Ovicidal, poupicidal and bactericidal effects of aminopyridinium-based ionic liquids on Culex pipiens and certain human pathogenic bacteria

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1. Introduction

Mosquito-borne diseases are widely distributed among human populations and transmitted to more than 700 million people per year [1–7]. Culex pipiens is one of the most prevalent mosquitoes particularly in the Al-Madinah Al-Munawwarah region, Kingdom of Saudi Arabia, transmitting numerous diseases such as arboviruses, avian malaria, elephantiasis, dirofilariasis, and encephalitis. Worldwide, Culex pipiens has developed resistance to numerous insecticides; therefore, considerable efforts have been done to introduce new mosquito control candidates. Due to their unique physical, biological, and eco-friendly properties, ionic liquids (ILs) have been recently considered as promising agents in controlling a variety of organisms. Six aminopyridinium-based ionic liquids (IL1–IL6) were assayed against eggs and pupae of C. pipiens. Percentages of non-hatched eggs, non-emerged pupae, the lethal concentrations (LC50 and LC90) of both C. pipiens eggs and pupae were recorded, after exposure, to different ILs. The effects of ILs against eggs and pupae were concentration and IL-dependent. The percentages of egg unhatchability were much higher than those of pupal mortality, as the percentage of egg unhatchability reached 99% after treatment with IL6 at 0.5 g/L, while the percentage of pupal mortality reached 40.8% after treatment with IL4 at 0.5 g/L. The LC50 and LC90 of ILs against pupae were much higher than those of ILs against eggs. Effects of ILs on Staphylococcus aureus (Bacillales: Staphylococcaceae) and Escherichia coli (Enterobacterales: Enterobacteriaceae) were also studied; some ILs showed a considerable effect on both bacteria species. This is the first study to show the ovicidal, poupicidal, and bactericidal effects of aminopyridinium-based ILs in controlling C. pipiens, S. aureus, and E. coli.
enterotoxins that target human intestines, causing food poisoning and are considered one of the most threatening bacteria in the globe [29,30]. On the other hand, E. coli could be either commensal in the intestinal tract or pathogenic. According to their virulence factors, E. coli are classified into six pathotypes [31]. Both S. aureus and E. coli can be detected in DWW, particularly coliforms (lactose fermenters) [27].

Microbial multidrug resistance is a major global issue. Hence, scientists investigate antimicrobial activity through screening natural products that might lead to a promise inhibiting level [32]. The relationship between chemical compounds and their biological activity against bacteria has been documented previously as the cell wall and plasma membrane disruptors, inhibitor of nucleic acid synthesis and negatively affecting enzymatic proteins structurally [33,34]. The bacterial ability to resist several antibiotics is a worldwide concern. Therefore, investigation of new chemical compounds challenging microbial pathogens is a key need. A variety of chemical compounds have been extracted from plants such as flavonoids, quinones, terpenoids, tannins, coumarins, saponins, steroids, glycosides and alkaloids can affect bacterial infections and/or also as antibacterial agents [35–41].

Ionic liquids became attractive for use in numerous chemical and physical applications [42–45]. Furthermore, exciting biological activities of ILs against a wide range of microorganisms have been shown [46–49]. Several ILs have antifungal and antimicrobial effects [50–53]. Ionic liquids also have repulsive and antifeedant effects against some insect pests [51,54] and show lethal effect against Aedes aegypti and Culex pipiens larvae [55,56].

Numerous mosquito surveys [ex. 57–59] were performed in some Saudi Arabia regions, demonstrating various mosquito species (Aedes caspius, Anopheles multicolor, Culex perexiguus, Culex pippins, Culex pusillus, Culiseta langiareolata, and Culiseta subochrea). Culex pipiens considered among the most abundant mosquitoes in these studies. However, a previous study by [56] determined the efficacy of novel aminopyridinium-based ILs on C. pipiens larvae. The present hypothesis is to evaluate the ovicidal, pupicidal effects of these novel compounds as useful mosquito-cidal agents. Lethal concentrations (LC50 and LC90) of ILs against eggs and pupae will be determined as well. Eventually, the bactericidal effects of the same IL derivatives against E. coli (Gram-negative) and S. aureus (Gram-positive) were also assessed in Al-Madinah Al-Munawwarah, Saudi Arabia.

2. Materials and methods

2.1. Ovicidal and pupicidal bioassay

Pupae alongside larvae of C. pipiens were collected from the DWW drain area of Saad Al-Ghab [56]. Numerous water samples were collected and poured into 5L containers. The sampled water with larvae and pupae was then transferred into plastic trays (25 × 25 × 15 cm) which were 2/3 filled and kept at 24 ± 2°C for further experiments.

Larvae, pupae, and adults of C. pipiens were identified according to [60,61]. Larvae and pupae were reared in the laboratory; larvae were fed on fish food. Field-collected pupae and pupae transformed from field-collected larvae were kept in cages containing quail birds. Each cage was divided into two chambers separated by a net that allows emerged female mosquitoes to move through for blood-feeding on quail birds. Feathers on the quail’s back were removed to facilitate blood-feeding. Adults were also allowed to feed on supplied 20% sucrose solution through soaked cotton pads placed in Petri dishes and on top of the cage nets. Daily fresh laid eggs were collected using a fine brush from white plastic cups that were half-filled with water. Collected egg rafts (about 100–200 eggs/raft) were counted and used directly in the experiments.

