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EFFICACY AND RESIDUAL EFFECT OF MOSQINOK (NOVALURON 0.8 P) AGAINST *CULEX QUINQUEFASCIATUS* LARVAE FOR THE CONTROL OF LYMPHATIC FILARIASIS IN SEMI-FIELD CONDITIONS IN BANGLADESH

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ABSTRACT: Insect Growth Regulators (IGRs) or Chitin Synthesis Inhibitors (CSIs) are supposed to inhibit the formation of chitin microfibrils in newly synthesized cuticle during molting by the retardation of larval growth and developmental processes during their life cycle. There are relatively few data on the morphological effects of CSI or IGR on mosquitoes in Bangladesh. In this study, the impact of Mosqinok (0.8P Novaluron) on mortality and pupation of *Cx. quinquefasciatus* was evaluated. Toxicity bioassays showed that Novaluron is toxic to 4th instar larva when exposed in 50L plastic drums at a dose rate of 1/4th of 1 tablet (10g) in 25L water. The larvae that were treated with Novaluron exhibited reduced activity levels and started to die after 24h of exposure, with a mortality rate of 6.67%, which reached 100% within 10 days. Novaluron-treated larvae exhibited toxic effects that inhibited molting, resulting in the failure of pupation. One-way ANOVA analysis revealed significant differences in larval mortality ($p < 0.05$) and pupal emergence ($p < 0.01$) between the treatment and control groups. This product also exhibited residual activity for up to 91 days after a single application. To conclude, the IGR larvicide has the potential to significantly contribute to vector control programs, particularly in terms of its efficacy and strategies for managing insecticide resistance in vector mosquitoes. It is recommended that additional field studies be conducted on the effectiveness of Novaluron against various mosquito species in diverse habitats throughout Bangladesh

Key words: Novaluron, IGR, Culex, Mosquito, Bangladesh

INTRODUCTION

Mosquitoes play a significant role in the transmission of numerous diseases, both for humans and animals, making them crucial vectors from a medical and veterinary perspective (Grech *et al.*, 2007). *Cx. quinquefasciatus*, the southern house mosquito (Bhattacharya *et al.*, 2016; Hill *et al.*, 2009),

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represents the most common mosquito species in Bangladesh (Bashar *et al.*, 2016). Over half of the global population resides in areas where the *Cx. quinquefasciatus* mosquito species is present (WHO, 2019). This species serves as the primary vector for a variety of diseases, including filariasis, avian malaria, and numerous arboviruses such as Western equine encephalitis virus, Zika virus, West Nile virus and St. Louis encephalitis virus (Duguma *et al.*, 2020; Azarm *et al.*, 2021). Effective vaccines and treatments for vector-borne diseases have either not been developed or are not widely available. (Fiaz *et al.,* 2021). Biological (Ndava *et al.,* 2018) and genetic vector control measures (Alphey *et al.,* 2013) are also growing in popularity but face logistical, fiscal, and regulatory obstacles that limit their deployment (Cuthbert *et al.,* 2018). Consequently, current vector control strategies rely heavily on synthetic insecticides (Olliaro *et al.,* 2018), bacteria (Ruiu *et al.,* 2007), plant products (Souza *et al.,* 2011), and essential oils (Park *et al.,* 2011). But mosquito populations have become resistant to these chemicals. (Metcalf, 1989; Cuervo *et al.,* 2016). However, mosquito populations have grown resistant to these chemical agents, underscoring the critical need for the development of new products and agents to control disease-carrying mosquito species (Poopathi *et al.,* 2015). Due to the high expenses involved in developing and launching a new product aimed at controlling public health insects, it is more convenient to explore the possibility of utilizing agents and products already in use in the field of agriculture (NourElhouda *et al.,* 2013).

