LABORATORY EVALUATION OF ANDALIN, AN INSECT GROWTH REGULATOR INTERFERING WITH CUTICLE DEPOSITION, AGAINST MOSQUITO LARVAE

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Abstract

Andalin, a benzoylphenylurea (BPU) derivative, was evaluated on *Culex pipiens* L. (Diptera: Culicidae). Treatment was made on newly 3rd- and 4th instar larvae for 24 h. The compound exhibited insecticidal activity and mortality occured after earlier inhibition of their development or by their inability to complete their ecdysis. Treatment resulted in a significant larvicidal effect and in a inhibition of adult emergence. Moreover, the compound disturbed growth and development since several morphological types and an increase in the duration of larval stage were observed. Histological study conducted on 4th instar larval integument, showed that Andalin caused a significant reduction in the thickness of cuticles secreted compared to controls. Thus, Andalin prevent molting in *C. pipiens* by interfering with cuticle deposition confirming the primary mode of action of this BPU insecticide.

Key words: *Culex pipiens, mosquito, control, Andalin, flucycloxuron, development, cuticle.*

Résumé

Andalin, un dérivé de la benzoylphenylurée (BPU), a été évalué su*r Culex pipiens* L. (Diptera: Culicidae). Les traitements ont été réalisés pendant 24 h sur les larves nouvellement exuviées des troisième et quatrième stades. Le composé manifeste une activité insecticide et la mortalité est due, soit à une inhibition précoce du développement, soit à une exuviation incomplète. Le traitement provoque une mortalité larvaire significative et une inhibition de l'émergence des adultes. De plus, il perturbe la croissance et le développement. On observe plusieurs types morphologiques et un allongement de la durée du stade larvaire. L'étude histologique, menée sur le tégument du quatrième stade larvaire, montre que l'Andalin cause une réduction significative des épaisseurs des cuticules secrétées comparativement aux témoins. Ainsi, l'Andalin empêche le processus de mue chez *C. pipiens* par interférence avec le dépôt de la cuticule, confirmant le mode d'action primaire de cet insecticide dérivé de la BPU.

Mots clés: *Moustique, Culex pipiens, lutte, Andalin, flucycloxuron, developpement, cuticule.*

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ملخص

الأندلين, مشتق من مجموعة البنزويلفينيليري BPU, تم تقييمه على البعوض من نوع *Culex pipiens* L. (Diptera Culicidae). تمت المعالجة وهذا لمدة 24 ساعة على يرقات الطور الثالث والرابع حديثة الانسلاخ. إن المركب يظهر نشاط مبيدي وموت إما ناتجة عن تثبيط مبكر للنمو, أو انسلاخ غير كامل. إن المعالجة تسبب موت معنوية وتثبيط لخروج الكاملات. زيادة على هذا يحدث اضطراب للنمو والتطور. كما نلاحظ عدة ظواهر مرفولوجية و زيادة في مدة الطور اليرقي. إن الدراسة النسيجية التي تمت على جليد الطور الرابع, توضح أن الأندلين يسبب نقص معنوي في سمك الجليد المفرز مقارنة مع الشاهد. كذلك الأندلين يمنع عملية الانسلاخ عند *C.pipiens* بتداخل مع تراكم الجليد, الذي يؤكد طريقة التأثير الأولي لهذا المبيد المنشق من BPU.

الكلمات المفتاحية: *Culex pipiens, بعوض, مكافحة, الأندلين, flucycloxuron, نمو, جليد.*

Because of secondary effects of conventional insecticides in secondary effects of conventional insecticides in environment, the of conventional insecticides in environment, the insect growth regulators (IGRs) have shown promise in controlling insects of agricultural, medical and veterinary importance. Among these compounds the benzoylphenyl urea derivatives (BPUs) prevent molting in insects by interfering with cuticle deposition and are considered as inhibitors of chitin biosynthesis [1, 2]. Diflubenzuron (DFB), the first insecticide based on interference with cuticle deposition [3], have been the subject of intensive investigation. It was found to be effective on several insect species [4-7]. Since the introduction of DFB, a number of other BPU derivatives have been developped such as Flucycloxuron (FCX) and Triflumuron (TFM). These two compounds have recently been found to interfere with chitin biosynthesis [8-10]. Other IGRs were reported to inhibit ecdysteroid synthesis [11, 12] or to mimic the action of 20-hydroxyecdysone [13]. Several compounds have been tested on mosquito species other than *Culex pipiens* L. [14-20]. In Algeria, mosquitoes are generally controlled by conventional insecticides. Thus, some IGRs were tested against *C. pipiens* under laboratory conditions. Alsystin, the common name of TFM, was found to disturb the growth and development in *C. pipiens* [21, 22] while RH-0345, a non steroidal ecdysteroid agonist belonging to a new class of compounds, benzoylhydrazines, was reported to induce a precocious and incomplete moult [23]. Therefore, the aim of the present study was (a) to evaluate the effectiveness of Andalin, a novel BPU derivative, on larvae of *C. pipiens* under laboratory conditions and (b) to examine its effects on the cuticle secretion.

