Natural Insecticides from the Annonaceae: A Unique Example for Developing Biopesticides

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Abstract

The documented negative impacts of synthetic insecticides on the environment as well as on human health, the consumers' concerns over insecticide residues in foods, and the emergence of resistant insects call for new approaches to manage insect pests. In this context, research on the potential use of plant extracts and their constituents has grown dramatically in the past decade. Among terrestrial plant families, Annonaceae has drawn considerable attention since the 1980s, owing to the presence of acetogenins, a class of natural products with a broad range of insecticidal bioactivities. Crude extracts from seeds, leaves, bark, twigs, and fruits obtained from the plant species of Annonaceae have been extensively tested in recent years for bioactivity to pest insects and related arthropods worldwide. *Asimina triloba*, *Annona muricata*, and *Annona squamosa* are the species that have been most frequently examined for their insecticidal effects.

Keywords

Annonaceae • Acetogenins • *Annona squamosa* • Squamocin • Annonicin • Mitochondrial poison • Botanical insecticide

1 Introduction

Annonaceae is the largest plant family in the order Magnoliales (Westra and Maas 2012) and comprises around 2,500 species and 130 genera (Pirie et al. 2005). Except for two related North American genera (*Asimina* and *Deeringothamnus*), the family is entirely tropical (Thomas and Doyle 1996). Annonaceae enjoyed considerable attention from plant systematists in the twentieth century. The Swedish botanist Robert Fries spent a lifelong career studying herbarium specimens, mainly originating from the Neotropics. He contributed greatly to the flora of Central America, South America, and the West Indies, especially to the knowledge of the family of Annonaceae (Erkens 2007).

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Given the problems that synthetic insecticides have caused to the environment as well as to human health, there has been a surge of research on plant extracts and plant natural products for insect control (Castillo-Sánchez et al. 2010). The practice of using plant extracts in agriculture for pest control is not new. They have been used for at least two millennia, when botanical insecticides were considered important products for pest management in ancient China (Long et al. 2006), Egypt, Greece, and India (Isman 2006). Even in the United States and some European countries, botanical insecticides were widely used before the discovery of organochlorine and organophosphate insecticides in the late 1930s and early 1940s (Isman 1997). The Annonaceae family has drawn a lot of attention since the 1980s, due to the presence of acetogenins, a class of natural products with a broad range of biological activities, among which insecticidal activity stands out (Ocampo and Ocampo 2006).

2 Brief History of the Acetogenins and Their Pesticidal Activity

In the 1970s, the National Cancer Institute started funding Dr. Jerry McLaughlin at Purdue University to find botanical substances with anticancer potential. He tested and screened over 3,500 species of plants and found that the acetogenin compounds of the Annonaceae family had the most potential activity. McLaughlin (2008) thus focused his studies on 14 annonaceous species that yielded these novel substances. These were *Asimina triloba* (pawpaw), *Goniothalamus giganteus*, *Annona squamosa* L. (sugar apple or sweetsop), *Annona muricata* L. (soursop, graviola, guanabana), *Annona bullata* Rich., *Asimina parviflora* (Michx.) Dunal (dwarf pawpaw), *Annona longifolia* Kral. (long-leafed dwarf pawpaw), *Annona reticulata* L. (custard apple), *Annona glabra* L. (pond apple), *Annona jahnii* Saff., *Annona cherimola* Mill. (cherimoya), *Xylopia aromatica* (Mart.) Lam., *Rollinia mucosa* (Jacq.) Baill. (biriba), and *Rollinia emarginata* Schlecht.

Arguably, his most important contributions have been to our knowledge of the annonaceous acetogenins found in the pawpaw tree. From the pawpaw and these additional species, his research team isolated and characterized over 200 new annonaceous acetogenins. Following his observations in 1982 that the North American pawpaw tree was potently bioactive, his group identified some 50 acetogenins in its seeds and bark with antitumor and pesticidal properties. The acetogenins are found in the leaves and branches and, predominantly, in the seeds of annonaceous plants. McLaughlin worked with Eli Lilly and Company (Greenfield, IN) and the USDA (Peoria, IL), demonstrating that the pawpaw acetogenins are potent inhibitors of a number of agricultural and other pests: mosquito larvae, two-spotted spider mites, Mexican bean beetles, striped cucumber beetles, European corn borers, melon or cotton aphids, blowfly larvae, and a nematode (*Caenorhabditis elegans*) (Alkofahi et al. 1989; Mikolajczak et al. 1989).

