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A Novel Mosquitocidal Bacterium as a Biocontrol Agent in Saudi Arabia: II- A promising Larvicide Against *Culex pipiens* Mosquito

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Abstract.- This study was conducted to test the toxicity of a novel bacterial product against the filaria vector, *Culex pipiens*, mosquito in Saudi Arabia for the first time. This bacterial product was extracted from the soil bacterium, *Pseudomonas frederiksbergensis* isolated from the contaminated Saudi soil that was proved to be eco-friend and safe to living organisms. *P. frederiksbergensis* extract identified as glycolipid that afforded a potent mosquito larvicide and has surface and histological activities against 3rd instar larvae of *Cx. pipiens*. Larvicidal bioassay showed that the LC_{50} and LC_{90} against larvae of *Cx. pipiens* were 434.45 µl/l, and 767.50 µl/l, respectively, 24 hr post-treatment. Scanning electron microscopy revealed morphological symptoms of the toxic effect of *P. frederiksbergensis*-extract. External shrinking of larval cuticle was clearly noted compared to untreated control which may be attributed to the surface activity characteristic *P. frederiksbergensis* extract. Moreover, a significant shrinkage in the whole body size was detected in treated larvae compared to control ones 24h post-treatment. In addition, the histological studies with the light microscopy showed *P. frederiksbergensis* extract destructive effects on the treated larvae midgut epithelial cells. Epithelial cells first appeared with cytoplasmic extensions followed by cellular and nuclear degradation, as well as peritrophic membrane devastation that lead to septicemia. Starting from 8 h post-treatment, larvae ceased feeding resulting in starvation and death at 24 h post-treatment. The current study introduces *P. frederiksbergensis* bacterial extract may a safe bio-larvicide candidate for use in the battle against *Cx. pipiens* mosquito. This may help in reducing the spread of this mosquito vector, that could limit mosquito-borne diseases in Saudi Arabia.

Keywords: *Pseudomonas frederiksbergensis*, biocontrol, *Culex pepiens*, histological effects, morphological alteration, bio-larvicide

INTRODUCTION

Mosquitoes of the genera *Aedes*, *Culex* and *Anopheles,* found in tropical and subtropical zones throughout the world, are the primary, worldwide arthropod vectors for fevers and parasitic diseases (Nasci and Miller, 1996). They have a cosmotropical distribution between 30ºN and 20ºS (Christophers, 1960; Knight and Stone, 1977), and exhibit a distinct preference for human habitats, including artificial oviposition sites, *e.g.*, tires, flower vases and water storage containers (Tabachnick, 1991).

Vector-borne diseases affect two-thirds of the world's population and kill millions annually (Gubler, 1998). The cost of combating these diseases and loss of productivity has crippled the economic growth of endemic countries. Considering the importance of these diseases and their vectors, it is surprising that control strategies, consisting primarily of insecticides to reduce vector populations and drugs to kill the parasites, have changed little over the decades. Unfortunately, both vectors and parasites of some mosquito-borne diseases like (for example malaria) have developed resistance against many commonly used pesticides and drugs, respectively.

Thus, there is an urgent need to identify new strategies for controlling the spread of vector-borne diseases. Therefore, number of mosquito vectors should be kept below the level of economic injury. However, it is well known that the use of broad spectrum chemical insecticides in the battle against insect pests had left both the soil and ground water contaminated with hazardous chemicals. This is a further main cause of some human diseases, which

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vary from mild allergy to severe cases of cancer, thus, the urgent need for a clean and safe environment has forced the majority of scientists to focus on the utilization of environmentally safe biocontrol agents to manage mosquito vectors and to keep their numbers down the threading level. That is why we urgently in need of other methods or control measures to keep mosquito vector below threatening level, and in the same time, to be environmentally safe. Given the success of conventional pesticides, and the real concerns about resistance, the simplest, and perhaps still the most effective and readily deployable technique, is to find alternative safe biological agents to reduce mosquito transmission of disease.

