

The cellular reforms caused by any of the tested compounds are clearly visualized in the midgut region where digestion and absorption processed [40]. There are scanty studies which focused on the histological effects and ultrastructure changes followed the treated midgut region of mosquitoes with plant extracts [41-43].

Hence, the objective of this study is to investigate and compare the toxic effect and detect the ultrastructure alterations after treatment of *Cx. pipiens* larvae with doses of and *A. indica, .E. regnans*, and *P. nigrum.*

2. Materials and Methods

2.1 Mosquito (*Culex pipiens*) rearing:

The laboratory colony of *Cx. pipiens* were raised under a lab. Conditions, at 25–30oC and 80–90% relative humidity and 11/13 h (light/dark) photoperiod, at Entomology Department, Faculty of Science, Ain- Shams University, Cairo, Egypt. Eggs were collected in plastic cups 10X10X7 cm with clean dechlorinated water until hatching. Hatched larvae were fed on tetramine and utilized for bioassay. Plant oils extraction

2.2 Three plant species were used in this study, *Azadirachta indica* (neem) mahogany family Meliaceae, *Eucalyptus regnans* (Eucalyptus) family Myrtaceae and *Piper nigrum* (Black pepper) family Piperaceae. The herb and its oil were obtained from the faculty of Pharmacy, Cairo University, Egypt. The oils of the three plants were extracted from dried and powdered herbs through Clevenger apparatus by hydrodistillation process according to Anderson *et al*. [44]. Extracted oils were kept at -20 ^{o}C until use in bioassay tests.

Bioassay test:

Larvicidal toxicity of three plant oils extracts was tested against third larval instar of *Cx. pipiens***.** Susceptibility test was carried according to WHO standard method [45]. Each extracted oil was dissolved in absolute ethanol for preparation the following concentrations (0.25, 1, 3, 5 ppm). 20 newly-moulted 3rd larval instar of *Cx. pipiens* were transferred to 100 ml plastic cups exposed to these serial concentrations of plant oils. Each test for each concentration was repeated four times. Positive control received ethanol only, while larvae in negative control were maintained in distilled water, with three replicates for each. The mortality was recorded after 24 hours. Mortality percentage was adjusted according to Abbott's formula [46].The LC_{50} & LC_{95} values of extracted oils were estimated by log-probit analysis [47].

2.3 Light and Transmission electron microscopy:

The changes in the mid gut region of treated third larval instar with LC_{50}value of each oil were detected by light and transmission electron microscopy and compared to control.

For transmission electron microscopic study, twenty-four hrs treated midgut was fixed in glutaraldehyde (2.5%) and paraformaldehyde (4%) in phosphate buffer 0.1M (pH7.3), fixation in 1% osmium tetroxide solution takes place in the same buffer and dehydration in a series of acetone solutions, after that the specimens were embedded in epoan. Ultra-thin specimens were stained with uranyl acetate and lead citrate according to Reinbold *et al*. [48]. Thin sections were taken under Reichert Supernova Ultra microtome. Treated and untreated specimens were examined under SEO PEM-100TEM. Electron Microscopy Unit, Faculty of Science, Ain Shams University, Abbasya, Cairo, Egypt.

3. Results

3.1 Bioassay test

Data from the table (1), represent the LC values of *Cx. pipiens* exposed to the three tested oils (*A.indica, E.regnans and P. nigrum)*. Based on LC_{50} values, the most potent oil was that of *P. nigrum* followed by *E.regnans* and *A. indica*, (LC_{50} values = 6.99 X 10 $^{-3}$, 2.24 X 10 $^{-2}$ and 0.44 ppm , respectively). In fact, the LC_{50} value of *P. nigrum* decreased by 62.9 and 3.4 folds than LC_{50} values of *A. indica and E. regnans* respectively

The 95% confidence limits of the three evaluated extracted oils were overlapped

According to LC_{95}values*, P. nigrum* was the most effective tested oil, since *Cx. pipiens* mosquito larvae showed the highest susceptibility to *P. nigrum* followed by *A. indica* and finally to *E. regnans*, (0.11, 9.47 and 149.9 ppm respectively). The 95% confidence between *A. indica* and *E. regnans* and between *E. regnans* and *P. nigrum* was substational overlapped. While 95% confidence between and *A. indica* and *P. nigrum* was not overlapped.

