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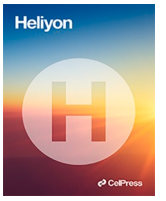

Research article

Assessment of biorational larvicides and botanical oils against *Culex quinquefasciatus* Say (Diptera: Culicidae) larvae in laboratory conditions

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ABSTRACT

Mosquitoes are known vectors that transmit deadly diseases to millions of people across the globe. The reliance on synthetic insecticides has been the sole way to combat mosquito vectors for decades. In recent years, the extensive use of conventional insecticides in mosquito suppression has led to significant pesticide resistance and serious human health hazards. In this light, investigating the potential application of biorational compounds for vector management has drawn significant attention. We, hereby, evaluated the efficacy of three microbial derivative biorational insecticides, abamectin, spinosad, and buprofezin, and two botanical oils, neem (*Azadirachta indica* A. Juss) and karanja oil (*Pongamia pinnata* Linn.) against the *Culex quinquefasciatus* under laboratory conditions. The fourth-instar *C. quinquefasciatus* larvae were exposed to different concentrations of the selected larvicides and lethality was estimated based on LC_{50} and LT_{50} with Probit analysis. All larvicides showed concentration-dependent significant effects on survival and demonstrated larvicidal activity against *C. quinquefasciatus* larvae. However, abamectin exerted the highest toxicity (LC_{50} = 10.36 ppm), exhibited statistically significant effects on *C. quinquefasciatus* larval mortality, followed by spinosad (LC_{50} = 21.32 ppm) and buprofezin (LC_{50} = 56.34 ppm). Abamectin caused larval mortality ranged from 30.00 to 53.33 % and 53.00–70.00 % at 06 and 07 h after treatment (HAT), respectively. In the case of botanicals, karanja oil (LC_{50} = 216.61 ppm) was more lethal (more than 1.5 times) and had a shorter lethal time than neem oil (LC_{50} = 330.93 ppm) and showed a classic pattern of relationship between concentrations and mortality over time. Overall, the present study highlighted the potential of deploying new generation biorational pesticides and botanicals in mosquito vector control programs.

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1. Introduction

Mosquitoes are considered to be one of the most nuisance species of the Diptera order in terms of importance to public health. They transmit a comprehensive number of diseases such as malaria, filariasis, dengue, chikungunya, and certain types of encephalitis such as West Nile fever, and cause millions of deaths each year [1–3]. The species *Anopheles, Aedes*, and *Culex* are the three most important mosquito causes of malaria, dengue, and filaria, respectively. The *Anopheles* species spreads malaria, poses a threat to 1.61 billion people in Southeast Asia. At the same time, about 2.5 billion people in the world are at a high risk of *Aedes* sp. transmitting dengue fever, of which about 1.3 billion reside in dengue-prone areas in Southeast Asia [4,5]. The *Culex* sp. is considered to be the primary vector for spreading the Japanese encephalitis virus in Southeast Asian countries [6]. They are also vectors of the parasitic worm *Wuchereria bancrofti*, the causative agent of lymphatic filariasis in Asia [7,8].

Bangladesh is a subtropical country characterized by a hot and humid environment. Owing to the presence of numerous stagnant water bodies, it has become an ideal place for mosquito breeding and population establishment [9], and therefore, the population of Bangladesh is highly prone to infestation of various mosquito vector-borne diseases. A 12-month-long survey revealed the occurrence of 13 different mosquito species in Dhaka, the capital of Bangladesh; among them, the *C. quinquefasciatus* was recorded as one of the most predominant species [10]. The *C. quinquefasciatus* was also found as the key species in the other urban and suburban areas of Bangladesh [11]. It is the vector insect responsible for spreading bancroftian filariasis in the country [12].

The primary tool for mosquito control in Bangladesh has been the sparing use of synthetic pesticides of various groups. In recent years, scheduled control measures have been implemented only in specific metropolitan areas by fogging in combination with pyrethroid insecticides [13]. Habitat destruction is also occasionally performed through community engagement as part of public and private initiatives [14,15]. People largely use mosquito coils, mats, and aerosols in their residences that use pyrethroids and organophosphates as active ingredients [16]. Coil smoke and aerosol solvents are health hazards and can cause severe headaches, asthma, persistent wheezing in children, lung cancer, and heart, liver, and central nervous system damage [17,18]. In addition, chemical insecticides have caused a cascade of problems related to residues, resistance, pollution, and resurgence in many countries, including Bangladesh [19–21]. Since 1980, the *Anopheles vagus* has been resistant to DDT in Bangladesh [22], which has also been found to be resistant to permethrin and deltamethrin, with mortality of only 29 % and 55 %, respectively, after 30 min [23]. The populations of *A. aegypti* sourced from Dhaka, Bangladesh displayed high levels of permethrin resistance with mortality rates ranging from 0 to 14.8 % [13]. Pyrethroids are also frequently used as fogging to combat *Culex* sp. mosquitoes in urban and suburban areas. With the sole reliance on synthetic insecticides, it is likely that pesticide resistance genes and reduced sensitivities to a variety of pesticides are already present in the *Culex* population. Resistance of the *Culex quinquefasciatus* to several groups of chemical insecticides has been reported in several countries, including Thailand, France [23], Colombia [24], West Africa [25], the USA [26], and Malaysia [27]. However, our previous report with fenitrothion, deltamethrin, and chlorpyrifos has shown high potency against *C. quinquefasciatus* in Bangladesh [28].