There are different types of mosquito vectors spread all over the Kingdom of Saudi Arabia (Mattingly and Knigh, 1956; Büttiker, 1981; Abdoon and Ibrahim, 2005; Al-Khreji, 2005; Ahmed *et al.*, 2011). These vectors transmit common mosquito-borne diseases including dengue fever (Fakeeh and Zaki, 2003; Ayyub *et al.,* 2006; Khan *et al.,* 2008), filaria (Hawking, 1973), malaria (Warrel, 1993; Abdoon and Alsharani, 2003), and Rift valley fever (Jupp *et al.,* 2002; Al-Hazmi *et al.,* 2003; Balkhy and Memish, 2003; Madani, 2005). Three filarial cases were reported from Saudi residences in Armed Forces Hospital, Riyadh in 2002 (Haleem *et al.,* 2002). Omar (1996) reported that local *Culex pipiens* mosquitoes might act as a potential vector of introduced Bancroftian filariasis in Saudi Arabia. Thus, the Saudi Ministry of Health developed and implemented strict plans to prevent the appearance of mosquito-borne diseases specially in Hajj (pilgrimage) time. Hence, and in support of these governmental plans, we propose this study here as a support of utilizing environmentally safe strategies for controlling mosquito vectors.

Researchers have pointed at the use of entomopathogenic microorganisms to control insects since long time ago. Microbial biopesticides such as fungi, *Bacillus thuringiensis* and *Bacillus sphaericus* bacteria, are widely used to control mosquitoes (Becker and Margalith, 1993; Margalith and Ben-Dov, 2000; Kay *et al*., 2002; Ahmed, 2013). *P. frederiksbergensis*, a decomposer of organic matter in soil, water and food products (Palleroni, 1993) is used for a biological control of fungal diseases in plants (Nielsen *et al.*, 1998). Abdel-Megeed *et al.* (2006) found that the surface active agents produced by these bacteria could have biotechnological application for insect control. Thus, the current study evaluated the efficiency of a bacterial extract, the extracellular glycolipids of *P. frederiksbergensis* bacterium, as an environmentally safe biocontrol agents, against the larval stages of the filaria vector, *Cx. pipiens* in the laboratory as a first step towards utilizing it in the biocontrol measure in a large scale Saudi Arabia.

MATERIALS AND METHODS

Mosquito rearing

A pure strain of the mosquito vector, *Cx. pepiens* from the cyclic colony (lab-reared for more than 25 generation) in mosquito Research Lab, Zoology Department, College of Science, King Saud University was used for the current study. This mosquito is considered as the filarial vector in Saudi Arabia (Omar, 1996; Haleem *et al*., 2002) and worldwide (Hawking, 1973), and widely distributed in most regions of Saudi Arabia. Mosquitoes were reared under standard insectarium conditions (26ºC, 12 h/12 h light/dark period and 80–82% humidity). Larvae were reared in tap water (or distilled water for experimental purposes) in the insectary of Zoology Department, College of Science, King Saud University, as previously detailed in Ahmed *et al.* (1999). Adults emerging within a 24h period were maintained in rearing cages (30 × 30 × 30 cm each) with continuous access to a 10% glucose solution (w/v). After adult emergence, mosquitoes of the same age were used for the relevant experiments in this study. To maintain a stock of mosquito colony, they were kept accessing 10% glucose until being allowed to feed on CD mouse blood for triggering vitellogenesis as detailed in Ahmed *et al.* (1999).

Bacterial isolation and culturing

The bacterium used in this study was *P. frederiksbergensis*. It has been isolated from contaminated soil in Riyadh, Kingdom of Saudi Arabia according to Abdel Megeed and Mueller (2009). Bacteria were grown aerobically in a mineral salt medium containing 0.5 % Na

HPO_4.$(12H_2O)$, 0.1 %KH_2PO_4, 0.05% $(NH_4)_2$ SO_4, $MgSO_4$.$(7H_2O)$, (pH 7.0) in Erlenmeyer flasks with a working volume of 1 liter. The enrichment of bacteria was carried out using Luria Bertani (LB) media. One mM docosane was used as a sole source of carbon and energy. Bacteria-seeded media was incubated for 24h at 36°C until visible colonies are formed in the media. A sample of bacteria was moved onto a suitable nutrient solid agar medium then incubated for 24 h at 36°C. Resulting colonies, observed as identical in morphology, size and colour, were used in the current study as detailed below.

