Journal of the Egyptian Society of Parasitology, Vol.49, No. 1, April 2019
J. Egypt. Soc. Parasitol. (JESP), 49(1), 2019: 221 – 226

BIOLOGICAL EFFECTS OF SOME CHITIN SYNTHESIS INHIBITORS ON *CULEX PIPIENS* (DIPTERA: CULICIDAE)

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Abstract

This work evaluated the biological effects of three chitin synthesis inhibitors; Novaluron (Roxy), Teflubenzuron (Nomolt) and Hexaflumuron (Consult) against the second larval instar of *Culex pipiens* at different concentrations (0.000001, 0.00001, 0.0001, 0.001 & 0.01 ppm).

The results showed that all three tested CSI's were toxic to the larvae as effective dose dependant. Novaluron was more effective followed by teflubenzuron and hexaflumuron. The toxicity index was 100, 50 & 25 with Novaluron, teflubenzuron and hexaflumuron, respectively. All tested CSI's induced a significant prolongation in larval and pupal duration. Also, pupation rate decreased compared with control as well as reduction in adult emergence.

Keywords: Chitin synthesis inhibitors (CSI's), Novaluron, Teflubenzuron, Hexaflumuron, *Culex pipiens*.

Introduction

Mosquitoes are able to carry and spread disease to humans causing millions of deaths every year. In 2015 malaria alone caused 438 000 deaths. The worldwide incidence of dengue has risen 30-fold in the past 30 years, and more countries are reporting their first outbreaks of the disease. Zika, dengue, chikungunya, and yellow fever are all transmitted to humans by the *Aedes aegypti* mosquito. More than half of the world's populations live in areas where this mosquito species is present (WHO, 2019). In Egypt, mosquitoes as *Culex* 8 species, *Anopheles* 12 species and *Aedes* 4 species were encountered (Mikhail *et al*, 2009). *C. pipiens* plays the main role in transmission of filariasis *bancrofti* as in Egypt (Harb *et al*, 1993) and Rift Valley Fever (El Gebaly, 1978), West Nile virus (Wilson, 1991), and Sindbis fever Egypt (Monath, 1991). Anopheles plays the main role in malaria transmission (Saleh *et al*, 2016). *Aedes aegypti* and dengue hemorrhagic was encountered in Egypt (Morsy, 2018). Consequently, the sustained mosquito control efforts were important to prevent risky outbreaks from these diseases (El-Bahnasawy *et al*, 2013). The most common and widespread mosquitoes are members of *C. pipiens* complex.

The environmental friendly insect growth regulators (IGR's) have been used as agents that elicit their primary action on insect metabolism, ultimately interfering and disrupting the process of growth, development and metamorphosis of the target insects, particularly when applied during the sensitive period of insect development (Ghanim and Ishaaya, 2011).

The biological effects of hexaflumuron on *C. pipiens* were studied by Nassef *et al.* (2008) as well as other *Culex* species by Mulla *et al.* (1989), Amalraj and Velayudhan (1989), Amalraj and Das (1996) and Refaie (2008). The biological effects of novaluron on *C. pipiens* were studied by Djeghader *et al.* (2013) as well as other *Culex* species by Su *et al.* (2003), El-Barky *et al.* (2009) and Rajaskar and Jebanesan (2011). The biological effects of triflumuron on *C. pipiens* were studied by Rehimi and Soltani (1999) and Soltani *et al.* (1999). The biological effects of cyromazine on *C. pipiens* were studied by Cohen (1986) and Assar *et al.* (2016). The biological effects of other CSI's on *C. pipiens* were studied by Saleh and Ali (1987) using chlorfluazuron, Soliman *et al.* (2003) using Bay Sir and altosid and by Rajaskar and Jebanesan (2012) using buprofezin.

The present study evaluated the biological effects of the CSI's, Novaluron, teflubenzuron and hexaflumuron on the widely distributed *Culex pipiens* larvae.

Materials and Methods

Origin of *Culex pipiens*: The strain of *C. pipiens* was obtained from the Research Institute of Medical Entomology, Ministry of Public Health and Population, Dokki, Giza, Egypt.