Building on the above understanding, the viability of continued pesticide applications is in question. Therefore, developing a safer and more effective alternative method of controlling mosquitoes is currently receiving a lot of attention. Microbial biopesticides and botanicals among biorational compounds have demonstrated significant promise in this regard [29–34]. The extracts, oils, and secondary metabolites originating from diverse plants have been used as an alternative mosquito control strategy since ancient times [3,34]. They are non-toxic, readily available at reasonable costs, biodegradable, and exhibit broad-spectrum, target-specific actions against multiple species of mosquito vectors and other insects. They can act at multiple and novel target sites in susceptible insects, reducing the potential for resistance development. Amongst microbial biopesticides and botanicals, spinosad, abamectin, buprofezin, and nonedible oils such as neem oil (*Azadirachta indica* A. Juss) and karanja oil (*Pongamia pinnata* Linn.) are considered a novel class of insecticides. They are commonly used against many insects of agricultural importance but rarely against mosquitoes of medical importance. Considering that puddles, stagnant ditches, clogged drains and gutters, containers, construction sites with still water are the ideal breeding sites for mosquitoes very common in urban and peri-urban areas, deploying softer and selective chemistry remains the sustainable ways to combat mosquitoes. Although buprofezin or abamectin have been reported to raise concerns on some aquatic organisms [35,36], the compounds we selected in our study are merely selective in their action with minimal impact on the non-targeted organisms. They are particularly suitable for urban and semi-urban areas having large aquatic ecosystems. Accordingly, in this current study, we evaluated the performance of three biopesticides, namely spinosad, abamectin, buprofezin, and two botanicals, namely neem and karanja oil, for their effectiveness against the fourth-instar larvae of *C. quinquefasciatus* mosquito under laboratory conditions with a view to using for sustainable mosquito control.

2. Materials and methods

2.1. Collection of mosquito larvae and rearing

The larvae *Culex quinquefasciatus* Say (Diptera: Culicidae) mosquito species were used as the testing organism. The *C. quinquefasciatus* larvae were collected from stagnant water bodies around the campus area of Bangladesh Agricultural University, Mymensingh, for a period of three months from March to May 2017. The district is located between latitude 24° 02′ to 25° 02′ N and 89° 04′ to 90° 02′ E with annual rainfall of 2250 mm and relative humidity varies from 60 to 90 %. It has a hot climate with a yearly temperature of 28.8 °C ranging from 12 °C to 40 °C. Mosquito larvae were identified following the identifying characteristics suggested by Bram [37] and maintained in a plastic tray at 25–28°C and 70–80 % RH under a photoperiod of 12:12 h (light: dark) in the Insect Biotechnology and Biopesticide Laboratory of the Department of Entomology, Bangladesh Agricultural University. They were fed on

goldfish feed, Osaka 2000 mini pallet (ingredients: soybean meal; corn gluten meal; yellow corn; astaxanthin as a color enhancer; vitamins: A, D3, E, B1, B2, B6, B12, Niacin, Pantothenic Acid, Choline; and minerals: calcium, phosphorus, iron, copper, cobalt, sodium). The 4th instar mosquito larvae (around 0.5 cm) were used for mortality bioassay test.

2.2. Tested biorational products

In Bangladesh, it is authorized to use spinosad, abamectin, and buprofezin to control crop pests [38]. We collected spinosad (Tracer 45SC, Auto Crop Care Ltd.), abamectin (Ambush 1.8 EC, Haychem Ltd., Bangladesh), and buprofezin (Award 40SC, Square Pharmaceuticals Ltd., Bangladesh) from local pesticide dealer outlet. Two commercially available botanical oils were natural neem oil, *Azadirachta indica* A. Juss. (Family: Meliaceae) and karanja oil *Pongamia pinnata* Linn. (Family: Fabaceae) were sourced from the stock collection of the Insect Biotechnology and Biopesticide Laboratory, Department of Entomology, Bangladesh Agricultural University, Mymensingh 2202. An emulsion formulation with a concentration of 3 % (v/v) was prepared by adding the required amount of each oil with a nonionic surfactant, Tween 20 in ratio of 1:3 and then added dropwise to water by stirring the system at room temperature used for successive experiments.

2.3. Preparation of test solutions

Stock concentration solution (5000 ppm on a v/v basis) was prepared for each tested biopesticide and botanical oil using 100 ml of water in a standard flask. Using the stock solution, several diluted concentrations (10, 20, 50, and 100 ppm) for all bioinsecticides and 100, 400, 1000, and 2000 ppm for the botanical oils were made by adding a known volume of stock solution for the bioassay study. To prepare emulsified oil-water stocks, a non-ionic surfactant, Tween 20 (polyoxyethylene (20) sorbitan monolaurate) was added and the solutions were stirred intensely to confirm the uniform mixing of oil in water [39].

