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# RESEARCH ARTICLES

## EFFECTS OF DIFLUBENZURON AGAINST THE LARVAL STAGES OF *ANOPHELES (CELLIA) DIRUS* AND *ANOPHELES (CELLIA) MACULATUS* (DIPTERA : ANOPHELINAE)

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### ABSTRACT

*The larvicidal effects of Diflubenzuron (a chitin synthesis inhibitor) on the larval stages of four anopheline species,* Anopheles dirus *species A,* An. dirus *species B,* An. sawadwongporni *and* An. maculatus *form E were studied. The second instar larvae of the four species were the most sensitive instar, with the* $EC_{50}$ *value of 0.000340, 0.000349, 0.000498, and 0.00050 mg/l for* An. dirus *species A,* An. dirus *species B,* An. sawadwongporni *and* An. maculatus *form E respectively. The same instar larvae of* An. dirus *species A and* An. dirus *species B were more sensitive to Diflubenzuron than those of* An. sawadwongporni *and* An. maculatus *form E. Diflubenzuron induced various degrees of morphological abnormalities in all four anopheline species. Under laboratory conditions, the residual effect of Diflubenzuron at the concentrations of* $EC_{50}$ *on the same instar larvae of* An. dirus *species A and* An. maculatus *form E lasted for 20 to 28 days and 18 to 34 days respectively.*

### INTRODUCTION

Diflubenzuron [1-(4-chlorophenyl)-3- (2,6-difluorobenzoyl,) urea], or DFB, is one of the chitin synthesis inhibitors which belongs to the insect growth regulator compounds (IGRs). This compound acts as interference of normal development and blocks the mechanism of ecdysis in insects by inhibiting the chitin synthesis in the cuticle formation during moulting process.[1] It inhibits chitin synthetase - the final enzyme that synthesizes chitin from glucose. Thus, higher animals such as fishes, amphibian, birds, and mammals which do not produce chitin should not (or little if anything) be affected by this inhibitor.[2] Diflubenzuron induced a wide range of morphological abnormalities in *Culex pipiens fatigans* when the larval stages were exposed to this chemical.[3]

Mulla and Darwazeh[4] found that formulations of Diflubenzuron were effective on larval stages of four asynchronous mosquito species, i.e., *Culex pipiens quinquefasciatus* (under laboratory conditions), *Cx. peus*, *Cx. tarsalis*, and *Culiseta inornata* (under field conditions). The compound inhibited adult emergence for 15 to 18 days. Grosscurt[5] reported that Diflubenzuron provided a mean of controlling an organophosphorus - resistant strain *Cx. tarsalis*, a vector of encephalitis in California.

In Thailand, *Anopheles (Cellia) dirus* species A and species B are affirmed as the primary vectors of human malaria with the very high rate of infection of human malarial parasites.[6,7,8] *Anopheles (Cellia) maculatus sawadwongporni* and *An. maculatus* form E are widely distributed in the Oriental region. The *An. maculatus* form E has been recognized as an important vector of human malaria in some parts of Indonesia, Malaysian Peninsular and in southern Thailand.[7,9,10,11]

Since member species within the taxon of *An. dirus* and *An. maculatus* complex are important vectors of human malaria and there is no study on the effect of Diflubenzuron on them, it is therefore, the aim of this study to investigate the activity and longevity of Diflubenzuron against *An. dirus* species A, *An. dirus* species B, *An. sawadwongporni*, and *An. maculatus* form E.

## MATERIALS AND METHODS

### 1. Maintenance of mosquitoes

The mosquitoes used in the present study were *An. dirus* A, *An. dirus* B, *An. sawadwongporni*, and *An. maculatus* form E. These anopheline mosquitoes were colonized in the laboratory at the Center for Applied Malacology and Entomology, Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand. Since anopheline mosquitoes cannot copulate naturally under laboratory conditions, an artificial mating[12] technique was applied to induce copulation. The maintenance of mosquito eggs, larvae, pupae and adults in the present study was carried out under laboratory conditions at 28±2°C, 86±3 % RH, and 12-h light dark photophase. The temperature of water in rearing trays and bowls was 22° ± 1°C and the pH values ranged from 6.6 in the freshwater in which the eggs were floated to 6.9 in the pupal habitat.

### 2. Chemical

Diflubenzuron used for this study was a wettable powder formulation, with 25% active ingredient. The concentrations used and the effects of Diflubenzuron on different instar larvae of each mosquito species were expressed as mg A.I/l. One hundred mg of Diflubenzuron was dissolved in 1000 ml distilled water to yield a stock solution of 25 mg/l. From the stock solution, the designated concentrations were prepared by serial dilutions in distilled water.