Table 1: Susceptibility test of 3rd larval instar of *Culex pipiens* to *Azadirachta indica*, *Eucalyptus regnans* and *Piper nigrum* oils (24hrs post-treatment).

Plant extracted oils	LC_{50} (95% C.I.)	LC_{95} (95% C.I.)	slope
Azadirachta indica	0.44 ppm (0.02 -2.04)	9.47 ppm (2.54 - 123.38)	1.242514 ± 0.317444
Eucalyptus regnans	2.24 X 10^{-2} ppm (8.64 10^{-4} -1.39 X10^{10})	149.9 ppm (5.43 10^{-9} -2617.59)	0.4299841 ±0.2127478
Piper nigrum	6.99 X 10^{-3} ppm (5.53 10^{-4} -5.78 10^{-2})	0.11 ppm (0.05 - 0.26)	1.34333 ±0.3028325

(95% C.I.)= Ninety-five percent confidence limit

3.2 Light and Transmission electron microscopy

3.2.1 Semithin sections and ultrastructure of midgut region of untreated *Cx. pipiens* sample (Control):

The midgut of untreated *Cx. pipiens* 3rd instar larva consisted of one layer of columnar epithelial cells resting on an intact basement membrane. Circular and longitudinal muscle layers are well-developed and surrounded by a basement membrane (basal lamina) externally. The epithelial cells contained relatively large centric rounded nuclei and each nucleus was lined by an intact nuclear envelope (Figs.1:a&b). Ribosoms, endoplasmic reticulum, and mitochondria are embedded in the cytoplasm. The peritrophic membrane was distinct. The gut lumen was wrinkled with a distinct normal adhesive brush border membrane with long microvilli.

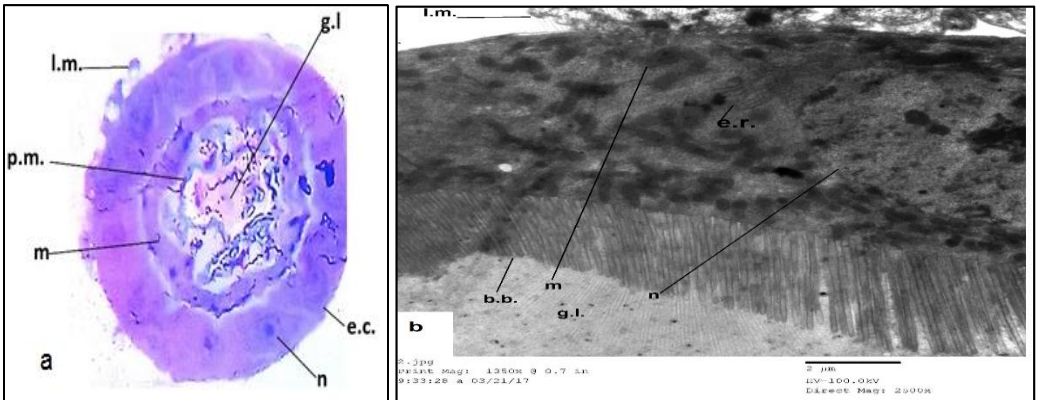

Fig 1: Transverse section (a) (400 X) and electron micrograph (b) (4000X) in the midgut region of untreated 3rd larval instar of *Culex pipiens*.(e.c.= epithelial cell. g.l.= gut lumen. n= nucleus. l.m.= longitudinal muscle. b.b.= brush border, p.m. Peritrophic membrane, m =microvilli, m=mitochondria, e.r.= endoplasmic reticulum)

3.2.2 Semithin sections and ultrastructure of midgut region of 3rd larval instar of *Cx. pipiens* treated with *A. indica*.

The histopathological effect of *A. indica* on midgut of 3rd larval instar of *Cx. pipiens* was demonstrated in Fig. 2. The Peritrophic membrane was disintegrated (Fig. 2a). Mid gut epithelial cells were elongated, swelled and detached from each other in certain places. Nuclei were elongated compared to control, as shown in Figs. (2a &2b). Microvilli were damaged, became shorter and retracted in certain places (Fig. 2b). Numerous vacuoles appeared in the cytoplasm as shown in (Fig.2c). It was remarkable also that gut lumen contained food (2a).