During the developmental stages of insects, the hemolymph undergoes metabolic modifications, and carbohydrates and lipids represent the primary forms of stored energy. Insects have things inside them that're really important for how their bodies work. The amount of glycogen in insect tissues is connected to things that happen in their bodies like when they fly shed their skin and have babies. When insects are, around IGRS products it can change how they make things that their bodies need. (Kaufmann and Brown, 2008). (Rodríguez-Ortega *et al.,* 2003). The use of IGRs, for control is being investigated thoroughly. (Boudjelida *et al.,* 2005; Cetin *et al.,* 2006). Novaluron is something that can really mess with the hormones of insects. It affects how they grow and develop especially when they are still young. (Ghanim *et al.,* 2011). In the last few years, Novaluron has become a promising alternative for managing several types of insect pests. This compound, also known as (±)-1-3-chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy)phenyl-3-(2,6-difluorobenzoyl) urea, is classified as a chitin synthesis inhibitor that falls under the benzoylphenylurea category(Cutler and Scott-Dupree, 2007; Arredondo-Jimenez *et al.,* 2006; Cetin *et al.,* 2006; Jambulingam *et al.,* 2009). Novaluron works on the insects metabolism, which's the way their bodies work. This means that Novaluron disrupts the process of growth and development in the target insects. (Dhadialla, 2012). Studies have shown that Novaluron is highly effective against larval Coleoptera, Homoptera, and Lepidoptera, which are significant pests of crops and forestry (Malinowski and Pawinska, 1992; Malinowski, 1995; Ishaaya *et al.,* 1996; Ishaaya *et al.,* 2001). In laboratory conditions, some studies showed that Novaluron can kill the larvae of *Ae. aegypti* (Mulla et al, 2003) and *Cx.* mosquitoes (Su *et al.,* 2003; Tawatsin *et al.,* 2007). No studies have specifically evaluated the mode of action, the general mechanisms and effects of Novaluron against mosquitoes in Bangladesh. This study aims to evaluate the activity and residual effects of Novaluron against 3rd instar larval stages of *Cx. quinquefasciatus* and its impact on pupal emergence from larvae. The findings will provide a better understanding of Novaluron mode of action including its duration of residual activity. In addition, data obtained from this study can be used globally to provide better insights into the use of Novaluron in Integrated Pest Management (IPM) programs.

MATERIAL AND METHODS

Larvae Collection: Culex quinquefasciatus mosquitoes were used for efficacy testing. The larvae of *Cx. quinquefasciatus* were obtained from field collection in the Savar area. Collected larvae were brought to the Insect Rearing and Experimental Station (TRES), Department of Zoology, Jahangirnagar University, Dhaka-1342, Bangladesh, for processing and identification. Larvae of the mosquitoes were identified morphologically under stereo microscopes using taxonomic keys (Barraud, 1934; Bram, 1967).

Tested IGR Product: A commercial formulation of Insect Growth Regulators, named Mosqinok, was provided by Russell Bio Solutions Ltd, United Kingdom. This formulation is a new product manufactured by the same company. It is a solid tablet that appears white to pale yellow with no detectable odor. The active ingredient is Novaluron at 0.8%, with the remaining 99.2% consisting of other ingredients. The tablet has a melting range of 80-85°C, a density of 1.2 g/cm^3 at 23°C, and is 100% solids by weight. It's a controlled-release tablet containing both soluble and insoluble ingredients, and it does not contain any volatile organic compounds. The tablet also specifies its thermal decomposition properties, with no decomposition occurring up to 200°C, as well as recommended storage conditions. The patented formula of Novaluron has been developed to provide the controlled release of the active ingredient, Novaluron. This is a larvicide that was designed to be released at a slower and steadier pace in the area of the water that stands still. This product was trialed in different countries. It has not been previously evaluated against any mosquito species in Bangladesh.

Bioassay in Semi-Field Conditions: Mosqinok (0.8P Novaluron) was tested in semi-field conditions. A total of 4 plastic drums capable of containing 50L of water were placed in a location with a maintained temperature between 25-27 °C, RH between 70-80%, and a photoperiod of 14L:10D. Each drum was filled with 25L tap water, and one 10g Novaluron tablet was divided into equal 4 parts (2.5g). Then, an equal amount of tablet (2.5g) was added to the water of 3 drums to achieve a dose rate of 100 ppm, as recommended by Russell Bio Solutions Ltd., which suggested using a 10g tablet in 100L of water (1g for 10L of water). The experiment was conducted with 3 replicates, and the other one was regarded as a control. Newly ecdysed third instar larvae (20) of *Cx. quinquefasciatus* were released into the water of the treatment and control drums. Larvae were fed daily with meshed fish feeds. After the exposure time, the growth and development of the larvae were examined, and mortality, as well as pupal emergence, of the larvae were recorded daily until all the given larvae were dead. The time from releasing 20 larvae in each drum until their death was considered a batch completion time. A total of 13 batches (A-M) of larvae were released in each drum during the total study period. When one batch was completed, another batch of larvae was again released following the same procedure till 93 days (January-April, 2023) as EPA, 2017 recommended to use this tablet for 90 days. During the study period, the water in the drums was never replaced; instead, it was added when the water started to dry up.