2.2. Aminopyridinium-based ILs

Derivatives of 4-dimethylaminopyridinium-based ionic liquids (Table 1) were synthesized in the Department of Chemistry, College of Science, Taibah University. The synthesis process was based on three criteria, firstly, keeping the same cationic nucleus for all tested ILs: secondly, the introduction of different length alkyl chains containing two to six carbons as in the case of IL1–IL4 or a phenyl group as in IL5. Thirdly, introduction a different functional group such as ketone for IL6.

2.3. Screening of aminopyridinium-based ILs for ovicidal and pupicidal activity

Based on [56], ILs concentrations, 0.0156, 0.0312, 0.0625, 0.125, 0.25, 0.5 g/L, were tested for their effect against eggs. Concentrations, 0.125, 0.25 and 0.5 g/L, showed an effect against C. pipiens eggs, so they were selected for ovicidal bioassay. However, all those and higher IL concentrations had no effect against pupae, therefore, much higher concentrations (10, 15, and 20 g/L) were used to test the pupicidal efficacy of ILs. The ovicidal and pupicidal effects of six synthesized ILs (Table 1) were monitored daily for 4 days post-treatment to determine egg un-hatchability and pupal mortality.

2.4. Toxicity of selected aminopyridinium-based ILs on eggs and pupae

Aminopyridinium-based ILs were weighed up, then added separately into 250 ml glass beakers and dissolved in 100 ml of DWW to simulate the natural environmental conditions faced by C. pipiens eggs and
Table 1. Ionic liquids (IL1–IL6) used in the bioassay experiments.

| Code | Ionic liquid | Structural formula | Chemical structure |
|------|--------------|--------------------|-------------------|
| IL1  | 4-(dimethylamino)-1-ethylpyridinium bromide | C_{9}H_{15}BrN_{2} | ![Chemical structure](image) |
| IL2  | 4-(dimethylamino)-1-propylpyridinium bromide | C_{10}H_{17}BrN_{2} | ![Chemical structure](image) |
| IL3  | 4-(dimethylamino)-1-pentylpyridinium bromide | C_{12}H_{21}BrN_{2} | ![Chemical structure](image) |
| IL4  | 4-(dimethylamino)-1-hexylpyridinium iodide | C_{13}H_{23}IN_{2} | ![Chemical structure](image) |
| IL5  | 4-(dimethylamino)-1-(3-phenylpropyl) pyridinium bromide | C_{16}H_{21}BrN_{2} | ![Chemical structure](image) |
| IL6  | 1-(4-chlorobenzoyl)-4-(dimethylamino) pyridinium chloride | C_{14}H_{14}Cl_{2}N_{2}O | ![Chemical structure](image) |

pupae in the study area. Concentrations of 0.125, 0.25, 0.5, 10, 15 and 20 g/L for each IL were prepared. The bioassays were conducted in triplicates by using *C. pipiens* eggs (one egg raft/replicate) and pupae (25/replicate) at 24 ± 2°C in the insectary. Both egg un-hatchability and pupal mortality were recorded daily. For each concentration, one egg raft and 25 untreated pupae served as controls. *Culex pipiens* eggs were considered un-hatched when they did not transform into larvae within a 4-day period. Pupae were considered dead when they settled motionless at the beakers’ bottoms and did not respond to visual or mechanical stimuli, consequently failed to emerge to adults in a 4-days period. According to [62,63] eggs and pupae of *C. pipiens* transformed to the next stages in about 1.1 and 2.7 days respectively at ∼28°C. Similar observations were noticed for hatching and eclosion of eggs and pupae respectively; so, a 4-days period was adopted to completely guarantee the un-hatchability and non-emergence of eggs and pupae, respectively.

### 2.6. Bactericidal activity of ILs

The biological activity of ILs against both bacterial species was tested using agar disc diffusion methods. The 6 mm discs filter paper was soaked with 0.25 g/L of different ILs solutions (10 µl each) before mounting on the agar surface. All plates were incubated for at least 16 h at 37°C. Then, the inhibition zones were measured by calculating the diameter in (mm) to determine the bactericidal activities of ILs.

### 2.7. Data analysis

Minitab® software 17 (a trial version) was used to analyze data. The mean percentages of un-hatched eggs and non-emerged pupae of *C. pipiens* were analyzed using one-way ANOVA to determine the differences among concentrations of each IL and the differences among ILs for each concentration. When significant differences were existing, this was followed by a Tukey’s test for pairwise comparisons of means. The alpha value was set at 5%. Probit analysis [64] was used to determine LC_{50} and LC_{90} values of different ILs used against eggs and pupae of *C. pipiens*.

### 3. Results

#### 3.1. Oval and pupal mortality related to IL side chain:

##### 3.1.1. Alkyl side chain

In the present study, *C. pipiens* eggs and pupae were exposed to five synthesized ILs (IL1–IL5) that contained an alkyl chain with carbon atoms or a phenyl group (Table 1) to identify their effect on eggs and pupae. Different concentrations of ILs showed significant differences in the toxicity to *C. pipiens* eggs and pupae (*P* < 0.05).

