



Exploring the Insecticide and Acaricide Potential of Development Regulators obtained from Restinga vegetation from Brazil

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Manuscript received on April 20, 2018; accepted for publication on July 25, 2018

How to cite: FEDER D, GONZALEZ MS, MELLO CB, SANTOS MG, ROCHA L, KELECOM A AND FOLLY E. 2019. Exploring the Insecticide and Acaricide Potential of Development Regulators obtained from Restinga vegetation from Brazil. *An Acad Bras Cienc* 91: e20180381. DOI 10.1590/0001-3765201920180381.

Abstract: As a part of our continuing search for insect and arthropod development regulators from Brazilian restinga vegetation of the Rio de Janeiro State, crude extracts, purified fractions and essential oils were submitted to screening tests seeking for biological activities on the development of the insects *Rhodnius prolixus*, *Dysdercus peruvianus*, *Oncopeltus fasciatus* and *Rhipicephalus (Boophilus) microplus*. Up to now, 102 secondary metabolites have been detected in the fractions, among them monoterpenes, sesquiterpenes and two triterpenes which were obtained from the species, *Eugenia sulcata*, *Pilocarpus spicatus*, *Manilkara subsericea*, *Myrciaria floribunda* and *Zanthoxylum caribaeum*. These secondary plant metabolites are considered of interest for the use of studies related to arthropod endocrinology, vector-parasite interaction system, and population control of vector insect and agricultural pest. The observed biological activities were surprisingly high, involving increased mortality, molting and metamorphosis inhibition, paralysis, corporeal deformities, apparition of permanent nymphs, of adultoids and juvenoids, partial or total inhibition of oviposition and egg hatching. These compounds are now being studied further to determine if they may or may not be useful in controlling insect populations and/or interfere with the life cycle and vector transmission of parasites to animal and human populations.

Key words: arthropods, essential oil, green pesticide, insect pests, insect vectors, insect growth regulators

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INTRODUCTION

Restinga vegetation is a mosaic of plant communities growing at sandy coastal plains found along much of the tropical, subtropical and temperate Brazilian coast (Araujo and Henriques 1984, Araujo 1992).

They are formed by marine, fluvio-marine or wind sediments originated from the Quaternary Period. (Marques and Oliveira 2004, Kelecom et al. 2002a). Such environments display many geomorphological formations, invariably with low availability of resources, mainly due to the low capacity of retention of water and nutrients in sandy soils. (Rosado and De Mattos 2007). The estimative for the Brazilian restinga vegetation is more than 4,000 species, only to Rio de Janeiro state, 1,005 species have already been registered (Sá 2014, Araujo 1992).

According to the Chico Mendes Institute for Biodiversity Conservation (ICMBio, 2017), the Restinga de Jurubatiba National Park, created on April 29, 1988, is a federal conservation unit with 14,922.39 hectares, 44 km of coast and 18 coastal lagoons. It is located between latitudes 22° and 22°23'S and longitudes 41°15' and 41°45'W (Fig. 1) incorporating the cities of Macaé, Carapebus, and Quissamã in the state of Rio de Janeiro (Santos et al. 2004). According to Araújo (2000), the Restinga of Jurubatiba possesses a great diversity of vegetal species, almost completely unknown from the chemical and pharmacological points of view. It is thus reasonable to expect that new metabolites with very uncommon structures may be encountered, and that a number of them may be endowed of biological activities. Hence, due to the biological potential of secondary metabolites, plants of such a peculiar biome were investigated looking for insecticide and acaricide potential, aiming agriculture pests control.

The agricultural sector is nowadays one of the main sources of income and productivity in the world economy. According to the Food and

Agriculture Organization of the United Nations (FAO 2013), about 75% of the world agricultural economy is generated in developing countries, and in many of these this sector contributes 30% of gross domestic product (GDP).

In Brazil, agrobusiness plays a major role in the economic scenario, being a fundamental component for economic performance and integration (Campanhola, 2005). In the year 2013, Brazilian agrobusiness, from the agricultural raw material to its industrialization and commercialization, including the sectors that supply feedstock, machinery, and implements, accounted for 22.5% of the GDP, that is about USD 300.000.000.000 (Barros et al. 2014). According to the literature, the increase in agricultural production in Brazil is directly related to technological development, which is derived from studies and directed research (Campanhola, 2005).