Data Analysis: Assessments were made by counting the mortality of mosquito larvae and the emergence of pupae in treatments and controls after 24 hours, until all larvae in each batch were dead. Mortality data were recorded in a tabular format and then entered and cleaned in Microsoft Excel 2019. Mean and standard error (SE) values for the immature populations (larvae and pupae) for each day were determined. Analysis of variance (ANOVA) was performed on the mortality data using a 95% significance level to determine effectiveness at different time intervals.

RESULTS AND DISCUSSION

Effects on larval mortality: The study was performed to confirm whether Novaluron affects the duration of larval mortality before adult emergence. The product exhibited larvicidal activity against the treated mosquito species. In this experiment, 3rd instar larvae of *Cx. quinquefasciatus* exhibited abnormal behavior, including sluggishness, tremor, and convulsion, followed by paralysis at the bottom of the container, resulting in death after exposure to Novaluron at a dose rate of 100 ppm. Moribund and dead larvae were observed after 24 hours of treatment, whereas the control group contained alive and active larvae with normal motion. Novaluron inhibits growth by extending the larval development stage, resulting in larval death. The death larvae after treatment showed that most mortality occurred after an incomplete molting. The new cuticle synthesis in treated larvae was completely inhibited in this case, and as a consequence, the larvae died, trapped within their old exuvia (Fig. 1). The chitin synthesis inhibitor showed to affect the development in *Cx. quinquefasciatus*. A significant difference ($p < 0.05$) in larval mortality was found between Novaluron-treated and untreated plastic drums.

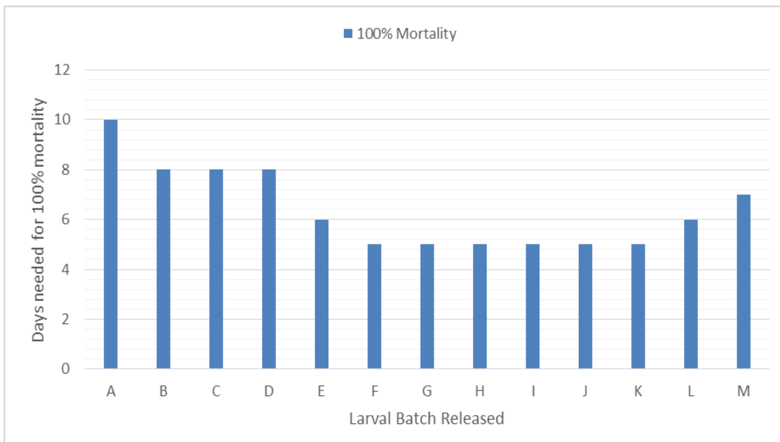
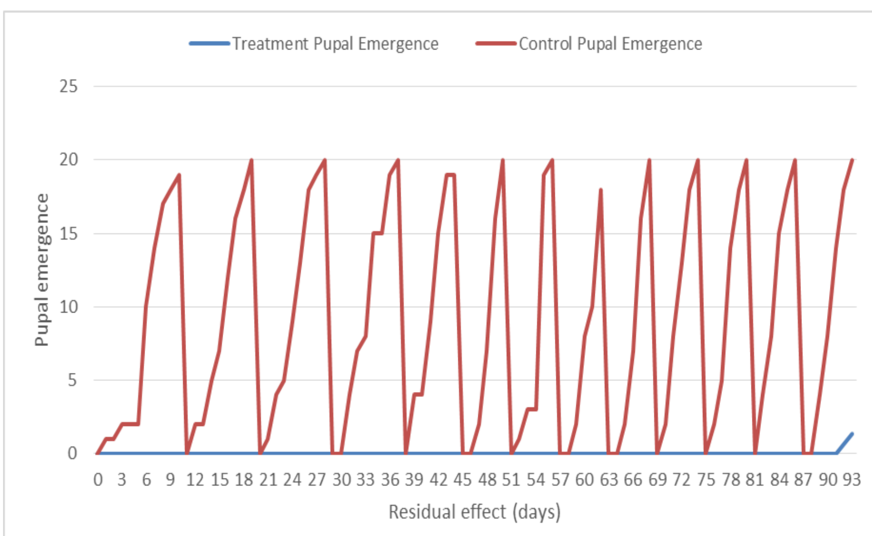