We noticed that the length of the alkyl side chain was directly proportional to the efficacy of ILs. The data showed that the longer the side chain, the greater the effect against *C. pipiens* eggs and pupae (Table 2). For example, IL4 (side chain with six carbon atoms) caused high significant egg un-hatchability (96.7%) if compared to lower egg un-hatchability (88%) of IL1 (side chain with two carbon atoms) at 0.5 g/L (*P* < 0.05). Pupal mortality was significantly higher when exposed to IL4 (23.7%) than to IL1 (13%) at 15 g/L (*P* < 0.05).

Concerning IL1, 76.8% of eggs were un-hatched at 0.125 g/L and this percentage increased gradually reaching 83.4% egg un-hatchability at 0.25 g/L and the highest egg un-hatchability was 88%, which achieved at 0.5 g/L (Table 2 and Figure 1A). For pupal mortality, IL1 showed 7.4% mortality at 10 g/L. This effect increased

#### 2.5. Bacterial growth

Both *S. aureus* and *E. coli* were clinical isolates, which were kindly obtained from King Fahad hospital. Both bacterial species were also abundant in DWW of Saad Al-Ghab. Gram-positive *S. aureus* was grown on nutrient agar and Gram-negative *E. coli* was grown on MacConkey agar before incubating for at least 16 h at 37°C.

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Figure 1. Percentage of unhatched eggs after exposure to 0.125, 0.25, and 0.5 g/L of IL1 (A), IL2 (B), IL3 (C), IL4 (D), IL5 (E), and IL6 (F). Different letters indicate significant difference among concentrations within the same IL.

In the case of IL2, unhatched eggs represented 80.3% at 0.125 g/L, while at 0.25 g/L, unhatched eggs recorded 85.4%. At the highest concentration (0.5 g/L), 92.6% of eggs did not hatch (Table 2 and Figure 2A). At 10 g/L, un-emerged pupae attained 10.7% and this percentage increased to 18.3% at 15 g/L and finally reached 26.4% at 20 g/L (Table 2 and Figure 2B).

When eggs were treated with IL3 at 0.125, 0.25 and 0.5 g/L, unhatchability reached 85.1, 88.3 and 94.2% respectively (Table 2 and Figure 1C). Whereas 18.1, 22.3, and 30.5% of pupae did not emerge after treatment with 10, 15, and 20 g/L, respectively (Table 2 and Figure 2C).

Among eggs exposed to IL4, 89.4, 94.8 and 96.7% did not hatch when treated with 0.125, 0.25, and 0.5 g/L respectively (Table 2 and Figure 1D). Consequently, higher pupal mortalities were recorded; 19.5, 23.7, and 40.8% of pupae died when treated with 10, 15, and 20 g/L of IL4 respectively (Table 2 and Figure 2D).

The IL5 showed a higher ovicidal efficiency as more than 90% of eggs did not hatch at 0.125 g/L and 98% did not hatch at 0.5 g/L (Table 2 and Figure 1E). Concerning
Figure 2. Percentage of non-emerged pupae after exposure to 10, 15, and 20 g/L of IL1 (A), IL2 (B), IL3 (C), IL4 (D), IL5 (E), and IL6 (F). Different letters indicate significant difference among concentrations within the same IL.

pupal mortality, at 10, 15, and 20 g/L of IL5 recalled in 15.3, 21.5, and 31.9% mortality, respectively (Table 2 and Figure 2E).

3.1.2. Halides containing an acyl group
Among the ILs tested, only IL6 had a functional group (acyl group) on its side chain. At concentrations of 0.125, 0.25, and 0.5 g/L of IL6, the ovicidal activity ranged from 93.4% to 99% (Table 2 and Figure 1F). On the other hand, at 10, 15, and 20 g/L, pupicidal activity of IL6 ranged from 16.1% to 35.7% (Table 2 and Figure 2F).

From the previous results, both ovicidal and pupicidal activities of tested ILs increased proportionally with concentrations (Table 2 and Figures 1 & 2). For egg un-hatchability, significant differences among ILs were reported at each concentration tested (P < 0.05). At 0.125 g/L, the percentages of egg un-hatchability of IL1 and IL2 were significantly lower than those of IL3 – IL6. It was also reported that IL3 was significantly lower than IL6. At 0.25 g/L, the percentages of egg un-hatchability of IL1, IL2, and IL3 were significantly lower than those of IL4, IL5, and IL6. At 0.5 g/L, the percentages of egg un-hatchability of IL1 were significantly lower than those of IL3, IL4, IL5, and 6. It was also found that the percentages of egg un-hatchability at the same concentration of IL2 were significantly lower than those of IL5 and IL6.

For the pupal mortality percentages, significant differences among ILs were also recorded at each concentration used (P < 0.05). At 10 g/L, for IL1, the mortality percentages were significantly lower than those of IL3
IL6, respectively (Figure 3A). For LC90 values, they were 28.84, 27.54, and 37.15 g/L for IL1, IL2, IL3, IL4, IL5, and IL6, respectively for treated eggs. Concerning treated pupae, LC90 values were increased to 48.98, 47.86, 51.29, 28.84, 27.54, and 37.15 g/L for IL1, IL2, IL3, IL4, IL5, and IL6, respectively. Concerning treated pupae, LC90 values were increased to 194.98, 234.42, 467.74, 123.03, 64.56, and 181.97 g/L for IL1, IL2, IL3, IL4, IL5, and IL6, respectively.

