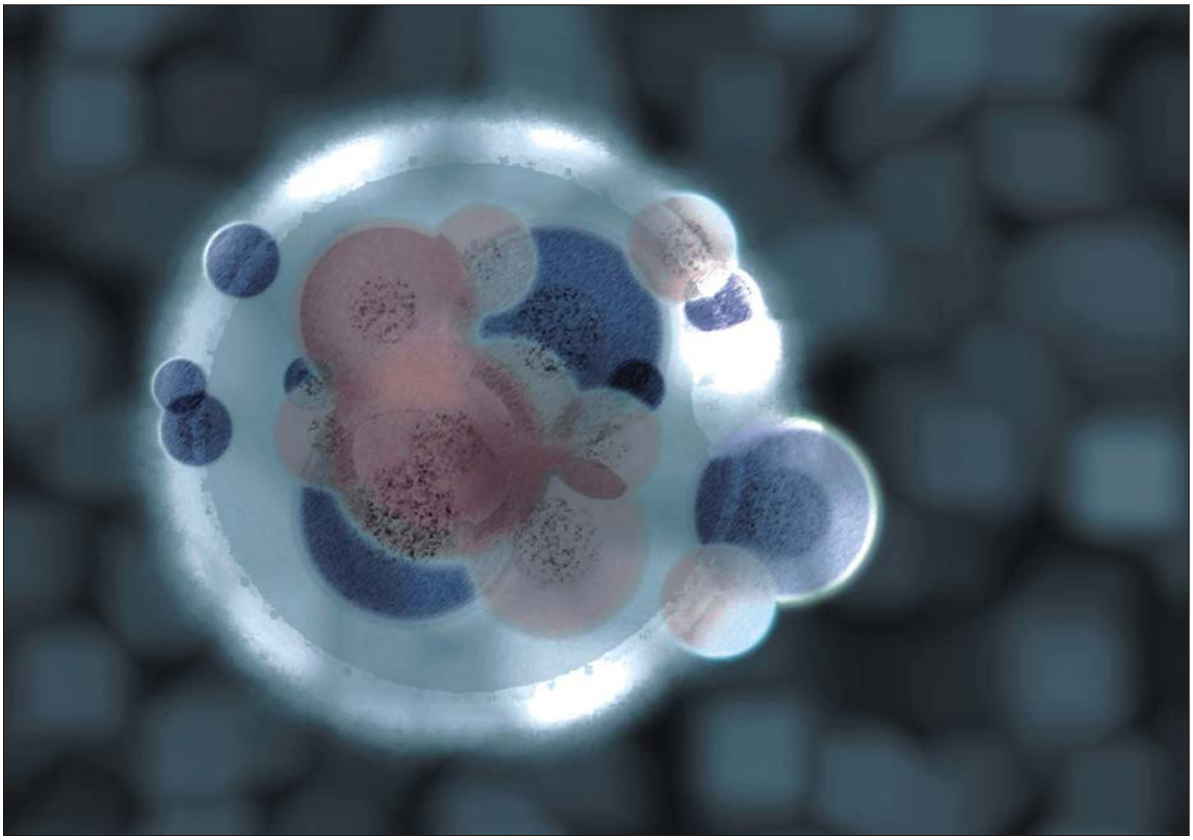

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Insecticidal Activities of *Averrhoa carambola* on *Drosophila melanogaster* and *Syntermes grandis*

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Abstract: In this study, we show the toxic effect of *A. carambola* Aqueous Extract (*Ac*AE) on *Drosophila melanogaster* life cycle and its paralyzing effect on termites (*Sintermes grandis*). In our assays, *Ac*AE, at concentrations of 0.5, 5, 50 and 500 μg of protein/mL, caused a dose-dependent decrease of, respectively 28±1.96, 55±3.85, 73±5.11 and 91±6.67% of adult emergence from *D. melanogaster* pupae. After 180 min following application, the same series of concentrations produced a significant and irreversible dose-dependent paralysis of termites. This study presents the first description of star fruit insecticidal activity and the relevance of the toxic effect and/or growth development retarding effects of *Ac*AE could acquire greater significance once observed following application in natural habitats.

Key words: *Averrhoa carambola*, star fruit, termites, *Drosophila melanogaster*, insecticidal activity

INTRODUCTION

Plants growing in our environment are frequently exposed to a wide range of microorganism and insect attacks. Nevertheless, they are able to resist those aggressions and depending on their tolerance toward a particular pathogen or phytogen, plants may continue to grow, overcoming the stress induced by the action of these agents[1] .

For many years, it was assumed that certain secondary metabolites were useless by-products. More recently however, it was demonstrated that these compounds might be important components for plant defense against pathogens, plagues and herbivores[2].