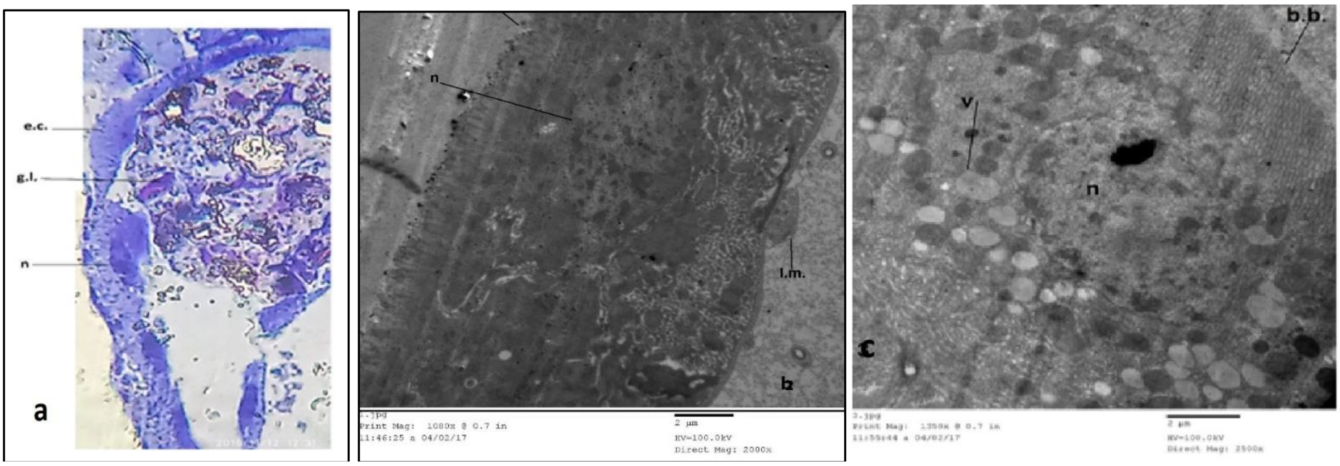

Fig 2: Transverse section (a)(400X) and electron micrograph (b&c) (2000X & 2500X) in the midgut region of *Culex pipiens larvae* treated with *Azadirachta indica*.(e.c.= epithelial cell. g.l.= gut lumen. n= nucleus. L.m.= longitudinal muscle. b.b.= brush border. v= vacuoles).

3.2.3 Semithin sections and ultrastructure of midgut region of 3rd larval instar of *Cx. pipiens* treated with *E. regnans*.

Histopathological examinations of *E. regnans* treated *Cx. pipiens* midgut showed that epithelial cells were enlarged and protruded in the lumen. The epithelial cells were detached from the basement membrane. The brush border layer was remarkably damaged. Epithelial cells and the nuclei were vacuolated as shown in Fig. (3.a).The plasma membrane was folded as shown in Fig.(3.b). Muscle layers were disappeared. Gut lumen seems normal and contains food.