The presence of populations of the phylum Arthropoda, mainly of the class Insecta and order Ixodida that act as agricultural plagues or parasites of animals are considered as a serious economic problem since the annual losses of productivity of crops and animal breeding reach of significant proportions. The use of chemicals to control populations of insects and ticks to improve economic investment may result in toxicological impacts on animals, man and the environment, and are often more damaging than the pests intended to be controlled (Faria et al. 2007). Among the main damages observed are: (i) increased resistance by pests to current insecticides due to changes in the rate of penetration and inactivation of the biochemical mechanisms of these insecticides, (ii) the emergence of secondary pests as a result of the elimination of natural enemies, and (iii) the elimination of non-target organisms, *e.g.* insects useful for pollination. However, because of their proven effectiveness and rapid action, conventional pesticides (insecticides) are still the most important tool for population control programs of disease



Figure 1 - Map showing the coordinates of Restinga de Jurubatiba National Park.

vectors, mites and agricultural pests, even though the arsenal of effective products has been decreasing due to the development of resistance to the most commonly used insecticides (Zaim and Guillet 2002).

Resistance development is a skill acquired by an organism's lineage in tolerating doses of a toxic product that is lethal to most of the normal population of the same species. Ticks and insects have a rapid life cycle and accelerated reproduction, where one female breeds hundreds to thousands of individuals. This favors the emergence of populations with new genetic characteristics. The spread of insecticide resistance in insect populations is related to the frequency of their use and results not only from the selective pressure of these toxic compounds on these populations but also from the inherited characteristics of the insect species involved (Hemingway and Ranson 2000).

Individuals with advantageous mutations related to the resistance phenotype are more likely to survive insecticide treatments and contribute to

offspring than susceptible individuals, resulting in increased frequency of the gene conferring resistance in the next generations (Beaty and Marquard 1996).

Nowadays, liquid solutions and sprays containing organochlorines, organo-phosphates and synthetic pyrethroids are commonly used in the control of arthropods important for public health and management of modern agriculture (Mello et al. 2007).

In the specific case of acaricides, such products are classified as "systemic" or "by contact". The formers are applied by injections into the bovine host, while the latter's can be applied by spraying or immersion of the cattle or by the *pour on* method (application on the animal's back) (Rajput et al. 2006).

Since the use of insecticides and acaricides of first generation (inorganic compounds) and second generation (organochlorines, organophosphates, carbamates and pyrethroids) for the chemical control of agricultural pests and insect vectors is

banned in many countries throughout the World, a public demand for safe technologies became a major concern, and this implies in the development of products with more selective target sites and low risk for non-target organisms and the environment (Graf 1993, Faria 2009). Integrated management of agricultural and arthropod pests of medical and veterinary interest should now be considered, consisting of the use of controlled techniques capable of increasing efficiency in combating these organisms, simultaneously improving the possible use of economic investment and avoiding harmful effects on the environment (Kogan 1998, Smith and Raupp 1986).

The European Union (EU) regulatory system is the strictest in the world and Pesticides in the EU are constantly monitored. Only substances for which there is objective evidence of safe use are approved. (European Commission access in 17 04 2018). Organochlorine and organophosphates pesticides are synthetic pesticides widely used all over the world, which have vast application in the chemical industry and in agriculture. Residues of pesticides can be found in a great variety of everyday foods and beverages. These compounds are known for their high toxicity, slow degradation and bioaccumulation. (Jayaraj et al. 2016).

Alternative strategies for the control of arthropod, tick and insect populations are available, for example the use of fungi, BT toxin, nematodes etc. Secondary metabolites participate in physiological processes essential to plants. They are also responsible for the relationship of plants with the environment in which they interact, defending against microorganisms, herbivores or ultraviolet rays, attracting pollinating agents, or even assisting in symbiotic relationships with other beings. They are also responsible for several pharmacological activities that the plants present. Their production is modulated according to the needs of the plant itself, which is synthesized and degraded (Taiz and Zeiger 1991).

Studies on insect physiology and plant chemistry have revealed defensive plant strategies against insects that do not use the intrinsic toxicity of the secondary metabolites produced (Rattan 2010). These strategies promote a direct interruption in the specific physiological mechanisms necessary for the insect to successfully perform its metamorphosis, reproduction, diapause and behavior through interference with its endocrine system, regulating these and other important vital functions, thus representing a vulnerable point in the life cycle of these insects and ticks. Borges et al (2003) showed that extracts of *Melia azedarach* L. (Meliaceae) inhibit egg production of immersed *Rhipicephalus (Boophilus) microplus* ticks and Feder et al. (1988) showed azadirachtin inhibits egg production in *Rhodnius prolixus*. Such compounds are classified as Insect Growth Regulators (IGRs), since they interfere or are capable of inducing changes in growth and/or differentiation processes throughout the insect/ticks life cycle. These chemicals are of special interest because of their ability to act specifically on target organisms and absence or minimal biological activity for non-target organisms, in addition to the low environmental impact associated with their application, which qualifies them for use in integrated programs for the control of vectors of diseases, agricultural and livestock pests (Modal and Parween 2001).

Our investigation of IGRs has been focused on the flora of Brazilian restinga vegetation. This is a mosaic of plant communities growing at the sandy coastal plains (Magnago et al. 2011, Araujo 1992).

