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# Synergistic action of octopamine receptor agonists on the activity of selected novel insecticides for control of dengue vector *Aedes aegypti* (Diptera: Culicidae) mosquito

Mohamed Ahmed Ibrahim Ahmed [a,b], Christoph Franz Adam Vogel [b,c,*]

[a] Plant Protection Department, Faculty of Agriculture, Assiut University, Assiut 71526, Egypt
[b] Center for Health and the Environment, University of California Davis, Davis, CA 95616, USA
[c] Environmental Toxicology Department, University of California Davis, Davis, CA 95616, USA

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ABSTRACT

Studying insecticide resistance in mosquitoes has attracted the attention of many scientists to elucidate the pathways of resistance development and to design novel strategies in order to prevent or minimize the spread and evolution of resistance. Here, we tested the synergistic action of piperonyl butoxide (PBO) and two octopamine receptor (OR) agonists, amitraz (AMZ) and chlordimeform (CDM) on selected novel insecticides to increase their lethal action on the fourth instar larvae of *Aedes aegypti* L. However, chlorfenapyr was the most toxic insecticide ($LC_{50}$ = 193, 102, and 48 ng/ml, after 24, 48, and 72 h exposure, respectively) tested. Further, PBO synergized all insecticides and the most toxic combinatorial insecticide was nitenpyram even after 48 and 72 h exposure. In addition, OR agonists significantly synergized most of the selected insecticides especially after 48 and 72 h exposure. The results imply that the synergistic effects of amitraz are a promising approach in increasing the potency of certain insecticides in controlling the dengue vector *Ae. aegypti* mosquito.



## 1. Introduction

The current insecticide-based strategy for vector control of *Aedes aegypti* L. relies heavily on the use of pyrethroids on bed nets, indoor spraying and larvicidal applications [1–3]. Such a heavy reliance on only one type of insecticide is likely to lead to future problems of resistance development that would create intractable predicaments in the control efforts of *Ae. aegypti* [4]. Knowing the history of many insecticide resistance problems, most researchers in this field agree that such a scenario is not farfetched in this case. However, it is also necessary to point out that insecticidal chemicals are the best proven approach among other methods of pest control that works well in controlling the dengue vector mosquitoes as shown by the earlier success of DDT [5]. Importantly, there is no question about the great need to continue searching for novel approaches of mosquito control, such as development of vaccines, biological control measures, and introduction of molecular biological means of mosquito control [6].

Chemical insecticides are developed by pesticide manufacturing companies through mostly empirical screening and biological testing efforts. In more recent years, however, most pesticide companies rely on the known mode of action of each chemical class of insecticides, such as chemicals targeting the sodium channel, the nicotinic acetylcholine receptor, cholinesterase etc., because such knowledge-based approaches increase the chance of discovering the 'blockbuster' pesticide more quickly [7]. The major classes of pesticides used today can be distinguished by the biochemical target through which each group of pesticides attacks. Accumulation of knowledge in this regard has helped tremendously in selecting flexible approaches to control pests, such as rotating insecticides with a totally different mode of action to ameliorate the damages resulting from very stubborn cases of recalcitrant resistant pests in the past. Thus, a useful approach to preclude rapid development of resistance has been found to be avoiding excessive, uniform, and region-wide uses of high dose application of the main type of insecticide throughout all stages of the mosquito life cycle [8]. Unfortunately, the heavy reliance on pyrethroids in controlling vector mosquitoes as larvicides and adulticides is now reaching the stage where resistance problems are expected to grow [9–11]. The most effected type of strategy is an integrated pest management (IPM)

*Abbreviations:* PBO, piperonyl butoxide;; AMZ, amitraz; CDM, chlordimeform; IPM, integrated pest management; OR, octopamine receptor; cAMP, cyclic adenosine monophosphate; IGRs, insect growth regulators; IRR, Institutional Review Board; SR, synergistic ratio; $LC_{50}$, lethal concentration, 50%; 95% CL, 95% confidence limit.

* Corresponding author. Center for Health and the Environment, Environmental Toxicology Department, University of California Davis, Davis, CA 95616, USA. Fax: +1 530 752 5300.
*E-mail address:* cfvogel@ucdavis.edu (C.F.A. Vogel).

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program in order to mitigate or reduce the chance of rapid development of resistance. In this regard, many different types of efforts have been made to minimize the use of the main insecticide of the control program including limiting the percentage used in terms of the spray areas, using mixtures of insecticides with different modes of action, using the right insecticide formulation and targeting only the critical time or area of vulnerability [12].

There are a number of examples of successful applications of mixtures of pesticides in resistance management [8,13]. At least there is one demonstration of the effectiveness of combinatorial effects (permethrin plus imidacloprid) reducing the number of mosquito bites when topically applied to dogs [14].