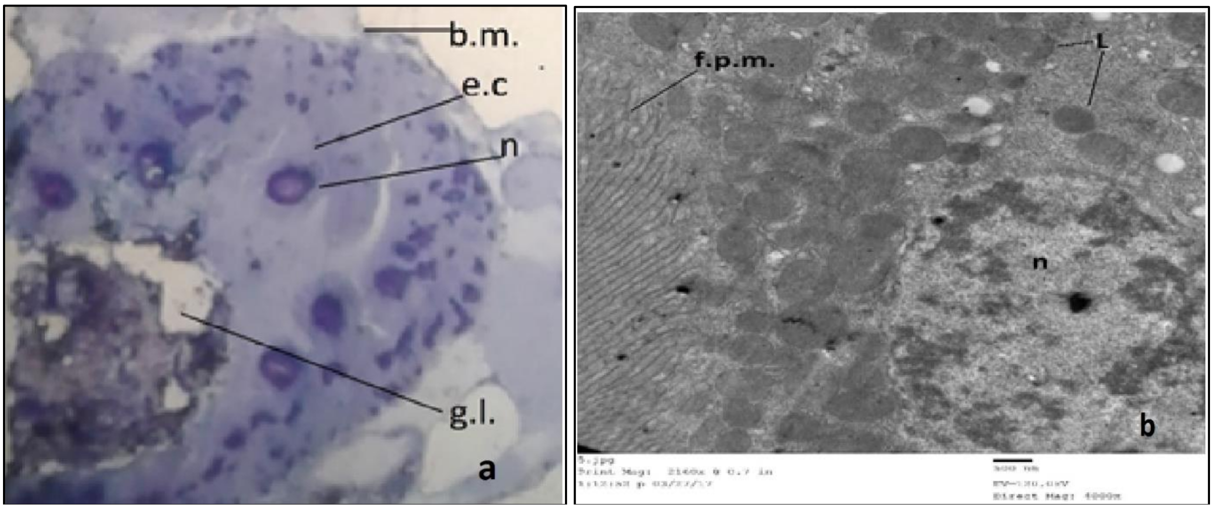

Fig 3: Transverse section (a) (400X) and electron micrograph (b) (4000X) in the midgut of 3rd larval instar of *Culex pipiens* treated with *Eucalyptus regnans*.(e.c.= epithelial cell. g.l.= gut lumen. n= nucleus. b. m. = basement membrane. L= lipids, f.p.m = folded plasma membrane

3.2.4 Semithin sections and ultrastructure of midgut region of 3rd larval instar of *Cx. pipiens* treated with *P. nigrum*.

As shown in Fig. 4, treatment with *P. nigrum* gave rise to dramatic alterations in the midgut of treated *Cx pipiens*3rd larval instar. The epithelial cells were greatly vacuolated. They were detached from the basement membrane and some cells were ruptured. The peritrophic membrane was completely disintegrated (Fig. 4.a). Brush border was irregularly scattered and destroyed completely in some locations (Fig. 4.b). The nuclei lost their characteristic oval appearance, enlarged, and some of them had taken a spindle shape appearance, (Fig. 4.c). The chromatin was concentrated in the center of the nucleus and sometimes clumped into a large irregular structure. Mitochondria became less dense and cell organelles were hardly detected. Muscle layer greatly degenerated.

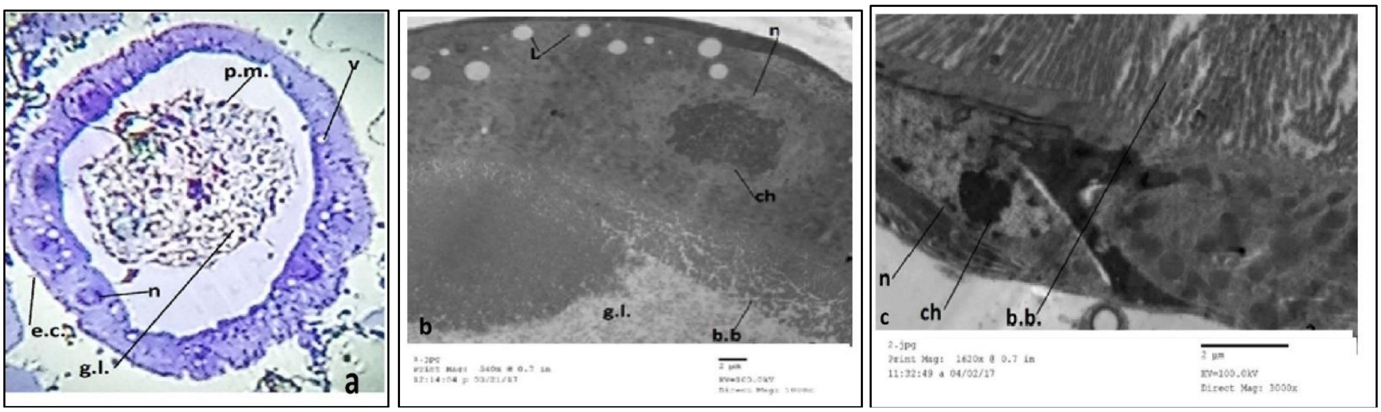

Fig 4: Transverse section (a) (400X) and electron micrograph (b & c) (1000X &3000X) in the midgut of treated 3rd larval instar of *Culex pipiens* treated with *Piper nigrum*.(e.c.= epithelial cell. g.l.= gut lumen. n= nucleus. b.b.= brush border, ch= chromatin. p.m.=peritrophic membrane.

4. Discussion

The aim of this study is defining new plant-based insecticides which will be operative, eco-friendly and cheap, and simply available. Data proved that *P. nigrum* oil had the highest potential activity against 3rd larvae of *Cx. pipiens*, followed by *E. regnans* and *A. indica* oils. Our histopathological results on midgut of treated mosquito larvae had explained the mode of its action on a cellular basis and confirmed its lethal effect.