### 3. Biological assay

The biological assay methods used in the present study was modified from those described by Ratanatham *et al.*[13] The $EC_{50}$ values and 95% confidence intervals within instars of all species were comparatively determined using probit analysis.[14]

Biological effects of Diflubenzuron were carried out by exposing lots of 25 larvae of each instar (2nd,3rd,4th) of each mosquito species in 500 ml glass beaker which contained 250 ml test solution. A series of concentrations chosen for the critical range were 0.00025, 0.0005, 0.001, 0.002, and 0.004 mg/l. Each concentration was replicated four times with concurrent controls. All live larvae were daily fed with a small amount of ground hamster food. Dead mosquitoes were recorded daily and preserved in 70% alcohol for their morphological observation.

### 4. Persistence of Diflubenzuron

The residual effects of Diflubenzuron were studied in glass beakers under laboratory conditions. At the concentrations of $EC_{50}$ of the second instar larvae, 0.00034 mg/l for *An. dirus* A and 0.00050 mg/l for *An. maculatus* form E, four replicates for each dose with untreated

control were prepared, and 25 second instar larvae of each mosquito species were used in each replicate. On daily basis, the larvae were fed and the dead specimens were removed. The mortality was recorded.

After all the treated larvae in the first batches died and the untreated larvae developed into adult stage, the second batches of 25 second instar larvae of each species were introduced into each of the above beakers. The introduction of larvae was continued until the treated batches had the same mortality rate as those in control ones.

Test with a control effect of 20% or more were unsatisfactory and repeated.

## RESULTS

### 1. Morphological changes induced by Diflubenzuron

Diflubenzuron induces delayed morphological changes in larval, pupal and adult stages. It was observed that the treated larvae which did not die during their development upto adult stage required longer time to develop than the untreated ones. In many cases, the larvae could not grow and develop normally, but they took double or more time than the controls to develop into adults. It was also observed that at the concentrations of 0.0002 and 0.0004 mg/l, most of the mosquitoes died as larvae when the second instar larvae were treated; they could not reach even the pupal stage.

The effects of Diflubenzuron on mosquitoes exhibited various degrees of morphological abnormalities. Dead specimens were grouped and modified according to those described by Ratanatham *et al.*[13] and they were classified into eight groups as follows:

Group 1. Normal larva. The larvae died before reaching the pupal stage with normal appearance (Fig. 1A).

Group 2. Deformed larva. The larvae died with abnormal appearance including those which had shorter bodies when compared to the normal ones (Fig.1C), and the twisted larvae (Figs. 1B).

Group 3. Black larva. The dead larvae with part of or whole body were black in color (Fig. 1D).

Group 4. Dead normal brown pupa. The dead pupae with normal figures and were brown in color (Fig. 2A).

Group 5. White pupa. The pupae died immediately after moulting before darkening and hardening of the cuticle. Thus, they were very pale in color which were similar to "albino type" described by Arias and Mulla[15] (Fig. 2B).

Group 6. Deformed pupa. The pupae with abnormal appearance (Fig. 2C).

Group 7. Adult attached to the pupal case. A wide range of adults were attached to the capsules. Some adults emerged only partly while most of their bodies still remained within the pupal cases except for the main trunks that could exuviate. Some adults were almost completely free from the pupal exoskeleton but their legs, tarsi or wings were still attached to the exuvia (Fig.2D).

Group 8. Normal adult. Adults emerged completely with normal appearance.

Morphogenetic effect of Diflubenzuron on the four *Anopheles* species treated at the dosage of $EC_{50}$ of their second instar larvae were also recorded (Table 1).

