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The role of octopamine receptor agonists in the synergistic toxicity of certain insect growth regulators (IGRs) in controlling Dengue vector *Aedes aegypti* (Diptera: Culicidae) mosquito

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ABSTRACT

The synergistic action of octopamine receptor agonists (OR agonists) on many insecticide classes (e.g., organophosphorus, pyrethroids, and neonicotinoids) on *Aedes aegypti* L. has been reported recently. An investigation of OR agonist's effect on insect growth regulators (IGRs) was undertaken to provide a better understanding of the mechanism of action. Based on the IGR bioassay, pyriproxyfen was the most potent IGR insecticide tested (EC_{50} = 0.0019 ng/ml). However, the lethal toxicity results indicate that diafenthiuron was the most potent insecticide (LC_{50} = 56 ng/cm^2) on *A. aegypti* adults after 24 h of exposure. The same trend was true after 48 and 72 h of exposure. Further, the synergistic effects of OR agonists plus amitraz (AMZ) or chlordimeform (CDM) was significant on adults. Among the tested synergists, AMZ increased the potency of the selected IGRs on adults the greatest. As results, OR agonists were largely synergistic with the selected IGRs. OR agonists enhanced the lethal toxicity of IGRs, which is a valuable new tool in the field of *A. aegypti* control. However, further field experiments need to be done to understand the unique potential role of OR agonists and their synergistic action on IGRs.

1. Introduction

Aedes aegypti L., a mosquito vector that transmits Dengue disease, is globally distributed in tropical and subtropical areas, which have increased since 1970 (Chandrashekhar et al., 2015; Govindarajan and Rajeswary, 2015; Poole-Smith et al., 2015). Insecticides play a central role in managing *A. aegypti* (Ahmed and Matsumura, 2012; Bara et al., 2014; Bissinger et al., 2014; Ahmed and Vogel, 2015), such as field controlling of larvae and adults, indoor controlling of adults through direct spraying, residual applications, and use of insecticide coated mosquito bed-nets. An unfortunate result of the extensive reliance on insecticides for mosquito control is the eventual rise in broad-spectrum insecticide resistance (Maestre-Serrano et al., 2014; Pocquet et al., 2014; Saavedra-Rodriguez et al., 2015). Thus, it is very important to employ contrastive methods to avoid or at least reduce the development of insecticide resistance. Insect growth regulators (IGRs) provide this option. IGRs inhibit chitin synthesis during insect development. Various features of IGRs make them attractive as alternatives to broad-spectrum of insecticides, especially conventional insecticides: IGRs are more selective, less harmful to the environment, and more compatible with pest management systems that include biological controls, such as natural predators. Furthermore, intelligent use of IGRs should reduce the likelihood of resistance developing (Brabant and Dobson, 2013; Suman et al., 2013; Thavara et al., 2013; Seccacini et al., 2014).

Formamidine insecticides are related to a unique group of insecticides that have numerous mode of actions on insect pest species. Their effect on monoamine–mediated production of cyclic adenosine monophosphate (cAMP) induces adverse behavioral changes in treated insects (Harrison et al., 1973; Hollingworth, 1976; Beeman and Matsumura, 1978; Evans and Gee, 1980; Nathanson and Hunnicutt, 1981). Formamidines are known to interact with several types of receptors by binding to octopamine receptors (OA receptors) and acting as octopaminergic agonists. This has attracted the attention of many scientists to elucidate the pathways of formamidines' insecticidal actions (Hollingworth and Murdock, 1980; Evans and Maqueira, 2005; Balfanz et al., 2014; Wu et al., 2014).

OA receptor agonists, amitraz (AMZ) and chlordimeform (CDM), have been extensively studied as targets of synergism of many insecticides. However, increasing the rate of uptake of these insecti-

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cides has been proposed to be a mechanism of OA receptor agonists that synergistic pyrethroid, organophosphate, and neonicotinoid insecticides. However, they remain a promising target for novel insect pest control agents (Casida and Durkin, 2013; Ahmed et al., 2015).

In this study, we assessed the toxicity of five IGR insecticides to *A. aegypti* fourth instars and the acute toxicity to *A. aegypti* adults. In addition, we investigated the role of OA receptor agonists, AMZ and CDM, on the activity of selected IGRs against *A. aegypti* adults.

2. Material and methods

2.1. Mosquitoes

The field strain (Fresno, CA) of *A. aegypti* was obtained from the laboratory of Dr. Thomas W. Scott, University of California, Davis, and was used for all experiments. The rearing regime is described in detail by Ahmed and Vogel (2015). Because the UC Davis Institutional Review Board (IRB) ruled that this study did not meet the requirements for human subject research; therefore, IRB approval was not required.

2.2. Chemicals

Chlordimeform (99.8%), amitraz (96.8%), pyriproxyfen (99%), diafenthiuron (99.9%), lufenuron (99.7%), diflubenzuron (98.1%), and novaluron (99.6%) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA).