4.1 Bioassay

Results presented in the introduced study showed that LC_{50} of *A. indica* oil accounted for 0.44 ppm against 3rd larva instar of *Cx. pipiens*. Our findings coincide with many studies concerning the evaluation of neem activities against mosquitoes. Kaewnang-O *et al*. [43] reported that fixed oil : n-hexane extract, gotten from *A. excelsa* seed kernel has more power activity as a larvicide against *A. aegypti* larvae than the methanolic extract (crude extract)does. However, they documented that both oil and crude extract were actual in suspending larval development to pupal stage and diminished adult emergence.

Alouani *et al*. [49] documented that *A. indica* extracts LC_{50} is calculated as 0.35 mg/L against *Cx. pipiens* collected from Annaba in Algeria. Another study concerning the neem oil of seed and leaf extract suggests that they enclose belongings that could be established and handled in control of *Anopheles* sp. mosquito in Tropics [14].

Neem seed acetone extract was also tested against 3rd larval instars of *Corcyra cephalonica* (Lepidoptera) and 0.16% dose level caused 100% insect mortality [18]. The authors concluded that Azadirachtin (the basic insecticidal constituent of neem seed) prevents the release of prothoracic hormones and thus affecting metamorphosis in insects. It also alters insect behavior because of its antifeedant and repellent action.

In fact, crude neem extracts were used locally in countries wit h neem crops or plantations. However, in the fore most of the world western states, a small number of commercial neem insecticides have to get to marketplace [15].

The main fundamental of neem (Azadirachtin) has an indirect action on the neurosecretory system of insects since it causes obstruction of the relief of morphogenic peptide hormones and allostatins [15] .The function of the prothoracic glands and *corpora allata* are governed by these hormones respectively. The new cuticle formation and ecdysis are switched by prothoracic gland molting hormone, but the creation of juvenile stages at each molt is switched by juvenile hormone. In adults, the two hormones are tangled in eggs yolk deposition. Therefore, some interference in these Azadirachtin cascade proceedings can affect molt disruption, molt defects, and sterility effects. As a growth regulator for many insect pests, Meliaceae herbal family is used [49].

Generally, Azadirachtin has been documented to be environmentally-safe with no toxicity to vertebrates [50].

In the current study, the LC_{50} of *E. regnans* against 3rd larval instars of *Cx. pipiens* accounted for 2.24×10^{-2} ppm. Indeed, earlier studies by different workers on the botanical-based products have reported the repellency, larvicidal, adulticidal, and anti-malarial activity of different Eucalyptus species against mosquitoes.

For example, the essential oil extract from *E. tereticorn is* showed high bioactivity (LC_{50} = 18.3 ppm) against *An. stephensi*[20]. Lucia *et al*. [22] reported that *E. grand is* essential oil $LC_{50}$was of 32.4 ppm in contradiction of *A. aegypti* larvae. As documented by the study of Singh *et al*. [21], the LC_{50} value of hexane extract of *E. citriodora* accounted for 69.8, 81.1 and 91.7 ppm contrary to *An. stephensi*, *Cx. quinquefasciatus* and *A. aegypti*, respectively 24 hrs post-treatment. The previous study on the hibernating adults of Chinese pear psylla (*Cacopsylla chinensis*) had documented the efficacy of *E. robusta* essential oil from its leaves with LD_{50} value of 10.61 µg/adult [23].

The larvicidal action of *Eucalyptus* sp. could be attributed to growth inhibition effect as Singh *et al*. [21] had remarked different morphological abnormalities and delayed mortality. Corbet *et al*. [51] illustrated that the mosquito larvae susceptibility to surface materials inflowing through their tracheal system, and observed that essential oils of *Eucalyptus* sp. enlarged the attraction to tracheal flooding and chemical poisonousness.

Eucalyptus species are frequently used in medicine in the treatment of infections like asthma, phthisis, upper respiratory corruptions, and skin diseases. *Eucalyptus* species have a pronounced perspective for the development of an ecologically-safe herbal insecticide product for the regulation (control) of mosquito-breeding in supplied waters which could be used even for drinking purposes [21].