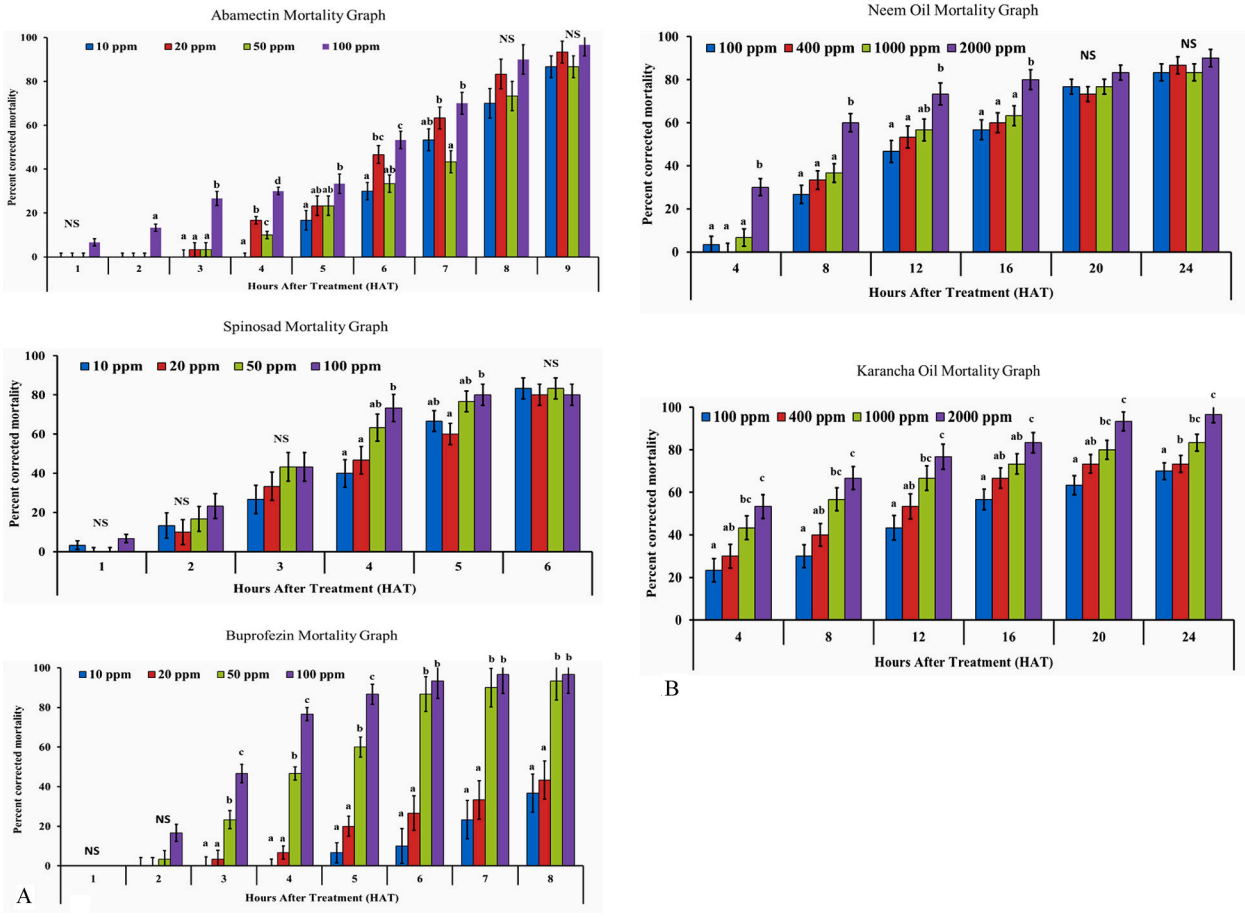

Fig. 1. The effects of the concentration-mortality relationship for the abamectin, spinosad, buprofezin (Fig. 1A), neem oil and karanja oil (Fig. 1B) against the 4th instar larvae of *C. quinquefasciatus*.

2.4. Mortality bioassay and data collection

To test the mosquito larvicidal activity, a slightly modified method of Rahman and Howlader [28] was adopted. Ten 4th instar mosquito larvae were added into a beaker containing 100 ml along with one of the respective tested concentrations. Each concentration was replicated three times. Three untreated controls were also prepared with water only. The experimental conditions were maintained at an ambient room temperature environment (12 L: 12D photoperiod, 25–28°C temperature, and 80–85 % RH) in the laboratory during the test. The number of larvae that died at each concentration was recorded hourly up to 100 percent mortality, and the percentage mortality was calculated as follows.

$$\%Larval\ mortality = \frac{\text{Number of dead insect}}{\text{Number of total insect}} \times 100$$

Table 1
Analysis of variance (ANOVA) of mortality analysis of biorational compounds.

(A) Abamectin.

Hours After Treatment (HAT)	Df	Sum of square	Mean square	F-value	Pr(>F)	Signif. codes
01 HAT	3	100.00	33.33	4.000	0.070	NS
02 HAT	3	400.00	133.333	16.000	0.003	*
03 HAT	3	1366.667	455.556	14.909	0.003	*
04 HAT	3	1425.000	475.000	57.000	0.000	*
05 HAT	3	425.000	141.667	2.429	0.164	NS
06 HAT	3	1091.667	363.889	7.706	0.018	*
07 HAT	3	1225.000	408.333	5.444	0.038	*
08 HAT	3	758.333	252.778	1.857	0.238	NS
09 HAT	3	225.000	75.000	1.000	0.455	NS

(B) Spinosad.

Hours After Treatment (HAT)	Df	Sum of square	Mean square	F-value	Pr(>F)	Signif. codes
01 HAT	3	91.667	30.556	2.200	0.189	NS
02 HAT	3	291.667	97.222	0.795	0.540	NS
03 HAT	3	600.000	200.000	1.263	0.368	NS
04 HAT	3	2091.667	697.222	4.736	0.050	**
05 HAT	3	758.333	252.778	2.935	0.012	*
06 HAT	3	33.333	11.111	0.129	0.939	NS

(C) Buprofezin.

Hours After Treatment (HAT)	Df	Sum of square	Mean square	F-value	Pr(>F)	Signif. codes
01 HAT	3	0.000	0.000	0.000	0.000	NS
02 HAT	3	566.667	188.889	3.400	0.094	NS
03 HAT	3	4166.667	1388.889	21.739	0.001	*
04 HAT	3	11625.000	3875.000	116.250	0.000	*
05 HAT	3	12133.333	4044.444	52.000	0.000	*
06 HAT	3	15891.667	5297.222	22.976	0.001	*
07 HAT	3	12891.667	4297.222	15.317	0.003	*
08 HAT	3	9158.333	3052.778	10.990	0.008	*

(D) Neem Oil.

Hours After Treatment (HAT)	Df	Sum of square	Mean square	F-value	Pr(>F)	Signif. codes
04 HAT	3	1666.667	555.556	11.765	0.006	*
08 HAT	3	1891.667	630.556	11.350	0.007	*
12 HAT	3	1158.333	4.633	0.037		*
16 HAT	3	966.667	322.222	4.833	0.033	*
20 HAT	3	158.333	52.778	1.583	0.268	NS
24 HAT	3	91.667	30.556	0.611	0.627	NS

(E) Karancha Oil.

Hours After Treatment (HAT)	Df	Sum of square	Mean square	F-value	Pr(>F)	Signif. codes
04 HAT	3	1625.000	811.111	9.419	0.011	*
08 HAT	3	2433.333	644.444	6.270	0.028	*
12 HAT	3	1933.333	377.778	5.440	0.038	*
16 HAT	3	1133.333	475.000	8.143	0.015	*
20 HAT	3	1425.000	430.556	9.118	0.012	*
24 HAT	3	1291.667	811.111	9.419	0.011	*

'*' $p \leq 0.05$.
'*' $p \leq 0.05$, 'NS' not significant.
'**' $p \leq 0.01$, '*' $p \leq 0.05$, 'NS' not significant.

