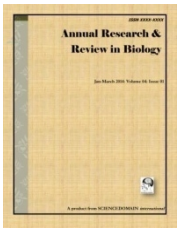

Annual Research & Review in Biology
4(14): 2263-2273, 2014

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Laboratory Evaluation of a Biorational Insecticide, Kinoprene, against *Culex pipiens* Larvae: Effects on Growth and Development

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Authors' contributions

This work was carried out in collaboration between both authors. Author NS conceived and designed the experiments. Authors KH and NS performed the experiment, analyzed the data and wrote the manuscript. Both authors read and approved the final manuscript.

Original Research Article

Received 25th February 2014
Accepted 23rd March 2014
Published 31st March 2014

ABSTRACT

Aims: In the present study, the objective was to estimate the toxicity of an insect growth regulator with juvenile hormone-like activity, kinoprene, on *Culex pipiens*, the most abundant and investigated mosquito species. Effects of this compound on growth and development were also evaluated.
Methodology: A commercial formulation (Enstar 65% EC) was tested at different concentrations ranging between 162.5 and 650µg/L on newly molted fourth-instar larvae that were exposed for 24 h under standard laboratory conditions according to World Health Organization recommendations.
Results: Kinoprene exhibited insecticidal activity by direct action on the treated fourth-instar larvae but also by differed action on the other following stages of development. Mortality occurred after earlier inhibition of their development or by their inability to complete their ecdysis. The LC_{50} values were 1287.4µg/L for the direct action on fourth instar larvae, and 246.8µg/L for the differed action until adult emergence. Moreover, the compound disturbed growth and development since several morphological types and an increase in the duration of larval and pupal stages were recorded. In a second series of experiments, the effects of kinoprene were examined on morphometric measurements of

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larvae, pupae and adults, respectively. The compound affected body volume.
Conclusion: The overall results are discussed in relation to previous reports and suggested that kinoprene have potential as a biorational insecticide for controlling of mosquitoes in an environmentally-friendly manner to the aquatic ecosystem.

Keywords: Mosquitoes; Culex pipiens; juvenile hormone analog; kinoprene; toxicity.

1. INTRODUCTION

Culex pipiens L. represents one of the most investigated organisms and is considered the first vector of West Nile Virus in Europe [1]. In Algeria, *Cx. pipiens* is the most abundant mosquito species, particularly in urban areas [2,3,4] and is generally controlled by conventional insecticides such as organophosphorous, carbamate and pyrethroids. However, these conventional neurotoxins possess strong secondary effects on the environment [5]. In this context, the insect growth regulators (IGRs) can be considered as an alternative source to conventional insecticides because of their specific activity against insect pests and their minimal toxic effects on the environment and human health [6,7]. In previous reports, we have tested some IGRs for mosquito control such as chitin synthesis inhibitors [2,8,9] or molting hormone agonists [10,11,12,13].

The sesquiterpenoid juvenile hormones (JH) are known to regulate many developmental and reproductive processes in insects [14,15,16,17]. Any interference with the two principle hormones, JH and moulting hormone, results in abnormal or detrimental growth, development, and reproduction [16]. Recently, some juvenile hormone analogues (JHA) have been tested. Thus, the larvicidal activity of (S)-methoprene was studied against *Aedes albopictus* under laboratory and field conditions [18], while pyriproxyfen was investigated on the susceptibility of freshly and embryonated eggs of *Aedes albopictus, Aedes aegypti, Aedes atropalpus* and *Culex pipiens* [19]. The information regarding the effectiveness of kinoprene a JHA are scare as compared to other mimics such as methoprene, fenoxycarb, pyriproxyfen or diofenolan [14].

In addition, they have not yet been tested against mosquito species in Algeria. Therefore, in the present study conducted under laboratory conditions on *Cx. pipiens*, a medically important mosquito species, we assessed the toxicity of kinoprene, a JHA, against larvae by determining the lethality parameters. In addition, we examined its effects on growth, development and morphometric measurements. The data obtained provide additional information on its toxicity and discuss its potential for use as a mosquito control agent.