- IL6. At the same concentration, it was also reported that the percentages of pupal mortality of IL2 were significantly lower than those of IL3, IL4, and IL6. A similar trend was also recorded when comparing IL1 with IL3–IL6 at 15 g/L concentration. At the same concentration, the mortality percentages of IL2 were significantly lower than those of IL6. At the highest concentration used in the present study (20 g/L), the percentages of pupal mortalities of IL1 were significantly lower than those of IL3–IL6. The percentages of mortality of IL2 were significantly lower than those of IL6. At the highest concentration (50 g/L), the percentages of pupal mortality of IL2 were significantly lower than those of IL3, IL4, and IL6. The percentages of mortality of IL2 were significantly lower than those of IL4 and IL6. The mortality percentages of IL3 were significantly lower than those of IL4. Eventually, the percentages of mortality of IL4 were significantly higher than those of IL5. From these results, it can be concluded that IL3–IL6 at most concentrations are more effective against eggs and pupae.

### 3.2. LC50 and LC90 of aminopyridinium-based ILs against eggs and pupae

LC50 values of the ILs were 0.012, 0.022, 0.008, 0.009, 0.013, and 0.011 g/L for IL1, IL2, IL3, IL4, IL5, and IL6, respectively for treated eggs. Concerning treated pupae, LC50 values were increased to 48.98, 47.86, 51.29, 28.84, 27.54, and 37.15 g/L for IL1, IL2, IL3, IL4, IL5, and IL6, respectively (Figure 3A). For LC90 values, they were 0.661, 0.429, 0.266, 0.132, 0.125 and 0.092 g/L for IL1, IL2, IL3, IL4, IL5, and IL6, respectively. Concerning treated pupae, LC90 values were extremely increased to 194.98, 234.42, 467.74, 123.03, 64.56, and 181.97 g/L for IL1, IL2, IL3, IL4, IL5, and IL6, respectively.

### 3.3. Bactericidal effect of ILs

The first ionic liquid (IL1) was the most effective on both bacteria with a wider inhibition zone on *E. coli*. IL2 came after IL1 in the effectiveness. The IL6 had the lowest effect on both bacteria. On the other hand, IL3, IL4, and IL5 had no effect on both bacteria (Table 3). Inhibition zones of IL1, IL2, and IL6 are illustrated in Figure 4. Furthermore, it was shown that IL3, IL4, and IL5 had no biological effect on the investigated bacteria (Figure 4). It was found that the Gram-positive bacteria, *S. aureus*, were more significantly resistant than Gram-negative bacteria, *E. coli*, (p < 0.05) to ILs used against both bacteria.

### 4. Discussion

Insecticides are well recognized for their instant action but have major drawbacks in their application as they are non-selective and may negatively affect other inhabitants in the environment [65–68]. Mosquitoes have become resistant to several categories of insecticides [ex. 69–74]. Consequently, searching for
new safe and degradable compounds should be encouraged for controlling mosquitoes. This study highlights the promising potential of ILs as practical alternatives to conventional insecticides [56]. Similar recent studies showed that numerous plant extracts and Eucalyptus oil have a potent effect at lower concentrations against larvae and pupae of different mosquito species [75,76].

Recently, larvicidal and pupicidal activities of Eucalyptus and neem oils against Aedes aegypti and Aedes albopictus were evaluated by [75] as they found that Eucalyptus oil was more effective against larvae and pupae. In the current study, ovicidal and pupicidal efficacies of ILs depended on volume rather than the surface area of water as compared to the application of Eucalyptus oil. On the other hand, the high ovicidal efficacy of ILs could be attributed to higher permeability of eggshells through de-waxing and disruption of endochorion tanning.

Comparable trends of ovicidal and pupicidal efficacy of ILs in the present investigation were observed, as high egg un-hatchability and moderate pupal mortality when using leaf extract of Annona senegalensis against C. quinquefasciatus were observed [77]. Cassia fistula fruit extract, carvacrol, thymol, and Anacardium occidentale nutshell extract against C. pipiens eggs, larvae, and pupae were effective [78–80]. It was also reported by [81] that the un-hatchability of freshly laid C. pipiens eggs significantly increased when treated with diflubenzuron, pyriproxyfen, and azadirachtin when compared to the effects of the same compounds on embryonated eggs.

El-Sheikh et al. [82] studied the effect of different concentrations of selected heavy metals’ salts against immature and mature stages of C. pipiens and they found that the potential survival of second instar larvae was highly affected by these salts. The number of eggs laid by females resulted from treated larvae decreased significantly and therefore, a lower fecundity was recorded for these females compared with control mosquitoes. Consequently, egg hatchability was significantly decreased compared to control eggs. The presence of such heavy metals in the ecosystem of C. pipiens could contribute to mosquito breeding reduction [82].