Thus, it has been reported that β-N-oxalyl-L-α, β-diaminopropionic acid (L-βOAA), isolated from the plant *Lathyrus sativus*, has an inhibitory effect on the growth of rice moth *Corcyra cephalonica* Staint larvae[3]. Balandrin and collaborators[4] showed that di-*n*-propyl disulfide, identified as the major volatile constituent from freshly crushed neem (*Azadirachta indica*) seeds, presents a larvicidal effect against *Aedes aegypti* (yellow fever mosquito), *Heliothis virecens* (tobacco budworm) and *Heliothis zea* (corn earworm). Furthermore, it has been reported that extracts from species of plants of the genus *Ocimum*, cultivated in Europe, Asia and Africa, are useful fungicides, nematicides, alelopaticides and larvicides[5,6]. In recent years, the study of insecticidal compounds obtained from plants and other biological sources has become a subject of great interest in view of the promising use of such compounds for the control of agricultural plagues and disease vectors. In fact, the development of insect resistance to chemical means of control encourages the search for new toxins and natural insecticides[7]. Moreover, one of the strategies to combat to tropical diseases has been to destroy their vectors; therefore, new bioassays have been described as crucial tools for the detection of plant extract activities that could be useful for the prevention of transmission of diseases like malaria, schistosomiasis and yellow fever[8].

The star fruit or carambola (*Averrhoa carambola*, Oxalidaceae) is originated from Asia and acclimatized to many tropical countries, including Brazil. Although *Anastrepha obliqua* and *Bactrocera carambolae* may infect star fruits[9,10], it has been suggested that fruits of some *A. carambola* subspecies are relatively more resistant to infestation of a variety of larvae and insects, indicating the presence of compound(s) that could inhibit infestation by some types of invertebrates. Thus, in this study, we show the effects of *A. carambola* aqueous extract (*Ac*AE) on the *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) life cycle and the *Ac*AE

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paralyzing effect on termites-*Syntermes grandis* Ramb. (Isoptera: Termitidae). Our assay methods were chosen for the sake of simplicity, suitable reliance criteria and reproducibility.

MATERIALS AND METHODS

Fruit collection and preparation of aqueous extract: *A. carambola* fruits (subspecies Golden Star) were harvested in pesticide-free trees from Campus of University of São Paulo, Ribeirão Preto/SP, Brazil. The fruits were washed and homogenized 1:1 (w/v) with distilled water. The *Ac*AE was boiled for 5 min, centrifuged at 3000 x g for 10 min and the supernatant used for the assays. In our studies, the protein content of *Ac*AE was measured by the Lowry method, as modified by Hartree[11].

Assay of the effects of *Ac*AE on *D. melanogaster* life cycle: For the bioassay of effects on *D. melanogaster*, the 4-24 instant drosophila medium from the Carolina biological supply company was employed. The medium (0.3 g/mL) was placed in vials containing 10 mL of distilled water (controls), or 10 mL of *Ac*AE at the following concentrations: 0.5, 5, 50 and 500 μg protein/mL. Four couples of adult flies were placed into the vials and removed 24 h later, a time interval sufficient for egg hatching. The assay was performed in triplicate, at room temperature. Percentages of successful adult emergence were determined by daily monitoring until all adults in the controls had emerged.

Bioassay of termites (*Syntermes grandis*): The bioassay using termites was performed according to Fontana[12]. Briefly, termites were collected in the Campus of Ribeirão Preto University of São Paulo and 2 μL of saline (NaCl; 0.9% w/v) (control) or *Ac*AE (at concentrations of 0.5, 5, 50 and 500 μg of protein/mL) were injected into the animals. Ten termites were used for each concentration of *Ac*AE and for the control group. The animals were observed for 5, 10, 15, 30, 60, 120 and 180 min following injections and their behavioral classification was as follows: normal (N): normal walking and body movements; paralysis (P): incapacity to shift their whole body and dead (D). Fisher's exact test was used to verify statistical significance of differences between numbers of animals in states P+D, or N and controls.

RESULTS AND DISCUSSION

In our assays of the effects of *Ac*AE on *D. melanogaster* life cycle, this extract, at the concentrations of 0.5, 5, 50 and 500 μg protein/mL, caused a dose-dependent decrease of, respectively 28±1.96, 55±3.85, 73±5.11 and 91±6.67% of adult emergence when compared to control group (Fig. 1). The EC_{50} value calculated by non-linear regression according to a sigmoidal dose-response curve was 6.1 μg protein/mL. *Ac*AE did not cause larvae and adult mortality, nor modified eggs hatching, since the same number of eggs was observed on the surface of the media in the control and experimental vials (data not shown). Insecticidal activity on larval or pupal stages is the major goal of mosquito and agricultural plague management programmes, because the adulticides may only temporarily reduce adult populations, allowing for their rapid upsurges within a few days[13].