Fig. 1. Number of days needed for 100% larval mortality or pupal emergence in each batch of larvae (Batch A-M)



Note: Every peak of the graph is a new batch of larvae

Fig. 2 The number of pupae that emerged during the different days after the release the Novaluron 0.8P in the larval breeding grounds both in control and treatment

Effects on the duration of mortality: The results of the mortality duration of the 3rd instar larvae of *Cx. quinquefasciatus* after treatment with Novaluron at a concentration of 100 ppm showed that mortality increased with increasing period of exposure. Generally, time-related mortality responses were observed in all treatments, resulting in nearly 100% mortality in the larvae, indicating that the duration of exposure had a more significant impact on larval mortality. Our observation showed that Novaluron effective against *Cx. quinquefasciatus* larvae. The days needed for giving 100% mortality of larvae of each batch decreased with increasing of exposure time. In the first batch (A), the death of Culex larvae began on the first day (1.33±0.67), and

100% mortality was recorded after 10 days of exposure (Table 1). Interestingly, the subsequent three batches (Batches B, C, and D) exhibited a faster rate of mortality, achieving complete larval death in 8 days (Table 2-4). Beyond 37 days post-application, the Novaluron tablet appeared to boost the release of its active ingredient. This resulted in even more rapid mortality in newer batches; Batch E achieved complete mortality within 6 days (Table 5), while all subsequent batches (Batches F through L) showed complete mortality in just 5 days (Table 6-12). At the end of the study, the effect of Novaluron on the exposed drum water began to diminish, and more days were required, resulting in 100% larval mortality. Batch M had taken 6 days to cause the mortality of all larvae (Table 13). In contrast, the larval population in the control group consistently remained at or near 20 live larvae throughout the entire 93-day observation period, with negligible mortality (Table 1-13). This indicates that natural mortality in the control environment was minimal.

Effects on molting and pupal emergence: After releasing the 4th instar larvae of *Cx. quinquefasciatus* in Novaluron-treated and untreated drums, the development of pupae from larvae was observed. Novaluron inhibited pupal emergence, as the treated larvae never reached the pupal stage in any of the three treatments during the entire study period. The new cuticle synthesis in treated larvae was completely inhibited in this case. In contrast, the emergence of pupae from larvae in this study reveals a significant difference ($p < 0.01$) compared to the control at various stages of larval development. The larvae in the control group underwent normal molting, whereas no pupae were observed in the Novaluron-treated water drums until 93 days (Fig. 2).

The present study evaluated the ability of Mosqinok (0.8% Novaluron) in preventing molting and reducing larval mortality by inhibiting them from becoming pupae as an IGR product, and also the duration of the residual effect on larvae of *Cx. quinquefasciatus*. The efficacy and residual bio-assay results against *Cx. quinquefasciatus* mosquito larvae in Bangladesh showed that Novaluron exhibits toxic effects via disturbing the growth and development of *Cx. quinquefasciatus*. The toxicity assays indicated that Novaluron presented a larvicidal property once applied to newly ecdysed 3rd instar larvae. The same results were found when the Novaluron was used against *Cx. quinquefasciatus* (Jambulingam *et al.,* 2009) in small and medium-scale trials, and in *Ae. aegypti* (Lau *et al.,* 2015; Arredondo-Jimenez *et al.,* 2006; Ahmed *et al.,* 2016; Farnesi *et al.,* 2012).