When the control mortality ranged between 5 % and 20 %, the percent mortality was corrected as per Abbott's formula [40].

2.5. Statistical analysis

The effects of treatment concentrations on larval mortality at different time intervals were compared by ANOVA followed by DMRT post hoc analysis to estimate the differences between concentrations using SPSS (Version 23.0). Results with $p < 0.05$ were considered to be statistically significant. The larval mortality data were subjected to Probit analysis to determine LC_{50}, LC_{90}, LT_{50}, LT_{90} values, chi-square values, and other statistics of the selected insecticides at 95 % confidence intervals using the Ldp software (http://www.ehabsoft.com/ldpline/). The survival data were analyzed by JMP (Version 16.0) (https://www.jmp.com/en_us/home.html).

3. Results

3.1. Concentration-treatment time-mortality relationship

The effects of the concentration-mortality relationship for abamectin, spinosad, buprofezin, neem oil and karanja oil on the 4th instar *C. quinquefasciatus* larvae are presented in Fig. 1A and B. All insecticides tested were found to be effective against *C. quinquefasciatus* and statistically significant differences between the insecticides and their concentrations were observed.

In the case of abamectin, larval mortality of *C. quinquefasciatus* significantly varied with concentrations across the observation time points. Larvae that were subjected to the highest concentration (100 ppm) had significantly greater mortality compared to lower concentrations in just 02 h after treatment (HAT) and this difference remained significant up to the 07 HAT time point (02 HAT: $F_{3,6} = 16.000$; $p < 0.001$; 03 HAT: $F_{3,6} = 14.909$; $p < 0.001$; 04 HAT: $F_{3,6} = 57.000$; $p < 0.001$; 05 HAT: $F_{3,6} = 2.429$; $p < 0.164$; 06 HAT: $F_{3,6} = 7.706$; $p < 0.05$; 07 HAT: $F_{3,6} = 5.444$; $p < 0.05$). At 06 and 07 HAT, the larval mortality ranged 30.00–53.33 % and 53.00–70.00 %, respectively (Fig. 1A). However, the effect was no more significant for 08 and 09 HAT, (Table 1, Fig. 1A).

Likewise, the spinosad concentration of 100 ppm exhibited a statistically significant effect on larval mortality of *C. quinquefasciatus* at 04 and 05 HAT time points (04 HAT: $F_{3,6} = 4.736$; $p < 0.05$; 05 HAT: $F_{3,6} = 2.935$; $p < 0.001$) but an insignificant effect noted at other time points (Table 1, Fig. 1A). At 04 and 05 HAT, the larval mortality ranged 40.00–73.33 % and 66.67–80.00 %, respectively (Fig. 1A).

As observed in other treatments, larval mortality significantly differed among the test concentrations of buprofezin at several observation time points (03 HAT: $F_{3,6} = 21.739$; $p < 0.001$; 04 HAT: $F_{3,6} = 116.250$; $p < 0.001$; 05 HAT: $F_{3,6} = 52.000$; $p < 0.001$; 06 HAT: $F_{3,6} = 22.976$; $p < 0.001$; 07 HAT: $F_{3,6} = 15.317$; $p < 0.001$; and 08 HAT: $F_{3,6} = 10.990$; $p < 0.001$) (Table 1, Fig. 1A). From 03 HAT onwards, the highest concentrations 100 ppm caused significantly greater mortality than other three tested concentrations. Also, from 03 HAT onwards, 50 ppm had an intermediate mortality that was significantly higher than lower two concentrations (10 and 20 ppm), but lower than that of higher concentration (Table 1). However, the difference between the mortality caused by 100 ppm and 50 ppm was no more significant after 5HAT onward (Fig. 1A).

In case of botanicals, the application of neem oil at 2000 ppm concentrations rendered significant effects on larval mortality ($p < 0.05$) from 04 HAT to 16 HAT (Table 1, Fig. 1B). Larvae that were exposed to 2000 ppm had significantly greater mortality compared to those received other doses in between 04 HAT and 16 HAT. However, the differences in mortality among the concentrations were non-significant at 20 and 24HAT. The effects of 100, 400, and 1000 ppm were found statistically identical from 4HAT to 16HAT (Table 1, Fig. 1B). At 20 and 24HAT, the larval mortality was 73.33–83.33 % and 83.33–90.00 %, respectively, which was statistically insignificant, meaning that there were no significant differences for the concentrations tested after 16 HAT (Table 1, Fig. 1B).

A classic example of the relationship between concentrations and mortality over time was observed with the application of Karanja oil to *C. quinquefasciatusi* larvae. Larval mortality increased with the increasing concentration and time. The tested concentrations showed a significant effect on mortality from 04 HAT to 24 HAT (Table 1, Fig. 1B). Larval mortality caused by 2000 ppm was significantly greater than that of 1000 and 100 ppm throughout the bioassay period ($p < 0.05$). After 04 HAT, the highest concentrations (2000 ppm) resulted in 53.33 % larval mortality and the lowest (100 ppm) in 23.33 % mortality. The effect of the other two concentrations was intermediate and not significantly different from each other (Fig. 1B and Table 1).

Table 2
Larvicidal activity (lethal concentrations) of selected biopesticides and botanical oils against 4th instar larvae of *Culex quinquefasciatus* (after 10 h for biopesticides and 24 h for botanicals, respectively).

Insecticides	LC_{50} (ppm)	(LCL – UCL) (ppm)	Regression Equation	Chi-square
Abamectin	10.36	0.94–19.89	$y = 0.43x - 0.440$	2.67
Spinosad	21.32	12.80–28.62	$y = 0.73x - 0.975$	9.00
Buprofezin	56.34	47.36–66.82	$y = 3.65x - 6.380$	45.80
Karanja Oil	216.61	49.54–375.66	$y = 0.54x - 1.260$	4.49
Neem Oil	330.93	111.97–513.9	$y = 0.53x - 1.340$	3.79

LC_{50} Lethal concentration that kills 50 % of the exposed larvae, *LCL* lower confidence limit, *UCL* upper confidence limit.