However, despite its relatively large size, this plant family had chemically been one of the least explored. The discovery of uvaricin, a *bis* (tetrahydrofuranoid) fatty acid lactone that was first isolated in 1982 from the roots of *Uvaria accuminata* – the first of the acetogenins – invigorated wide interest in this family (Jolad et al. 1982). The temperate American pawpaw (*A. triloba*; Fig. 2.1) and the tropical soursop (*A. muricata*; Fig. 2.2) and sweetsop (*A. squamosa* L.; Fig. 2.3) have been the species most intensively examined for their insecticidal effects. Each of these species contains complex mixtures of acetogenins comprising at least 30 compounds. Sesquiterpenes and monoterpenes are the main types of compounds present in essential oils of *Annona* species (Rios et al. 2003). From the wide variety of acetogenins, squamocin and annonacin have shown the greatest impact on insects (Álvarez et al. 2008) (Fig. 2.4). The annonaceous acetogenins are an important group of long-chain fatty acid derivatives found exclusively in the plant family Annonaceae. Nearly 400 compounds from this class have been published in the literature since the discovery of uvaricin. The potential application of acetogenin molecules is linked

Fig. 2.1 *Asimina triloba* (location unknown; Scott Bauer photo, courtesy of the United States Department of Agriculture)

also to their pesticidal properties (e.g., asimicin and annonin) (Gupta et al. 2011).

3 Mode of Action of Acetogenins

Acetogenins are mitochondrial poisons, inhibiting cellular energy production through a mode of action identical to that of the well-known botanical insecticide and fish poison, rotenone (Londershausen et al. 1991). More specifically, acetogenins block the respiratory chain at NADH-ubiquinone reductase (complex I) and cause a decrease in ATP levels, directly affecting electron transport in the mitochondria, causing apoptosis (Alali et al. 1999). Acetogenins also inhibit insect development and behavior (Table 2.1).

4 Research on Natural Insecticides from the Annonaceae in Canada

The majority of research on natural insecticides from Annonaceae conducted in Canada has taken place in our laboratory at the University of British

Fig. 2.2 *Annona muricata* growing in Saba, Netherlands Antilles (Mary Roduner photo)

Fig. 2.3 *Annona squamosa* growing at Cabocla Farm, Limoeiro do Norte County, State of Ceara, Brazil (Dr. Antonio Lindembergue Martins Mesquita photos)

Columbia. The first of our research in this area was published in 2004. Toxicity and antifeedant activities of crude seed extracts of *A. squamosa* from Maluku, Indonesia, against the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), and the cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), were determined using different bioassays. Aqueous seed extracts and an aqueous emulsion of ethanolic seed extracts were toxic to both species. A crude aqueous extract also deterred feeding of fourth-instar *P. xylostella* in a leaf disc choice bioassay. Toxicities of crude aqueous extracts to natural enemies, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), were investigated using direct spray and residual contact tests. *C. carnea* larvae were less susceptible to

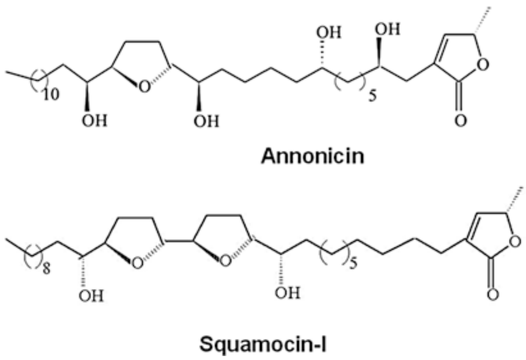

Fig. 2.4 Chemical structures of annonacin and squamocin, major insecticidal acetogenins from the seeds of commonly cultivated *Annona* species

the extracts than were *O. insidiosus* adults (Leatemia and Isman 2004a).