The 100% inhibition of pupal emergence in 4th instar larvae in the present study is in conformity with the findings of Mulla et al. (2003), as they found that the early instars of insects are more susceptible to the inhibitory and growth regulatory effects of this larvicide because they carried out this study on 2nd and 4th instar larvae of *Ae. aegypti.* A significant difference ($p < 0.01$) in the emergence of pupae from larvae compared to the control was observed at various stages of larval development. The final step of the chitin biosynthesis pathway is inhibited in the absence of chitin synthesis inhibitors. This reaction was not detected in the control. This information is used to support and explain that the use of this product increases the duration of the development stages. Besides the toxic effect, Novaluron may also affect the hormone level in the haemolymph, preventing metamorphosis, or may affect the feeding habits of the larvae, causing them to avoid food and increase their body metabolism, resulting in death. In the current study, 100% mortality was observed in each batch of larvae, and a significant difference in larval mortality was noted between the treatment and control groups. Mortality of the *Culex* larvae started from the first day (1.33±0.67), and 100% mortality was recorded after 10 days of exposure. There was an increase in the percentage mortality as the time progressed because of the residual effect of Novaluron. It provided 100% mortality of all 13 batches of larvae till 90 days post-application. So, it is obvious that this formulation of Novaluron has 3 3-month residual effect as claimed by the

Table 1. Efficacy of Novaluron 0.8 P against the larvae of *Culex quinquefasciatus* after 0-10 days (*batch A) of exposure

Day	Batch completed days	Treatment		Control	
		Mortality	Pupal Emergence	Mortality	Pupal Emergence
		Mean±SE	Mean±SE	Mean±SE	Mean±SE
0	0	0.00 ± 0.00	0 ± 0	0 ± 0	0 ± 0
1	1	1.33 ± 0.67	0 ± 0	0 ± 0	1 ± 0
2	2	4.67 ± 0.88	0 ± 0	0 ± 0	1 ± 0
3	3	7.33 ± 0.67	0 ± 0	0 ± 0	2 ± 0
4	4	9.00 ± 1.00	0 ± 0	0 ± 0	2 ± 0
5	5	10.33 ± 0.67	0 ± 0	0 ± 0	2 ± 0
6	6	12.67 ± 0.33	0 ± 0	0 ± 0	10 ± 0
7	7	14.67 ± 0.33	0 ± 0	0 ± 0	14 ± 0
8	8	16.33 ± 0.33	0 ± 0	0 ± 0	17 ± 0
9	9	18.33 ± 0.33	0 ± 0	1± 0	18 ± 0
10	10	20.00 ± 0.00	0 ± 0	1 ± 0	19 ± 0

Table 2. Efficacy of Novaluron 0.8 P against the larvae of *Cx. quinquefasciatus* after 11-19 days (*batch B) of exposure

Day	Batch completed days	Treatment		Control	
		Mortality	Pupal Emergence	Mortality	Pupal Emergence
		Mean±SE	Mean±SE	Mean±SE	Mean±SE
11	0	0.00 ± 0.00	0 ± 0	0 ± 0	0 ± 0
12	1	1.33 ± 0.33	0 ± 0	0 ± 0	2 ± 0
13	2	1.67 ± 0.33	0 ± 0	0 ± 0	2 ± 0
14	3	4.67 ± 0.33	0 ± 0	0 ± 0	5 ± 0
15	4	7.00 ± 0.58	0 ± 0	0 ± 0	7 ± 0
16	5	10.00 ± 1.15	0 ± 0	0 ± 0	12 ± 0
17	6	16.00 ± 1.15	0 ± 0	0 ± 0	16 ± 0
18	7	18.67 ± 0.33	0 ± 0	0 ± 0	18 ± 0
19	8	20.00 ± 0.00	0 ± 0	0 ± 0	20 ± 0
11	0	0.00 ± 0.00	0 ± 0	0 ± 0	0 ± 0
12	1	1.33 ± 0.33	0 ± 0	0 ± 0	2 ± 0

Table 3. Efficacy of Novaluron 0.8 P against the larvae of *Cx. quinquefasciatus* after 20-28 days (*batch C) of exposure