2. MATERIALS AND METHODS

2.1 Mosquito Rearing

Culex pipiens L. eggs and larvae were collected from untreated areas located at Sedrata, 36º7'42" N, 7º31'53" E, Souk-Ahras 36º17'11" N, 7º57'4" E. Larvae specimens were morphologically identified according to [20] and kept as previously described [2]. Pyrex storage jars (80 by 100mm) containing 150 ml of tap water were maintained at temperature 25ºC and a photoperiod of 14:10 (L:D). Larvae were daily fed with fresh food consisting of a mixture of Biscuit Petit Regal-dried yeast (75:25 by weight), and water was replaced every

four days. Adult females were fed with pigeon blood and the eggs raft produced were used for stock-rearing generations.

2.2 Toxicity Bioassay

Bioassays were conducted as previously described [9]. Enstar (65% EC, Wellmark International Inc., IL, USA) courtesy of Pr. G. Smagghe (Ghent University, Belgium) a trade formulation of kinoprene was added to treatment beakers at different final concentrations (162.5, 325, 487.5 and 650µg active ingredient per liter). Newly ecdysed fourth-instar larvae of *Culex pipiens* (<8h) were exposed to the different concentrations for 24h in accord with World Health Organization (WHO) criteria [21]. Controls were exposed to water only. After the exposure time of 24h, larvae were removed, washed with untreated water and placed in clean water. The test was carried out with 4 replicates containing each 25 larvae per concentration. Growth and development was examined and mortality was registered daily until adult emergence. The mortality percentage obtained was corrected [22] and toxicity data were studied by probit analysis [23]. Lethal concentrations (LC_{50} and LC_{90}) and 95% confidence limits (95% CL) were estimated, and slope of the concentration-mortality lines were calculated [24].

2.3 Morphometric Measurements

As above, newly moulted fourth instar larvae were treated with kinoprene at its LC_{50} and LC_{90} as determined before. The body size was recorded by measuring under a dissecting microscope the width of the thorax in larvae, and the wing length in adults as previously reported [11,4] on 3 replicates of 10 individuals from each stage (larvae, pupae and adults) and the body weight of individuals from different instars was also determined. The body size of larvae and adults was estimated by the cubic value of thorax width, and wing length, respectively [25].

2.4 Larval and Pupal Duration and Morphogenetic Aberrations

Kinoprene was applied on newly ecdysed fourth-instar larvae of *Cx. pipiens* at two concentrations: 246.8µg/L and 524.44µg/L corresponding to LC_{50} and LC_{90}, respectively. From the start of the experiment, the larvae were examined daily for aberrations and survival until adult emergence. The effect was also estimated on the duration of both larval and pupal development. The test was carried out with 4 replicates containing 10 individuals.

2.5 Statistical Analysis

The number of individuals tested in each series is given with the results. Data are presented as the mean ± standard deviation (SD).The significance between different series was tested using Student's *t* test. All statistical analyses were performed using MINITAB Software (Version 16, PA State College, USA) and $p \leq 0.05$ was considered to be a statistically significant difference.

3. RESULTS

3.1 Insecticidal Activity

Kinoprene applied for 24 h to newly ecdysed fourth-instar larvae of *Cx. pipiens* exhibited insecticidal activity with a dose-response relationship. Dose-response relationship was determined by direct action on the fourth-instar larvae (Fig. 1) and also by differed action on the other following stages of development until adult emergence (Fig. 2). The highest concentration tested 650 µg/L caused 10±4.90% mortality on fourth instar larvae against 98.00±4.62% mortality when scored until adult emergence (Table 1). With probit, the following LC_{50} were calculated: 1287.4µg/L for the direct action on fourth-instar larvae, and 246.8µg/L for the differed action until adult emergence (Table 2).