2.3. IGR bioassays

IGR bioassays were conducted with 20 fourth instars larvae placed in 140-ml glass cups containing 99 ml of distilled water and 1 ml of insecticide (pyriproxyfen, diafenthiuron, lufenuron, diflubenzuron, and novaluron) in acetone solution and only 1 ml of acetone for controls. The cups were covered with double layered white mesh cheesecloth to contain emerging adults form escaping. Emergence was determined after 10 days of insecticide exposure because controls had completed emergence at this time. The synergistic effects of CDM and AMZ were not included in the IGR assays because they prevent adult emergence at the concentrations used.

2.4. Adult bioassays

Adult bioassays were conducted in glass jars (600 ml) with internal surface area of 65 cm^2, which was evenly coated with 1 ml of insecticide solution in acetone solution. For controls, the internal surface of the jars was evenly coated with only 1 ml of acetone. Acetone was allowed to evaporate for 30 min, and then 20 adults (5–7 days post-emergence) were placed inside each jar. The jar opening was covered with double layered white mesh cheesecloth. Adults were considered dead if they were ataxic. Mortality was determined after 24, 48, and 72 h of the exposure.

2.5. Synergistic action bioassay

The synergistic action bioassay was conducted as described above for adult bioassays. Controls received only acetone and were run concurrently with each series of tests. Synergistic action was studied by testing the lethal actions of varying concentrations of tested insecticide alone or with the co-administered with 10 μg/ml of AMZ and CDM, all dissolved in 1 ml of acetone. After the addition of the insecticides, the test solution was allowed to evaporate for 30 min for the adult bioassays. Previous research on AMZ and CDM, published by our lab, demonstrated that a concentration of 10 μg/ml did not cause mortality during the 72-h test period on fourth instar larvae of *A. aegypti* (Ahmed and Matsumura, 2012; Ahmed and Vogel, 2015) Therefore, at least five insecticide concentrations were used for all bioassays, and every bioassay was held at 25 °C. Percentage mortality was recorded after 24, 48, and 72 h of exposure.

Table 1
Toxicity of five IGR insecticides to fourth instars of *Aedes aegypti*.

Compounds	EC_{50}[b] (95% CL)	Slope (±SE)	[a]*n*
Pyriproxyfen	0.0019 (0.00044–0.0046)	3.9 (±0.28)	360
Diafenthiuron	0.19 (0.03–0.57)	4.2 (±0.53)	360
Novaluron	0.63 (0.03–2.34)	4.7 (±0.81)	360
Lufenuron	2.85 (0.77–6.68)	4.9 (±0.34)	360
Diflubenzuron	5.30 (1.39–13.33)	5.1 (±0.62)	360

[a] *n*:number of larvae tested, including control.
[b] Effective concentration (ng/ml) to cause 50% of treated larvae to fail to emerge as adults. The toxicity was evaluated as percentage of adult emergence after 10 days.

2.6. Statistical analysis

The corrected mortality was calculated according to Abbott's formula (Abbott, 1925). Bioassay data (LC_{50} and 95% CL values) were analyzed by using IBM SPSS Statistics 22.0 software (SPSS Inc., Chicago, IL). Synergistic action was determined to be significant ($P \leq 0.05$) when the 95% CIs for the LC_{50} values for adults exposed to insecticide alone did not overlap with those for larvae exposed to insecticide + synergist mixture. Synergistic ratio (SR) was calculated by dividing the LC_{50} value of the test insecticide by that of the LC_{50} obtained for the combined treatment (insecticide + synergist). Plus, toxicity index calculated as [(LC_{50} of the most toxic tested IGR insecticide/LC_{50} of the tested IGR insecticide) × 100].

3. Results

The toxicity of diafenthiuron, novaluron, lufenuron, and diflubenzuron relative to pyriproxyfen is shown in Table 1. Pyriproxyfen was found to be the most potent insecticide (EC_{50} = 0.0019 ng/ml) followed by diafenthiuron (EC_{50} = 0.19 ng/ml). Novaluron and lufenuron had moderate toxicity (EC_{50} = 0.63 and 2.85 ng/ml, respectively). Least toxic was difluben-

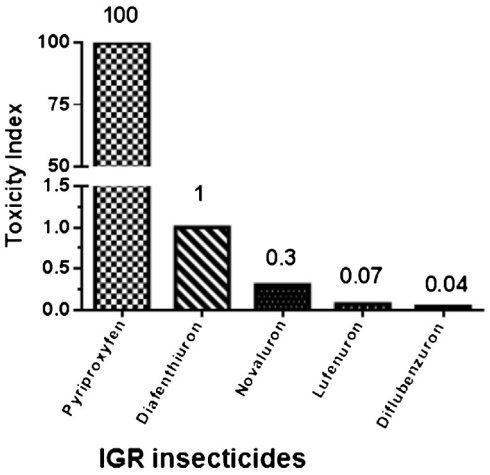

Fig. 1. Toxicity index of five IGR insecticides on 4th instar larvae of *Aedes aegypti* after 10 days of exposure. Toxicity index = [(LC_{50} of the most toxic tested IGR insecticide/LC_{50} of the tested IGR insecticide) × 100].