MATERIAL AND METHODS

Rearing method

Third and fourth instar larvae of *C. pipiens* were collected from a stock colony and kept in pyrex storage jars (80 by 100 mm) containing 200 ml of tap water as previously described [21]. The containers were held at 25 °C and photoperiod of 14: 10 (L:D). Daily larvae were supplied with fresh food which consists in a mixture of Biscuit Petit Régal-dried yeast (75/25 by weight) and water was replaced every four days.

Chemical

Andalin, the common name of flucycloxuron, was kindly provided by Solvay Duphar B.V. (The Netherlands). A dispersible concentrate formulation (250 g active ingredient/l) was used. Serial dilutions were prepared in distilled water and appropriate aliquots (0.1-1.0 ml) were added to the treatment beakers to give the following final concentrations: 14, 21, 28, 41 and 83 ng/l.

Insecticidal bioassay

The tests were conducted on third and fourth instars of *C. pipiens* larvae. Newly ecdysed larvae were exposed to 5 concentrations ranging from 21 to 83 ng/l for 24 h. Control larvae were reared in jars containing only water. After exposure, the larvae were removed, washed with untreated water and placed in clean water. The insecticidal assay was conducted with three replicates each of 25 larvae. The insects were examined daily until adult emergence. The percentage mortality obtained at various stages of development was corrected [24] and toxicity data were studied by probit analysis [25]. IC50 values (50% inhibition concentration), confidence limits and slope of concentration-mortality lines were calculated by the method of Swaroop [26]. The effects on exuviation (larval, pupal or adult exuviation) and duration of each instar were also recorded.

Histology

In order to take observations on the cuticle secretion, two doses (21 and 83 ng/l) were tested on newly ecdysed fourth instar larvae of *C. pipiens*. Histological techniques were conducted according to Martoja & Martoja [27]. Larvae were sampled from control and treated series at various times during the larval development. Then the abdomens were dissected and fixed in Bouin's solution. Finally, sections (5-7 µm) were stained with azocarmin-anilin and measurements made on sternal cuticle from transverse sections of abdomen. The thickness of both larval cuticle and the pre-ecdysial pupal cuticle was determined on five different larvae of the same age in treated and control series and averaged.

Statistical analysis

Data from insecticidal tests were subjected to analysis of variance after angular transformation of corrected mortality percentages. When the analysis of variance was significant ($p < 0.05$), mean values obtained were compared by Duncan's multiple range test at 5% level. In further experiments, data were calculated to be significant by the Student's t-test at 5% level.

RESULTS

Effect on growth and development

Andalin applied for 24 h to newly ecdysed larvae of *C. pipiens* affected growth and development. Morphological examinations of insects after treatment showed varying degrees of morphological aberrations depending upon the importance of larval-adult transformation. Moreover, larvae exposure to Andalin for 24 h resulted also in a significant increase in the duration of both third and fourth larval instars compared to untreated insects (Tables 1 & 2). However, there was no significant ($p > 0.05$) effect of Andalin in the duration of pupal development between control and treated series.

Concentrations (ng/l)	Duration (days)		
	3rd larval instar	4rd larval instar	pupal stage
0	4.1 ± 0.2 a	11.5 ± 1.1 a	2.8 ± 0.2 a
14	7.5 ± 0.6 b	14.5 ± 0.8 b	2.9 ± 0.1 a
21	6.9 ± 0.3 b	14.0 ± 0.7 b	2.9 ± 0.1 a
28	6.3 ± 0.5 b	13.8 ± 1.7b	2.9 ± 0.1 a
41	6.2 ± 0.8 b	12.8 ± 1.2 b	2.8 ± 0.1 a
83	6.2 ± 1.1 b	12.4 ± 0.8 b	2.9 ± 0.1 a

Table 1: Effect of Andalin applied to newly ecdysed 3rd instar larvae of *C. pipiens* on the duration of development (m±s, n= 21-75). For each stage, values followed by the same letter are not significantly different at the 5 % level by Duncan's multiple range test.

Concentrations (ng/l)	Duration (days)	
	4rd larval instar	pupal stage
0	11.4 ± 1.5 a	2.7 ±0.1 a
14	14.3 ± 0.9 b	2.7 ± 0.1 a
21	13.8 ± 0.3 b	2.8 ± 0.1 a
28	15.3 ± 1.1 b	2.8 ± 0.2 a
41	14.7 ± 0.6 b	2.8 ± 0.1 a
83	13.6 ± 0.9 b	2.8 ± 0.2 a

Table 2: Effect of Andalin applied to newly ecdysed 4th instar larvae of *C. pipiens* on the duration of development (m±s. n= 20-50). For each stage. values followed by the same letter are not significantly different at the 5 % level by Duncan's multiple range test.

