Acta Biologica Turcica 32 (2) 70-73 2019 Received 04 Apr. 2018; Accepted 16 Jan. 2019

ACTA BIOLOGICA TURCICA

E-ISSN: 2458-7893, http://www.actabiologicaturcica.com

Original research

Residual efficacy of diflubenzuron on mosquito larvae around Ankara province in Turkey

Nuri YIĞIT[1,*], Eda YAZICI[2], Nursel GÜL[1], Fulya SAYGILI YIĞIT[3], Duygu BAYRAMOĞLU[4], Derya ÇETINTÜRK[1], Hakan ESKIZENGIN[1]

[1]Ankara University, Faculty of Science, Department of Biology, Ankara/Turkey
[2]Kocaeli University, Institute of Health Sciences, Medical Microbiology Department, Kocaeli/Turkey
[3]Niğde Ömer Halisdemir University, Faculty of Science and Art, Department of Biotechnology, Niğde/Turkey
[4]Ankara University, Faculty of Science, Department of Chemistry, Ankara/Turkey
*Corresponding author email: nyigit@science.ankara.edu.tr

Abstract: The efficacy of diflubenzuron on mixed mosquito larvae (*Culex* sp., and *Anopheles* sp.) and it's deterioration in clean aquatic system were tested in the certain intervals by the HPLC. It was determined that the diflubenzuron (20 % wettable formula) deteriorated by 30 % at the end of three months and 60 % at six months respectively. Based on these finding, first 1,9 g (commercial product of 20 % WG) / 0.15 m^2 corresponding to the standard dosage (25 g / h) applied to mixed larvae and caused 100 % mortality in 96 hrs and then the application dosage reduced by 30 percent and then applied again. It was examined that the larvae mortality was observed as 80 % in 96 hours. When the dosage reduced 60 % corresponding six months residual efficacy, no sufficient larvae mortality were observed, and therefore our finding proved that the residual effectiveness of wettable diflubenzuron lasted to 30 days in the clean surface water.

Keywords: Diflubenzuron, residual efficacy, HPCL, mosquito larvae

Citing: Yiğit, N., Yazıcı, E., Gül, N., Saygılı Yiğit, F., Bayramoğlu, D., Çetintürk, D., & Eskizengin, H. 2019. Residual efficacy of diflubenzuron on mosquito larvae around Ankara province in Turkey. *Acta Biologica Turcica, 32*(2): 70-73.

Introduction

In recent studies, third lineage insecticide IGR (Insect Growth Regulator) that has minimal effects on environment and non-target organisms have been used in the control of insect larvae. The IGR insecticides consist of juvenile hormone analogues and chitin synthesis inhibitor known as diflubenzuron which was discovered by Philips and Duphar Laboratories in the early 1970s (Van Daalen et al., 1972; WHO 2008). Diflubenzuron [1-(4-chlorophenyl)-3-(2.6-flurobenzoyl) urea] is individual of benzoylphenylurea groups and has white crystal structure, scentless, melting point 230-232 °C and 0.08 mg/l resolution in water. When the diflubenzuron degraded in the soil, it breaks down into 2.6-difluorobenzoic acid (DFBA) and 4-chlorofenylurea (CPU) in large amount and parachloroanylin (PCA) in less amount (WHO, 2006a).

Diflubenzuron has been reported to be more larvacidal effect on mosquito larvae rather than on a lot of aquatic organisms, bees, fishes, birds and Mammalia (WHO, 2006a; Maduenko and Martinez, 2008; Marrs, 2012). Diflubenzuron and its metabolites (DFBA and CPU) showed no mutagenic effects during in vivo and in vitro assays (WHO, 2006a). Apart from these, 4-chloroanylin (PCA) is mutagen complex and minor metabolite of diflubenzuron. Diflubenzuron inhibits chitin synthesis during the cuticle occurring and moulting in insect development stages (Reynolds, 1987). Mortality existed in insects either due to failure of moulting (Ascher and

Nemy, 1976; Salokhe et al., 2012; Marrs, 2012) or loss of liquid during the moulting (Mulder and Gijswijt, 1973).