3.2. Lethal toxicity

The lethality of biorational insecticides investigated is shown in Tables 2 and 3. We found that the biorational insecticides tested had lethal toxicity to the larvae of *C. quinquefasciatus*. After exposure to the insecticides, the treated larvae reacted instantly and then settled at the bottom of the beakers, convulsing and paralyzed and slowly died. No development of pupal or adult stages occurred under the treatments applied.

The median lethal concentration (LC_{50}) value of abamectin, spinosad, and buprofezin were 10.36, 21.32 and 56.34 ppm, respectively. The toxicity of abamectin was highest followed by spinosad and buprofezin (Table 2). The two botanical oils, neem and karanja oil, were also found lethal to the *C. quinquefasciatus* larvae. The LC_{50} value of neem and karanja oil was 216.61 and 330.93 ppm, respectively. The karanja oil showed higher toxicity than neem oil (Table 2). The LC_{90} values (Table 3) also showed similar trend.

While considering the lethal period, it was found that spinosad required the shortest period ($LT_{50} = 3.36$ h) followed by buprofezin ($LT_{50} = 4.01$ h) and abamectin ($LT_{50} = 6.68$ h) (Table 4). This indicates that spinosad was quicker in exhibiting its effect compared to other two biorational compounds. In case of botanical oils, karanja oil provided much quicker lethality, about two times faster ($LT_{50} = 5.79$ h) than the neem oil ($LT_{50} = 11.15$ h).

3.3. Survival analysis

The concentration-dependent survival of the larvae differed significantly for the studied biorational compounds and botanical oils is shown to Figs. 2 and 3.

Abamectin significantly rendered substantial toxicity to the survival of mosquito maggots ($F_{4,\ 945} = 430.2307$; $p < 0.001$). Larvae tested against 10, 50, and 100 ppm of abamectin exhibited higher mortality compared to untreated larvae, which showed minimal mortality. The concentration of 20 ppm resulted in moderate toxicity. Survival of mosquito larvae varied significantly with spinosad concentration ($F_{4,\ 945} = 345.4532$; $p < 0.001$). Larvae exposed to the two higher concentrations had shorter survival, compared to untreated larvae. However, the lowest two spinosad doses had moderate toxicity (Fig. 2). Likewise, mosquito larvae survival also varied significantly with buprofezin concentrations ($F_{4,\ 945} = 499.4387$; $p < 0.001$). Larvae subjected to the two higher concentrations of buprofezin had a minimal survival rate, while untreated larvae had a longer survival rate. The lowest two concentrations had moderate toxicity (Fig. 2).

For botanical oils, the survival of the mosquito larvae varied significantly with the concentration of the neem oil ($F_{4,\ 705} = 18.8221$; $p < 0.001$). Larvae tested against 2000 ppm neem oil survived the shortest period, while other tested concentrations showed toxicity ranging between 2000 ppm neem and control treatment. The untreated control larvae more likely survived throughout the trial period (Fig. 3). Similarly, the survival of mosquito maggots also varied significantly with the concentration level for karanja oil treatment ($F_{4,\ 705} = 14.5323$; $p < 0.001$). Larvae were exposed to higher two concentrations (1000 and 2000 ppm) of Karanja oil had shorter survival, whereas untreated larvae had minimal mortality. The lower two concentrations had moderate toxicity (Fig. 3).

4. Discussion

Biorational compounds of microbial or botanical origin, also known as new generation insecticides, having minimal detrimental impact on non-target flora, have been found to be effective against a wide range of insects including mosquitoes and other crop pests [41–43]. In the present study, we have evaluated the efficacy of such compounds against the *Culex* mosquito, and the encouraging results found in this study reveal the promise of those compounds to serve as potential alternatives to synthetic chemical pesticides to manage mosquito populations.

4.1. Biorational compounds against mosquito

In the present study, abamectin exhibited the highest toxicity to the fourth-instar larvae of *C. quinquefasciatus*, which was consistent with the previous studies of Ali and Nayer [44] in which they found that abamectin caused the highest toxicity to both *C. quinquefasciatus* and *Aedes aegypti* larvae. Similarly, El-Kady et al. [45] also reported significant effects of abamectin on the mortality of *C. pipiens* mosquito larvae. Further, abamectin and Sulfoxaflor + spinetoram were effective against *C. pipiens* after 24 h of exposure [46]. Abamectin also serves as an adulticide against *Aedes aegypti*, *C. pipiens*, and *Anopheles quadrimaculatus* [47]. Unlike other

Table 3
Larvicidal activity (lethal concentration, LC90) of selected biopesticides and botanical oils against 4th instar larvae of *Culex quinquefasciatus* (after 10 h for biopesticides and 24 h for botanicals, respectively).

Insecticides	LC_{90} (ppm)	(LCL – UCL) (ppm)	Regression Equation	Chi-square
Abamectin	10626.42	1296.42–597678.03	$y = 0.43x - 0.440$	2.67
Spinosad	1187.56	488.09–7528.44	$y = 0.73x - 0.975$	9.00
Buprofezin	126.547	98.56–202.42	$y = 3.65x - 6.380$	45.80
Karanja Oil	59219.56	14249.59–3888583.56	$y = 0.54x - 1.260$	4.49
Neem Oil	90561.54	19132.97–8550022.45	$y = 0.53x - 1.340$	3.79

LC_{90} Lethal concentration that kills 90 % of the exposed larvae, *LCL* lower confidence limit, *UCL* upper confidence limit.

Table 4
Lethal time (LT_{50}) of selected biopesticides and botanical oils against 4th instar larvae of *Culex quinquefasciatus* using fixed concentrations (for biopesticides, 50 ppm and 1000 ppm for botanical oils).