Rearing technique: The colony was maintained under the laboratory conditions of 27±2°C and 75±5% R.H. (El-Bokl and Mowad 1996). The 2nd instar larvae were collected for the bioassay tests. Five concentrations (0.000001, 0.00001, 0.0001, 0.001 & 0.01ppm) of the tested CSI's were tests. In each test, 25 larvae were put in clean plastic cup with 50ml stored tap water and treated with the CSI's. Each test was replicated four times; control experiments were performed by using tap water only. A mixture of ground dried bread and Brewer's yeast pellets (3:1) were added daily larval food. Dead larvae were daily recorded and removed. Larval mortality was corrected according to Abbott's formula (1987).

Statistical analysis: Data were subjected to probit analysis (Finney 1971) and Le Ora Soft Ware (1987) to give values of LC_{50}. The toxicity index of the CSI's was calculated (Sun, 1950). Pupae were collected and transferred to other cups with cloth covers in emerging cages for adult emergence. Pupal duration, percent pupation and adult emergence were determined. The adult emergence reduction was calculated (Khazanie, 1979).

The tested chitin synthesis inhibitors: 1- Novaluron (Rimon) (Roxy) 10 % E C. (RS)-1-[3-chloro-4-(1,1,2-trifluoro 2-trifluoromethoxyethoxy) phenyl]-3-(2,6-difluorobenzoyl) urea (Sigma-Aldrich Chemicals). 2- Teflubenzuron (Nomolt 15% SC) 1-(3,5-dichloro-2,4-difluo-rophenyl)-3-(2,6 difluorobenzoyl) urea (Basev Limited, Egypt). 3- Hexaflumuron (consult 10% EC)1-[3, 5-dichloro-4-(1, 1, 2, 2-tetrafluoroethoxy) phenyl]-3-(2, 6- difluorobenzoyl) urea (Dow Agroscience, Egypt).

Statistical analysis: Data were analyzed by one way analysis of variance (ANOVA) using SPSS (Ver. 21) software.

Results

The results were shown in tables (1, 2, 3, 4, 5, 6 & 7).

Table 1: Effect of chitin synthesis inhibitors (CSI's) on *C. pipiens* larval mortality treated as 2nd larval instar.

Concentrations (ppm)	Novaluron	Teflubenzuron	Hexaflumuron	*p*
Control	5	5	5	
0.000001	62	60	50	NS
0.00001	84	70	62	*
0.0001	90	82	74	*
0.001	93	95	85	*
0.01	98	99	92	NS
P	*	*	*	

NS: Non Significant (p>0.05) *Significant (p<0.05)

Table 2: Effect of the tested CSI's on *C. pipiens* larval mortality# treated as 2nd larval instar.

Concentrations (ppm)	Novaluron	Teflubenzuron	Hexaflumuron	*p*
0.000001	60.00	57.89	47.36	NS
0.00001	83.15	68.42	60.00	*
0.0001	89.47	81.05	72.63	*
0.001	92.63	94.73	84.21	*
0.01	97.89	98.49	91.57	NS
P	*	*	*	

#Corrected larval mortality

Table 3: LC_{50}, LC_{75} and Toxicity index* of tested CSI's

CSIs	Novaluron	Teflubenzuron	Hexaflumuron
LC_{50} (ppm)	0.0000005	0.000001	0.000002
Toxicity index	100	50	25

Table 4: Effect of CSI's on *C. pipiens* larval duration treated as 2nd larval instar.

Concentrations (ppm)	Novaluron (days)	Teflubenzuron (days)	Hexaflumuron (days)	*p*
Control	11.32 ±0.10	11.32 ±0.10	11.32 ±0.10	
0.000001	12.81± 0.19	12.40± 0.30	11.92 ± 0.21	*
0.00001	13.16 ± 0.65	12.75 ±0.49	12.20 ± 0.37	*
0.0001	15.50 ± 0.53	13.68 ± 0.51	14.29 ± 0.59	*
0.001	19.50 ± 0.49	14.55 ± 0.54	15.77 ± 0.49	*
0.01	19.80 ± 0.50	14.92 ± 0.58	15.96 ± 0.51	*
P	*	*	*	

*Significant (p< 0.05)

Table 5: Effect of CSI's on percent pupation of *C. pipiens* larvae treated as 2nd larval instar.