Comparing with synthetic products that exert commonly neurotoxic effects and promote the development of cross-resistance in insects, ILs may have some modes of action, such as the generation of reactive oxygen species and disruption of embryonic and pupal development. A recent study by [83] showed that buprofezin and azadirachtin affected embryonic development and egg hatchability through hormonal alterations. The current study showed that egg unhatchability and pupal mortality were ILs concentration dependent. The same trend was observed in the response of freshly laid eggs of C. pipiens when treated with different insect growth regulators [81].

The toxic effects of ILs were tested against C. pipiens larvae and a specific cell line of the fall armyworm, Spodoptera frugiperda [56,84]. They showed that the ILs caused death in the treated larvae and cells; the toxicity of ILs was dependent on concentration, structure, and exposure time [56,84]. Goellner et al. [55] obtained comparable findings but against Aedes aegypti larvae with
aqueous solutions of two imidazolium salts, recording up to 90% mortality after 48 h exposure. These results are like those of the present study, as the ovicidal effect was around 90% at a higher concentration (0.5 g/L).

Based on the obtained results by [56], it can be clearly conclude that alkyl chain length played a major role in the toxicity caused by different ILs and that the efficiency of the tested ILs increased when the length of the attached alkyl chain increased. The concentrations of tested IL contributed to their toxicity. Accordingly, a possible correlation between the chemical structure of ILs and their oxicidal and pupicidal effects may be present.

It was recorded in the present work that the percentages of mortalities in treated eggs were higher than their counterparts in treated pupae and this was also expressed in the LC50 and LC90 values. These values were much higher for ILs used for treating pupae than those of ILs used for treating eggs; this indicates the higher susceptibility of eggs (~77–99% un-hatchability) treated with different ILs. In contrast, pupae can highly tolerate the efficacy of ILs. Similar results for the un-hatchability of eggs were obtained by [85] when they treated eggs of C. quinquefasciatus, Aedes aegypti, and Anopheles stephensi with methanol extract of Asparagus racemosus. It was also reported by [86] that the oxicidal activity of ethanol extract of Gliericidia sepium reached 100% in Anopheles stephensi. They also reported the pupicidal activity against the same mosquito species and they found moderate pupal mortality of about 40%. It was also recorded by [79] that LC50 of phenolic lipid products from Anacardium occidentale against pupae of C. quinquefasciatus was very high if compared to that of the same products against larvae. The same trend was also observed by [77], as high egg un-hatchability and moderate pupal mortality recorded when using leaf extract of Anonna senegalensis against C. quinquefasciatus. This difference of tolerance between eggs and pupae may be due to the presence of special structures/modifications found in both non-feeding stages (eggs and pupae). It was concluded by [56,87] that the effects of ILs on C. pipiens larvae and adults occurred after the ingestion of ILs; and they also attributed the larval mortality to the damage of the intestinal epithelium. [88] noted in the early embryogenesis of different mosquito species including C. quinquefasciatus, that the water passes freely through the transparent eggshell, which at this moment is composed of exochorion and endochorion layers. Concerning, the pupal stage, it was reported by [89], the presence of a ring of hydrofuge cuticle encircles the spiracular aperture which resists the water entrance inside the pupal body. These explanations may reveal the cover of why eggs were much susceptible to ILs than pupae. This resistance of pupae to ILs could be also due to 1) pupal stage is preceded by a feeding stage (larvae) which could possibly gain cross-resistance throughout exposure to other relevant insecticides and/or chemicals and 2) stored fats in pupae may enhance their resistance to different pupicidal substances.

Concerning the bactericidal effect of ILs in the present study, we noticed that the treated water had both bacterial species (E. coli and S. aureus); so it seemed that the water may be contaminated after discharging in the valley (study area). Human pathogens have been found in the water body of the study region (data not shown) indicating pollution with sewage was merged at some points. The illegal discharge could be one of the reasons.

It was found that E. coli, were more significantly susceptible than S. aureus. This finding parallels the effects of previous antibacterial compounds’ activity against S. aureus and E. coli [90]. The difference in the cell wall structure could differentiate between Gram-positive and Gram-negative bacteria. The resistance of S. aureus to ILs may be due to the presence of peptidoglycans, which are 70–100 layers in the cell wall of Gram-positive bacteria. Another reason for the resistance of Gram-positive bacteria is the structure of peptidoglycans, as they are consisting of two types of polysaccharides, N-acetyl-muramic acid, and N-acetyl-glucosamine cross-linked by cross bridges and peptide side chains. The difference of resistance against ILs may be also attributed to the differences in modifications of fatty acid composition in Gram-positive and Gram-negative bacteria [91]. The Gram-negative bacteria can resist some of the antibiotics such as penicillin by the lactamase enzyme secretion which exists in the periplasmic space between the cytoplasmic membrane and the thin outer membrane [90].

5. Conclusion

Overall, our findings show that the oxicidal and pupicidal effects of ILs are concentration dependent. Percentages of egg un-hatchability and pupal mortality are proportionally increased with concentrations of different ILs. Generally, the pupae are more tolerant to ILs than eggs, as the pupal mortalities reached 40.8% at the maximum, while egg un-hatchability reached 99%. Concerning the bactericidal effect of ILs, E. coli was more significantly susceptible than S. aureus. This is the first study to display the oxicidal and pupicidal effects of aminopyridinium-based ILs in controlling C. pipiens; as well as their effects against certain human pathogenic bacteria. Further studies are required to test the applications of ILs under field circumstances and to characterize their probable fate, residual traces, and side effects against C. pipiens and other non-target inhabitants.