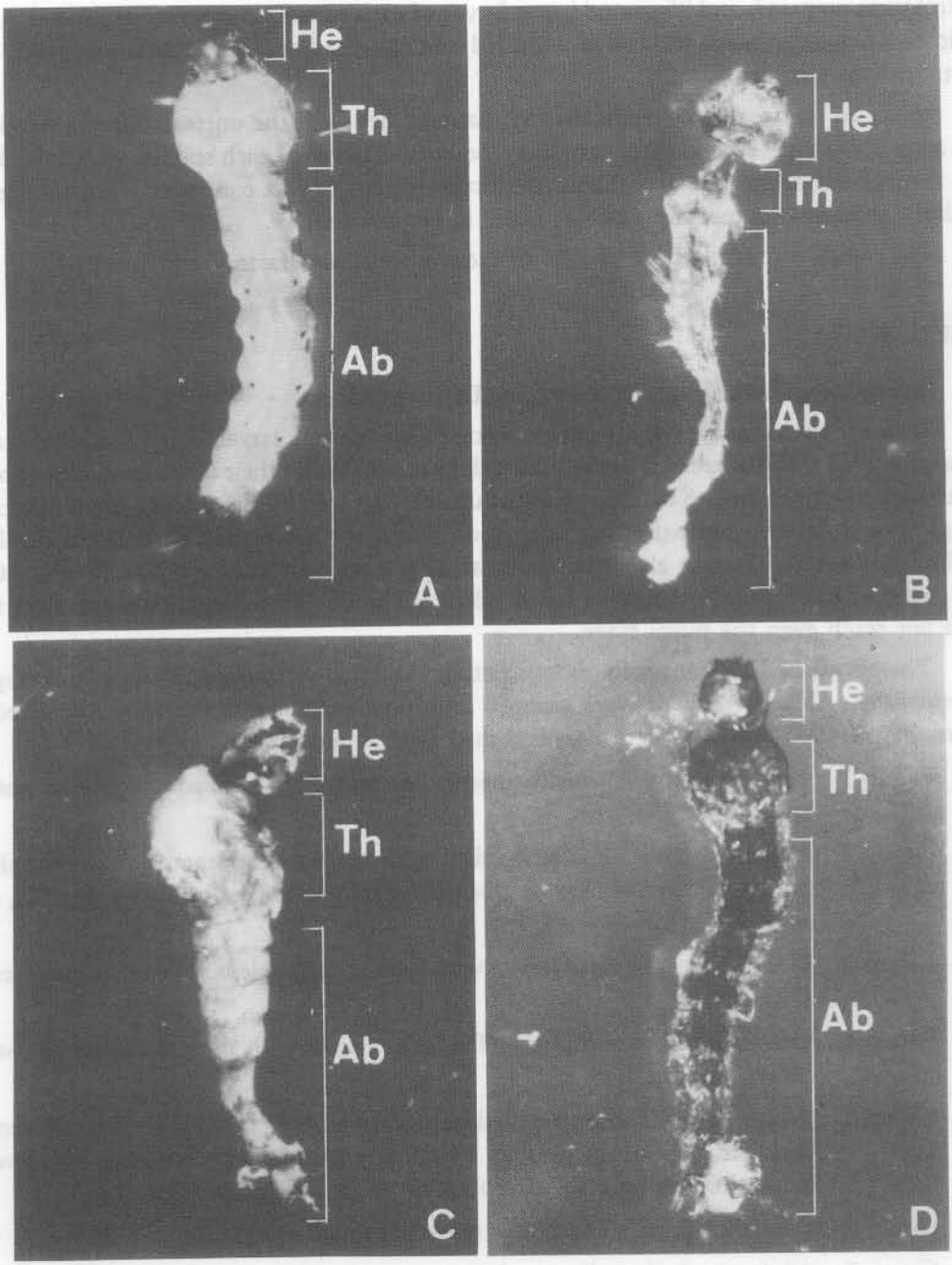


**Fig. 1.** Various forms of morphological aberration induced by Diflubenzuron in larvae of *An. dirus* species A: A, normal fourth instar larva; B and C, deformed fourth instar larvae; D, black larva showing deformed exoskeleton. (He = head, Th = thorax, Ab = abdomen; (A-D x 16).