Preparation of bacterial extract

A biosurfactants compound was extracted and purified from *P. frederiksbergensis* according to Abdel-Megeed and Majhadi (2009). Briefly, 200 ml liquid bacterial culture were centrifuged at 10,000 rpm for 10 min at 4°C. The floating materials resulting from the treatment of the supernatant by 40% ammonium sulphate were collected by centrifugation and then dissolved in a small fraction of water. Chilled acetone was added to the solution to remove the protein and acetone-insoluble materials. This step was critical in the purification procedure because the biosurfactant was soluble in acetone at high concentration. Any decane that may have remained in the acetone fraction was removed by extracting it three times with hexane. For sugar fatty acid esters (SFAE) separation, the mixture was subjected to extraction with warm hexane (50°C) and filtration. This step resulted in fatty acids in the organic phase and sugar esters, enzymes and sugars onto the filter.

Hydrophilic moiety was determined *via* dinitrosalicyclic assay (DNSA) that depends mainly on reducing ends of carbohydrates. Reagent composition was 1 % of 3,5-dinitrosalicylic acid (DNSA), 30 % of sodium potassium tartrate, and 0.4 M NaOH. Equal volumes of the sample and the previously mentioned reagent were mixed and heated in a boiling water bath for 10 min. After rapid cooling to room temperature and diluting with 10 volumes of water, the absorbance at 570 nm will be measured. Sucrose was used as a reference for the calibration curve. For further identification of the sugar moiety, the analysis was carried out by HPLC. Methyl esters of fatty acids were prepared as described by Morrison and Smith (1964). Equal amounts from the sample and 1M of methanolic NaOH were mixed well in a glass vial. The mixture was incubated in a water bath at 60°C for 20 min in order to cleave off fatty acids. Equal amount of BF_3/ methanol was then added after cooling in ice for 1 min in order to create methyl esters. After incubation at 60°C for 10 min, 200 µl of saturated NaCl was added and mixed well. The sample was transferred to a 1.5 ml Eppendorf tube, then, 300 µl hexane was added and finally centrifuged at 13,000 rpm for 10 min. The hexane layer was separated. The extract was dried over anhydrous Na_2SO_4 before analysis with GC-MS. The supernatant was transferred to a fresh tube. The water layer was extracted for second time and the hexane layer was combined with the previous extract, then subjected to GC-MS. The System parameters: inlet temperature was 250°C and detection temperatures was 280°C, a temperature program with an initial oven temperature 160°C for 1 min, final temperature 240°C and heating rate 10°C/min to 240°C with Helium as carrier gas at P = 0.37 atm.

Larvicidal activity bioassay

Different concentrations of the *P. frederiksbergensis* extract were used for carrying out the bioassay to etermine the mosquito-larvicidal activity against *Cx. pipiens*. Fractions were dissolved in hexane and made up to different concentrations in ascending serial dilutions from the stock solution of the extracts. Preliminary bioassay was performed using nine ascending concentrations ranging from 3.5–1000 µl/l. Based on the obtained results, a narrower range of the lethal concentrations ranging from 31.25 – 1000 µl/l in five replicates each (N = 5) were used for carrying out the main bioassay experiment in rearing plastic trays (30 × 15 × 10 cm each). On hundred 3rd instar larvae were put in each tray containing 1000 ml dH_2O infused with various concentrations of the bacterial extract according to (Rey *et al.*, 1999) in the presence of food (ground Tetramin flakes). In parallel, control larvae were treated in the same manner with hexane, the solvent of the bacterial extract. Food was added as normal, so that larvae were feeding normally until feeding cessation was noticed starting from 8h

