The potential use of *Annona* (Annonaceae) by products as a source of botanical insecticides

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INTRODUCTION

The structural and functional diversity of secondary metabolites (allelochemicals) is a key factor for the survival and evolutionary success of plant species inhabiting an environment with an abundance of natural enemies. Therefore, the tropical flora, with its unique biodiversity, is a promising natural reservoir of bioactive substances. In this context, Brazil has the highest plant genetic diversity in the world offering enormous potential for the development of novel active substances based on natural products.

In the agricultural context, the study of defense mechanisms of plants comprises an important approach for the selection of new insecticides/acaricides compounds that meet the requirements of effectiveness, safety and selectivity, which are essential precepts of the integrated pest management (IPM) programs. Plant-derived compounds show great potential for the management of populations of arthropod pests, both through homemade preparations for direct use in the field and in the development of botanical insecticides (non-synthetic), as well as templates molecules for the synthesis of new synthetic insecticides.

Among the botanical families that occur in the Neotropical region, Annonaceae is the main family of the order Magnoliales and is one of the most specious families of angiosperms comprising 135 genera and approximately 2,500 species (Chatrou et al., 2004). Annonaceae exhibits a pantropical distribution with 40 genera and 900 species in the Neotropical region. In Brazil, this family is represented by 29 genera (1endemic) and 386 species, and a large proportion of this richness is found in the Amazon Rain Forest and Atlantic Forest (Maas *et al.*, 2013).

In addition, some species of *Annona* genera (e.g.: *Annona muricata, Annona squamosa, Annona cherimolia, and Annona cherimolia* x *Annona squamosa*) have great economic importance due to their edible fruits of ample commercial interest. Consequently, a considerably cultivated area (~ 14,000 hectares) with these species is observed in Brazil. However, most of *Annona* fruits production are destined for fruit-processing industries and commercialized as frozen pulps for juice preparations due to its small shelf life. Thus, the seeds, which composed between ~7% of total weight of fruits, are discarded and could serve as an inexpensive and readily available source of biomass for botanical insecticides development. In light of this potential, some studies have been conducted in order to changing these industrial wastes into ecofriendly solutions for pest control.

BIOACTIVE COMPOUNDS FROM *Annona* SPECIES

Despite the limited number of studies, a large number of compounds of diverse chemical natures have been isolated in several structures of the genus *Annona*, including alkaloids, acetogenins, diterpenes, and flavonoids. Among the compounds, annonaceous acetogenins stand out because of their structural abundance and the wide range of biological activities they exhibit, such as powerful insecticidal and acaricidal activities (Colom *et al.* 2010). The acetogenins comprise a series of natural products (C-35/C-37) derived from long-chain fatty acids (C-32/C-34) combined with a unit of 2-propanol (Alali *et al.*, 1999), which are found only in some genera [*Annona, Anomianthus, Asimina, Desepalum, Goniothalamus, Rollinia* (now *Annona*), *Polyalthia, Porcelia, Uvaria,* and *Xylopia*] of the Annonaceae family (Johnson, 2000) and with a high concentration in their seeds.

Acetogenins are potent mitochondrial poisons, inhibiting the cellular energy production (Isman & Seffrin, 2014). More specifically, acetogenins block the respiratory chain at complex I (NADH: ubiquinone oxidoreductase) of the mitochondrial electron-transport system and of the enzyme NADH:oxidase in the cell membrane of target arthropods, directly affecting electron transport in the mitochondria and causing apoptosis as result of ATP deprivation (Alali *et al.*, 1999). Moreover, recent study suggest that acetogenins at sublethal doses cause damage in the insect midgut epithelium and digestive cell, decreasing the expression of genes associated with transport and absorption of nutrients, metabolites and nonelectrolytes as well as increasing the expression of genes linked with autophagy induction (Costa *et al.*, 2016).

In addition to lethal toxicity, acetogenin-based extracts or isolated compounds also affect the insect development and feeding and oviposition behavior (**Table 1**). In light of the promising acute and chronic effects on pest species of agricultural importance, some acetogenin-based formulations (Anosom®, BioRakshak®, AnonaCin®) were recently released on the market in eastern countries (e.g.: India).

BIOPROSPECTING STUDIES WITH NEOTROPICAL ANNONACEAE – THE BRAZILIAN CASE

Bioprospecting studies carried out with diverse flora species constitute a strategic action for the creation of differentiated products with high-added value, such as agricultural pesticides. In addition, the medicinal, economic, and ecological importance of native species, as well as the risk of extinction by human predatory action, has supported the studies of these plants for their preservation and sustainable use.