We here review the screening tests carried out by our group on crude extracts, purified fractions and essential oils seeking for biological activity on the development of arthropods of agricultural, veterinary or medical interest. Several fractions obtained from 5 plant genera were selected containing a number of active secondary metabolites. These metabolites are considered of interest for the use of studies related to insects and

ticks endocrinology. We also discuss their scientific relevance in the current state of the art in the field of IGRs use for the population control in insect/ticks of agricultural, medical and veterinary interest.

MATERIALS AND METHODS

STUDIED MATERIAL

Native plants of the Restinga de Jurubatiba National Park, Rio de Janeiro state, Brazil were located, identified and collected by a botanist (Prof. Dr. Marcelo Guerra Santos). Voucher specimens were deposited at the Herbarium of the Faculdade de Formação de Professores da Universidade do Estado do Rio de Janeiro (RFFP). Five species will be discussed: *Eugenia sulcata* Spring ex Mart. (Myrtaceae), with the local name “murtinha” or “murta-preta” (Santos et al. 2009); *Pilocarpus spicatus* A.St.-Hil. (Rutaceae), with the local name “jaborandi-da-restinga” (Mello et al. 2007); *Manilkara subsericea* (Mart.) Dubard (Sapotaceae), with the local name “guracica” (Santos et al. 2009); *Myrciaria floribunda* (H. West ex Willd.) O. Berg (Myrtaceae), with the local name “camboim” (Tietbohl et al. 2014); and *Zanthoxylum caribaeum* Lam. (Rutaceae), with the local name “guando-do-mato” (Nogueira et al. 2014a, b).

Extraction purification and identification of Manilkara subsericea constituents

Fresh fruits were crushed and macerated in ethanol (EtOH) 96% (v/v) at room temperature. Filtration and concentration under vacuum afforded the ethanolic crude extract, which was then dissolved in EtOH/H₂O 90% (v/v) and partitioned with hexane to obtain, after evaporation, the hexane-soluble fraction (FH). Silica gel column chromatography (0.063-0.2 mm particle size, Vetec®) of FH. This hexane-soluble fraction (FH) was chromatographed on a silica gel column (0.063-0.2 mm particle size, Vetec®) using mixtures with increasing amounts of

ethyl acetate in n-hexane. The fraction eluted at the ratio of 98:2 (v/v) n-hexane: ethyl acetate provided, after repeated crystallizations in acetonitrile, a white amorphous powder (PFT) (459.6 mg, yield 0.27%). Identification of PFT as a mixture of α and β -amyrin acetates resulted from ¹H (300MHz, CDCl₃) and ¹³C-NMR (75MHz, CDCl₃) recorded on a Varian VNMRS 300MHz spectrometer, and by GC/MS performed with a SHIMADZU GCMSQP5000 equipment (Fernandes et al 2013).

Extraction of the essential oils

Each material (*E. sulcata* - leaves and stems, *M. floribunda* - leaves, stems and flowers, *P. spicatus* - leaves and *Z. caribaeum* - leaves) was placed in a 5L distillation flask and submitted to hydrodistillation during 3-4h using a Clevenger-type apparatus. At the end, the oils were collected, dried over anhydrous sodium sulphate and stored at 4 °C until further analysis. (Rocha et al. 2012)

Chemical analysis of the essential oils

Essential oils were analyzed on a GCMS-QP5000 (SHIMADZU) gas chromatograph equipped with an electron impact mass spectrometer. The gas chromatographic (GC) conditions were as follows: injector temperature, 260°C; FID temperature, 290°C; carrier gas (helium), flow rate 1 mL/min and split injection with split ratio 1:40. Oven temperature was raised from 60°C to 290°C at a rate of 3°C/min. One microliter of each sample, dissolved in CH₂Cl₂ (1:100 mg/μL), was injected on a DB-5 column (i.d. = 0.25mm, length 30m, film thickness = 0.25μm). The mass spectrometry (MS) conditions were voltage 70 eV and scan rate 1 scan/s. The retention indices (RI) were calculated by interpolation to the retention times of a mixture of aliphatic hydrocarbons (C₉-C₃₀) analyzed in the same conditions. The identification of the substances was performed by comparison of their retention indices and mass spectra with

those reported in literature. The MS fragmentation pattern of compounds was also checked with NIST mass spectra libraries. Quantitative analysis of the chemical constituents was performed by flame ionization gas chromatography (CG/FID), under same conditions of GC/MS analysis and percentages obtained by FID peak-area normalization method. (Lima et al. 2012; Mello et al. 2007, Tietbohl et al. 2012, Nogueira et al. 2014a).