Diflubenzuron is commonly used against aquatic or terrestrial insect larvae (Grosscurt, 1978; Schroeder, 1996; Baruah and Das, 1996; Romeo et al. 2009; Silva et al., 2009; Saleh et al., 2013, Djordjevic et al., 2015, Caputo et al., 2015). Diflubenzuron was applied to *Leptinotarsa decemlineata* larvae and it caused disordered cuticle of larvae (Grosscurt, 1978). The commercial product of diflubenzuron residues on citruses were reported to be decreased the egg opening of *Diaprepes abbreviatus* after 46 days of diflubenzuron exposure (Schroeder, 1996). After application of another commercial diflubenzuron product (Micromite® 4L, 80 WG; wettable granules) to citrus trees, 80 WG residues caused a development deterioration of *D. abbreviatus* eggs with the mortality of 60 - 64 % (Adair and Mehta, 2001). Also the dosage (0.10 µl/louse) of diflubenzuron had harmful effects on reproduction (eggs hatch and nymph produce) of sheep body lice (*Bovicola ovis*) (Levot and Sales, 2008). Apart from these, the acute, sub lethal dosages with residual efficacy of diflubenzuron on the various mosquito species were investigated by many researchers (Silva et al., 2009; Romeo et al., 2009; Saleh et al., 2013; Djordjevic et al., 2015; Caputo et al., 2015).

Diflubenzuron is available under the different brand names as a tablet (TB), granular (GR, WG) and liquid (SC) formulations as well as in Turkey. Diflubenzuron is also commonly used in every kind of aquatic systems 'clear, pond and sewage' to struggle larvae management as considering the safe to non-target animals such as mammals in Turkey. However, the companies claim the different residual effects for their products and cause the unrealistic competition on the product marketing. Therefore, the present study aimed to determine the residual activity of diflubenzuron on mosquito larvae based on the deterioration rate in clean water.

Materials and Methods

The mosquito~~s~~ larvae in experiment were obtained from Çubuk-2 Dam lake, and cultured in laboratory conditions (kept under 14 hour light cycle at 26 ± 1 C° and 60-65 % relative humidity). The mosquitos were identified at the genera level (*Culex* sp. and *Anopheles* sp.) in accordance with the criteria given by Rozendaal, 1997~~,~~ using Stereomicroscope (Leica).

Residual tests

The active substance (diflubenzuron) of the commercial product (20 % wettable granule "WG") and its deterioration rate were determined in three months intervals by HPLC analyses after applied to clean water. It was determined that the diflubenzuron was deteriorated 30 % in three months and 60 % in six months. The standard application dosage is between 25 - 100 mg ai / h (WHO 2006b). The lower limit of the dosage was used to the standard application dosage from the product of 20 % WG and this dosage was firstly reduced 30 % and then secondly 60 %. These two doses were applied to the clean water with mosquito larvae (*Culex* sp. and *Anopheles* sp.).

Diflubenzuron Applications to Mosquitos Larvae

For this purpose, larvae were divided into 4 categories (each one has 40 larvae (stages L_3 and L_4) in the equal numbers for each genera);

1) First group: Control
2) Second group: Standard application dose (1.9 mg / 0.15 m^2 corresponding to 25 g ai /h)
3) Third group: 30 % Reduced dose (1.3 mg / 0.15 m^2 corresponding to 3 months residual activity)
4) Fourth group: 60 % Reduced dose (0.95 mg / 0.15 m^2 corresponding 6 months residual activity)

In the test procedure, the water containers with the surface area of 0.15 cm^2 were checked every day to determine larval mortality, the experiments were repeated 3 times, and the results were analysed by one-way ANOVA.

HPLC Analysis

The commercial product contained 20 % diflubenzuron WG were used for determining the degradation rate of product. The first commercial product was tested to confirm the content of 20 %. After then, the diflubenzuron was prepared in different concentration from 20 % WG formulation in the clean water to determine the degradation rate in pH; 6.5 - 6.7. The solutions were kept in room temperature and 12-hour light/12hour dark period. The test materials in the clean water (surface water of pool) were stored up to six months, then monthly analysed by the Essence HPLC. Degradation rates were detected by using ACE5 C18 colon in 75 % methanol solvent and the proportion of mobile phase flow was 1.5 ml/ min at room temperature. As a result, the peak emerged between 3-6 minutes has been affiliated with diflubenzuron. (Fig.). Quantitative calculation of diflubenzuron was performed standard protocol of WHO (http://www.WHO.int/WHOpes/quality/en/Diflubenzuron.pdf).