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References

[1] Hay SI, Guerra CA, Tatem AJ, et al. Urbanization, malaria transmission and disease burden in Africa. Nat Rev Microbiol. 2005;3:81–90.
[2] Sinka ME, Bangs MJ, Manguin S, et al. The dominant Anopheles vectors of human malaria in the Asia-Pacific region: occurrence data, distribution maps and bionomic precics. Parasites and Vectors. 2011;4:89.
[3] Ali Khan M, Ghamdi K, Mahyoub J. Aedes mosquito species in western Saudi Arabia. Journal of Insect Science. 2014;14(69):1–7. http://www.insectscience.org/14.69.
[4] Lucey D, Gostin L. A yellow fever epidemic: a new global health emergency? JAMA. 2016;315:2661–2662.
[5] Petersen L, Jamieson D, Powers A, et al. Zika virus. N Engl J Med. 2016;374:1552–1563.
[6] Vest KG. Zika virus: a basic overview of an emerging arboviral infection in the western hemisphere. Disaster Med Public Health Prep. 2016;10:707–712.
[7] Rana M, Singh SJ, Yadav S. Effect of microencapsulated plant extracts on mosquito repellency. Journal of Applied and Natural Science. 2017;9:2127–2131.
[8] Kim HC, Wilkerson RC, Pecor JE, et al. New records and reference collection of mosquitoes (Diptera: Culicidae) on Jeju Island, Republic of Korea. Entomol Res. 2005;35(1):55–66.
[9] Scott G, Yoshimizu M, Shinji K. Pyrethroid resistance in Culex pipiens mosquitoes. Pestic Biochem Physiol. 2015;120:68–76.
[10] Farajollahi A, Fonseca DM, Kramer LD, et al. “Bird biting” mosquitoes and human disease: a review of the role of culex pipiens complex mosquitoes in epidemiology. Infect Genet Evol. 2011;11(7):1577–1585. doi:10.1016/j.meegid.2011.08.013.
[11] Carballo H, King K. Emergency department management of mosquito-borne illness: malaria, dengue, and West Nile virus. Emerg Med Pract. 2014;16:1–23.
[12] Benelli G. Green synthesized nanoparticles in the fight against mosquito-borne diseases and cancer—a brief review. Enzyme Microb Technol. 2016;95:58–68. https://doi.org/10.1016/j.enzymtec.2016.08.022.
[13] Hamer GL, Anderson TK, Donovan DJ, et al. Dispersal of Adult Culex mosquitoes in an urban West Nile Virus hotspot: a mark-capture study incorporating stable isotope enrichment of natural larval habitats. PLoS Negl Trop Dis. 2014;8(3):e2768. doi:10.1371/journal.pntd.0002768.
[14] Hemingway J, Ranson H. Insecticide resistance in insect vectors of human disease. Annu Rev Entomol. 2000;45:371–391.
[15] Strode C, Donegan S, Garner P, et al. The impact of pyrethroid resistance on the efficacy of insecticide-treated bed nets against African anopheline mosquitoes: systematic review and meta-analysis. PLOS Med. 2014;11:e1001619.
[16] Naqash M, Gokce A, Bakhsh A, et al. Insecticide resistance and its molecular basis in urban insect pests. Parasitol Res. 2016;115:1363–1373.
[17] Ranson H, Lissenden N. Insecticide resistance in African Anopheles mosquitoes: a worsening situation that needs urgent action to maintain malaria control. Trends Parasitol. 2016;32:187–196.
[18] Fouad EE, Saad M, Adlau EB, et al. Resistance of Culex pipiens (Diptera: Culicidae) to organophosphate insecticides in centreal Morocco. International Journal of Toxicological and Pharmacological Research. 2016;8(4):263–268.
[19] World Health Organization. Global programme to eliminate lymphatic filariasis: progress report on mass drug administration. Wkly Epidemiol Rec. 2010;35:377–388.
[20] World Health Organization. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. Geneva: Switzerland; 2016.
[21] Knecht H, Richards S, Balanay A, et al. Impact of mosquito age and insecticide exposure on susceptibility of Aedes albopictus (Diptera: Culicidae) to infection with Zika Virus. Pathogens. 2018;7:67.
[22] Benelli G, Jeffries C, Walker T. Biological control of mosquito vectors: past, present, and future. Insects. 2016;7:52. doi:10.3390/insects7040052.
[23] Jayapriya G, Shoba F. Larvicidal, ovicidal, adulticidal and repellent activity of Justicia adhatoda Linn (acanthaceae) against Aedes aegypti linn and Culex quinquefasciatus say. Int J Recent Sci Res. 2014;5:2321–2327.
[24] Sakthivadivel M, Saravanan T, Tenzin G, et al. Laboratory evaluation of two meliaceae species as larvicides against Culex quinquefasciatus Say (Diptera: Culicidae). Vector Biology Journal. 2016;1:2–10.
[25] Rajesh A, Shamshudin M. Evaluation of ovicidal and larvicidal potential of Kalanchoe pinnata leaf extracts against filarial mosquito vector, Culex quinquefasciatus. International Journal of Mosquito Research. 2017;4:142–147.
[26] Ganesan P, Stalin A, Paulraj M, et al. Biocontrol and non-target effect of fractions and compound isolated from Streptomyces rimosus on the immature stages of filarial vector Culex quinquefasciatus Say (Diptera: Culicidae) and the compound interaction with Acetylcholinesterase (AChE1). Ecotoxicol Environ Saf. 2018;161:120–128.
[27] Cui Q, Huang Y, Wang H, et al. Diversity and abundance of bacterial pathogens in urban rivers impacted by domestic sewage. Environ Pollut. 2019;249:24–35.
[28] Amirsoleimani A, Brion GA, Diene SM, et al. Prevalence and characterization of Staphylococcus aureus in wastewater treatment plants by whole genomic sequencing. Water Res. 2019;158:193–202.
[29] McCarthy H, Rudkin JK, Black NS, et al. Meticillin resistance and the biofilm phenotype in Staphylococcus aureus. Front Cell Infect Microbiol. 2015;5:1.
[30] Ansari S, Rajesh KJ, Mishra SK, et al. Recent advances of Loureiro and their antimicrobial activity. Pharm Biol. 2001;39:221–225.
[31] Bardiau M, Grégoire F, Muylle F, et al. Entero-pathogenic (EPEC), enterohemorrhagic (EHEC) and verotoxigenic (VTEC).Escherichia coli in wild cervids. J Appl Microbiol. 2010;109(6):2214–2222.
[32] Zgoda JR, Porter JR. A convenient microdilution method for screening natural products against bacteria and fungi. Pharm Biol. 2001;39:221–225.
[33] Araujo MG, Hilario F, Nogueira LG, et al. Chemical constituents of the methanolic extract of leaves of Leio-thrix spiralis Ruhdal and their antimicrobial activity. Molecules. 2011;16(12):10479–10490.
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[34] Cioch M, Satora P, Skotniczny M, et al. Characterisation of antimicrobial properties of extracts of selected medicinal plants. Pol J Microbiol. 2017;66:462–472.