Screening of biological activities

For *in vivo* analysis of the biological activity, the fractions, essential oils and isolated metabolites were administered to our arthropod models (see item 3.0) either orally (mixed with blood), topically (brushing the dorsal cuticle) or by continuous treatment (*i.e.*, contact with the previously treated substrate). Periodically after the treatments, the following biological parameters were quantified: preference or refusal by untreated fertilized females to deposit their eggs on substrates treated with different concentrations of the tested compounds; viability and hatchability of the eggs deposited or

relocated to these substrates; differential sensitivity of different molting stages to treatments; attraction and repellency; anatomical malformations; phagoinhibition; feed rate and excretion; weight gain and body growth; rate, time and period of moulting; death after 24 hours, and at different times of the development and longevity; oviposition (fecundity); viability and egg hatching (Mello et al. 2007, 2008, Vinturelle et al. 2017) (Fig. 2).

ARTHROPODS AND THEIR AGRICULTURAL, MEDICAL OR VETERINARY IMPORTANCE.

ORDER HEMIPTERA

According to Gullan and Cranston (2008), the order Hemiptera is divided into two main groups: (i) Homoptera, represented by cicadas, leafhoppers, aphids and cochonilas, and (ii) Heteroptera, consisting of bedbugs, such as Stinkbugs, water cockroaches, barbers, notonectomids and reduviids. The latter group is characterized by the presence of metapleural odoriferous glands, among which are the families Reduviidae, Pyrrhocoridae and Lygaeoidea.

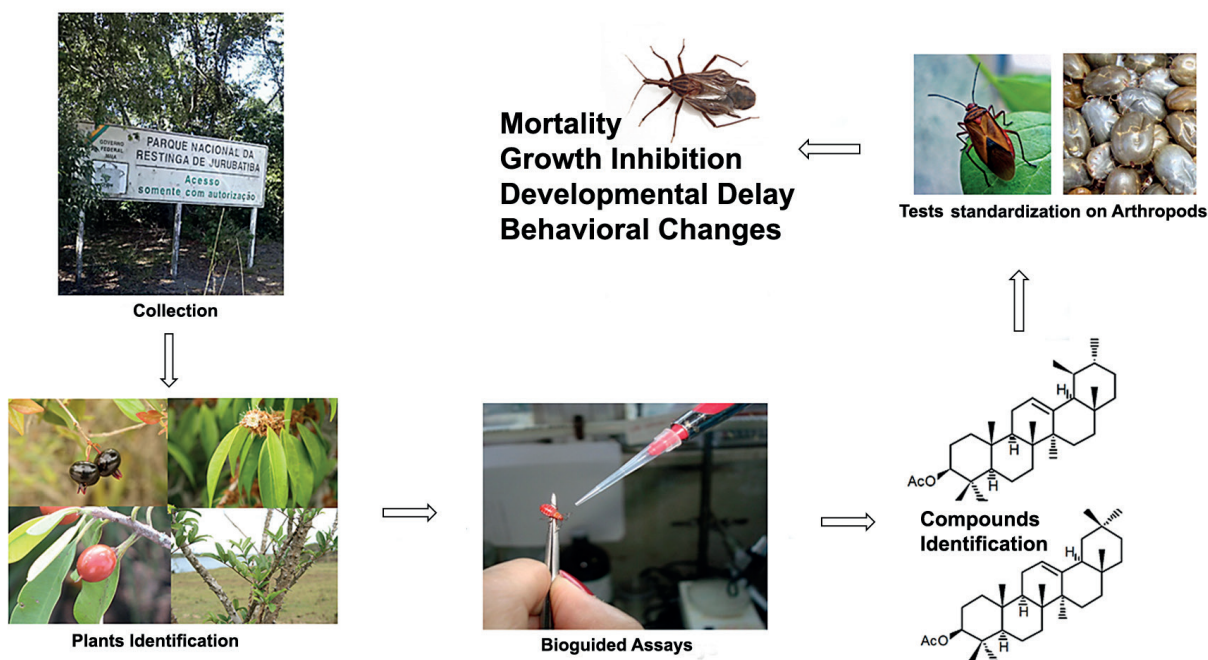


Figure 2 - Methodological Approach and Experimental Design.

RHODNIUS PROLIXUS AND CHAGAS DISEASE

More than 100 years after its discovery, Chagas disease (Chagas, 1909, 1911) is still recognized by the World Health Organization as one of the thirteen most important neglected tropical diseases in the world, is a scourge for humanity and a relevant social and economic issue in many Latin American countries. It produces important social losses in terms of mortality, absenteeism, uselessness for vital activities, and medical-social costs. Such facts are generally ignored by politicians and governments, due to poor visibility of the disease and anonymity of the population affected by the disease. Probably over 10,000 people die every year from clinical manifestations of Chagas disease, and more than 25 million people risk acquiring the disease. An estimated 8 million people are infected worldwide, mostly in Latin America. The main mechanism of transmission is through insect vector such as hematophagous Hemipterans of the subfamily Triatominae. They infect people by depositing their infected feces under the skin and mucosae after the bite. Other modalities of transmission are by transfusion, organ transplantation, orally and congenitally (WHO, 2006, OPAS, 2014). *Rhodnius prolixus* Sthal, 1856 (Hemiptera: Reduviidae) (Fig.