Insecticidal activity

Andalin applied for 24 h to newly ecdysed larvae of *C. pipiens* exhibited insecticidal activity with a dose-response relationship. Mortality occured after earlier inhibition of their development or by their inability to complete their ecdysis. Moreover, this compound presented a toxicity by direct action on the treated larval instars but also by differed action on the other following stages of development (Tables 3 & 4). There was a significant larvicidal effect on both third instar ($p < 0.001$) and fourth instar larvae ($p < 0.01$). The mortality varied as a function of

Types of ecdysis	Doses (ng/l)				
	14	21	28	41	83
Larval ecdysis	29.5 ± 2.1	33.7 ± 2.2	39.1 ± 2.9	47.5 ± 4.1	57.1 ± 4.9
Pupal ecdysis	3.9 ± 0.3	13.6 ± 1.9	14.1 ± 0.7	18.0 ± 0.8	15.8 ± 0.4
Adult ecdysis	0.0 ± 0.0	1.3 ± 0.2	1.3 ± 0.2	2.7 ± 0.1	1.3 ± 0.1
Total	33.4 ± 2.8	48.6 ± 7.8	54.6 ± 2.9	68.2 ± 3.2	74.2 ± 1.9

Table 3: Effects of Andalin applied to newly ecdysed 3rd instar larvae of *C. pipiens* on the inhibition of different ecdysis until adult emergence (mean of corrected percentages ± s. established on three replicates each of 25 larvae).

Types of ecdysis	Doses (ng/l)				
	14	21	28	41	83
Pupal ecdysis	24.2 ± 2.6	38.0 ± 1.3	14.1 ± 0.7	45.4 ± 4.2	51.5 ± 6.1
Adult ecdysis	4.3 ± 1.2	4.5 ± 0.8	1.3 ± 0.2	14.1 ± 1.4	17.4 ± 1.5
Total	28.5 ± 7.9	42.5 ± 7.5	54.6 ± 2.9	59.5 ± 5.9	58.9 ± 5.9

Table 4: Effects of Andalin applied to newly ecdysed 4th instar larvae of *C. pipiens* on the inhibition of different ecdysis until adult emergence (mean of corrected percentages ± s. established on three replicates each of 25 larvae).

the larval instar treated. However, death as larvae was relatively important compared to the mortality recorded for the other stages of development (Tables 3 & 4). Comparison of responses of 3rd (Inhibition of larval ecdysis) and 4th instars (Inhibition of pupal ecdysis) of *C.pipiens* larvae according to their IC50 values indicated that Andalin was more toxic to 3rd instar larvae (IC50= 52.7 ng/l; Fiducial limits = 29.4-94.4 ng/l; Slope= 10.6) than to 4th instar larvae (IC50= 94.8 ng/l; Fiducial limits = 49.5-181.6 ng/l; Slope= 13.9). The inhibition of exuviation was also recorded at various developmental stages, following treatment until adult emergence. Results revealed that the compound produced a dose-dependent reduction in adult emergence when treatment was made on 3rd (IC50= 23.9 ng/l; Fiducial limits = 15.9-35.8 ng/l; Slope= 5.1) or on 4th (IC50= 31.1 ng/l; Fiducial limits = 20.4-47.3 ng/l; Slope= 5.5) instars larvae. Andalin treatment on 3rd instar larvae appeared more efficient.

Effect on the cuticle deposition

The histological study of sternal cuticles from 4th instar larvae of *C.pipiens* showed that Andalin treatment affected the cuticular secretion. In controls, the thickness of larval cuticle, e.i. the old cuticle, increased until apolysis (day 4) to reach a maximum of about 3 µm. The pre-ecdysial pupal cuticle, i.e. the new cuticle, was secreted starting day 4 after larval ecdysis in control series (Fig. 1A). Andalin applied at two concentrations (21 and 83 ng/l) to newly ecdysed 4th instar larvae reduced the cuticle thickness (Fig. 1B & 1C). According to the doses tested, the compound led to a 43 to 44% decrease in the thickness of the larval cuticle measured at apolysis and 34 to 69% decrease in the thickness of the pre-ecdysial pupal cuticle measured just before the pupal ecdysis compared to respective controls. This reduction in the cuticle thickness was significant with two doses tested. In addition, the effect of Andalin on the cuticle deposition appeared more marked on the new cuticle since the decrease in the cuticle thickness was significant in a dose-response relationship. In treated series the apolysis occured also at day 4 and the pupal ecdysis was slightly delayed.