[35] Das K, Tiwari RK, Shrivasatva DK. Techniques for evaluation of medicinal plant products as antimicrobial agents: current methods and future trends. J Med Plants Res. 2010;4(2):104–111.

[36] Fernebro J. Fighting bacterial infections—future treatment options. Drug Resist Updates. 2011;14:125–139. doi: 10.1016/j.drup.2011.02.001.

[37] Teixeira B, Marques A, Ramos C, et al. European pennyroyal (Mentha pulegium) from Portugal: chemical composition of essential oil and antioxidant and antimicrobial properties of extracts and essential oil. Ind Crops Prod. 2012;36:81–87.

[38] Srivastava J, Chandra H, Nautiyal AR, et al. Antimicrobial resistance (AMR) and plant-derived antimicrobials (PDAMs) as an alternative drug line to control infections. Biotechnol. 2014;4(5):451–460.

[39] Guiotti AM, Cunha BG, Paulini MB, et al. Antimicrobial activity of conventional and plant-extract disinfectant solutions on microbial biofilms on a maxillofacial polymer surface. J Prosthet Dent. 2016;116(1):136–143. doi:https://doi.org/10.1016/j.prosdent.2015.12.014.

[40] Lee YS, Lee YJ, Park SN. Synergistic antimicrobial effect of Lonicera japonica and Magnolia obovata extracts and potential as a plant-derived natural preservative. J Microbiol Biotechnol. 2018;28(11):1814–1822. doi:10.4014/jmb.1807.07042.

[41] Lee YS, Lee YJ, Park SN. Synergistic antimicrobial effect of Lonicera japonica and Magnolia obovata extracts and potential as a plant-derived natural preservative. J Microbiol Biotechnol. 2018;28(11):1814–1822. doi:10.4014/jmb.1807.07042.

[42] Srivastava J, Chandra H, Nautiyal AR, et al. Antimicrobial properties of extracts from two Mediterranean species of parasitic plant Cynitus. BMC Complement Altern Med. 2019;19(1):82. doi:10.1186/s12906-019-2487-7.

[43] Maisetta G, Batoni G, Caboni P, et al. Tannin proline: potential as a plant-derived natural preservative. J Microbiol Biotechnol. 2019;29(11):2801–2806. doi:10.1016/j.jmb.2018.07.042.

[44] Mollaret Le, Dubent G, Brotot S, et al. Tannin profile, antioxidant properties, and antimicrobial activity of extracts from two Mediterranean species of parasitic plant Cynitus. BMC Complement Altern Med. 2019;19(1):82. doi:10.1186/s12906-019-2487-7.

[45] Levillain J, Dubant G, Abrunhosa I, et al. Synthesis and physico-chemical properties for their toxicity and antimicrobial properties of thiazoline based ionic liquids derived from two Mediterranean species of parasitic plant Cynitus. BMC Complement Altern Med. 2019;19(1):82. doi:10.1186/s12906-019-2487-7.

[46] Levillain J, Dubant G, Abrunhosa I, et al. Synthesis and properties of thiazoline based ionic liquids derived from the chiral pool. Chem Commun. 2003;23:2914–2915.