Day	Batch completed days	Treatment		Control	
		Mortality	Pupal Emergence	Mortality	Pupal Emergence
		Mean±SE	Mean±SE	Mean±SE	Mean±SE
20	0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
21	1	6.33 ± 1.2	0 ± 0	0 ± 0	1 ± 0
22	2	7.67 ± 1.2	0 ± 0	0 ± 0	4 ± 0
23	3	8.33 ± 0.88	0 ± 0	0 ± 0	5 ± 0
24	4	11.67 ± 0.88	0 ± 0	0 ± 0	9 ± 0
25	5	15.33 ± 0.67	0 ± 0	0 ± 0	13 ± 0
26	6	18.33 ± 0.33	0 ± 0	0 ± 0	18 ± 0
27	7	18.33 ± 0.33	0 ± 0	0 ± 0	19 ± 0
28	8	20 ± 0	0 ± 0	0 ± 0	20 ± 0

Table 4. Efficacy of Novaluron 0.8 P against the larvae of *Cx. quinquefasciatus* after 29-37 days (*batch D) of exposure

Day	Batch completed days	Treatment		Control	
		Mortality	Pupal Emergence	Mortality	Pupal Emergence
		Mean±SE	Mean±SE	Mean±SE	Mean±SE
29	0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
30	1	1.67 ± 0.33	0 ± 0	0 ± 0	0 ± 0
31	2	6 ± 0.58	0 ± 0	0 ± 0	4 ± 0
32	3	7.67 ± 0.33	0 ± 0	0 ± 0	7 ± 0
33	4	8 ± 0	0 ± 0	0 ± 0	8 ± 0
34	5	15 ± 0.58	0 ± 0	0 ± 0	15 ± 0
35	6	16.33 ± 0.33	0 ± 0	0 ± 0	15 ± 0
36	7	18.33 ± 0.33	0 ± 0	0 ± 0	19 ± 0
37	8	20 ± 0	0 ± 0	0 ± 0	20 ± 0

Table 5. Efficacy of Novaluron 0.8 P against the larvae of *Cx. quinquefasciatus* after 38-44 days (*batch E) of exposure

Day	Batch completed days	Treatment		Control	
		Mortality	Pupal Emergence	Mortality	Pupal Emergence
		Mean±SE	Mean±SE	Mean±SE	Mean±SE
38	0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
39	1	5.33 ± 0.67	0 ± 0	0 ± 0	4 ± 0
40	2	9.33 ± 0.67	0 ± 0	0 ± 0	4 ± 0
41	3	11.33 ± 0.33	0 ± 0	0 ± 0	9 ± 0
42	4	14.67 ± 0.33	0 ± 0	1 ± 0	15 ± 0
43	5	18.67 ± 0.33	0 ± 0	1 ± 0	19 ± 0
44	6	20 ± 0	0 ± 0	1 ± 0	19 ± 0

Table 6. Efficacy of Novaluron 0.8 P against the larvae of *Cx. quinquefasciatus* after 45-50 days (*batch F) of exposure

Day	Batch complete d days	Treatment		Control	
		Mortality	Pupal Emergence	Mortality	Pupal Emergence
		Mean±SE	Mean±SE	Mean±SE	Mean±SE
45	0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
46	1	2 ± 0.58	0 ± 0	0 ± 0	0 ± 0
47	2	5.33 ± 0.33	0 ± 0	0 ± 0	2 ± 0
48	3	11.67 ± 0.88	0 ± 0	0 ± 0	7 ± 0
49	4	18 ± 0.58	0 ± 0	0 ± 0	16 ± 0
50	5	20 ± 0	0 ± 0	0 ± 0	20 ± 0

Table 7. Efficacy of Novaluron 0.8 P against the larvae of *Cx. quinquefasciatus* after 51-56 days (*batch G) of exposure

Day	Batch completed days	Treatment		Control	
		Mortality	Pupal Emergence	Mortality	Pupal Emergence
		Mean±SE	Mean±SE	Mean±SE	Mean±SE
51	0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
52	1	1.33 ± 0.33	0 ± 0	0 ± 0	1 ± 0
53	2	2.33 ± 0.33	0 ± 0	0 ± 0	3 ± 0
54	3	5 ± 0.58	0 ± 0	0 ± 0	3 ± 0
55	4	19 ± 0.58	0 ± 0	0 ± 0	19 ± 0
56	5	20 ± 0	0 ± 0	0 ± 0	20 ± 0

manufacturing company. This may elucidate why WHO suggested its use as a mosquito larvicide (WHO, 2005). EPA (2017) suggests applying the treatment at the beginning of the mosquito season and continuing up to the last brood of the season. But in actual field conditions, residual activity may vary in flowing water and may not provide 100% effectiveness for longer periods using the same dose since we've used it in small water-storage containers compared to field. Therefore higher dosages of this formulation can be used in *Aedes* and *Culex* breeding habitats to obtain better result. For application to large shallow surface area (up to 6”-12” depth), it is recommended to apply 320 to 400 grams of large tablet of Mosqinok® Novaluron Insecticide per 1000 sq. ft. of surface area (EPA, 2017).