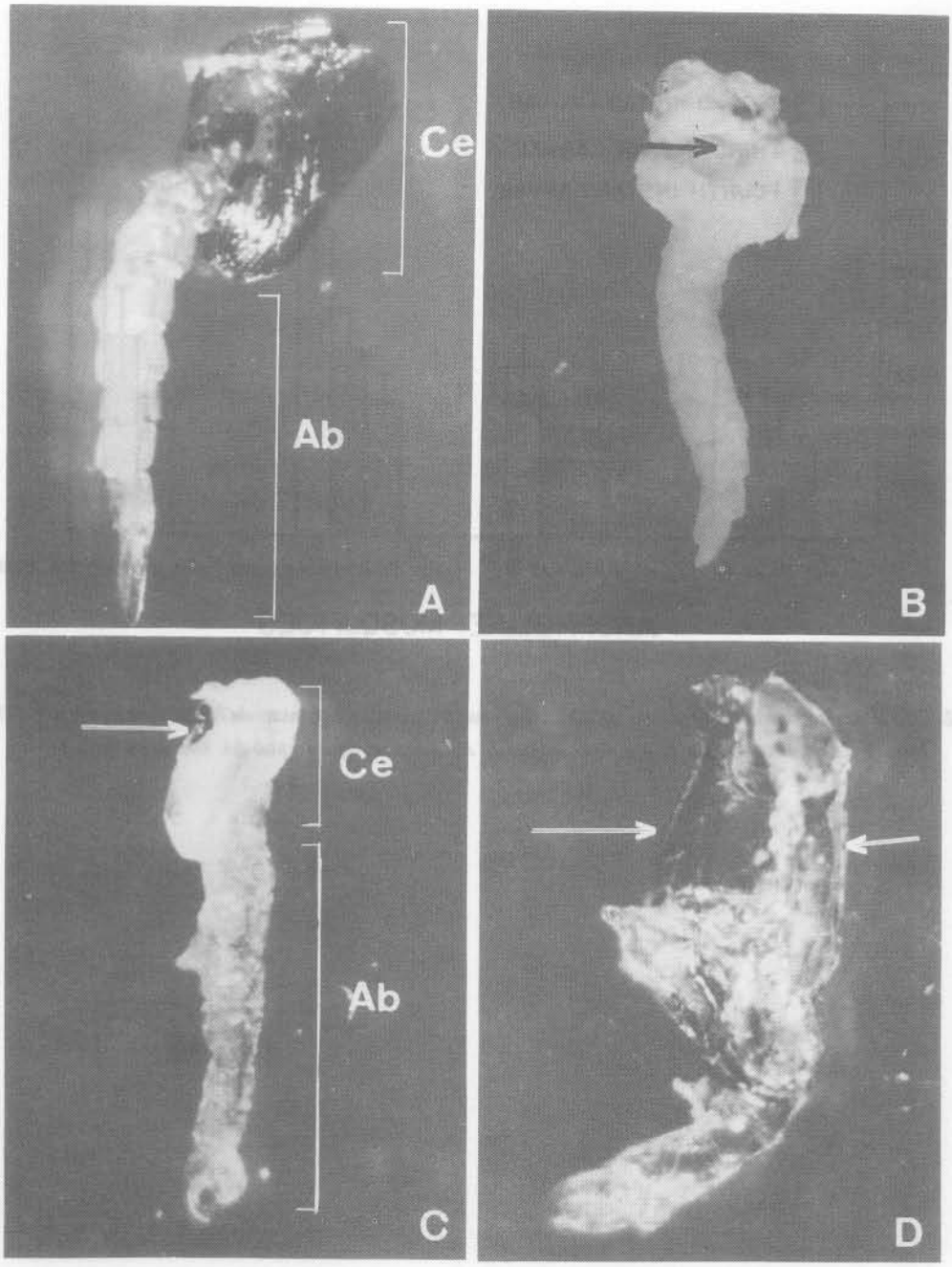


**Fig. 2.** Gradation of morphological changes induced by Diflubenzuron in pupae of *An. dirus* species A: A, normal appearance of pupa (Ce = cephalothorax, Ab = abdomen; B, white pupa showing deformed cephalothorax (arrow); C, deformed pupa showing abnormal cephalothorax (Ce) (arrow) (Ab = abdomen); D, adult attached to the pupal case (arrows = partly exuviated adult. (A-D x 16)

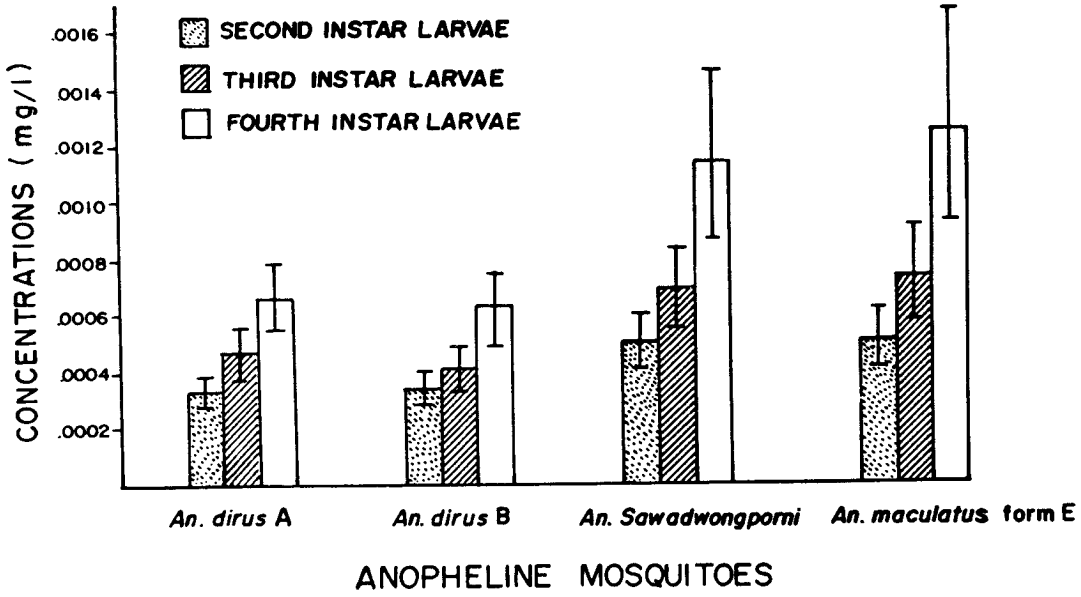


**Fig. 3.** The 50% effective concentrations ($EC_{50}$) as mg/l and 95% confidence intervals of Diflubenzuron to 3 different instars of *An. dirus* species A, *An. dirus* species B, *An. sawadwongporni*, and *An. maculatus* form E.