[47] Messali M, Alamir MN, Abderrahman B, et al. New pyridinium-based ionic liquids: an eco-friendly ultrasound-assisted synthesis, characterization and biological activity. S Afr J Chem. 2015;68:219–225.

[48] Lee S, Chang W, Choi A, et al. Influence of ionic liquids on the growth of Escherichia coli. Korean J Chem Eng. 2005;22:687–690.

[49] Ventura S, Gonçalves A, Gonçalves F, et al. Assessing the toxicity of [C3H3][N] to aquatic organisms of different trophic levels. Aquat Toxicol. 2010;96:290–297.

[50] Aljumani A, El-Sayed W, Sahu P, et al. Microwave-assisted synthesis of novel imidazolium, pyridinium and pyridinium-based ionic liquids and prediction of physico-chemical properties for their toxicity and antibacterial activity. J Mol Liq. 2018;249:747–753.

[51] Aljumani A, El-Sayed W, Aljumani A, et al. Microwave-assisted synthesis of some potential Bioactive imidazolium-based room temperature ionic liquids. Molecules. 2018;23:1727.

[52] Fang B, Zhou C, Rao X. Synthesis and biological activities of novel amine-derived bis-azoles as potential antibacterial and antifungal agents. Eur J Med Chem. 2010;45:4388–4398.

[53] Pernak J, Nawrot J, Kot M, et al. Ionic liquids based stored product insect antifeedants. RSC Adv. 2013;3:25019–25029.

[54] Dalla Lana D, Donato R, Bundchen C, et al. Imidazolium salts with antifungal potential against multidrug-resistant dermatophytes. J Appl Microbiol. 2015;119:377–388.

[55] Pendleton J, Gilmore B. The antimicrobial potential of ionic liquids: a source of chemical diversity for infection and biofilm control. Int J Antimicrob Agents. 2015;46:131–139.

[56] Fink G, Thakur D. Insecticidal potential properties of citronellol derived ionic liquid against two major stored grain insect pests. J Entomol Zool Studies. 2016;4:365–370.

[57] Goel N, Schmitt A, Coutou J, et al. Larvicidal and residual activity of imidazolium salts against Aedes aegypti (Diptera: Culicidae). Pest Manag Sci. 2018;74:1013–1019.

[58] Alahmadi S, Ibrahim R, Messali M, et al. Effect of aminopyridinium-based ionic liquids against larvae of Culex pipiens (Diptera: Culicidae). J Taibah Univ Science. 2020;14(1):863–872. doi:10.1080/16586365.2020.1782601.

[59] Ahmed AM, Shaalan EA, Aboul-Soud MAM, et al. Mosquito vectors survey in the AL-Ahsaa district of eastern Saudi Arabia. J Insect Sci. 2011;11(176):1–11.

[60] Al-Ahsa Oasis, Saudi Arabia. Asian Pacific J Tropical Disease. 2017;7(2):106–111. doi:10.12980/apjtd.7.2017D6-340.

[61] Babeker AHI, Elhadi FEM, Alshahrani AM, et al. Adult mosquito entomological survey (Diptera: Culicidae) in Aseer region. Kingdom of Saudi Arabia. BioMed Res J. 2020;4(2):209–216.

[62] Aseer region. Kingdom of Saudi Arabia. BioMed Res J. 2020;4(2):209–216.

[63] Thielman AC, Hunter FF. A photographic key to adult female mosquito species of Canada (Diptera: Culicidae). Can J Arthropod Identification. 2007, no. 4.

[64] Dehghan H, Sadraei J, Moosa-Kazemi SH, et al. A pictorial key for Culex pipiens complex (Diptera: Culicidae) in Iran. J Arthropod-Borne Dis. 2016;10(3):291–302.

[65] de Meillon B, Sebastian A, Khan ZH. The duration of egg, larval and pupal stages of Culex pipiens fatigans in Rangoon, Burma. Bull World Health Organ. 1967;36(1):7–14.

[66] Kiace-Makura MW, Ngumbi PM, Lee D. Effects of temperature on the growth and development of Culex pipiens complex mosquitoes (Diptera: Culicidae). IOSR J Pharm Biol Sci. 2013;10(6):1–10.

[67] Singh A, Zahra K. Le50 assessment of cypermethrin in Heteropeustes fossilis: probit analysis. Int J Fisheries Aquatic Studies. 2017;5(5):126–130.

[68] Satoh T, Hosokawa M. Organophosphates and their impact on the global environment. Neurotoxicology. 2000;21(1-2):223–227.

[69] Aardema H, Meertens JH, Ligtenberg JJ, et al. Organo-phosphorus pesticide poisoning: cases and developments. Neth J Med. 2008;66(4):149–153.

[70] Ansari M, Moraiti E, Ahmad S. Insecticides: impact on the environment and human health. In: Malik A, Grohmann E, Akhtar R, editors. Environmental deterioration and human health. Dordrecht: Springer; 2014. p. 99–123.

[71] Lushchak VI, Matvishyn TM, Husak WV, et al. Pesticide toxicity: a mechanistic approach. EXCLI J. 2018;17:1101–1136. doi:10.17179/excli2018-1710.