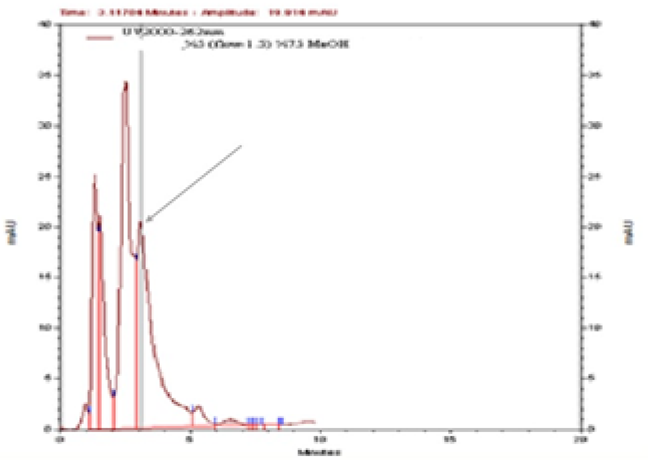

Figure. Peak of the diflubenzuron (arrow) by HPLC analyses.

Results and Discussions

According to the HPLC analysis, diflubenzuron deteriorated 30 % at the end of three months and 60 % at the six months. Therefore, the application dosage given by WHO (2006b) was reduced 30 % and 60 % accordingly, and then it applied to mixed mosquitos larvae to determine the effectiveness. In this aspect, the larvae were divided into 4 groups. No mortality has been observed in control group larvae (transition to pupas successfully occurred). In the second group which exposed to the application dose, the larvae mortality was 100 %. In the third group which were exposed to 30 % reduced dose, the larvae mortality was recorded as 80 % at the end of 4 days (96 hours). No mortality rate was determined in the fourth group (exposed to 60 % reduced dose) after 96 hours (Table). According to the results, the application dosage was notably effective on the mosquito populations of Ankara province. The differences between the second, third and fourth groups were found to be statistically significant according to ANOVA test ($p < 0.05$). This statistical result is mostly originated from the fourth group in which no mortality was observed. In spite of the application dosage in the present study is 25 g/h, the lower dosage such as 0.2 ppm (0.020 kg/ha) for both diflubenzuron and methoprene were found to eliminate 92-96 % *Culex quinquefasciatus* and *Aedes albopictus* larvae (Baruah and Das 1996). In consistent with this finding, when the considering 30 % reduced dosage, this dosage can cause 80 % mortality corresponding to 17.1 ai/h. The last dosage (69 % reduced) corresponds to 11.8 ai/h and has no efficacy on the larvae.

Table. Survival rate of larvae exposed to different diflubenzuron doses (G: Groups).

G	Doses	≠	Mortality rate (%)				
			24 h	48 h	72 h	96 h	Σ
1	Control	40	100	100	100	100	0
2	Application dosage (1.9 mg/0.15m^2)	40	30	80	90	100	100
3	30 % reduced dosage (1.3 mg/0.15m^2)	40	20	50	70	80	80
4	60 % reduced dosage (0.95 mg/0.15 m^2)	40	0	0	0	0	0

Our results confirmed that the residual activity of diflubenzuron lasted at least three months in the clean water (surface water without or very small amounts of organic load). However, it is a well-known fact that the residual activity strictly depends on the organic load of the water body. Other factor for efficient larvae managements in aquatic systems is water temperature that determine the development success of the larvae, the metamorphosis normally occurs up to two week in suitable water temperature (Rozendaal, 1997). The efficacy of diflubenzuron with different formulas have been studied on different Diptera larvae for different purposes (Schroeder, 1996; Baruah and Das, 1996; Romeo et al., 2009; Silva et al., 2009; Saleh et al., 2013; Djordjevic et al., 2015; Caputo et al., 2015).

Saleh et al. (2013) reported that slow-release tablet of diflubenzuron controlled the adult emergence of *Aedes aegypti* in the 10 weeks post-treatments. This effectiveness is consistent with our findings for *Culex Anopheles* larvae. The three diflubenzuron-based formulations (GR, TB and SC) were reported to be good efficacy on *Culex pipiens* and *Aedes albopictus* until minimum week 5 post-treatment in the water with organic load (Romeo et al., 2009). Two different granule formulations of 1% diflubenzuron (100g / h) were tested on *C. pipiens* larvae on surface water in Belgrade. These formula with different granule size were reported to be good residual effect on the lower larval stages, and it was also pointed out to the different effects on the larval stages (Djordjevic et al., 2015). Schroeder (1996) also stated that the diflubenzuron residue also affects reproduction of Coleopteran species (*D. abbreviatus*) from the leaves treated. These findings supported the broad ranges of residual efficacy on different insect larvae. Caputo et al.