post-treatment. Larvae were observed each 3 h for detecting feeding cessation and death until 24 h post-treatment. The number of larvae revived was counted at 24 h. Death or lack of reaction to gentle prodding with a glass pipette was the measured mortality according to (Brown *et al*., 1998). Larval mortality percentages were calculated at 24h post-treatment in five independent replicates (N = 5) for each concentration according to the WHO guide lines (2005) using the Abbott's formula (Abbott, 1925) as following:

$$\text{Mortality } (\%) = [(X - Y)/X]100$$

where X is % survival in control, and Y is % survival in treated mosquitoes.

The relationship between concentrations and mean mortality % was plotted *via* a Regression Plot using MINITAB software (MINITAB, Stat College, PA, version 13.1, 2001), and the resulting Linear Equation was used for determining the fifty percent and ninety percent lethal concentrations (LC_{50} and LC_{90}, respectively). LC_{50} (434.45 µl/l, see results) was used in the subsequent experiments of the current study.

Scanning electron microscopy

Third instar larvae of control or infected (alive, but sluggish) larvae were routinely prepared, at 24 h post-treatment, for investigating the morphological alterations on larval body under scanning electron microscope according to the instructions of the Electron Microscopy Unit in Zoology Department, King Saud University. Briefly, control or *P. frederiksbergensis* extract-treated larvae were immediately fixed in 2.5% glutaraldehyde in 0.1 M PBS. Following a minimum of 24 h of fixation, larvae were rinsed with 0.1 M PBS three times at 10 min intervals then fixed in 2% osmium tetroxide in distilled water. Larvae were then rinsed three times in 0.1 M PBS, dehydrated in ascending ethanol series (30%, 50%, 70%, 95%) for 10 min each, then rinsed three times in 100% ethanol. This was followed by treatments with 50% ethanol:50% acetone, 100% acetone, and 50% acetone: 50% hexamethyldisilazane (HMDS), each for 15 min. Finally, larvae were rinsed twice in100% HMDS for 15 min and allowed to air dry overnight. Larvae were then gold coated using a sputter machine (SPI Module Sputter Coater, USA) and observed under the SEM (JSM-6380 LA, Japan). Measurements of body size, in terms of head and thorax widths, were performed under SEM. Measurements of larval heads and thoraces were performed directly under the microscope using SEM Control User Interface Program (Version 7.06, 2004). Five different heads and thoraces measurements were performed using five different control or infected larvae (N = 5). Resulting images from control or treated larval preparations were imported into Adobe Illustrator Cs, 2003 Software and adjusted for contrast and suitable qualities

Histological examination of larval midgut tissues

After treatment with LC_{50} (434.45 µl/l) of *P. frederiksbergensis* extract, alive, but sluggish, 3rd instar *Cx. pipiens* larvae were used for investigating histological alterations at 24h post-treatment under light microscopy according to Villalon *et al.* (2003) and Ahmed *et al.* (2010). Briefly, the midgut sections were fixed overnight in cold 2.5% glutaraldehyde in 100 mM phosphate buffer (pH 7.2) and post-fixed for 1 h at room temperature in 1% OsO_4 in the 100 mM phosphate buffer. Midgut sections were then dehydrated through an ascending ethanol series, treated with propylene oxide and embedded in Poly/Bed 812 (Polysciences Inc., Warrington, PA). Thin 10 µm sections were mounted on slides, stained with hematoxylin and eosin (Sigma-Aldrich) and mounted with Paramount (Fisher) and examined by light microscopy using a Zeiss Axioskop 50 compound microscope (Carl Zeiss, Inc., Thornwood, NY). Resulting images from control or treated larval preparations were imported into Adobe Illustrator Cs, 2003 Software and adjusted for contrast and suitable qualities.