Concentrations (ppm)	Novaluron	Teflubenzuron	Hexaflumuron	*p*
Control	95	95	95	
0.000001	38	40	50	*
0.00001	16	30	38	*
0.0001	10	18	26	*
0.001	7	5	15	*
0.01	2	1	8	*
P	*	*	*	

Table 6: Effect of CSI's on pupal duration of *C. pipiens* larvae treated as 2nd larval instar.

Concentrations (ppm)	Novaluron (days)	Teflubenzuron (days)	Hexaflumuron (days)	*p*
Control	7.53 ±0.06	7.53 ± 0.06	7.53 ± 0.06	
0.000001	8.93 ±0.14	8.41 ± 0.49	9.06 ± 0.26	*
0.00001	10.12 ± 0.55	9.76 ± 0.62	11.43 ± 0.46	*
0.0001	11.43 ± 0.51	11.00 ± 0.45	12.88 ± 0.42	*
0.001	12.85 ± 0.49	13.52 ± 0.51	14.90 ± 0.49	*
0.01	13.12 ± 0.52	13.84 ± 0.46	15.00 ± 0.50	*
P	*	*	*	

Table 7: Effect of CSI's on adult emergence of *C. pipiens* larvae treated as 2nd larvae instar.

Concentrations (ppm)	Novaluron		Teflubenzuron		Hexaflumuron		*P*
	% AE	%R	% AE	% R	% AE	% R	
Control	79	-	79	-	79	-	
0.000001	36	54.43	30	62.02	40	49.36	*
0.00001	12	84.81	26	67.08	30	62.02	*
0.0001	7	91.13	20	74.68	19	75.94	*
0.001	1	98.73	2	97.46	10	87.34	*
0.01	0	100	0	100	4	94.93	*
P	*		*		*		

% AE = percent of adult emergence, % R = percent reduction in adult emergence, NS: Non Significant (p>0.05)

The corrected larval mortality treated with different CSI's concentrations was given (Tabs. 1 & 2). Larval mortality was dose dependent with a significant difference in each CSI's. Novaluron was more toxic than teflubenzuron and/or hexaflumuron (Tab. 3). Treatment of 2nd larval instar with CSI's significantly prolonged duration. This was dose dependent especially with novaluron than hexaflumuron and/or teflubenzuron (Tab. 4). Pupation of 2nd instar larvae treated with different concentrations was highly decreased especially at higher concentrations (0.001 & 0.01 ppm). CSI's caused a significant prolongation in pupal duration; hexaflumuron was more effective than novaluron and/or teflubenzuron (Tab. 6). All CSI's affected adult emergence (Tab. 7).

Discussion

In the present study, corrected larval mortality of *C. pipiens* treated with different CSI's concentrations showed larval mortality as dose dependent with significant difference for each CSI's. The LC_{50} was 0.0000005, 0.000001 and 0.000002ppm with novaluron, teflubenzuron and hexaflumuron, respectively. CSI's toxicity index was 1, 0.5 & 0.25, respectively. So, novaluron was more toxic

than teflubenzuron, followed by hexaflumuron. These data agreed Mulla *et al.* (1989) who found that hexaflumuron induced larval mortality in *C. quinquefasciatus* and *C. pipiens molestus*. Vasuki and Rajavel (1992) reported that hexaflumuron were toxic to *C. quinquefasciatus* larvae at 0.01 & 0.1mg/L. Also, Vasuki (1992; 1993) found that hexaflumuron gave a high toxicity against all larval instars of *C. quinquefasciatus, Anopheles stephensi* and *Aedes aegypti.* Nassef *et al.* (2008) reported that hexaflumuron was toxic to *C. pipiens* larvae.