(2015) also developed a new technique for mosquito control using the adhesive traps treated with diflubenzuron, and these traps have been successfully used to mosquito management in Italy. Apart from these sublethal concentrations of diflubenzuron (2-3.5 ppb) had negative effect on the fitness of *A. aegypti* (Silva et al. 2009). As a result, the diflubenzuron has residual efficacy on the larvae management, and its efficacy lasts up to three months in still and surface clean water. The application frequencies should be minimum in every two weeks and the suitable formula such as (slow-release tablet) can be chosen to use for long-term residual control instead of SC formula.

References

Adair R.C.J.R., Mehta N.K. 2001. Residual effects of micromite (diflubenzuron) treated citrus leaves on eggs deposited by *Diaprepes abbreviatus*. Proceedings of the Florida State Horticultural Society, 114: 101-106.

Ascher K.R.S., Nemy N.E. 1976. Contact activity of diflubenzuron against *Spodoptera littoralis* larvae. Pesticide Science, 7: 447-452.

Baruah I., Das S.C. 1996. Evaluation of Methoprene (Altosid) and Diflubenzuron (Dimilin) for control of mosquito breeding in Tezpur (Assam). Indian Journal of Malariology, 33: 61-66.

Caputo B., Ienco A., Manica M., Petrarca V., Rosa R., Della Torre A. 2015. New adhesive traps to monitor urban mosquitoes with a case study to assess the efficacy of insecticide control strategies in temperate areas. Parasite & Vectors, 8: 134-156.

Djordjevic M., Mirilovic M., Stajkovic N., Jankovic L., Pešic B., Bokonjic D. 2015. Statistical evaluation of the larvacidal effect of diflubenzuron on *Culex pipiens* larval stages. Acta Veterinaria, 65 (4): 496-509.

Grosscurt A. C. 1978. Diflubenzuron: some aspects of its ovicidal and larvacidal mode of action and an evaluation of its practical possibilities. Pesticide Science, 9 (5):373-386.

Levot G., Sales N. 2008. Resistance to benzoylphenyl urea insecticides in Australian populations of the sheep body louse. Medical and Veterinary Entomology, 22: 331-334.

Maduenho L.P., Martinez, C.B.R. 2008._Acute effects of diflubenzuron on the freshwater fish *Prochilodus lineatus.* Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology, 148 (3): 265-272.

Marrs, T.C. 2012. Toxicology of insecticides to mammals. Mini-Review Pest Manag Sci., 68(10):1332-1336.

Mulder R., Gijswijt M.J. 1973. The laboratory evaluation of two promising new insecticides which interfere cuticle deposition. Pestic. Sci., 4: 737-745.

Reynolds S.E. 1987. The cuticle, growth and moulting in insects: the essential background to the action of acylurea insecticides. Pesticide Science, 20: 131-146.

Romeo B., Alessandro A., Marco C., Roberta C., Luciano D., Maurizio M., Roberto P., Rodolfo V., Giancarlo C., Nazario, L. 2009. Efficacy and lasting activity of four IGRs formulations against mosquitoes in catch basins of northern Italy. European Mosquito Bulletin, 27: 33-46.

Rozendaal J.A. 1997. Vector Control-Methods for Use by Individuals and Communities. World health Organisation (WHO), Geneva, xii + pp 412.

Salokhe S.G., Deshpande S.G., Mukherjee S.N. 2012. Evaluation of the insect growth regulator lufenuron (Match[R]) for control of *Aedes aegypti* by simulated field trials. Parasitology Research, 111 (3):1325.

Schroeder W.J. 1996. Diflubenzuron residue: reduction of *iaprepes abbreviates* (Coleoptera: Curculionidae) neonates. Florida Entomologist, 79: 462-463.

Silva J. J., Mendes J., Lomonaco C. 2009. Effects of sub lethal concentrations of diflubenzuron and methoprene on *Aedes aegypti* (Diptera: Culicidae) fitness. International Journal of Tropical Insect Science, 29 (1): 17-23.

Van Daalen J.J., Meltzer J., Mulder R., Wellinga K. 1972. A New insecticide with a novel mode of action. Naturwissenchaften, 59: 312-313.

WHO, 2006a. Diflubenzuron. WHO specifications and evaluations for public health pesticides.

WHO, 2006b. Pesticides and their application. Sixth Edition, Department of Control and Neglected Tropical Diseases, WHO Pesticides evaluation scheme (WHOPES).

WHO, 2008. Diflubenzuron in drinking-water: Use for vector control in drinking-water sources and containers. Background document for preparation of WHO, Guidelines for drinking-water quality. Geneva.