Table 1. Efficacy of kinoprene applied on fourth-instar larvae of *Cx. pipiens*: corrected mortality (m±SD, n=4 repeats each containing 25 individuals)

Doses (µg/L)	162,5	325	487,5	650	p
Mortality (%)*	1.75±0.96	2,25±1,70	4.00±2.16	10±4.90	p<0.05
Mortality (%)**	32.00±12.20	54.00±2.31	81.00±10.58	98.00±4.62	p≤0.001

**: Mortality scored against fourth-instar larvae; **: Mortality scored until adult emergence*

Table 2. Efficacy of kinoprene against fourth-instar larvae of *Cx. pipiens*: probit analysis

Regression curve	Slope	R^2	LC_{50} (µg/L) (FL 95%)	LC_{90} (µg/L) (FL 95%)
Y=1.83 X-0.69*	3.49	0.756	1287.4 (876.27-1891.44)	6446 (3420.58-12146.71)
Y=3.91 X- 4.36**	1.79	0.877	246.8 (206.13-295.47)	524.44 (395.16-695.99)

**: Mortality scored against fourth instar larvae; **: Mortality scored until adult emergence*

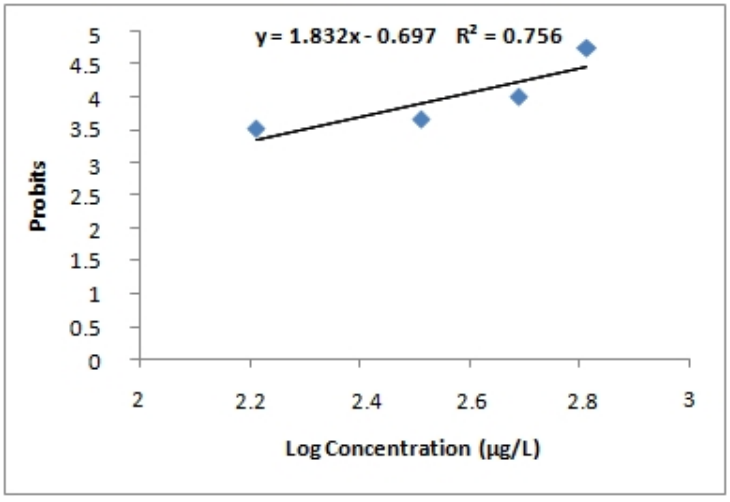

Fig. 1. Dose-response relationship of kinoprene applied for 24h to newly ecdysed fourth-instar larvae of *Culex pipiens*: direct action on fourth-instar larvae (R^2 = coefficient of determination)

Moreover, the compound disturbed growth and development since several morphological aberrations were observed. Mortality occurred, after earlier inhibition of their development or by their inability to complete their ecdysis. Morphological examination revealed that intoxicated fourth-instar larvae by kinoprene had stiff body with curved head which prevented normal jerky movements of the entire body, thus they were get stuck in the bottom enable to dive up to the surface to breathe. Exposure to kinoprene resulted in supernumerary instars and larval-pupal mosaics that do not survive. Moreover, some pupae at larval-pupal intermolt were unable to escape from the larval exoskeleton and other pupae had one air trumpet. Also, the surviving adults were unable to detach their legs and wings from the exuvium and died (Fig. 3).

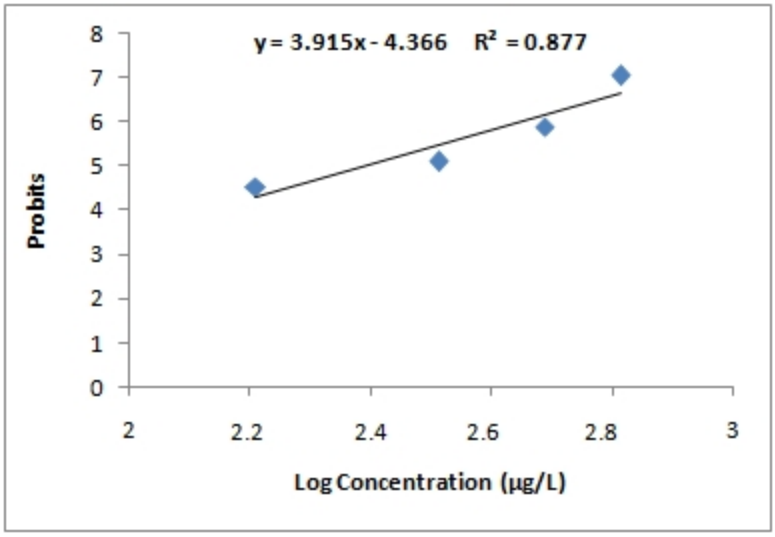

Fig. 2. Dose-response relationship of kinoprene applied for 24h to newly ecdysed fourth-instar larvae of *Culex pipiens*: differed action on the other following stages of development until adult formation (R^2 = coefficient of determination)