Leatemia and Isman (2004b) assessed the efficacy of crude seed extracts of *A. squamosa* against larvae of the diamondback moth, *P. xylostella* L., feeding on cabbage. Three greenhouse trials were carried out using aqueous seed extracts and an aqueous emulsion of ethanolic seed extracts. At a concentration of 0.5 % (w/v), an aqueous emulsion of an ethanolic seed extract was 2.5-fold more effective than 1 % rotenone, a commercial botanical insecticide. Crude aqueous seed extracts showed efficacy compared to pyrethrum, the most widely used botanical insecticide. Crude ethanolic seed extracts of *A. muricata*, *A. squamosa* (Annonaceae), *Lansium domesticum*, and *Sandoricum koetjape* (Meliaceae) collected from different locations and years in Maluku, Indonesia, were screened for inhibition of larval growth against the polyphagous lepidopteran *Spodoptera litura* (Noctuidae). Extracts of *A. squamosa* were significantly more active (20-fold) than those of *A. muricata* (Leatemia and Isman 2004c). A similar study of *A. squamosa* and *Annona atemoya* from Brazil indicated that crude methanolic seed extracts of *A. squamosa* were ~10 times more active as a feeding deterrent than *A. atemoya* against third-instar *T. ni* larvae in a leaf disc choice bioassay. *A. squamosa* was ~ three times more active as a growth inhibitor than *A. atemoya*. Methanolic seed extracts of *A. squamosa* and *A. atemoya* were toxic to third-instar *T. ni* larvae following either topical or oral application. *A. squamosa* was more toxic through feeding (LC_{50} = 167.5 ppm vs. 382.4 ppm), whereas *A. atemoya* exerted greater toxicity via topical application (LC_{50} = 301.3 mg/larva vs. 197.7 mg/larva). Both *A. squamosa* and *A. atemoya* extracts reduced leaf area consumption and larval growth in a greenhouse experiment (De Seffrin et al. 2010) (Fig. 2.5).

Regarding public health pests, a crude ethanolic extract obtained from seeds of *A. squamosa* was evaluated for its larvicidal effect against the mosquitoes *Aedes aegypti* and *Aedes atropalpus*. The extract produced >70 % mortality in both young (late 1st to 2nd instar) and older larvae (3rd to early 4th instar) of *A. aegypti* at concentrations of 250–500 ppm. In most cases, mortality was greater at 48 h compared to 24 h. At a concentration of 100 ppm, the extract produced complete mortality of young instar larvae of *A. atropalpus* at 24 h. *A. atropalpus* is significantly more susceptible than *A. aegypti* (Srikrishnaraj and Isman 2006).

5 Research on Natural Insecticide from the Annonaceae Worldwide

Crude extracts from seeds, leaves, bark, twigs, and fruits from Annonaceae have been extensively tested in recent years for bioactivity to pest insects and related arthropods.

Among agricultural pests, Dadang and Prijono (2009) assessed the effectiveness of two botanical insecticide formulations: mixtures of *Piper retrofractum* (Piperaceae) and *A. squamosa* extracts and *Aglaia odorata* (Meliaceae) and *A. squamosa* extracts at 0.05 and 0.1 %. These concentrates were compared to the synthetic pyrethroid insecticide deltamethrin at 0.04 % and the microbial insecticide *Bacillus thuringiensis* at 0.15 % in the field of two major cabbage insect pest populations. The application of both mixtures and conventional formulations decreased populations of *Crocidolomia pavonana* (F.) (Lepidoptera: Pyralidae) and *P. xylostella* (L.) (Lepidoptera: Yponomeutidae).

Table 2.1 Biological activity of different plant species from the Annonaceae family on arthropods