CSI's triflumuron (Dieghader *et al,* 2013) and novaluron and cyromazine (Assar *et al,* 2016) were toxic to *C. pipiens* larvae. Medina *et al.* (2002) reported differences in the toxicity of IGR's depend upon the cuticle penetration, distribution and excretion

In the present study, treatment of 2nd larval instar of *C. pipiens* with CSI's resulted in a significant prolongation in larval duration, which was dose dependent with novaluron than with hexaflumuron and/or teflubenzuron. The larval duration with novaluron was 12.81, 13.16, 15.5, 19.5 & 19.8 days at 0.000001, 0.00001, 0.0001, 0.001 & 0.01 ppm, respectively, as 11.32 days in controls. This result agreed with Vasuki (1993) who reported that exposure of 3rd & 4th instar larvae of *C. quinquefasciatus* to sublethal hexaflumuron doses significantly prolonged larval duration. Nassef *et al.* (2008) found that hexaflumuron prolonged *C. pipiens* larval duration. But, Triflumuron induced prolongation of *C. pipiens* larval period (Rehimi and Soltani, 1999; Soltani *et al,* 1999). Novaluron delayed *A. aegypti* larval development (Farnesi *et al,* 2012).

In the present study, pupation emerged from of 2nd instar larvae treated with CSI's different concentrations was highly decreased compared to control, especially at higher concentrations (0.001 & 0.01 ppm). Teflubenzuron and novaluron were more effective than hexaflumuron. This agreed with Amalraj and Das (1996) who used hexaflumuron against *C. quinquefasciatus, A. aegypti* and *A. stephensi*. Hexaflumuron decreased *C. pipiens* pupation (Nassef *et al,* 2008).The pupation of *C. pipiens* decreased by diflubenzuron (Kelada *et al,* 1981), and by cyromazine (Cohen, 1986; Assar *et al,* 2016).

In the present study, the CSI's caused a significant prolonged pupal duration ($P < 0.05$). Hexaflumuron was more effective than novaluron and/or teflubenzuron. Pupal duration was 9.06, 11.43, 12.88, 14.90 & 15 days at 0.000001, 0.00001, 0.0001, 0.001 & 0.01ppm with hexaflumuron, respectively, as as 7.53 day in controls. Such increase in pupal duration may reflect the disruption in metamorphosis. This agreed with Vasuki (1993) and Refaie (2008) using hexaflumuron on *C. pipiens*, Georghiou *et al.* (1975) and Kelada *et al.* (1981) using diflubenzuron and Assar *et al.* (2016) by using cyromazine.

In the present study, all the tested CSI's affected the emergence of adult *C. pipiens*. This effect was dose dependent. Novaluron and teflubenzuron completely inhibited adult emergence at 0.01ppm. The adult emergence reduction was 54.43, 84.81, 91.13, 98.73 & 100% at0.000001, 0.00001, 0.0001, 0.001 & 0.01 ppm of novaluron, while with teflubenzuron, reduction was 62.02, 67.08, 74.68, 97.46 & 100%, respectively. This reduction agreed with Nassef et al. (2008). Also, hexaflumuron reduced adult emergence of *C. tarsalis* (Mulla *et al,* 1989) and *C. quinquefasciatus* (Amalraj and Velayudhan 1989; Vasuki and Rajavel, 1992; Vasuki 1993; 1997). Novaluron inhibited adult *C. quinquefasciatus* emergence (Su *et al,* 2003; El-Barky *et al,* 2009; Rajasker and Jebansan, 2011; Rajasker and Jebansan, 2012). Soliman *et al.* (2003) found that LC_{30} of Bay Sir and altosid reduced adult emergence of *C. pipiens*. Diflubenzuron caused inhibition of adult emergence of *C. pipiens* (Mulla *et al,* 1975; Takahoshi and Ohtaki, 1976; Eshita and Kurihara, 1977; Alizaev, 1986; Luna and Daza, 1992; Cetin *et al,* 2006). Generally, the adult emergence decrease could be due to the fact that IGR's block the maturation of imagine discs that primordial of many adult integu-

mentary structures in endopterygote insects or due to deformation of adult chitin (Schnidermann, 1972; Degheele, 1990).

Conclusion

In fact, mosquitoes can transmit different bacteria, viruses or parasites diseases worldwide. Climate, travel and trade can influence their spread. The present data proved that the CSI's possess a potent insecticidal activity on *C. pipiens* larvae and can be successfully used with other mosquito larvae.

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