The effectiveness of formamidine, type octopamine receptor (OR) agonist, in synergizing the insecticidal actions of pyrethroids on houseflies (dipterous insects) was first reported by Liu and Plapp [15] with the most effective synergistic effect using amitraz. In view of the significant synergism between octopamine and cypermethrin, the molecular base of this synergism is likely activation of the OR signaling. The subject of the mechanism of action of this class of OR agonists, particularly that of formamidines, has been extensively reviewed [16–18]. Briefly in insects octopamine acts like epinephrine, and OR in this regard serves as the receptor mediating the sympathetic route, equivalent of the mammalian autonomic controlling system. As in the case of epinephrine, one of the major consequences of its signaling in insects is excitation coupled with loss of appetite. While there are qualitative differences in the actions between octopamine and epinephrine, the above two basic actions of OR signaling serves as two of the main modes of their pesticidal actions. While studies on the action of octopamine are rare in mosquitoes, in the brain of adult *Culex pipiens* mosquitoes, octopamine causes activation of cyclic adenosine monophosphate (cAMP), indicating this basic mechanism does indeed operate in this species. Among dipterous insects, Drosophila is the best studied species in which octopamine has been shown to induce cAMP. In some insects elevation of cAMP by octopamine directly causes the loss of appetite [19].

Neonicotinoids are a relatively new group of powerful insecticides that clearly shows a wider spectrum of insect controlling property especially mosquito control [20–22]. The molecular bases of their diverse action spectra are gradually becoming elucidated [23,24]. Their basic mode of action is to attack the nicotinic acetylcholine receptor (nAChR), which is totally different from the action sites of any of the major insecticides frequently used for the control of mosquitoes in the past. While the original types of neonicotinoids were relatively polar, suited mostly on sucking type insect pests, the spectra of effectiveness of neonicotinoids have been steadily expanding including chewing type of insect pests because of their increased hydrophobic properties [25].

Recently, new promising tools have been used in mosquito control. For instance, chlorfenapyr is a protoxin requiring activation by cytochrome P450, mediates its toxic effects by disrupting the production of ATP resulting in cellular death and ultimately mosquito mortality [26].

Another example are insect growth regulators (IGRs) which inhibit chitin synthesis during the development of mosquitoes [27,28]. The advantage of the IGRs is causing less detrimental effects to beneficial insects. For instance they do not affect an insect's nervous system and are less toxic to beneficial insects, more compatible with pest management systems that use biological control components, and are less likely to become resistant to IGRs [29–32].

Herein, we studied the synergistic action of OR agonists, AMZ and CDM, plus PBO on the insecticidal activity of selected novel insecticides against fourth instar larvae of *Ae. aegypti*. However, the potential of this study is to shed light on new promising tools to control the dengue vector *Ae. aegypti* mosquitoes.

## 2. Materials and methods

### 2.1. Mosquitoes

The FIELD strain (Fresno, CA) of *Ae. aegypti* was obtained from the laboratory of Dr. Thomas W. Scott, University of California Davis, and was used for all experiments. The rearing regime has been described earlier [8]. Briefly, the eggs were hatched by placing a piece of dried paper towel loaded with previously deposited eggs in a flask filled with 750 ml of distilled water that been held under vacuum for 45 min. The vacuum was released and 50 mg of larval diet (dog food powder) added to the water. The hatched larvae were held overnight in the same flask, and then 200 larvae were transferred to each 600-ml beaker containing 400 ml of distilled water. Larval diet was added to each beaker according to the following regime: day 1, 75 mg; day 3, 38 mg; day 4, 75 mg; day 5, 113 mg; and day 6, 150 mg. Adult mosquitoes were reared in an environmental chamber with a temperature ranging from 22 to 30 °C, 80% relative humidity (RH), and a photoperiod of 14:10 (L:D) h. Adults were held in a $60 \times 60 \times 60$-cm screened cage and provided 10% sucrose ad libitum. Human blood was provided to adults twice a week. Eggs were collected on paper towels lining the rim of water containers. The papers with eggs were air dried at 27 °C and 80% humidity for 24 h and stored in containers with 100% humidity for 3–30 d.

The UC Davis Institutional Review Board (IRR) determined that feeding laboratory-reared mosquitoes on people in this experiment did not meet the requirements for human subject research, and thus, did not require IRR approval.

### 2.2. Chemicals

Piperonyl Butoxide (99%), chlordimeform (99.8%), amitraz (96.8%), nitenpyram (99.9%), chlorfenapyr (99.6%), lufenuron (99.7%), diafenthiuron (99.9%), diflubenzuron (98.1%), and novaluron (99.6%) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

### 2.3. Larval bioassays

Twenty of fourth instar larvae were placed in 140-ml glass cups containing 99 ml of distilled water, 1 ml of each insecticide (chlorfenapyr, nitenpyram, diafenthiuron, diflubenzuron, novaluron, and lufenuron) in acetone solution, and 1 ml of acetone as vehicle for controls. Larvae were considered dead if they were unresponsive to touching with a probe or if they could not reach the surface of the water. Because of the slow-acting nature of some of these insecticides, mortality was determined after 24, 48, and 72 h of exposure.

### 2.4. Synergistic action bioassay

The synergistic action bioassay was conducted as described above. Controls received acetone only and were run concurrently with each series of tests. Each series of synergistic action tests was carried out by testing the lethal action of varying concentrations of a test insecticide alone or the test insecticide co-administered with 10 μg/ml of PBO, AMZ, or CDM. After the addition of the insecticides, the test solution was stirred briefly to ensure uniform mixture. Preliminary tests showed that PBO at 10 μg/ml was the maximum sublethal concentration where no mortality was observed of fourth instar larvae. Previous study on AMZ and CDM, which was published by our lab, demonstrated that concentration of 10 μg/ml did not cause mortality during the 72-h test period on fourth instar larvae of *Ae. aegypti* [8]. At least five concentrations were used for each bioassay. Every bioassay was held at 25 °C. Percentage mortality was recorded after 24, 48, and 72 h.