Table 8. Efficacy of Novaluron 0.8 P against the larvae of *Cx. quinquefasciatus* after 57-62 days (*batch H) of exposure

Day	Batch completed days	Treatment		Control	
		Mortality	Pupal Emergence	Mortalit y	Pupal Emergence
		Mean±SE	Mean±SE	Mean±SE	Mean±SE
57	0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
58	1	1.33 ± 0.33	0 ± 0	0 ± 0	0 ± 0
59	2	3.67 ± 0.33	0 ± 0	0 ± 0	2 ± 0
60	3	7 ± 0.58	0 ± 0	0 ± 0	8 ± 0
61	4	12.33 ± 0.88	0 ± 0	0 ± 0	10 ± 0
62	5	19.67 ± 0.33	0 ± 0	2 ± 0	18 ± 0

Table 9. Efficacy of Novaluron 0.8 P against the larvae of *Cx. quinquefasciatus* after 63-68 days (*batch I) of exposure

Day	Batch completed days	Treatment		Control	
		Mortality	Pupal Emergence	Mortality	Pupal Emergence
		Mean±SE	Mean±SE	Mean±SE	Mean±SE
63	0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
64	1	1.67 ± 0.33	0 ± 0	0 ± 0	0 ± 0
65	2	3 ± 0.58	0 ± 0	0 ± 0	2 ± 0
66	3	12 ± 0.58	0 ± 0	0 ± 0	7 ± 0
67	4	17.67 ± 0.67	0 ± 0	0 ± 0	16 ± 0
68	5	20 ± 0	0 ± 0	0 ± 0	20 ± 0

Table 10. Efficacy of Novaluron 0.8 P against the larvae of *Cx. quinquefasciatus* after 69-74 days (*batch J) of exposure

Day	Batch completed days	Treatment		Control	
		Mortality	Pupal Emergence	Mortality	Pupal Emergence
		Mean±SE	Mean±SE	Mean±SE	Mean±SE
69	0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
70	1	2 ± 0.58	0 ± 0	0 ± 0	2 ± 0
71	2	7.67 ± 0.33	0 ± 0	0 ± 0	8 ± 0
72	3	13 ± 0.58	0 ± 0	0 ± 0	13 ± 0
73	4	17 ± 0.58	0 ± 0	0 ± 0	18 ± 0
74	5	20 ± 0	0 ± 0	0 ± 0	20 ± 0

Table 11. Efficacy of Novaluron 0.8 P against the larvae of *Cx. quinquefasciatus* after 75-80 days (*batch K) of exposure

Day	Batch completed days	Treatment		Control	
		Mortality	Pupal Emergence	Mortality	Pupal Emergence
		Mean±SE	Mean±SE	Mean±SE	Mean±SE
75	0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
76	1	1.33 ± 0.33	0 ± 0	0 ± 0	2 ± 0
77	2	4.67 ± 0.67	0 ± 0	0 ± 0	5 ± 0
78	3	7.33 ± 0.67	0 ± 0	0 ± 0	14 ± 0
79	4	13 ± 0.58	0 ± 0	0 ± 0	18 ± 0
80	5	20 ± 0	0 ± 0	0 ± 0	20 ± 0

Table 12. Efficacy of Novaluron 0.8 P against the larvae of *Cx. quinquefasciatus* after 81-86 days (*batch L) of exposure