Some members of the family Piperaceae were recognized for their use as food spice agents for food and also for containing insecticidal possessions [52]. In the present study, the LC_{50} of *P. nigrum* accounted for6.99×10^{-3} ppm. Indeed, our findings agreed to some former studies which evaluated the insecticidal properties of Piperaceae against several types of mosquitoes. Chaithong *et al*. [33] reported that the LC_{50} values of ethanolic extracts that originated from 3 species of Piperaceae were (2.23, 4.06 and 8.13 ppm) for the ethanolic extracts of *P. longum*, *P. sarmentosum* and *P. ribesoides,* respectively in contradiction of early 4th larval instars of *A. aegypti*. Another study disclosed that the methanol extract of *P. longum* fruit was vigorous against larvae of *Cx. pipiens pallets* at 10 µg/ml after 24 hr. post-treatment, [27]. Moreover, Samuel *et al*. [30] proved that the late instar larvae of *An. gambiae* complex showed marked toxic effect induced by the commercial ground black pepper and piperine. Furthermore, Lija-Escaline [29] observed larval mortality of *A. aegypti* after 3 hours of exposure to *P. nigrum* L. leaves and recorded LC_{50}value as 34.97ppm. Amer and Mehlhorn [5] study the effect of black pepper on *A. aegypti* and the results showed that ratio mortality of 3rd instar larvae in 50 ppm solution of black pepper was 100% after 24 hrs exposure.

The efficacy of Piperaceae species was tested also against other orders of insect species. Scott *et al*. [31] showed that Colorado Potato beetle *Leptinostarsa decemlineata* (Say) early larvae and neonates were susceptible to 0.064% extract of *Piper tuberculatum* and that 0.05% extract of *P. nigrum* lessened larval survival to 70% during one week. Black pepper was tested also against *Sitophilus granarius* at a concentration of 0.5% and a 100% mortality in the primary 5 days produced [35]. Black pepper showed repulsive activity against adult *Callosobruchus chinensis* and adult *Callosobruchus maculatus* [39].

Lija-Escaline *et al*. [29] suggests that *P. nigrum* may have an in vitro inhibitory outcome on Carboxylesterase. Another study of Choocote *et al*. [53] suggest that *P. nigrum* species may have a lethal effect on neuromascular system, a theory which has been reinforced by Chaithong *et al*. [33] cause of they noticed certain symptoms on treated mosquito larvae with this plant such as excitation, paralysis and finally death. “A potential advantage of utilizing Piperamides for larval control is that they may act as neurotoxins, but in a manner distinct from Pyrethroids” - Samuel *et al*. [30] reported, and suggested that Piperamides have a novel mode of action. Jensen *et al*. [54] have reported that the inhibitor effect of Piperamides may also be on enzymes, and synergistic effects are showed when operated in combination with pyrethrum. They do not persist in the environment; they rapidly degrade in the most sunlight and have little mammalian toxicity. They may be instantly needed for alternative methods of vector control [30].

In the present study, data obtained on larvicidal effects of *P. nigrum*, *E. regnans*and *A. indica* (neem) oilsa gainst *Cx. pipens* mosquito larvae could be regarded as a provision to the search of new biodegradable larvicides of natural source.

4.2 Histopathology

The midgut region of *Cx. Pipiens* larvae is separated into four regions (anterior and posterior midgut, cardia, and gastric caeca,). In all mentioned regions, the epithelium comprises a single layer of cuboidal digestive cells that comprises apical microvilli. Plentiful mitochondria are scattered in the cytoplasm. Nuclei include polytene chromosomes. At the base of digestive cells, regenerative cells are spread. A cellular well-built peritrophic membrane barrier lines the midgut epithelium and separates it from digested food [55]. The midgut retains well-developed microvilli which enhance the rate of absorption [56].

The semithin and ultrastructure sections of midgut of *Cx. pipiens* larvae treated with the studied three types of tested oils (*P. nigrum, E. regnans* and *A. indica*) indicated that histopathological effects of these three oils were in parallel with their toxicological effect. The most characteristic signs were epithelial cells elongation and vacuolization, peritrophic membrane rupture, detachment of basement membrane, damage and disorganization of microvilli, and furthermore, degeneration of the muscle layer. In the case of black pepper, the midgut alterations were more dramatic.