Fig. 3. Morphogenic aberrations after treatment with kinoprene in *Cx. pipiens* (A: Untreated pupa; B: Pupa from treated series with one air trumpet; C: Interrupted metamorphosis; D: Larva-pupa intermediate from treated series)

3.2 Effect of Kinoprene on Growth and Development

Data revealed that larvae exposure to kinoprene for 24 h resulted in a significant increase in the duration of both fourth larval and pupal stages compared to untreated insects (Table 3). Indeed, the compound at the two tested doses increased significantly the larval duration (LC_{50} vs control p=0.035; LC_{90} vs control p=0.001) with a dose-response relationship (LC_{50} vs LC_{90} p=0.002). The treatment exhibited also a differed action on the pupae since a significant increase in the duration of the pupal stage was recorded with the highest dose (LC_{90} vs control p=0.004).

Table 3. Effect of kinoprene applied at two concentrations (LC_{50} and LC_{90}) on the duration (days) of the fourth larval and pupal stages of *Cx. pipiens* (m±SD; n=4 pools each containing 10 individuals; for each instar, mean values followed by the different letter are significantly different (p≤0.05)

Stages	Control	LC_{50}	LC_{90}
4th instar larvae	3.25±0.50 a	4.75±0.50b	7.50±0.57c
Pupae	3.50±0.57 a	4.50±0.57a	5.75±0.50b

The whole body weight measurements of *Cx. pipiens* larvae, pupae, male and female adult presented in Table 4 showed that kinoprene had no significant effect on weight (P<0.05) as compared with control.

Table 4. Effect of kinoprene applied at two concentrations (LC_{50} and LC_{90}) on the body weight (mg) of different stages in *Cx. pipiens* (m ± SD; n=3 pools each containing 10 individuals; for each stage, mean values followed by the same letter are not significantly different (p>0.05)

Instars	Control	LC_{50}	LC_{90}
4th instar larvae	6.02±0.38a	5.37±0.07a	5.23±0.72a
Pupae	6.63±0.59a	5.49±0.38a	4.59±0.98a
Male adults	2.03±0.04a	1.90±0.14a	1.70±0.07a
Female adults	3.52±0.26a	3.44±0.34a	3.11±0.57a

Table 5. Effect of kinoprene applied at two concentrations (LC_{50} and LC_{90}) on the body volume (mm^3) of different stages in *Cx. pipiens* (m ± SD; n=3 pools each containing 10 individuals; for each stage, mean values followed by the same letter are not significantly different (p>0.05)

Instars	Control	LC_{50}	LC_{90}
4th-instar larvae	5.09±0.22a	4.22±0.50a	3.42±0.17b
Pupae	8.30±0.58a	6.18±0.16b	5.94±0.41b
Male	50.20±0.78a	43.62±0.11b	35.68±1.82c
Female	86.35±5.97a	79.80±5.72a	69.31±9.74b

As shown in Table 5, the body volume of controls and treated series of *Cx. pipiens* significantly increased during different developmental stage (fourth-instar larvae, pupae and female and male adult) (p<0.001). Kinoprene significantly reduced the body volume at fourth

instar larvae (p=0.0005) and female adult (p=0.037) only with the highest concentration (LC_{90}) compared to controls of the same stage. On the other hand, on pupae, kinoprene showed a significant effect with LC_{50} (p=0.036) and LC_{90} (p=0.041) as compared to control but there was no significant difference (P>0.05) between the tested doses. Also, the body volume of male adult decreased following treatment with the two tested doses (LC_{50} vs control p=0.005; LC_{90} vs control p=0.010) with a dose-response relationship (LC_{50} vs LC_{90} p=0.010).

4. DISCUSSION

4.1 Insecticidal Activity

IGRs and *Bacillus thuringiensis* were used against larvae [26,27,28]. But synthetic insecticides are primarily used to regulate mosquito's populations by targeting adult stage [2,3]. However, continual reliance on these insecticides may result many drawbacks, including resistance even to methoprene [27,28] or target pest resurgence [29,30].