DISCUSSION

Morphological effects

The BPUs have been the subject of intensive investigations and are considered as inhibitors of chitin biosynthesis [1]. Andalin has not been tested on *C. pipiens*. As reported previously in *Tenebrio molitor* pupae [8], Andalin was found to disturb the growth and development in *C. pipiens*. The morphogenetic aberrations observed after exposure *C.pipiens* larvae to this compound were similar to those commonly induced by other BPUs in several insect species [5, 6, 9, 18, 20]. Moreover, treatment resulted also in an increase of the duration of development of larval instars tested confirming the effects induced by this class of insecticides [6, 9, 28].

Insecticidal activity

The bioassays conducted on *C. pipiens* indicated that Andalin exhibited insecticidal activity when applied to newly ecdysed larvae for 24 h. Mortality occurred after earlier inhibition of the development or by failure in ecdysis during the different stages of development. Andalin proved to be highly efficient to larvae of *C. pipiens* since the IC50

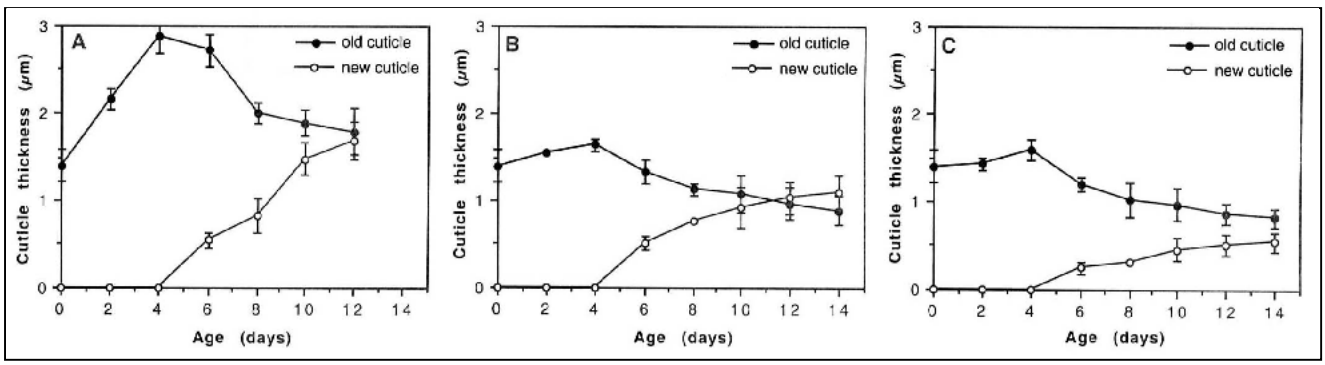

Figure 1: Effect of Andalin applied to newly ecdysed fourth instar larvae of *C. pipiens* on the cuticle thickness in controls (**A**) compared to that in 21 (**B**) and 83 ng/l (**C**)-treated series, respectively (m ± s, n= 5).

values against 3rd- and 4th-instar larvae after 24 h exposure were found to be about 52 and 94 ng/l, respectively. In our study, the compound appeared less effective on older larval stages of *C. pipiens* as observed after treatment of several species with other BPUs [4, 29]. Our results on C. pipiens showed also that Andalin at a concentration of 83 ng/l applied to newly ecdysed larvae for 24 h, resulted approximately in a 68 to 74 % inhibition of adult emergence according to the larval instar treated. In a insecticidal experiment conducted with Alsystin on 4th instar larvae for 24 h completely inhibited normal emergence of adult in *C. quinquefasciatus* at a concentration of 50 µg/l [16] and in *C. quinquefasciatus*, *C. tarsalis*, *Aedes taeniorhynchus*, *A. nigromaculis* at concentrations ranging between 1 and 40 µg/l [17].

Effect on cuticle secretion

Andalin was found to interfere with chitin biosynthesis as demonstrated by inhibition of the incorporation of radiolabelled precursor into chitin in *Spodoptera littoralis* [30] and in *T. molitor* [8]. Histological examinations done on 4th instar larvae of *C. pipiens* exposed for 24 h to two concentrations of Andalin revealed reduction in the thickness of secreted cuticles as compared to untreated series. The compound affected both the old- and the new cuticle secreted during the 4th larval instar. These observations are in agreement with those commonly reported after treatment by BPU insecticides [1, 8, 9, 21, 31]. Thus, Andalin exhibited insecticidal activity in *C. pipiens* and mortality occured by interference with cuticle deposition confirming the primary mode of action of the BPU insecticides. The comparison of the potencies of some BPU insecticides against mosquitoes showed that Andalin appeared more toxic against mosquito larvae than Alsystin.

Conclusively, Andalin prevent molting in *C. pipiens* by interfering with cuticle deposition confirming the primary mode of action of this BPU insecticide.

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