## 2. Toxic effects of Diflubenzuron on mosquito larvae

Mosquito mortalities varied and relatively changed with increased concentrations of Diflubenzuron. The majority of mosquito death was in the larval stage. The $EC_{50}$ value and 95% confidence limit, Probit regression line (Y) and chi- square test of Diflubenzuron against the second, third, and fourth instar larvae of all four anopheline species were tabulated (Table 2).

The $EC_{50}$ values when testing with the second instar larvae of *An. dirus* A, *An. dirus* B, *An. sawadwongporni* and *An. maculatus* form E were 0.00034, 0.00035, 0.00050, and 0.00050 mg/l, respectively. The $EC_{50}$ values for the third instar larvae were 0.00047 (*An. dirus* A), 0.00041 (*An. dirus* B), 0.00070 (*An. sawadwongporni*) and 0.00074 mg/l (*An. maculatus* form E). The $EC_{50}$ values for the fourth instar larvae were 0.00067 (*An. dirus* A), 0.00063 (*An. dirus* B), 0.00113 (*An. sawadwongporni*) and 0.00124 mg/l (*An. maculatus* form E). When the concentrations were increased, the larval mortalities increased correspondingly. At high concentrations of 0.002 and 0.004 mg/l, most of the mosquitoes died as larvae, whereas at lower concentrations, larval and pupal mortalities and incomplete emergence rates did not reveal any great differences.

The comparative studies of the $EC_{50}$ values of Diflubenzuron against the three different instar larvae of the four mosquito species were illustrated (Fig. 3). No significant difference was shown between the $EC_{50}$ values of Diflubenzuron against the second instar larvae of all species. Only a slight difference of the $EC_{50}$ values among the third instar larvae of all species was observed. On the other hand, there were marked differences between the $EC_{50}$ values of *An. dirus* A and *An. dirus* B, and those of *An. sawadwongporni* and *An. maculatus* form E. The same instar larvae of *An. dirus* A and *An. dirus* B were more sensitive to Diflubenzuron than *An. sawadwongporni* and *An. maculatus* form E.

## 3. Persistence of Diflubenzuron

The residual effect of Diflubenzuron on the second instar larvae of *An. dirus* A and *An. maculatus* form E, representing the two anopheline species complex, were tested (Tables 3, 4). The residual effects of Diflubenzuron at the $EC_{50}$ values of 0.00034 mg/l for *An. dirus* A and of 0.00050 mg/l for *An. maculalus* form E were 20-38 days and 18-34 days, respectively.

The mortality rates of the exposed larvae of *An. dirus* A decreased markedly from 54% for the first batch to 16% for the second batch (N=100). Similarly, the mortality rates of the exposed *An. maculatus* form E also decreased from 51% for the first batch to 14% for the second batch (N=100). The mortality rates of the third batches of larvae did not differ significantly between the control and the treated groups of both species.

## DISCUSSION

There were several morphogenetic changes in the anopheline mosquitoes induced by Diflubenzuron in the present study. The dead specimens were catagorized into eight major groups : dead normal larvae, deformed larvae, black larvae, dead normal brown pupae, white pupae, deformed pupae, adults attached to the pupal cases (exuvia), and dead as adult after separating from the exuvia. Hoying and Riedl[16] reported that larvicidal activity of the Diflubenzuron depended on continuous ingestion or extended contact of the insects. The present study shows that younger (second) instar larvae of both anopheline species, *An. dirus* complex and *An. maculatus* complex were more susceptible to Diflubenzuron than older instar larvae. In the present study, the distortion of cuticle in the treated specimen from all four species of anophelines were observed. This result agreed with Binnington[17] that Diflubenzuron

TABLE 1. Percentage morphogenetic effects of Diflubenzuron on the four anopheline species treated at the dosage of $EC_{50}$ of their second instar larvae.

| Morphogenetic groups | Mosquito species | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| 1. Normal larva | 19.5 | 21.5 | 24.4 | 26.2 |
| 2. Deformed larva | 8.0 | 5.0 | 6.0 | 4.0 |
| 3. Black larva | 6.0 | 5.5 | 4.5 | 5.0 |
| 4. White pupa | 1.8 | 1.4 | 1.3 | 1.5 |
| 5. Deformed pupa | 0.9 | 0.5 | 0.7 | 0.4 |
| 6. Brown pupa | 11.0 | 9.0 | 10.0 | 6.0 |
| 7. Adult attached to pupal case | 12.4 | 13.6 | 11.5 | 14.7 |
| 8. Normal adult | 40.4 | 43.5 | 41.6 | 42.7 |
| Total | 100 | 100 | 100 | 100 |