In this context, there is a search for new insect-selective insecticides with minimal toxic effects on the environment and human health. Therefore, JHAs were considered as alternative in pest control management [14,31]. Kinoprene was commercialized in 1975 for the control of aphids and whiteflies in greenhouses on ornamental plants and vegetable seed crops [32]. Moreover, methoprene is used against mosquitos [33] and for grain protection [34]. Methoprene tested against *A. aegypti* caused 50% mortality with around 50µg/L [35], while with *s*-methoprene an LC_{95} of 1.35µg/L against *Culex annulirostris* was reported [36]. Finally, pyriproxyfen evaluated against the late 3rd instar larvae of *Culex quinquefasciatus* showed an LC_{50} of 0.84µg/L [37].

In the current study, after using kinoprene against fourth-larval instar of *Cx. pipiens* we found an LC_{50} of 246.8µg/L. These results may suggest that kinoprene is somewhat less effective than methoprene. However, activity depends on test methodology, the mode of delivery and the formulation and possible use of bait. In addition, the chitin synthesis inhibitors appeared more potent against *Cx. pipiens* larvae as compared to molting hormone agonists and juvenile hormone analogues.

Kinoprene has been shown to be indirectly harmful to natural enemies, nevertheless this JHA did not indirectly affect percent parasitoid emergence from citrus mealybug (*Planococcus citri*) mummies [38]. Kinoprene (ZR-777; commercial trademark Enstar ^{TM} II) was efficacious against homopterous species [39]. Another study examined the lethal and sublethal effects of kinoprene on the beneficial insect *Bombus terrestris*; results revealed that only the maximum field recommended concentration (MFRC) caused a toxic effect on the larval development and lower concentration (0.0650 mg ai/L) had a stimulatory effect on brood production (more eggs contained in ovaries of treated workers than in the controls) [40]. Also, there were no consistent patterns to suggest that kinoprene had any effect on egg production of *Planococcus citri* [41]. Field experiments indicated that kinoprene was very active against nymphs of *Aphis gossypii* (Homoptera: Aphididae) but without complete control [42] because of its non-persistence [6].

4.2 Effect on Growth and Development

Application of kinoprene to fourth instar larvae of *Cx. pipiens* showed several abnormalities by interfering with normal metamorphosis, failure of adult eclosion, supernumerary instars and larval-pupal mosaics and pupae had one air trumpet. Same morphologic aberrations were recorded after treatment of *M. domestica* and *Cx. pipiens* with pyriproxyfen [43], with methoprene on *Cx. quinquefasciatus* [44].

The body size is a crucial trait for mosquitoes, because it can influence their blood-feeding ability, host attack rate and fecundity. All of these traits are important determinants of their potential to transmit diseases [45]. In the present study, kinoprene was applied with two lethal concentrations (LC_{50} and LC_{90}) against fourth-instar larvae of *Cx. pipiens*. The compound was found to decrease the body volume in all considered stages. Though, there was no significant effect on body weight as compared with control series. In *Cx. pipens,* kinoprene stretch out the larval and pupal duration confirming previous a report using pyriproxyfen on *Thrips tabaci* larvae [46].

5. CONCLUSION

This study was conducted to offer a preliminary understanding of the role played by kinoprene as IGR against *Cx. pipiens*. The results showed that this juvenile hormone analogue disrupt the different morphometric measurements of different developmental stages of *Cx. pipiens*. This finding provides appreciable evidence that this IGR have potential for controlling of mosquitoes in an environmentally-friendly manner to the aquatic ecosystem. It constitutes an alternative to other JHAs. Indeed, methoprene rapidly photodegrades and is metabolised in soil under both aerobic and anaerobic conditions, with a half-life of 10 to 14 days [29]. While pyriproxyfen has demonstrated high toxicity to aquatic organisms and the US EPA issued a cancellation order for all fenoxycarb product registrations at the end of 2010 [47].

ACKNOWLEDGEMENTS

The authors wish to thank Pr. G. Smagghe (Ghent University, Belgium) for providing kinoprene. This study was financed by the Algerian Fund for Scientific Research and by the Ministry of High Education and Scientific Research of Algeria (CNEPRU and PNR projects, Pr. N. Soltani).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=486&id=32&aid=4203