3), is an important vector of Chagas' disease in South and Central America, traditionally used as a model for studies on insect physiology (Garcia and Azambuja 1991), strategies of vector control (Garcia and Azambuja 2004), and parasite-vector interactions (Cortez et al. 2002, Garcia and Azambuja 1991, Gonzalez et al. 2000, Kollien and Shaub 2000, Kelecom et al. 2002b, Azambuja et al. 2017). All these studies provide us with a significant amount of information about molecular factors in the neuroendocrine system and digestive tract that may be involved with fundamental stages of its life cycle (*i.e.*, molting, reproduction) and with the development of *Trypanosoma cruzi* in these hosts.

DYSDERCUS PERUVIANUS AND ONCOPELTUS FASCIATUS

Dysdercus peruvianus Guerin-Méneville (1831) (Heteroptera: Pyrrhocoridae) is popularly named cotton stainer bug. This insect is of great economic importance, being considered as the pest that causes serious economic damages in cotton plantations (*Gossypium hirsutum* L. - Malvaceae). Among damages, this phytophagous damage seeds and stain the cotton fibers with its waste, besides introducing fungi and bacteria (Chiang et al. 1970).

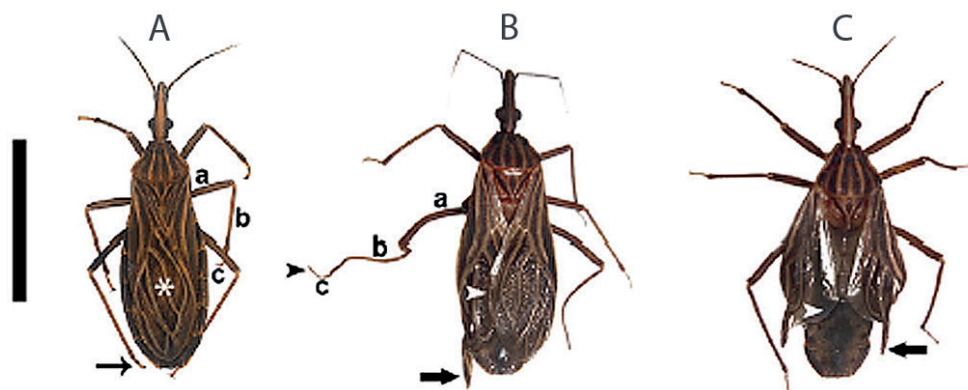


Figure 3 - Malformation of adult males of *Rhodnius prolixus* after continuous treatment with *Z. caribaeum* essential oil. (A) Normal legs of control insects (arrow), normal wings (asterisk). (B) and (C) Short wings (white arrowhead) and deformed (broad black arrow). Elongated legs (black arrowhead). Femur (a), tibia (b) and tarsus (c). Insect (B) shows an absence of the left claw. (Nogueira et al. 2014a). The bar is equal to 1cm.

O. fasciatus (Dallas, 1852), belongs to the family Lygaoidea and, among the species of the genus *Oncopeltus*, it is undoubtedly one of the most used as a model in tests of both physiology and insect biochemistry, in addition to harboring protozoan species of the genus *Phytomonas* that cause damage to plant species of several families (Alves e Silva et al. 2013).

Both *D. peruvianus* and *O. fasciatus* (Fig. 4) are laboratory-friendly species that can be naturally found from the tropical regions of South America to the temperate ones in North America (Dingle et al. 1980, Dingle et al. 2003).

ORDER IXODIDA

The bovine tick *Rhipicephalus (Boophilus) microplus* (Canestrini, 1887) belongs to the phylum

Arthropoda, class Arachnida, order Ixodida, subclass Acari, superfamily Ixodoidea and family Ixodidae (Figure 5). Cattle are the main host for this tick, though it also parasitizes sheep, horses, and even humans (Kerber et al. 2009). These species used are the only ones tested with extracts from the Restinga Plants by our group.