Insecticides	Lethal times (hours)			
	LT_{50} (95 % FL)	LT_{90} (95 % FL)	Slope±SE	Chi-sq
Abamectin	6.68 (6.43–6.95)	10.43 (9.72–11.43)	6.62 ± 0.48	11.076
Spinosad	3.36 (3.12–3.59)	6.915 (6.16–8.12)	4.09 ± 0.39	0.429
Buprofezin	4.01 (3.79–4.22)	7.35 (6.82–8.06)	4.87 ± 0.33	9.746
Karanja Oil	5.79 (4.18–7.16)	44.38 (31.78–77.99)	1.45 ± 0.21	1.650
Neem Oil	11.15 (10.16–12.15)	30.12 (26.02–36.54)	2.97 ± 0.25	3.89

LC_{50} Lethal concentration that kills 50 % of the exposed larvae, *LCL* lower confidence limit, *UCL* upper confidence limit.

broad-spectrum pesticides, abamectin has a unique mode of action through interfering with neurophysiological activity, stimulating the nerve endings to release neurotransmitter inhibitor γ-aminobutyric acid (GABA), prompting the extensive opening of the GABA-gated chloride channel with chloride channel activating effect possibly resulting higher toxicity than others. Therefore, a large influx of chloride ions causes nerve membrane potential to be hyperpolarized, resulting in the inhibition of the nerve membrane and thereby block the contact between nerve endings and muscle, thus causing insect paralysis, poor feeding, and death [48,49].

We found spinosad lethal to *C. quinquefasciatus* larvae in our assays as second best compared to abamectin. It is particularly effective against *Aedes* and *Anopheles* species larvae in both the laboratory and field trials [50–52]. Spinosad, derived from *Saccharopolyspora spinosa,* is also effective against a broader range of important agricultural insect pests. It primarily targeting binding sites on nicotinic acetylcholine receptors (nAChRs) of insect nervous systems, leading to involuntary neuronal excitation that initiates tremors, muscle contraction, paralysis, and finally death [53,54] It has been reported to be effective against *C. quinquefasciatus* in both suspension concentrate (SC) and granular (G) formulations [55]. Even spinosad has shown efficacy as a larvicide to control the temephos-resistant population of *Ae. Aegypti* from Brazil [56] and against *Ae. albopictus* and *Ae. Aegypti* from Mexico [57]. Similar observations were also made on controlling mosquito species, *Culex* and *Anopheles* with spinosad by Jiang and Mulla [58] and Prabhu et al. [51]. Recently, it has been revealed that the combined use of spinosad with the insect growth regulator, pyriproxyfen has potentially inhibited the development of adult *Ae. aegypti* without posing any side effects to non-target organisms [59]. Not only mosquitoes affected, the combination of spinosad with other biopesticides proved to be an effective control strategy against other dipteran pest as well, such as the cucurbit fruit fly, *Bactrocera cucurbitae* [60].

We also tested the effectiveness of buprofezin as a representative of insect growth regulators (IGRs). It inhibit the incorporation of N-acetyl-[D-H3] glucosamine into chitin and interferes with cuticle formation, thereby resulting in immature mortality during molting [61,62]. Buprofezin was found lethal to the *C. quinquefasciatus* larvae and various synthetic chitin synthesis inhibitors including buprofezin were even highly effective against organophosphate-resistant larvae of *C. quinquefasciatus* [63]. Buprofezin caused greater toxicity to *Ae. aegypti* mosquito larvae as well than diflubenzuron [64]. The application of buprofezin on mosquito larvae also inhibited larval molting to their subsequent stages [65]. These studies, alongside our bioassay results, validate the effectiveness of buprofezin against mosquito larvae and its potential as a larvicide for mosquito control.

4.2. Botanical oils against mosquito

More than 2000 species of plants have been found to produce a diverse type of chemicals and metabolites deployed in various pest management programs. Botanicals consists of secondary metabolites such as alkaloids, steroids, terpenoids, essential oils, and phenolics, derived from different plants to defend themselves against herbivore predators and other environmental conditions.Recently, ursolic acid and its derivatives (acetate, formate, and benzoate) isolated from *Catharanthus roseus* (L) have been noted to be highly effective against the larvae of three different mosquito species [66]. The biosynthesis of silver nanoparticles from botanicals of *Leonotis nepetifolia* posing antifeedant, larvicidal, and pupicidal activity found potential against mosquito vectors and crop pests [67]. Considering the insecticidal properties of plant species, it is imperative to advocate that botanical oils can serve as alternative sources of environmentally friendly and biodegradable mosquito ovicides and larvicides [68,69].

Neem (*Azadirachta indica* A. Juss.) and karanja (*Pongamia pinnata* Linn.) are two well-known plants that produce diverse types of phytochemicals demonstrating medicinal and mosquitocidal properties. Neem oils and neem cakes are used to control different plant pests and diseases, while karanja oil is used in pharmacy and agriculture [34,70,71].

Neem is a multipurpose tree with a wide range of uses. The effects of neem in managing various insects of agricultural, medical, and veterinary importance, including mosquitoes, are well documented [70–74]. Neem seeds contain a complex set of biologically active compounds, including tetranortriterpenoids, limonoids, azadiractin, etc. Among them, azadirachtin is considered a new insecticide type, which is more ecofriendly than synthetic insecticides and possesses insecticidal efficacy against a diverse range of insects, including mosquitoes [73]. Primarily azadiractin restricts the release of prothoracicotropic hormone (PTTH) and allatotropins from brain-corpus cardiacum complex, ultimately causing detrimental effects on ovarian development, fecundity, and fertility of different insect species [74]. Apart from azadirachtin, neem seed oil also contains nimbin, nimbidin, and nimbolides, which are also effective as antifeedant, ovicide, fecundity suppressant, growth regulator, and repellent [75,76]. Although the LC_{50} values differed, the efficacy of azadiractin against *C. quinquefasciatus* reported in the present study supported by several previous studies [70–72]. Although azadiractin was found to be toxic to several mosquito species, variability in its performance was reported. This variation may be attributed