Day	Batch completed days	Treatment		Control	
		Mortality	Pupal Emergence	Mortality	Pupal Emergence
		Mean±SE	Mean±SE	Mean±SE	Mean±SE
81	0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
82	1	1.33 ± 0.33	0 ± 0	0 ± 0	4 ± 0
83	2	6 ± 0.58	0 ± 0	0 ± 0	8 ± 0
84	3	9.67 ± 1.45	0 ± 0	0 ± 0	15 ± 0
85	4	16.67 ± 0.88	0 ± 0	0 ± 0	18 ± 0
86	5	19.33 ± 0.33	0 ± 0	0 ± 0	20 ± 0

Table 13. Efficacy of Novaluron 0.8 P against the larvae of *Cx. quinquefasciatus* after 87-93 days (*batch M) of exposure

Day	Batch completed days	Treatment		Control	
		Mortality	Pupal Emergence	Mortality	Pupal Emergence
		Mean±SE	Mean±SE	Mean±SE	Mean±SE
87	0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
88	1	0.67 ± 0.67	0 ± 0	0 ± 0	0 ± 0
89	2	3 ± 1.53	0 ± 0	0 ± 0	4 ± 0
90	3	9 ± 3.06	0 ± 0	0 ± 0	8 ± 0
91	4	15.33 ± 0.88	0 ± 0	0 ± 0	14 ± 0
92	5	18.33 ± 0.33	0 ± 0	0 ± 0	18 ± 0
93	6	20 ± 0	0 ± 0	0 ± 0	20 ± 0

A study by Pest Management Regulatory Agency, Canada (2014), on Novaluron (Mosquiron 0.12P) found that it is very highly toxic to freshwater and marine invertebrates on an acute and chronic basis, showing negligible toxicity to freshwater and marine fish, algae, and aquatic vascular plants. An assessment of residues in water and food was not required for this registration as it does not involve dietary risk for any of the proposed use scenarios. An EC10 (emulsifiable concentrate containing 10% active ingredient) formulation of Novaluron was tested in field by Tawatsin *et al.,* (2007) and no negative impact on fishes or aquatic plants in the treated areas were detected during the study period. Therefore, environmental hazards must be assessed by testing the effect of Mosqinok (0.8P Novaluron) in field. Quantitative risk assessment for human subjects who come in contact with Mosquiron 0.12P products showed that the risk is not of concern for human subjects who use the product according to label instructions. Thus, the worker must wear a long-sleeved shirt, long pants, and chemical-resistant gloves when applying 0.12P to standing water (PMRA, 2014). In conclusion, Novaluron is an effective chemical in managing the immature stages of polluted water mosquitoes, especially Cx. quinquefasciatus in water bodies found in urban areas, and it has good potential in developing new IGR-based mosquito control agents in managing the developing stages of mosquitoes. Thus, it can be said that this chemical can be an integral part of Integrated Pest Management (IPM).

CONCLUSION

The present study has emphasized the need for Novaluron in controlling mosquito larvae through molting inhibition and causing their death. Due to the secondary effects of conventional neurotoxic insecticides on the environment, insect growth regulators (IGRs) are promising in controlling mosquito larvae with their unique mode of action on insect growth and development and with less toxicity to the environment in comparison to conventional insecticides. WHO (2005) has recommended the use of Novaluron as a larvicide in non-drinking water storage containers, temporary mosquito habitats, and polluted waters with a dosage of 10-50 µg a.i./l or 10-100 g a.i./ha; however, higher doses are needed for polluted and vegetated habitats and for longer residual activity.

In conclusion, our study reveals that Novaluron (10% EC) can be employed as an effective larvicide in the control of the larval stage of polluted water mosquitoes such as Cx. quinquefasciatus with residual effects lasting for three months after the administration of the first dose at the recommended dosage rate of 1 tab or 2 Tab of “Mosqinok (Novaluron 0.8 P)” per 100 liters of clear or polluted water. The experiment was carried out for 93 days, and the product remained 100% effective up to day 91. Therefore, retreatment with the second dose of the IGR can be recommended after 90 days from the first application in the same mosquito breeding habitat, depending on the environmental conditions at the sites of application. This IGR larvicide can be very instrumental in the operational management of mosquito populations, especially with regard to its effectiveness and environmental friendliness, as well as its application in the management of insecticide-resistant mosquito populations. Further studies on the effectiveness of Novaluron as an IGR larvicide against different mosquito species in different habitats in Bangladesh can be recommended.

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