* 1 = *An. dirus* species A, * 2 = *An. dirus* species B

* 3 = *An. sawadwongporni*, * 4 = *An. maculatus* from E

TABLE 2. Effect of Diflubenzuron on larvae of *An. dirus* species A, *An. dirus* species B, *An.sawadwongporni*, and *An. maculatus* form E.

| Species/Stage | $EC_{50}$ (mg/l) | 95% confidence limits of $EC_{50}$ (Lower-Upper) | Regression equation (Y) | Chi-Square |
|---|---|---|---|---|
| ***An. dirus* A** | | | | |
| Second instar | 0.00034 | 0.00028-0.00041 | 3.80+2.26 x | 2.20 |
| Third instar | 0.00047 | 0.00038-0.00059 | 3.79+1.92 x | 1.70 |
| Fourth instar | 0.00067 | 0.00055-0.00082 | 3.80+2.08 x | 0.43 |
| ***An. dirus* B** | | | | |
| Second instar | 0.00035 | 0.00029-0.00040 | 3.85+2.19 x | 1.60 |
| Third instar | 0.00041 | 0.00034-0.00049 | 3.78+1.83 x | 0.93 |
| Fourth instar | 0.00063 | 0.00049-0.00080 | 3.75+1.71 x | 1.82 |
| ***An.* sawadwongporni** | | | | |
| Second instar | 0.00050 | 0.00041-0.00061 | 3.83+2.12 x | 1.43 |
| Third instar | 0.00070 | 0.00056-0.00087 | 3.79+1.99 x | 0.05 |
| Fourth instar | 0.00113 | 0.00087-0.00146 | 3.71+1.62 x | 0.30 |
| ***An. maculatus* form E** | | | | |
| Second instar | 0.00050 | 0.00042-0.00060 | 3.84+2.17 x | 1.02 |
| Third instar | 0.00074 | 0.00058-0.00091 | 3.75+1.78 x | 1.56 |
| Fourth instar | 0.00124 | 0.00092-0.00 168 | 3.76+1.87 x | 0.62 |

TABLE 3. Residual effect of Diflubenzuron to *Anopheles dirus* species A after continuous exposure at the dosage of 0.00034 mg/l with larval food.

| Group | Batches of larvae | Testing period (days) | Time taken from the first day of the test (days) | Number of testing larvae | Mosquito emergence | |
|---|---|---|---|---|---|---|
| | | | | | Mortality | (%) |
| Treated | 1 | 20 | 20 | 100 | 54 | (46) |
| | 2 | 18 | 38 | 100 | 16 | (84) |
| | 3 | 19 | 57 | 100 | 8 | (92) |
| Control | 1 | 20 | 20 | 100 | 9 | (91) |
| | 2 | 18 | 38 | 100 | 7 | (93) |
| | 3 | 19 | 57 | 100 | 9 | (91) |

TABLE 4. Residual effect of Diflubenzuron to *Anopheles maculatus* form E after continuous exposure at the dosage of 0.00050 mg/l with larval food.

| Group | Batches of larvae | Testing period (days) | Time taken from the first day of the test (days) | Number of testing larvae | Mosquito emergence | |
|---|---|---|---|---|---|---|
| | | | | | Mortality | (%) |
| Treated | 1 | 18 | 18 | 100 | 51 | (49) |
| | 2 | 16 | 14 | 100 | 14 | (86) |
| | 3 | 17 | 51 | 100 | 9 | (91) |
| Control | 1 | 18 | 18 | 100 | 10 | (90) |
| | 2 | 16 | 34 | 100 | 8 | (92) |
| | 3 | 17 | 51 | 100 | 9 | (91) |

prevented the formation of normal lamella appearance in procuticle and interfered with deposition of epicuticle.

The primary effect of Diflubenzuron might include, 1. acting competitively on the chitin synthetase, and some enzymes that were corporated with chitin synthesis,[18] 2. reducing the activity of pre-ecdysone metabolizing enzyme and caused both morphological and reproductive failures, presumably by causing deficiencies in JH titer,[18,19] and 3. reducing the amount of chitin and the tensile strength of cuticle.[20,21]

The larval mortality rates varied and relatively changed with increased concentrations of Diflubenzuron. When the concentrations were increased from 0.00025 mg/l to 0.004 mg/l, the mortality rates increased correspondingly.