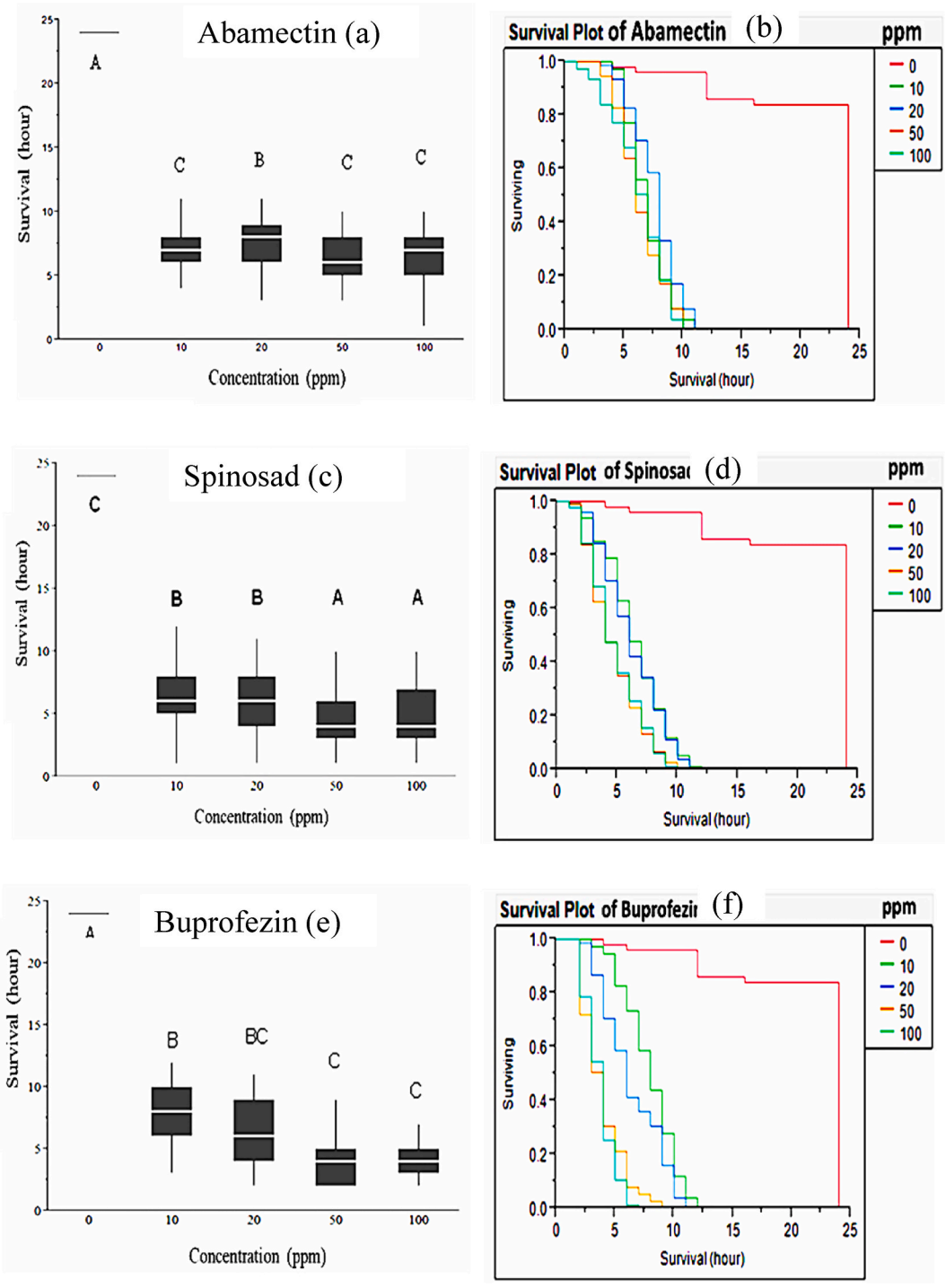

Fig. 2. Survival graphs of *C. quinquefasciatus* larvae treated with abamectin (a and b, upper panel), spinosad (c and d, mid panel), and buprofezin (e and f, lower panel).

to the differences in extraction methods, materials (e.g., seeds, leaves, barks, roots, etc.), larval instar, experimental methodology, and formulation used to test the bio-efficacy.

While both the neem and karanja oils were found to be effective in causing mortality to *C. quinquefasciatus* larval population, karanja oil was more toxic than neem oil (more than 1.5 times more potent and half lethal period compared to neem oil). Karanja plants produce different flavonoid compounds, such as karanjin and pongamol [77]. Karanja oils on average containing 2–4% karanjin