Statistical analysis

All statistical analyses were undertaken using MINITAB software (MINITAB, State College, PA, v: 13.1, 2001). Data pertaining to the mortality % were used for basic statistical analysis for assessing means and standard errors. Five replicates (five different mosquito groups: N = 5) were carried out for better statistical analysis. Data pertaining to body measurements were first tested for normality

using Anderson–Darling Normality Test (Morrison, 2002). Because these data were normally distributed, a two-sample t-test (for individual comparison) was used for comparing size-differences between treated and control larvae in each case.

RESULTS

Mosquito-larvicidal bioassay

This part of study was conducted to determine the LC_{50} and LC_{90} of *P. frederiksbergensis* extract against the 3rd larval instar of *Cx. pipiens* mosquito at 24 h post-treatment. Larvae were treated with wide range of ascending concentrations ranging from 3.5–1000 µl/l in a preliminary study. Concentration below 31.25 µl/l showed no mortality, and concentrations of 1000 µl/l and above showed 100% mortality 24h post-treatment (data not shown). Thus, ascending concentrations ranging from 31.25 – 1000.00 µl/l was assessed for determining LC_{50} and LC_{90} at 24h post treatment. The mean larval mortality percentage (calculated by Abbott's formula) was increased by increasing concentrations. Furthermore, cessation of feeding was noticed in treated larvae starting from 8 h post-treatment

A linear relationship between concentrations and mean mortality percentages was made *via* a regression plot (Fig. 1) using Minitab statistical program. The following resulting linear formula from the plot was used to calculate both LC_{50} and LC_{90}:

$$M = -0.804975 + 0.116942\ C$$

where M is mean larval mortality % and C is concentration of bacterial extract.

The concentration (C) was calculated from the formula in case of M = 50 or 90 (LC_{50} or LC_{90} respectively), which was equal to 434.45 or 767µl/l respectively. The LC_{50} (434.45µ/l) was used for the subsequent experimental purposes of the current study.

Morphological alterations of larval body

Both control and *P. frederiksbergensis* extract-treated larvae were observed under scanning electron microscope (SEM) for investigating the body size (in terms of head and thorax widths)

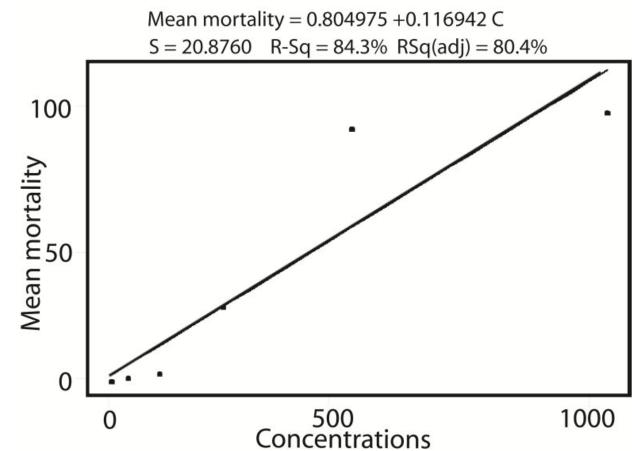

Fig. 1. A regression plot showing the linear relationship between the ascending lethal concentrations of *P. frederiksbergensis* extract, and mean mortality percentages of the 3rd larval instar of *Cx. pipiens* at 24h post-treatment. The resulting regression formula (on top of the graph) was used for calculating both LC_{50} and LC_{90}.

alterations at 24 h post-treatment. SEM investigation revealed that the size of thoracic regions were considered as an indication of the body size alteration in this study. As shown in Figure 2A and B and Figure 3, a significant shrinkage in the thoracic region of infected larva is obvious compared to that of control ones (Fig. 2A,C). Thorax of *P. frederiksbergensis* extract-treated larvae showed significant 46.8% reduction in size compared to that of the control ones (445± 13.0 *v* 950.8 ± 11.9 µm, respectively) ($P < 0.05$, N = 3, student t-test) (Fig. 2B, C, Fig. 3). However, head capsule was similar in size for both infected and control larvae (760.8 ± 12.1 *v* 755.4 ± 11.3 µm, respectively) ($P > 0.05$, N = 5, student t-test) (Figs. 2B, C, Fig. 3). Moreover, the mean thorax size was 1.25 folds of that of the head in control larvae, while it was 0.59 of that of the head in infected larvae (Fig. 2B, C, Fig. 3). These data clearly indicate a significant shrinkage in the body size of *P. frederiksbergensis* extract-treated larvae.