In termites assays, *Ac*AE, at concentrations of 0.5, 5, 50 and 500 μg protein/mL, produced a significant and irreversible dose-dependent paralysis within 180 min; the number of deaths increased with dose and time of exposure to the *Ac*AE (Table 1). This result shows that this extract may be a worthy source of biocides, useful as a termiticide or repellent in soil applications, capable of excluding termites from buildings and other structures in contact with the ground.

Termiticide-treated soil barriers have been used in subterranean termite control for the last 50 years[14]. Termiticides used to establish such barriers should be repellent, toxic, or both[15]. The broad use of organochlorine insecticides has been appointed as a public health concern due to their environmental

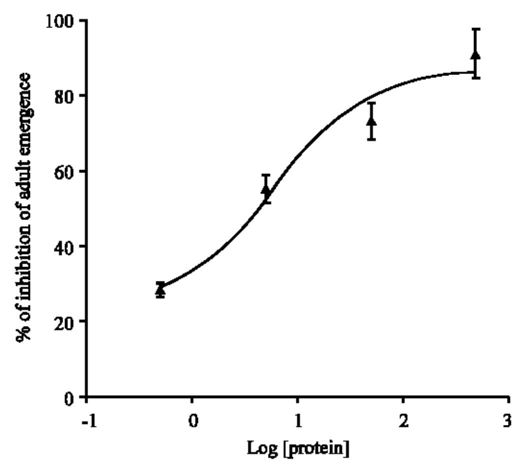

Fig. 1: Dose-dependent decrease of adult emergence from *D. melanogaster* pupae caused by *Ac*AE (0.5, 5, 50 and 500 μg of protein/mL). EC_{50} of 6.1 μg of protein/mL was calculated by non-linear regression, according to a sigmoidal dose-response curve

Table 1: Percent responses of Normal condition (N), Paralysis (P) and Death (D) of groups of 10 termites each, injected with 2 µL of saline (controls), or with, respectively 0.5, 5, 50 and 500 µg of protein/mL of *Ac*AE

Time (min)	Control			0.5 (µg/mL)			5 (µg/mL)			50 (µg/mL)			500 (µg/mL)		
	N	P	D	N	P	D	N	P	D	N	P	D	N	P	D
5	100	-	-	100	-	-	100	-	-	100	-	-	100	-	-
10	100	-	-	100	-	-	100	-	-	100	-	-	100	-	-
15	100	-	-	90	10	-	100	-	-	100	-	-	100	-	-
30	100	-	-	90	10	-	100	-	-	100	-	-	90	10*	-
60	100	-	-	90	10	-	100	-	-	100	-	-	60	40**	-
120	100	-	-	90	10	-	70	30**	-	60	40**	-	30	70**	-
180	90	10	-	70	30**	-	70	30**	-	50	50**	-	30	60**	10**

*$p<0.05$, ** $p<0.001$, one-side Fisher exact test

persistence and mammalian toxicity, indicating an urgent requirement for safer biocides[16,17]. Naturally occurring compounds may offer alternatives to the current reliance upon insecticidal treatments[18]. It has been demonstrated that terpenoids and essential oils are powerful repellent and toxic agents against termites, showing low mammalian toxicity[19]. Indeed, botanical insecticides formulated with isoborneol, cedarwood oil, plant oil or body oil did not affect termite mortality compared to control trials, however, they prevented termites from penetrating treated soil, suggesting that these formulations could be of value as termite repellents in soil applications, associated to adequate toxicological and environmental safety[20].

A large number of reports on the convulsive effects of *A. carambola* has been published in last years, however, little is known about its effects on insects[21]. The present study represents the first description of star fruit insecticidal activity; taken together, present results may explain star fruit resistance for some types of insect and larval infestations, at least of the subspecies assayed. Stable and probably non-proteic compound(s) in the star fruit may be responsible for its defensive action against insects, since the natural aqueous extract showed no significant difference in insecticidal activity prior to and after boiling (data not shown). Toxicological and environmental safety can be assumed for the tested material, since the star fruit enjoys wide human consumption over the world.

Finally, the relevance of the toxic effect on termites and/or growth development retarding influence on *D. melanogaster* of *Ac*AE would acquire greater importance if such effects could also be observed in natural habitats. This material could then have application in larval and pupal breeding sites like peri-domestic water and artificial containers at localities where the control of disease vectors is limited. In addiction, *Ac*AE could be useful as a new termiticide. Therefore, future investigations on the identity of its active component(s) and modes of action may result in applications of *Ac*AE to insect habitats, leading to promising mosquito and agricultural plague management programmes.

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