This ectoparasite brings great damages to Brazilian agriculture; it is present in tropical and subtropical areas, being distributed throughout America, Africa, Asia and Australia (Mathison and Pritt 2014). In Brazil, this parasite is found at high levels throughout the year in the Southeast and Center-West regions (Figueiredo et al. 1999). Depending on the degree of infestation, the parasite can cause damage such as blood spoliation and anemia in the cattle and consequently weight loss,

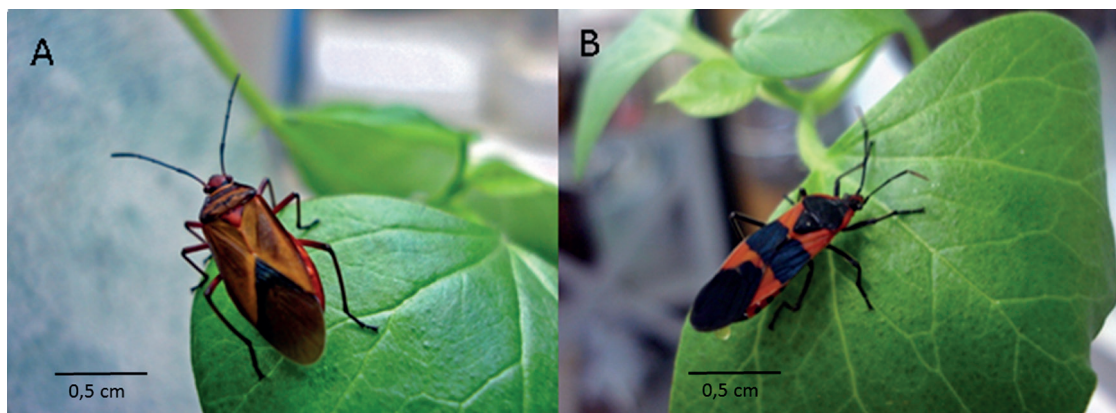


Figure 4 - Adults of: (a) *Dysdecus peruvianus* and (b) *Oncopeltus fasciatus*. The bar is equal to 1.2 cm. Photos Saulo Rigon e Felipe Leite.

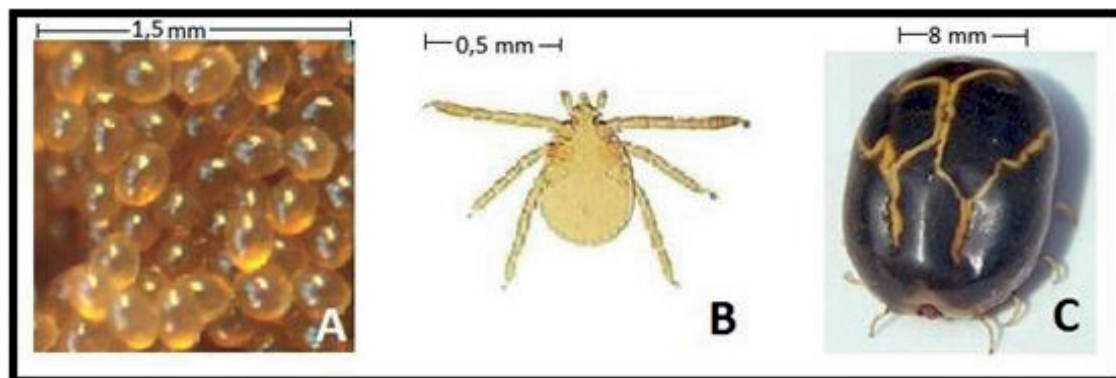


Figure 5 - Tick *Rhipicephalus (Boophilus) microplus*: (a) eggs; (b) larvae; (c) adult females. Photos Camila Mattos.

stress, decreased milk and meat production, as well as depreciation of the leather, since the parasite tears it to feed. In addition, perforations in the leather result in wounds that attract fly, compromising the quality of the material for the industry (Jonsson et al. 1998). Ectoparasitism is one of the major problems in the country's milk and beef cattle production, causing damage to the animal's health and consequent economic losses that reach \$ 3.24 billion per year (Grisi et al. 2014, Figueiredo et al. 1999, Agnolin et al. 2008), especially the tick *Rhipicephalus (Boophilus) microplus*, responsible for 75% of these losses to bovine herds in Brazil. The tick, in addition to the irritating, hematophagic and toxic actions, can transmit protozoans responsible for the set of diseases called "Parasitic Bovine Sadness" (PBS). Economic losses are due to animal morbidity and mortality, reduced meat and milk production, abortions and reduced fertility (Grisi et al. 2014). Thus, an effective strategy to control the tick population could be to interfere with the cycles of egg laying while lowering the larval densities, and survival adults females. Several strategies are being developed in the control of cattle tick, although few are effective. (Kluck et al. 2018).