Plant species	Arthropod species	Biological activity	Plant part tested	Lethal dosage (LD_{50}/LC_{50}/EC_{50})	Concentration	References
Annona squamosa	*Plutella xylostella*	T, FD	Seeds		0.5 %	Leatemia and Isman (2004a, b, c)
	Trichoplusia ni	FD	Seeds	167.5 ppm		De Seffrin et al. (2010)
	Spodoptera litura	GI	Seeds	191 ppm		Leatemia and Isman (2004a, b, c)
	Aedes atropalpus	T	Seeds		100 ppm	Srikrishnaraj and Isman (2006)
	Macrosiphum rosaeformes	T	Leaves		20 %	Dhembare et al. (2011)
	Bemisia argentifolii	T	Seeds		0.25 %	Lin et al. (2009)
	Aphis gossypii	T	Seeds		0.25 %	Lin et al. (2009)
	Tetranychus kanzawai	T	Seeds		0.125 %	Lin et al. (2009)
	Tribolium castaneum	T	Leaves		20 %	Anita et al. (2012)
	Callosobruchus chinensis	GI, OD	(+)-O-Methylarmepavine		1–4 μg/μL/larva	Konkala et al. (2012)
	Anopheles subpictus	T	Bark	93.80 mg/L		Kamaraj et al. (2011)
	Culex tritaeniorhynchus	T	Bark	104.94 mg/L		Kamaraj et al. (2011)
	Culex quinquefasciatus	T	Leaves	11.01 μg/mL		Magadula et al. (2009)
	Pediculus humanus capitis	T	Fruits		0.1, 1, and 10 % w/w	Kosalge and Fursule (2009)
	Musca domestica	GI	Seeds	345 mg/L		Begum et al. (2010)
	Sitophilus oryzae	T	Leaves		1 %	Kumar et al. (2010)
Asimina triloba	*Acalymma vittatum*	FD	Fruits		1 %–5 %	Sedlacek et al. (2010)
Annona muricata	*Plutella xylostella*	T	Seeds, leaves		5 ppm	Trindade et al. (2011)
	Anastrepha ludens	T	Stem, leaves		2,000 μg/mL	González-Esquinca et al. (2012)
	Bactericera cockerelli	GI	Seeds		2,500–5,000 ppm	Flores-Davila et al. (2011)
	Sitophilus zeamais	T	Seeds		0.4 %	Asmanizar and Idris (2012)
	Aedes aegypti	T	Seeds	93.48 μg/mL		Grzybowski et al. (2013)
Annona montana	*Spodoptera frugiperda*	T	Acetogenins		100 ppm	Di Toto Blessing et al. (2012)

FD feeding deterrence, *T* toxicity, *GI* growth inhibition, *R* repellency, *OD* oviposition deterrence

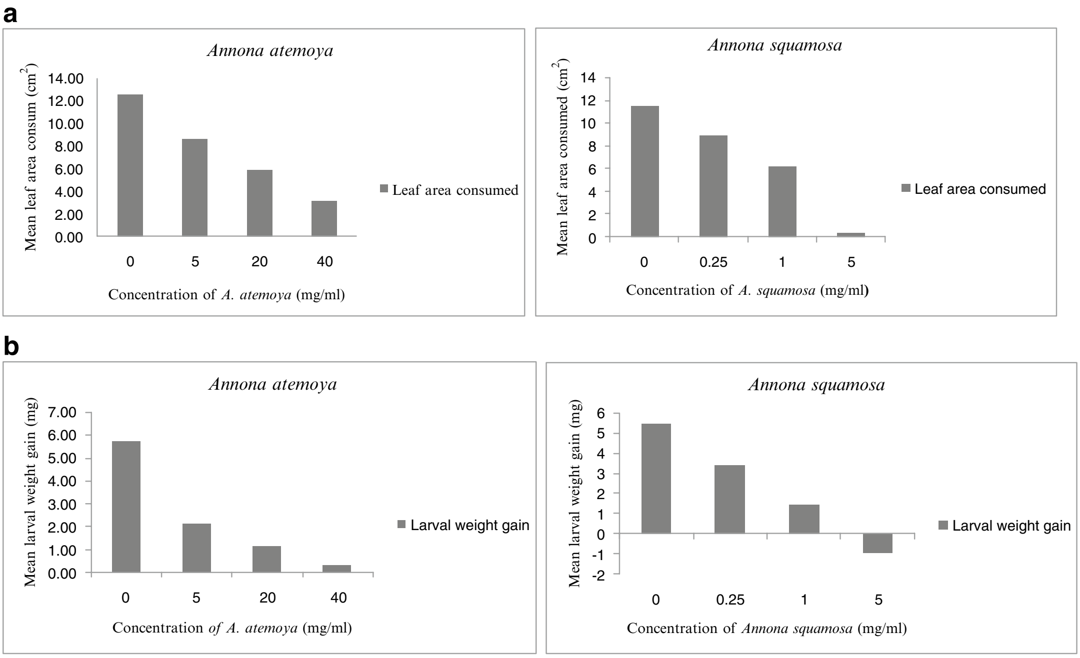

Fig. 2.5 Greenhouse trial measuring mean leaf area consumed (**a**) and mean larval weight gained (**b**) by third-instar *T. ni* when placed on cabbage plants sprayed with an increasing concentration of *A. atemoya* and *A. squamosa* crude extract