Our data were in agreement with many authors who considered the plant extracts and other agent's effect on mosquito and even on different insect's midgut sections. For example, Gusmao *et al*. [41] showed that *A. aegypti* larvae nourished with 150 µg/ml of *Derris urucu* (Fabaceae) extract have defective peritrophic matrix and wide injury of midgut epithelium. Whereas, Almehmadi *et al*. [57] reported that the most noticeable effect of *Matricharia chamomilla* (Asteraceae) against *Cx. pipiens* larvae were cell vacuolization, rupture of epithelium layer and microvilli injury. The effect of *Melia azedarach* (Meliaceae) on midgut of *Cx. Quinquefasciatus* was studied by Al-Mehmadi and Al-Khalaf [42]. They documented that the most characteristic signs were distraction of microvilli and midgut vacuolization manifestation, and sometimes columnar cells were protruded into the lumen as well. Our results also accord the study of Hamouda *et al*. [58] who stated the effect of *Artemisia judaica* (Asteraceae) against *Cx. pipiens* larvae midgut as they also show the vacuolated epithelium and ruin of peritrophic membrane. Moreover, Bawin *et al*. [59] showed similar effects while studying the effect of *Aspergillusa clavatus* (Ascomycota: Trichocomaceae) against *Cx. quinquefasciatus* larvae. Sharaby *et al*. [60] reviewed the histopathological effect of garlic oil on the alimentary canal of nymphs of the grasshopper *Heteracris littoralis*. They described similar marks of injury in both cytoplasm and nuclei of the epithelial layer; microvilli were ruptured and cytoplasm was vacuolated with large areas of necrosis, and additionally, peritrophic membrane rupture.

The histopathological effect of neem oil against other insects was studied as well. Nasiruddin and Mordue [61] showed that azadirachtin causes necrosis in epithelial cells and expansion of cytoplasm in locust midgut. Roel *et al*. [40] showed that epithelium coating of midgut of neem-treated larvae of *Spodoptera frugiperola* (Lepidoptera: Noctuidae) was disintegrated. These effects were described also for other insect species; for example: *Anticarsia gemmatalis* (Noctuidae) [62], *Apis mellifera* (Apidae) [63]. Levey *et al*. [62] suggest that the event of loss of cytoplasm is perhaps associated with the degeneration of cell in epithelial replacement. These patterns happen to owe the elevated activity of the midgut's emission of enzymes in turn to re-bring the lining attacked.

Indeed, several works have shown that pure azadirachtin gave rise to cytotoxic outcomes [64]. Furthermore, other compounds produced by *A. indica* like nimbolide and epoxy azadiradione have been remarked to be a reason of distraction in the plasma membrane and cause also cells bulge.

The midgut is the central part of the digestive tract of insect someplace food digestion and absorption take place, thus the cellular changes are more intense here as the midgut is the further most susceptible region for the act of external agents. But before epithelial cells interaction, the constitutions come across a barrier: the peritrophic membrane [65]. Destruction of the peritrophic membrane could enable the enhancement of the activity of insecticide of special mediators such as virus, bacteria, protozoans, toxic proteins [41]. Hence, botanic products such as our tested oils can be used as a branch of pest control management.

Out of four kinds of larval source management schemes (LSM) is Larviciding. The other types are habitation adjustment, habitation manipulation and biotic regulation [30]. Larviciding is recommended chiefly in areas where few, fixed and discoverable larval habitats. Wherever applied, LSM helps as a practical additional measure of vector control, direct mosquitoes at a time during their life time where their movement is restricted to the water body in which they live.

The presently used larvicides must fulfill the requirements of restrictive WHO (WHOPES) criteria. These include assessments of low risks to humans and environment, their storage resources, and shelf-life, local vector susceptibility and the related charges of utilization [30]. In terms of these criteria, botanical products such as *P. nigrum, E. regnans*, and *A. indica* may be predominantly recommended conditional their efficiency and residual activity under field conditions, which need further research.

5. Conclusion

To our knowledge, no study has yielded the effect of *P. nigrum* (black pepper) on mosquito midgut before. The present study is the first statement comparing the toxicological, and histopathological effects of the three tested oils (*P. nigrum*, *E. regnans* and *A. indica*) against *Cx. pipiens* in Egypt. Data proved that *P. nigrum* oil had the highest potential activity. Our data also showed that all tested oils had high efficacy as larvicides against *Cx. pipiens* in the laboratory. Field trials to investigate the stability of the products and safety assessments further research are needed, since today, the ecological safety of an insecticide is well though-out of principal importance. In particular, toxicity to non-target organisms and aquatic organisms should be more evaluated before application.

Indeed, our data give no signal about the persistence of black pepper overtime; nonetheless it is predicted that any pepper or peperine-based product for pest control would prerequisite formulation for augmented persistence. Formulated plant-based products would be required to guarantee sufficient spreading crosswise water surface in a situation of mosquito larval control.

6. References

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