PLANTS USED AND MAIN RESULTS

The screening process based on the assessment of the biological activity of the different extracts, fractions, essential oils and secondary metabolites, indicated that five of the investigated plants presented IGRs endowed of the capacity to interfere in the life cycle of selected insects or mortality of adult females for ticks, these are: *Eugenia sulcata* Spring ex Mart. (Myrtaceae), *Pilocarpus spicatus* A.St.-Hil. (Rutaceae), *Manilkara subsericea* (Mart.) Dubard (Sapotaceae), *Myrciaria floribunda* (H.West ex Willd.) O.Berg (Myrtaceae) e *Zanthoxylum caribaeum* Lam. (Rutaceae) (Figure 6). A total of 100 secondary metabolites were identified in the essential oils for a total of 203

occurrences (Table I) and two triterpenes, α and β -amyirin acetates, from the EtOH crude extract of *M. subsericea*. The essential oil components are in their majority monoterpenes and sesquiterpenes, present either as hydrocarbons or oxygenated compounds. In *E. sulcata*, among the 37 identified components, sesquiterpenes largely dominate both in leaves essential oils (58.2%) as in stem oils (85.3%) (Lima et al. 2012). In *Z. caribaeum*, 54 compounds were identified: sesquiterpenes (47.3%) and monoterpenes (41.2%) are the major constituents (Nogueira et al. 2014a). In *P. spicatus* sesquiterpenes dominate (58.64%) the monoterpenes (36.93%) (Mello et al. 2007). On the contrary, in *M. floribunda* monoterpenes (53.9%) overcome sesquiterpenes (39.6%); monoterpenes are in their majority oxidized when sesquiterpenes are present mainly as hydrocarbons (Gonzalez et al. 2014).

For several of the studied compounds, the biological activities observed are surprisingly high. One could observe increased mortality, molting and metamorphosis inhibition, paralysis, body deformities, the apparition of permanent nymphs, adultoids and juvenoids. Studies currently underway in our laboratories aim to define the chemical factors present in such extracts and their mechanism of action on the development and life cycle of these arthropods. The main results obtained are summarized in Table II.

DISCUSSION

Currently, IGRs represent an important strategy for the control of important vectors in public health, veterinary medicine and agricultural pests due to their selective action, high environmental safety and low risk for non-target organisms, especially when compared to conventional insecticides/acaricides (Mazzonetto and Vedramim 2003, Isman 2006, WHO, 2006, Khan et al. 2016, Zaman et al. 2012).



Figure 6 - Plants of Brazilian restinga vegetation. **a**-*Eugenia sulcata*, **b**-*Myrciaria floribunda*, **c**-*Manilkara subsericea*, **d**-*Zanthoxylum caribaeum*, **e**- *Pilocarpus spicatus*. Photos Marcelo Guerra Santos.

Among plant components acting as IGRs, the essential oils play an outstanding role. They are complex mixtures containing many individual compounds, among them volatile terpenes and phenolic compounds, which are highly differentiated in different plant species (Regnault-Roger and Philogene, 2008). They are derived from aromatic plants that, throughout evolution, have developed such secondary metabolites that have helped attract pollinators or repel predatory insects (Walling, 2000).

Isolated constituents of essential oils also demonstrate efficacy against arthropods in laboratory or field conditions. Eugenol for example, abundant in Indian clove [*Syzygium aromaticum* (L.) Merr.

& L.M.Perry – Myrtaceae], or cinnamaldehyde, abundant in cinnamon (*Cinnamomum verum* J.Presl - Lauraceae), exert toxic, ovicide, larvicide and adulticide activities on the Coleoptera *Acanthoscelides obtectus* (commonly known as the bean weevil) and inhibit its reproduction (Regnault-Roger et al. 1993). Eugenol is also highly toxic to mites *Sarcoptes scabiei* and *Psoroptes cuniculi* (Perrucci et al. 1995, Pasay et al. 2010), and is sold by a large number of suppliers under many different trade names for the domestic gardening market (Copping and Duke 2007).

In this sense, several products derived from plants also commercialized as insecticides and acaricides (George et al. 2008). Among the most

TABLE I
Composition of essential oils constituents of parts from: *Eugenia sulcata*, *Myrciaria floribunda*, *Pilocarpus spicatus*, *Zanthoxylum caribaeum* by GC/MS (% obtained by GC/FID peak-area normalization).