The mixtures of botanicals at 0.1 % were more effective than the synthetic insecticide, and they did not affect the performance of insect pest natural enemies. Effects of plant extracts were tested on the tomato leaf miner (*Tuta absoluta* [Meyrick]) (Lepidoptera: Gelechiidae), under laboratory and greenhouse conditions. Anosom EC (annonin), azadirachtin, and mixtures thereof were very effective in controlling this pest (Durmusoglu et al. 2011). Laboratory experiments examined the effects of pawpaw *A. triloba* (L.) Dunal, fruit extract on mortality, and feeding deterrence of the striped cucumber beetle, *Acalymma vittatum* (F). Pawpaw fruit extract reduced feeding by 89 % and 97 % at concentrations of 1 % and 5 %, respectively. The calculated LC_{50} value was 5.05 %, whereas the LCF_{10} (concentration at which only 10 % of the leaves were consumed) was 0.20 %. At 10 %, only 10 % of the beetles were killed; however, only 3 % of the leaf tissue was consumed. Thus, pawpaw fruit extract may be an effective insect feeding deterrent (Sedlacek et al. 2010).

The bioactivity of an ethanolic leaf extract of *A. muricata* on the development of the larvae and pupae of the diamondback moth *P. xylostella* was evaluated by Trindade et al. (2011). At the highest concentration tested (5 ppm), the most active extract caused 100 % larval mortality; at lower concentrations, the duration of the larval phase was increased by up to 2.6 days, and larval survival was significantly reduced. The pupal stage was far less affected by exposure to the extracts, although the duration was increased by up to 1 day in the presence of nonlethal concentrations. The major acetogenins from a Bolivian collection of *A. montana* (Fig. 2.6) – annonacin, cis-annonacin-10-one, densicomacin-1, gigantetronenin, murihexocin B, and tucupentol – were evaluated for their antifeedant and toxic effects on *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae), a serious pest affecting corn crops in Argentina and throughout the Americas. All the acetogenins produced 100 % mortality during the larval or pupal stages at 100 ppm in diet. In addition, the compounds annonacin, cis-annonacin-10-one,

Fig. 2.6 *Annona montana* growing at Gaspar, SC, Brazil (Anestor Mezzomo photo)

and densicomacin-1 deterred feeding by more than 80 % at the same concentration (Di Toto Blessing et al. 2012).

Among sucking insects, aqueous extracts of various annonaceous and other plants were tested for their insecticidal efficacy against the rose aphid, *Macrosiphum rosaeformes* (Davis), under laboratory conditions. Thirteen plant extracts were tested. Garlic, *Allium sativum* (Linn), was found to be the most effective, followed by custard apple, *A. squamosa* (Linn), and bullock heart, *A. reticulata* (Linn.), closely followed by neem, *Azadirachta indica* A. Juss. (Dhembare et al. 2011). Lin et al. (2009) tested the cold-pressed oil from the seeds of *A. squamosa*. The oil was effective in controlling the silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring (Homoptera: Aleyrodidae), infesting the leaves of tomato plants under greenhouse conditions. Sugar apple seed oil was also very effective in controlling the cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), on melon leaves and the Kanzawa spider mite, *Tetranychus kanzawai* Kishida (Acari: Tetranychidae), on soybean leaves. Nymphs of the potato psyllid, *Bactericera cockerelli* (Sulc), were treated with extracts of *A. muricata*, *Carica papaya*, *Euphorbia dentata*, *Thuja occidentalis*, *Sapindus saponaria*, and A. *indica*. After 72 h, *A. muricata* seed extract, at concentrations of 2,500 and 5,000 ppm, produced 98 and 100 % mortality of potato psyllid nymphs, followed by *A. indica* oil, causing 91 and 100 % mortality at 2,000 and 2,500 ppm, respectively. *A. muricata* seed extract was the most effective insecticide in the study (Flores-Davila et al. 2011).