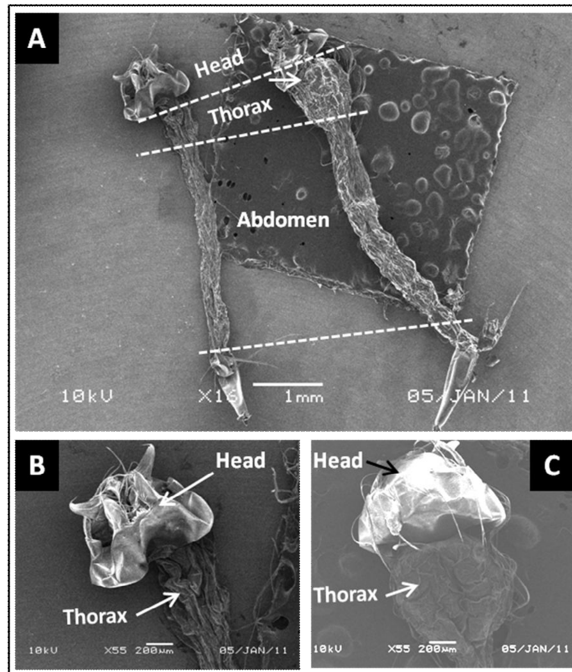

Fig. 2. Scanning electron micrograph of body regions of *Cx. pipiens* 3rd instar larvae at 24 h post-treatment with *P. frederiksbergensis* extract, or control larvae. A: A whole mounts of control (right) and infected (left) larvae at low magnifications showing clear shrinkage in thorax and abdomen of treated larva immediately after death. B & C: Heads and thoraces of control & treated larvae respectively, at higher magnifications showing shrinkage in head and thoracic region in treated larvae comparing to control ones.

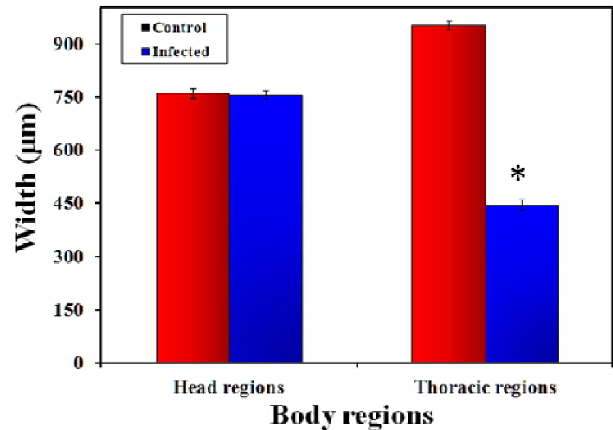

Fig. 3. Histogram showing the impact of *P. frederiksbergensis* extract on the body size, in terms of diameters (widths) of head and thoracic regions, in control and treated 3rd instar larvae of *Cx. pipiens* at 24 h post-treatment. An asterisk (*) indicates significant small size compared to that of control ($P<0.05$, n = 3, Student's t-test).

Histological impact on larval midgut tissues

Results from light microscopy revealed destructive effect of LC_{50} (434.45µl/l) of *P. frederiksbergiensis* extract on the internal integrity of the midgut of *Cx. pipiens* 3rd instar larvae (Fig.4), compared to the control one. This devastated effect was shown as numerous cytoplasmic extensions in epithelial cells, followed by cellular and nuclear degradation at 24 h post treatment. Although gut was still filled with nutritional contents, the peritrophic membrane, as well as the microvilli, were disappeared/destroyed by bacterial toxins, whereas those of the control retained their structural integrity (Fig. 4). Hence, this may indicate a high toxicity of *P. frederiksbergiensis* extract on both morphological and histological body integrity of treated larvae.