Given this potential, the National Science and Technology Institute for Biorational Control of Pest Insects (INCT-CBIP), which aggregate 7 Brazilian institutions from 5 States, established in 2011, a multidisciplinary research program aiming at obtaining bioactive allelochemicals from Neotropical Annonaceae. Using the maize weevil (*Sitophilus zeamais*) as bioindicator, a comprehensive initial screening was conducted in order to evaluated the bioactivity of ethanolic extracts of different parts from 29 Annonaceae species (7.5% of all Brazilian species) belonging to 11 different genera. As results, seeds from genus *Annona* were identified as the main sites of accumulation of compounds with activity against the maize weevil, being the ethanolic extracts prepared from seeds of *A. mucosa, A. sylvatica, A. montana*, and *A. muricata* the most active treatments (Ribeiro *et al.*, 2016). Among these derivatives, the extract prepared from the *A. mucosa* seeds – ESAM (**Figure 1**) demonstrated the lowest LC_{50} values and, consequently, the most pronounced insecticidal activity.

Figure 1. Tree, fruits, and seeds of *Annona mucosa*

Table 1. Lethal and sublethal toxicities of *Annona mucosa* seeds derivatives against some arthropod-pest of agricultural relevance in Brazil.

Arthropod pest	Tested derivative (local of bioassay)	LC_{50}/LD_{50} (exposure form)	Sublethal toxicities	References
Panonychus citri	Ethanolic crude extract (laboratory trial)	LC_{50}=2,608 ppm (residual contact)	Oviposition deterrence (EC_{50} = 3,194.80 ppm)	Ribeiro *et al.*, 2014c
Trichoplusia ni	Ethanolic crude extract and formulated extract (laboratory and greenhouse trial)	LC_{50}=328.86 ppm (ingestion); LD_{50} = 12.61 μg larva^{-1} (topical application)	growth inhibition (EC_{50}= 114.71ppm)	Ribeiro *et al.*, 2014a
Myzus persicae	Formulated extract (greenhouse trial)	--	Strong reduction of population growth rate (at 2,500 ppm)	Ribeiro *et al.*, 2014a
Spodoptera frugiperda	Ethanolic crude extract and isolated acetogenin (laboratory trial)	842.90 ppm (ingestion)	larval growth inhibition (EC_{50}= 580.4 ppm), interference in the insect development, and increase in the proportion of pupae and adults with morphological changes	Ansante *et al.*, 2015; Ribeiro *et al.*, 2016
Diaphorina citri	Ethanolic crude extract and formulated extract (laboratory, greenhouse, and field trial)	LC_{50}=57.76 ppm (residual contact)	Feeding and oviposition deterrence	Ribeiro *et al.*, 2015
Sitophilus zeamais	Ethanolic crude extract (laboratory trial)	621.70 ppm (residual contact)	Inhibition of the F1 progeny and reduction in grain losses	Ribeiro *et al.*, 2016
Atta sexdens rubropilosa	Seed ethanol Extract (laboratory trial)	MD = 3 days (2,0 mg mL-1, ingestion)	--	Bicalho, 2016
Helicoverpa armigera	Ethanolic crude extract, isolated acetogenin, and formulated extract (laboratory and greenhouse trial)	LC_{50}=1,479 ppm (ingestion)	larval growth inhibition (EC_{50}= 580.4 ppm), interference in the insect development, and increase in the proportion of pupae and adults with morphological changes	Souza *et al.*, 2017

In addition, ESAM caused promising insecticidal/insectistatic effects on other important pest species for tropical fruit crops, vegetables, and other commodities (cereals and oilseeds) in laboratory tests with crude extracts and in protected and field crops with formulated extracts (**Table 1**). Subsequently, biomonitored fractionations indicated that the acute and chronic toxicity of this botanical derivative is due to the synergistic interaction of structurally diverse acetogenins, being the acetogenin bis-tetrahydrofuran rolliniastatin-1 (**Figure 2**) the major active compound (Ansante *et al*., 2015). Interestingly, ESAM not affected the growth and development of entomopathogenic fungi species (Ribeiro *et al*., 2014b), which are important agents of natural and applied biological control.

Micro-encapsulated formulations (particles) containing the active compound encapsulated in materials such as gelatin, starch, cellulose, sodium alginate, etc (Burges, 1998) and studies using annonaceous extracts and acetogenins are in progress using methodologies developed by our group (Giongo *et al*., 2016, Carvalho *et al*., 2015, Rodrigues *et al*., 2016). Each particle can contain several molecules, and, after the drying process and having an increased storage period, it is ready for agricultural use, and can be applied directly on the soil or on the target pest, and even on plants, without the need for dispersion in an aqueous medium, facilitating the application (Rodrigues *et al*., 2016). Such encapsulated particles may be used to control various pests, due to the wide spectrum of the compound used, such neen against silverleaf whitefly *Bemisia tabaci* (Carvalho *et al*., 2015).

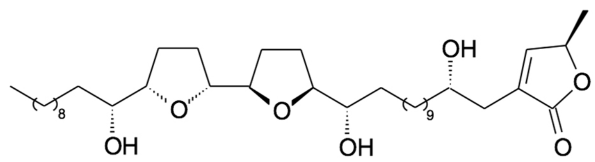

Figure 2. Chemical structure of acetogenin rolliniastatin-1, which is the primary compound of the ethanolic extract of *Annona mucosa* seeds (ESAM).

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