Increased concern by consumers over insecticide residues in food products, the occurrence of insecticide-resistant insects, and the precautions necessary to work with traditional chemical insecticides call for new approaches to control stored product insect pests (Konkala et al. 2012). Management of stored product pests using materials of plant origin is the subject that has received considerable research attention because of their minimal environmental hazards and low mammalian toxicity (Isman 1994). Chemical pesticides have often provided the first line of defense against insect pests of grain. Synthetic pesticide treadmills and inefficiencies have resulted in increased input costs for resource-poor farmers in developing countries. The need for cheaper but effective options for combating insect pests has resulted in the resurgent use of plant materials where the majority of the farmers in developing countries are resource constrained (Chikukura et al. 2011). Asmanizar and Idris (2012) tested the bioactivity of *Jatropha curcas* and *A. muricata* crude seed extracts against the maize weevil *Sitophilus zeamais* (Coleoptera: Curculionidae) by using dipping and surface protectant methods. Both *J. curcas* and *A. muricata* extracts had contact and stomach poison activities against *S. zeamais*. Using a dipping method, weevil mortality was 90 and 70 %, respectively, at a concentration of 20 % (v/v), whereas using a surface protectant method, weevil mortality was 100 % at 0.4 % (v/w) concentration for both crude extracts. *J. curcas* and *A. muricata* extracts applied to rice grain (surface protectant method) can reduce F_1 progeny production, weight loss, and rice grain damage.

The insecticidal and repellent activities of fruit extracts of *Xylopia aethiopica* (Dunal) A. Rich. and *Dennettia tripetala* (Baker f.) G.E. Schatz (both belonging to the family Annonaceae) were studied against the rice weevil *Sitophilus oryzae* (L.), an economic, primary postharvest pest of rice and other cereal products. The extracts of both plants caused significant adult weevil mortality and a reduction in F1 progeny emergence. Extracts were also significantly ($P < 0.001$) toxic to *S. oryzae* after 24 h with the highest dose (2 mg cm^{-2}) producing 100 % weevil mortality. Similarly, both male and female weevils significantly avoided the test arm compared to the control arm in a Y-tube olfactometer repellence test. These results suggest that *X. aethiopica* and *D. tripetala* natural extracts have potential use as part of an integrated pest management system for stored product protection against *S. oryzae* (Ukeh et al. 2012).

Pulverized leaves of *A. squamosa* (L.), *Moringa oleifera* (Lam.), and *Eucalyptus globulus* (Labill.) were tested for their insecticidal and seed protective effect against the confused flour beetle, *Tribolium castaneum* (Herbst), a stored grain pest of wheat. When larvae were introduced to pulverized leaves of *A. squamosa*, *M. oleifera*, and *E. globulus* separately, mortality increased with increasing concentrations and resulted in 100 % mortality within a short period of 8–10 days at the highest concentration. Larvae that were introduced to these cultures did not grow well or molt to the next developmental stage. These plants are also very effective at preventing seed damage. The seed protective effect ranged from 39 to 82 % for *A. squamosa*, 34–78 % for *M. oleifera*, and 42–88 % for *E. globulus*. Considering the insecticidal and seed protective effect, these three plant powders could be employed as alternatives to chemical and synthetic pesticides for smallholder farmers (Anita et al. 2012).

Topical application of (+)-O-methylarmepavine, a juvenile hormone analogue isolated from the leaves of *A. squamosa* L., caused inhibition of growth and development of fifth-instar larvae of the bruchid beetle *Callosobruchus chinensis*. Production of larval-pupal intermediates and pupal-adult intermediates was observed as well as adults with various ovarian abnormalities (Konkala et al. 2012).

Kamanula et al. (2011) conducted a survey on farmer ethno-ecological knowledge of pests of stored maize and bean and their pest management practices including pesticidal plant use in eastern Zambia and northern Malawi. They concluded that the rational use of pesticidal plants for insect pest management needs a constructive collaboration between scientists and farmers. Scientists can develop guidelines to ensure efficacious use of the pesticidal plants widely used by farmers. Some plants are required in large quantities, so cultivation may be an important consideration to increase their availability. Researchers can also develop guidelines for the propagation and cultivation of those plant species. This would have a positive impact on the availability of such plants and encourage more farmers to use them.