DISCUSSION

Control measures aim primarily at keeping numbers of mosquito vectors below the level of human health injury. However, it is well known that the use of broad spectrum chemical insecticides in the battle against insect pests contaminates the environment and causes some human diseases. Thus, the utilization of eco-safe biocontrol agents to manage mosquito vectors is the main goal nowadays. In this context, the simplest and perhaps still the most effective and readily deployable technique is to find alternative biological agents to reduce mosquito transmission of disease. Many studies have already been conducted for implementing biocontrol measures using different types of insect pathogens (Federici, 2005; Lord, 2005; Federici *et al*., 2007).

The ability to apply a biocontrol method using entomopathogenic bacteria against mosquito vectors, is of great deal for those who are interested in this field. This is because biocontrol method is save for environment, human and life stock, and overcoming mosquito resistant for most of chemical insecticides. Once an effective novel bacterial extract is ensured, it will be of a great help for stepping up an effective control measure against

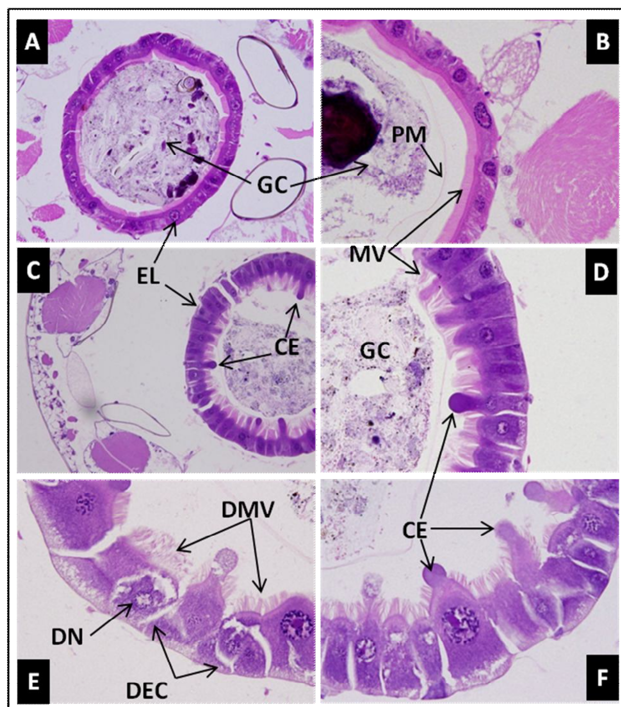

Fig. 4. Histopathological impact of *P. frederiksbergensis* extract on midgut epithelial tissue of *Cx. pipiens* larvae 24 h post-treatment. A & B (200 & 400X respectively): a cross-section of untreated control larvae showing normal gut epithelial layer (EL), peritrophic membrane (PM) and microvellii (MV), and nutritional gut contents (GC) filling the gut lumen. C & D: showing gut epithelium of treated larvae at 200 and 600X magnifications respectively with clear cytoplasmic extensions (CE) in the epithelial cells at 18h post-treatment. E & F: showing gut epithelium at 1000X magnification with clear degenerated epithelial cells (DEC), nuclei (DN) and microvellii (DMV) and traces of nutritional gut contents in the gut lumen.

mosquito vectors in the Kingdom of Saudi Arabia, specially, in the areas endemic with infectious diseases like Dengue fever in Eastern region.