Mulla and Darwazeh[4] studied the effects of Diflubenzuron on *Cx. pipiens quinquefasciatus*, *Cx. peus*, *Cx. tarsalis* and *Culiseta inornata*. They reported that the compound inhibited adult emergence for 15-18 days. Similar results were observed in the present study on both anopheline species. The residual effects of this chemical at their $EC_{50}$ values for *An. dirus* species A and *An. maculatus* form E under laboratory conditions were 20-38 days and 18-34 days, respectively. At the highest concentration of 0.004 mg/l, Diflubenzuron effectively blocked adult emergence of all four species of anopheline mosquitoes. Nevertheless, when mosquitoes were treated with Diflubenzuron in natural habitats, the effects and the persistence of the chemical were influenced by many factors, such as age, environmental conditions, formulation of the compound and the timing of application.[22] Diflubenzuron is stable between pH 2 and pH 8, unstable at pH 12, and is soluble in water at 1 mg/l. The half-life of Diflubenzuron in soil was up to 16 weeks.[23]

Diflubenzuron was readily degraded in various agricultural soils and in hydrosoil; 50% of the applied dose of 1 mg/kg was metabolized in two days or less.[2] Similar results were found by Schaefer and Dupras[24] who worked with two formulations of Diflubenzuron (25% wettable powder and 1% Attaclay granules). These two formulations did not persist in soil but showed limited persistence in water.

Diflubenzuron was very stable on foliage, but the agent showed much less tendency to build up in tissues of animals higher up in the food chain than in those of insects.[1] There had been many studies concerning the resistant development and cross-resistance to Diflubenzuron and the cross-resistance appeared at various levels when tested with many species of insects. [25,26,27]

In the present results, the residual effect of Diflubenzuron lasted more than 2 weeks in both species, *An. dirus* A and *An. maculatus* form E. Nevertheless, *An. dirus* complex species groups revealed more sensitivity to Diflubenzuron than *An. maculatus* complex species group.

From the results of this study and the evidence of the least toxicity of Diflubenzuron to animals higher up in the food chain and in the environment, it is worth suggesting, in conjunction with other suitable control strategies, that Diflubenzuron may be a potential compound to be used for the control of anopheline mosquitoes which are vectors of malaria.

## REFERENCES


1. Marx, J.L. (1977). Chitin synthesis inhibitors : new class of insecticides. *Science* **197**, 1170-1172.
2. Nimmo, W.B., De Wilde, P.C and Verloop, A. (1984). The degradation of Diflubenzuron and its chief metabolites in soils. Part I : Hydrolytic cleavage of Diflubenzuron. *Pest. Sci.* **15**, 574-585.
3. Sharma, V.P., Batra, C.P. and Brook, G.D. (1979). Laboratory and field evaluation of a growth - regulating compound (TH - 6040) against *Culex pipiens* fatigans (Diptera : Culicidae). *J. Med. Entomol.* **15**, 506-509.
4. Mulla, M.S. and Darwazeh, H.A. (1975). Activity and longevity of insect growth regulators against mosquitoes. *J. Econ. Entomol.* **68**, 791-794.
5. Grosscurt, A.C. (1978). Diflubenzuron : some aspects of its ovicidal and larvicidal mode of action and an evaluation of its practical possibilities. *Pest. Science* **9**, 373-386.
6. Baimai, V., Green, C.A., Andre, R.G., Harrison, B.A. and Peyton, E.L. (1984). Cytogenetic studies of some species complexes of *Anopheles* in Thailand and Southeast Asia. *Southeast Asian J. Trop. Med. Publ. Hlth.* **15**, 536-546.
7. Prasittisuk, C. (1985). Present status of malaria in Thailand. *Southeast Asian J. Trop. Med. Publ. Hlth.* **16**, 141-145.
8. Baimai, V., Kijchalao, U., Sawadwongporn, P. and Green, C.A. (1988). Geographic distribution and biting behaviour of four species of the *Anopheles dirus* complex (Diptera : Culicidae) in Thailand. *Southeast Asian J. Trop. Med. Pub. Hlth.* **19**, 151- 161.
9. Upatham, E.S., Prasittisuk, C., Ratanatham, S., Green, C.A., Rojanasunan, W., Setakana, P., Theerasilp, N., Tremongkol, A., Viyanant, V., Pantuwatana, S. and Andre, R.G. (1988). Bionomics of *Anopheles maculatus* complex and their role in malaria transmission in Thailand. *Southeast Asian J. Trop. Med. Pub. Hlth.* **19**, 259-269.
10. Green, C.A., Baimai, V., Harrison, B.A. and Andre, R.G. (1985). Cytogenetic evidence for a complex of species within the taxon *Anopheles maculatus* (Diptera : Culicidae). *Biol. J Linn.* **24**, 321-328.
11. Rattanarithikul, R. and Green, C.A. (1986). Formal recognition of the species of the *Anopheles maculatus* group (Diptera : Culicidae) occurring in Thailand, including the descriptions of two new species and a preliminary key to females. *Mosq. Syst.* **18**, 246-278.
12. Yang, C.K., Sta Maria, F.L. and Wharton, R.H. (1963). Maintenance of a laboratory colony of *Anopheles maculatus* Theobald by artificial mating. Mosq. News 23, 34-35.
13. Ratanatham, S., Rojanasunan W. and Upatham, E.S. (1994). Morphologicalaberrations induced by methoprene, juvenile hormone analogue, in *Anopheles dirus* s.s. and *An. sawadwongporni*(Diptera : Culicidae). *J. Sci. Soc. Thailand.* **20**, 171-182.
14. Finney, D.J. (1971). Probit analysis, 3rd ed. Cambridge University Press, London. 333 pp.
15. Aris, J.R. and Mulla, M.S. (1975). Morphogenetic aberrations induced by a juvenile hormone analogue in the mosquito *Culex tarsalis* (Diptera : Culicidae). *J. Med. Entomol.* **12**, 309-316.
16. Hoying, S.A. and Riedl, H. (1980). Susceptibility of the codling moth to Diflubenzuron diluted in three volumes of oils on boil weevil progeny. *J. Econ. Entomol.* **75**, 385-386.
17. Binningtion, K.C. (1985). Ultrastructure changes in the cuticle of the sheep flowfly, Lucillia, induced by certain insecticides and biological inhibitors. *Tissue Cell* **17**, 131-140.
18. Ker, R.F. (1978). The effects of Diflubenzuron on the growth of insect cuticle. *Pest Sci.* **9**, 265-295.
19. Yu, S.J. and Terriere, L.C. (1975). Activities of hormone metabolizing enzymes in house flies treated with some substituted urea growth regulators. *Life Sci.* **16**, 619-626.
20. Yu, S.J. and Terriere, L.C. (1977). Ecdysone metabolism by soluble enzymes from three species of diptera and its inhibition by the insect growth regulator TH- 6040. *Pest Bioch.* **7**, 48-55.
21. Hajjar, N.P. and Casida, J.E. (1978). Insecticidal benzoylphenyl ureas : structure-activity relationships as chitin synthesis inhibitors. *Science* **200**, 1499-1500.
22. Axtell, R.C., Dukes, J.C. and Edwards, T.D. (1979). Field test of Diflubenzuron, Methoprene, Flit MLO and Chlopyrifos for the control of Aedes taeniorhynchus larvae in diked dredged spoil areas. *Mosq. News* **39**, 527-529.
23. Phillips - Dupher, B.V. (1972). Experimental insecticides with a novel mode of action, pp 1-17. Technical information for testing purposes, Crop Protection Division, Wusp, 1230, The Netherlands.
24. Schaefer, C.H. and Dupras, E.F. Jr. (1977). Residues of Diflubenzuron [1 - (4 - chlorophenyl) - 3 - (2, 6 - difluorobenzoyl) urea] in pasture soil, vegetation, and water following aerial applications. *J. Agri. Food.* **25**, 1026-1030.