In the field of public health, mosquitoes such as *A. aegypti* L. are important pantropical vectors of dengue and yellow fever (Komansilan et al. 2012). Essential oils of *Guatteria hispida*, *G. blepharophylla*, and *G. friesiana* were tested against *A. aegypti*. GC-MS and NMR analyses confirmed the presence of caryophyllene oxide as the main constituent of the leaves of *G. blepharophylla*; in *G. friesiana*, the α- and β-geudesmols prevail; and in *G. hispida* α- and β-pinene and (*E*)-caryophyllene are the predominant compounds. The lethal concentrations LC_{50}, LC_{95}, and LC_{99} were, respectively, 85.74, 199.35, and 282.76 ppm for *G. hispida*; 58.72, 107.6, and 138.37 ppm for *G. blepharophylla*; and 52.6, 94.37, and 120.22 ppm for *G. friesiana.* The oil extracted from *G. friesiana* presented the best insecticidal effect (Aciole et al. 2011).

Costa et al. (2012) described morphological changes that occur in the midgut of third-instar *A. aegypti* L. (Diptera: Culicidae) following treatment with a methanolic extract of *Annona coriacea*. Insects exposed to the extract displayed intense, destructive cytoplasmic vacuolization in columnar and regenerative midgut cells. The apical surfaces of columnar cells exhibited cytoplasmic protrusions oriented toward the lumen, suggesting that these cells could be involved in apocrine secretory processes and/or apoptosis. *A. coriacea* extracts induced morphological alterations in the midgut of *A. aegypti* midgut larvae, supporting the use of plant extracts for control of this disease vector.

Cytotoxicity and larvicidal properties of the leaf extracts of *A. muricata*, *A. senegalensis* Pers, and *A. squamosa* L. were tested against brine shrimp larva and late 3rd instar of *Culex quinquefasciatus* Say. With the larvicidal properties of *A. senegalensis* being described for the first time, its value together with that of *A. squamosa* may prove to be the best natural source of larvicidal agents. The LC_{50} values for crude extracts of *A. senegalensis* and *A. squamosa* were 0.67 and 0.64 μg/mL, respectively, for shrimp larvae and 23.42 and 11.01 μg/mL, respectively, for *C. quinquefasciatus* (Magadula et al. 2009). An extract obtained from the fruits of Indian neem, *A. indica*, and seeds of *A. squamosa* L. was tested against the head louse *Pediculus humanus capitis*. Petroleum ether extracts of these plants produced high levels of mortality in adult lice. *A. squamosa* L. extract showed more potent activity than *A. indica* extracts at all concentrations (0.1, 1, and 10 % w/w) (Kosalge and Fursule 2009).

6 Solvents Used for Extracting Acetogenins

An ethanolic extract of *A. squamosa* leaves showed potent activity against the rice weevil *S. oryzae*. The extract produced significant knockdown (KDT_{50}) at 1 % (23.1 min) and 5 % w/v (11.4 min). Complete mortality was achieved at 39.6 ± 1.4 and 14.5 ± 1.1 min for 1 % and 5 % w/v, respectively (Kumar et al. 2010). Kamaraj et al. (2011) assessed the larvicidal activities of hexane, chloroform, ethyl acetate, acetone, and methanol dried leaf and bark extracts of *A. squamosa*, *Chrysanthemum indicum*, and *Tridax procumbens* against fourth-instar larvae of the malaria vector, *Anopheles subpictus* Grassi, and the Japanese encephalitis vector, *Culex tritaeniorhynchus* Giles (Diptera: Culicidae). All plant extracts showed moderate effects after 24 h of exposure; however, the most toxic were the methanolic bark extract of *A. squamosa*, leaf ethyl acetate extract of *C. indicum*, and leaf acetone extract of *T. procumbens* against the larvae of *A. subpictus* (LC_{50} = 93.80, 39.98, and 51.57 mg/L) and methanolic bark extract of *A. squamosa*, leaf methanol extract of *C. indicum*, and leaf ethyl acetate extract of *T. procumbens* against the larvae *of Cx. tritaeniorhynchus* (LC50 = 104.94, 42.29, and 69.16 mg/L) respectively. The acetone, chloroform, hexane, petroleum ether, and ethanol extracts *of A. squamosa* foliage were studied against the early fourth-instar larvae of *A. aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*. Larval mortality was observed after 24 h exposure. All extracts showed moderate larvicidal effects; however, the greatest larval mortality was obtained with a petroleum ether extract (Kumar et al. 2011).