The primary objective of the current study was to shed some light as to a new novel locally isolated mosquito larvicidal bacterium from contaminated soil that may be suggested as a new biological candidate in the battle against mosquito in Saudi Arabia. The target bacterium by the current study was *P. frederiksbergensis*, a rod-shaped Gram-negative phenanthrene-degrading bacterium that was first isolated from a coal gasification site in Frederiksberg, Copenhagen, Denmark (Anderson *et al.*, 2000). It has been used as biocontrol agent against plant pathogens (Haas and Defago, 2005). It has also been preliminarily tested against some Aedine and anopheline mosquito showing reasonable and promising effects (Prabakaran *et al.*, 2003, Ahmed *et al.*, 2013). Furthermore, this bacterium functions as an important decomposer of organic matter in soil, water and food products (Palleroni, 1993). Its biotechnological importance is partly due to its potential plant growth-promoting effects and application in biological control against fungal diseases in plants (Nielsen *et al.*, 1998). Abdel-Megeed and Majhadi (2009) found that this bacterial toxin could have biotechnological application for insect pest control. The toxic surface-active extract was tested against *Cx. pipiens* mosquito vector in the current study. Thus, it is important to clarify four points; a) a filaria mosquito vector *Cx. pipiens* in Saudi Arabia (Omar, 1996) was targeted by this study, b) a local native Gram negative bacterium, *P. frederiksbergiensis* (Abdel-Megeed and Majhadi, 2009) was isolated from soil in Riyadh region according to Abdel Megeed and Mueller (2009) and used for experimental purposes in this study and c) the LC_{50} (434.45µ/l) of the bacterial extract was used for carrying out the histological and morphological impact in treated larvae at 24h post-treatment and d) this bacterial product proved to be eco-friend and safe for living organisms (Nielsen *et al.*, 1998; Haas and Defago, 2005; Abdel-Megeed *et al.*, 2006; Abdel-Megeed and Majhadi, 2009).

Histopathological and morphological results obtained *via* light and scanning electron microscopy (SEM) revealed that *P. frederiksbergensis* extract treatments resulted in a massive destruction of the larval midgut epithelial cells in *Cx. pipiens* larvae as compared to control larvae. This destruction of epithelium tissue might be the main reason of the observed cessation in feeding by 12h post-treatment, septicemia oxidative stress and finally death at 24h post-treatment as previously recorded by Gill *et al.* (1992) and Ahmed (2012) in case of *B. thurengiensis*. On the other hand, the morphological alteration, in terms of severe shrinkage in the whole body was clearly shown as the mean thorax size was

0.59 of that of the head in infected larvae comparing to 1.25 folds of that of the head in control larvae. This, in fact, may be attributed to the surface activity of *P. frederiksbergensis* extract (Abdel Megeed *et al*., 2006). This may indicate that *P. frederiksbergensis* toxic extract targets and causes the destruction of the integument as well as the gut epithelium. It has been reported that destruction of the epithelial cells lining the midgut of mosquito larvae is often associated with midgut paralysis and cessation of feeding as recorded in *B. thurengiensis* (Goldberg and Margalit, 1977, Goettel *et al*., 1982; Clark *et al*., 2005; Bravo *et al*., 2007). The current study recorded similar symptoms which, may indicate high toxicity of *P. frederiksbergensis* against mosquitoes and being an effective biocontrol agent in the field of mosquito control.

In conclusion, our results provide solid evidence that the *P. frederiksbergensis* could be suggested as a mosquito larvicidal agent in the biocontrol measures against *Cx. pipiens* mosquito. This suggests the possibility of exploiting for the development of a commercial mosquito larvicide, a bacterium naturally occurring in Saudi Arabia soil. Moreover, testing this bacterial product against other mosquito vectors *e.g. Aedes caspius*, the Rift Valley Fever vector in Saudi Arabia (Balkhy and Memish, 2003; Madani *et al.,* 2003) showed higher larvicidal toxicity which may indicate a wide range of mosquitocidal effect amongst mosquito vectors. Finally, safety of *P. frederiksbergensis* toxic extract to environment and beneficial livestock has already been confirmed (Nielsen *et al*., 1998; Haas and Defago, 2005; Abdel-Megeed *et al*., 2006; Abdel-Megeed and Majhadi, 2009).

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