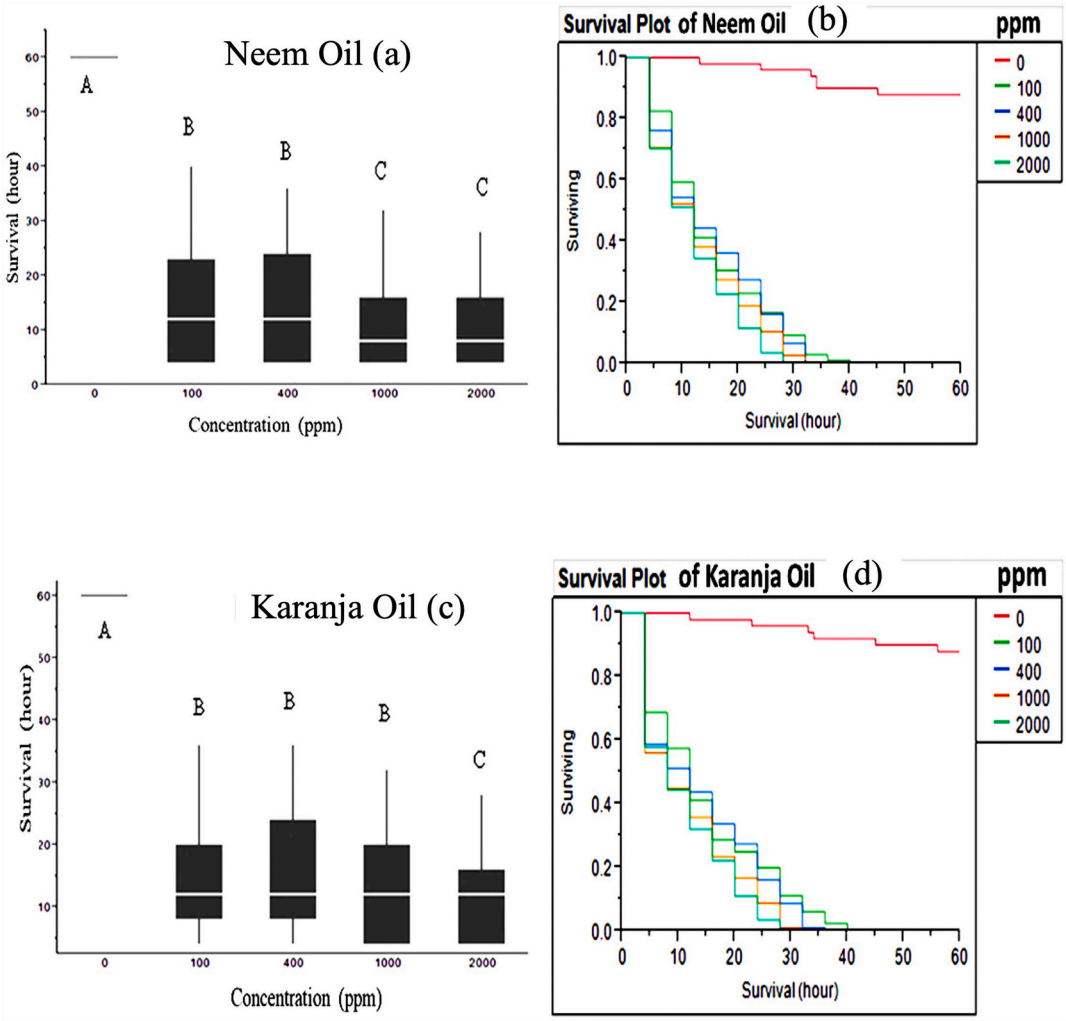

Fig. 3. Survival graphs of *C. quinquefasciatus* larvae treated with neem oil (a and b, upper panel) and karanja oil (c and d, lower panel).

and 0.3–0.9 % pongamol [78]. These flavonoids are reported to have insecticidal and acaricidal properties. Acetylcholinesterase (AChE) is the major site of action of the flavonoids [79]. Moreover, they have high potential to suppress the ecdysone hormone and inhibit the cytochrome P450 enzyme, eventually leading to developmental abnormalities in insects [80]. It is anticipated that due to the presence of these compounds, the karanja oils exhibited higher toxicity than neem oils, which mainly contain azadirachtin and other compounds. These findings are consistent with Perumalsamy et al. [81], who reported the potent toxicity of four flavonoids and two fatty acids isolated from karanja seeds not only against *C. pipiens* but also against the larvae of *Ae. aegypti* and *Ae. albopictus*. The individual LC_{50} of Karanjin and Pongamol against *Culex pipiens* were reported to be 14.61 and 16.13 mg/ml, respectively [81]. In our studies, the karanja oils demonstrated an LC_{50} of 216.61 ppm (i.e., 0.21661 mg/ml), indicating higher toxicity compared to the individual components. This could be attributed due to the presence of a mixture of all flavonoids, pongamol, and different oleic acids in our tested karanja oils, exerting their toxicity as neurotoxins possibly through inhibition of AChE and/or by stomach poison [81,82].

A similar result was also reported by Pant et al. [83], where karanja oil was found to be more potent than neem oil when applied individually against *A. aegypti*. Although, it was not assessed in the present study, it is evident that the performance of karanja oil cake was better than neem oil cake against *C. quinquefasciatus*, *A. aegypti*, and *An. stephensi* mosquitoes, as low LC_{50} value was estimated for the larvae of these three mosquito species [84]. While the combined use of both oils has been reported to be synergistic and larvicidal against different mosquito species [83,84].

The use of synthetic insecticides from various groups have been the primary method for mosquito control in many mosquito-prevalent countries including Bangladesh. In recent years, the extensive use of conventional insecticides in mosquito suppression has led to significant pesticide resistance and serious human health hazards. Our current observations suggest that biorational compounds of microbial or botanical origin, also known as new generation insecticides may be considered as potential alternatives to synthetic chemical pesticides to manage mosquito populations.

5. Conclusion

In a nutshell, our studies demonstrated the effectiveness of selected biorational chemicals and botanical oils in controlling *C. quinquefasciatus* larvae under laboratory conditions. Abamectin provided maximum efficacy, followed by spinosad, while karanja oil exhibited greater toxicity than neem oil. Overall, our findings suggest the potential application of biorational or new-generation insecticides for controlling mosquito larvae as larvicides in urban, suburban, and rural areas where mosquito-borne diseases are prevalent. We anticipate that the information presented in this manuscript brings valuable insights into the potential applicaton of new-generation chemistry to mosquito control in urban and suburban areas, possibly in case of pandemic situations. However, we recommend conducting detailed field trials to assess the effects on various non-target organisms and to formulate appropriate recommendations.

Declarations on competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All available data generated by experiments mentioned in this article are included either in the manuscript or in the supplementary material. Raw datasets used and/or analyzed during the current study are available from the corresponding authors upon reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors have given their consent for publication. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

CRediT authorship contribution statement

Md Mahfuzur Rahman: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Md Niaz Morshed:** Writing – original draft, Formal analysis. **Saleh Mohammad Adnan:** Writing – review & editing, Visualization, Formal analysis. **Mohammad Tofazzal Hossain Howlader:** Writing – review & editing, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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