González-Esquinca et al. (2012) used water and ethanolic extracts to determine the activity of stem and leaf extracts of *A. muricata* L., *A. diversifolia* Saff., and *A. lutescens* Saff. against larvae of *Anastrepha ludens* (Mexican fruit fly). Extracts of the three *Annona* species showed time-dependent larvicidal activity against *A. ludens*, with variable mortality rates at 72 h of exposure as follows: *A. lutescens* 87–94 %, *A. diversifolia* 70–90 %, *and A. muricata* 63–74 %. Grzybowski et al. (2013) tested crude ethanolic extracts of *A. muricata* L. seeds and *Piper nigrum* L. fruits against *A. aegypti* larvae. The LC_{50} value for *A. muricata* was 93.48 µg/mL and for *P. nigrum* 1.84 µg/mL. Begum et al. (2010) investigated the toxic effects of ethanol extracts of seeds of *A. squamosa* and *Calotropis procera* (Asclepiadaceae) against different developmental stages of the housefly *Musca domestica* L. (Diptera: Muscidae). LC_{50} values for the extracts of *C. procera* and *A. squamosa* seeds were 870 and 345 mg/L, respectively. The high concentration (10 %) of extract from the seeds of *A. squamosa* exhibited maximum inhibitory effects (56 %) on acetylcholinesterase activity from all three developmental stages of the fly. The extracts can be dissolved in other solvents such as dichloromethane, dimethyl sulfoxide, or emulsified in water with Tween 20® (Castillo-Sánchez et al. 2010). From this information, it can be inferred that acetogenins can range from very polar, such as those extracted by water and ethanol, to nonpolar, i.e., those extracted by hexane; however, environmental considerations would suggest the use of more polar solvents (Bobadilla et al. 2005).

7 Commercial Pesticides Based on the Annonaceae

Acetogenins for insect control will probably continue to be based on crude or partially refined extracts obtained from plant sources (Leatemia and Isman 2004c), at least in developing countries or for use in organic food production in industrialized countries. The seeds are powdered and mixed with water or alcohol for application in Indian tea plantations. They can be useful against stem borers, sucking pests, and scale insects (Mamun and Ahmed 2011). The North American pawpaw *A. triloba* is a tree fruit in the early stages of commercial production in the United States and Canada. This plant contains acetogenins with pesticidal properties in the twigs, unripe fruit, seeds, roots, and bark tissues. However, commercial development of these compounds, based on twig extracts, has been problematic due to limited availability of biomass for extraction (Pomper et al. 2009). Commercially formulated botanicals are often more expensive than synthetic insecticides and not as widely available (Rajashekar et al. 2012).

Globally, the rapid increase in the human population and limited availability of arable land are becoming key factors stimulating market growth for agrochemicals. Also since 1990, the world market for organic produce has grown. Pressure from consumers for organic produce and other foods is leading even conventional growers to reduce their use of synthetic pesticides and consider alternatives, creating greater market space for botanical insecticides.

8 Conclusion

Asimina triloba, *Annona muricata*, and *Annona squamosa L.* are the species that have been most frequently examined for their insecticidal effects. Extracts from Annonaceae have been tested for control of Lepidoptera, Hymenoptera, Coleoptera, and Diptera, especially against *Spodoptera frugiperda*, *Plutella xylostella*, *Aedes aegypti*, and stored grain insects. Crude extracts from seeds, leaves, and fruits have been frequently tested as biopesticides to control arthropods. At present, there are two commercial insecticides in India based on an *Annona*: Anosom™ (seed extracts of *A. squamosa* and *A. reticulata*, containing 1 % squamocin as the active ingredient) and Bio Rakshak™ (a seed extract of *A. squamosa*). The registration and large-scale production of standardized botanical pesticide products are important barriers to commercialization of botanical pesticides. Smallholder farmers in developing countries are using their empirical familiarity with plant properties to protect their crops from pests (Perez et al. 2008).

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