25. Cerf, D.C. and Georghiou, G.P. (1974). Cross resistance to an inhibitor of chitin synthesis, TH 60-40, in insecticide resistant strains of the house fly. *J. Agri. Food* **22**, 1145-1146.

26. Brown, T.M., DeVries, D.H. and Brown, A.W.A. (1978). Induction of resistance to insect growth regulators. *J. Econ. Entomol.* **71**, 233-229.

27. Furlong, M.J. and Wright, D.J. (1994). Examination of stability of resistance and cross-resistance patterns to acylurea insect growth regulators in field populations of diamondback moth *Plutella xylostella*, from Malaysia. *Pest. Sci.* **42**, 315-326.

## บทคัดย่อ

สารไดฟลูเบนซูรอน (สารยับยั้งการสร้างไคติน) มีฤทธิ์ฆ่าลูกน้ำยุงก้นปล่องทั้งสี่สปีชีส์ (*Anopheles dirus* species A, *An. dirus* species B, *An. sawadwongporni* และ *An. maculatus* form E) สารไดฟลูเบนซูรอนมีฤทธิ์ฆ่าลูกน้ำยุงระยะที่สองมากที่สุด และมีค่า EC50 ต่อยุง *An. dirus* species A, *An. dirus* species B, *An. sawadwongporni*, และ *An. maculatus* form E เท่ากับ 0.00034, 0.000349, 0.000498 และ 0.00050 mg/l ตามลำดับ กลุ่มยุงก้นปล่องไดรัสมีความไวต่อฤทธิ์ของสารดังกล่าวมากกว่ากลุ่มยุงก้นปล่องแมคคูลาตัส ซึ่งอยู่ในระยะเดียวกัน สารไดฟลูเบนซูรอนชักนำให้เกิดความผิดปรกติของยุงแบบต่างๆ ในทั้ง สี่สปีชีส์ ฤทธิ์ตกค้างของสารไดฟลูเบนซูรอนที่มีต่อลูกน้ำยุงระยะที่สองของ *An. dirus* species A และ *An. maculatus* form E คือ 20-28 วัน และ 18-34 วันตามลำดับ