#	Compounds	<i>Eugenia sulcata</i>		<i>Myrciaria floribunda</i>			<i>Pilocarpus spicatus</i>	<i>Zanthoxylum caribaeum</i>
		Leaves	Stems	Leaves	Stems	Flowers	Leaves	Leaves
1	α -amorphene		0.7					
2	δ -amorphene			4.0	4.2	1.4		
3	aristolochene-4,5-di- <i>epi</i>							0.8
4	<i>allo</i> -aromadrendene		2.0					
5	biciclogermacrene						6.2	
6	α -bulsene						1.4	
7	<i>trans</i> -cadin-1,4-diene	3.4	3.6				4.2	
8	<i>trans</i> -cadin-1(6),4-diene						0.3	
9	δ -cadinene						3.7	
10	γ -cadinene	4.3	5.8				0.5	
11	α -cadinol		2.3				0.9	1.7
12	<i>epi</i> - α -cadinol		1.1					
13	<i>trans</i> -calamenene	4.4	7.0					
14	camphene							0.4
15	camphoric acid		1.0					
16	α -carene							3.7
17	<i>trans</i> -carveol							0.3
18	β -caryophyllene	24.7	18.8				3.3	4.3
19	(E)-caryophyllene			1.9		2.6		
20	9- <i>epi</i> -E-caryophyllene				4.7		0.6	
21	caryophyllene oxide	1.9	5.6					
22	1,8-cineol	5.7						
23	citronellal							0.3
24	citronellol							0.5
25	α -copaene	3.8	3.0	3.8			3.2	
26	β -copaene	0.5	0.6		1.8			
27	α -cubebene	1.2	1.1		1.7		0.4	0.4
28	cubebol							1.2
29	1- <i>epi</i> -cubebol						0.3	
30	cubenol							0.6
31	1- <i>epi</i> -cubenol		1.7					0.9
32	b-cyclocitral							0.9
33	θ -cymene							0.5
34	dauca-5,8-diene		0.6				1.7	
35	<i>isodaucene</i>							8.3
36	β -elemene		1.0				1.3	4.0

#	Compounds	<i>Eugenia sulcata</i>		<i>Myrciaria floribunda</i>			<i>Pilocarpus spicatus</i>	<i>Zanthoxylum caribaeum</i>
		Leaves	Stems	Leaves	Stems	Flowers	Leaves	Leaves
37	δ -elemene						0.2	0.7
38	elemol							0.8
39	eudesm-7(11)-en-4-ol			0.6				
40	γ -eudesmol				3.3		0.3	
41	10-epi- γ -eudesmol						0.3	
42	geranyl acetate			1.5		0.9		
43	(2E,6Z)-farnesol			1.4	0.8	16.1		
44	(2E,6E)-farnesyl acetate			3.7	13.1	13.4		
45	germacra-4(15),5,10(14)-trien-1 α -ol				19.9			0.7
46	germacrene B						0.3	0.7
47	germacrene D	1.0	2.2					
48	globulol		1.2					
49	α -guaiene							1.4
50	guaiol						0.5	
51	α -gurjunene						0.5	
52	γ -gurjunene							0.6
53	n-heneicosane		0.4					
54	hexanal							0.2
55	hexanol							0.4
56	n-hexanol							0.3
57	γ -himachalene	2.0	3.2	7.0				
58	α -humulene	5.2	5.6	0.9	5.9		0.7	1,1
59	neo-intermedeol			0.8	1.1			
60	ledol				1.7			0.8
61	limonene	1.1		2.6		1.6	14.7	
62	linalool		0.5	2.6		12.7		2.0
63	p-trans-mentha-2-en-ol				7.0			0.3
64	cis-muurool-3,5-diene	0.6	0.9					0.6
65	trans-muurool-3,5-diene						2.7	
66	cis-muurool-4(14),5-diene	1.3	1.1					
67	trans-muurool-4(14),5-diene						4.4	8.4
68	α -muurolene						0.8	
69	γ -muurolene						7.0	0.9
70	α -muurolol						0.3	0.4
71	myltayl-4(12)-ene							0.4
72	myrcene	0.6		0.7		0.6	0.3	4.0
73	(E)- β -ocimene						0.1	2.0
74	(Z)- β -ocimene			0.8		7.6	0.3	0.4

#	Compounds	<i>Eugenia sulcata</i>		<i>Myrciaria floribunda</i>			<i>Pilocarpus spicatus</i>	<i>Zanthoxylum caribaeum</i>
		Leaves	Stems	Leaves	Stems	Flowers	Leaves	Leaves
75	(E)-ocimene							0.4
76	β -phytol							1.0
77	α -pinene	17.3	0.3	0.4			5.1	7.6
78	β -pinene	11.0	0.3				1.4	0.7
79	pogostol			1.7				
80	premnaspirodiene							0.8
81	sabinene						10.8	0.8
82	selin-11-en-4- α -ol		0.5					
83	selina-3,7(11)-diene			3.8	4.6	1.6		
84	α -7- <i>epi</i> -selinene				4.5			2.8
85	β -selinene	2.3	4.2	3.6				
86	spathulenol	1.2	8.8		2.2			0.6
87	sylvestrene							11.3
88	α -terpinene					0.4	1.0	1.0
89	γ -terpinene			0.8		2.8	1.5	1.8
90	terpinen-4-ol			0.6		0.6	3.0	0.6
91	α -terpineol	1.3	0.6	5.5		5.4		1.0
92	terpinolene				2.7		2.0	0.4
93	n-tetradecanol		0.9					
94	n-tricosane		0.3					
95	α -tujene							0.3
96	valencene			1.8				
97	viridiflorene							3.0
98	viridiflorol		1.4					
99	zonarene			4.6		2.1	1.6	0.4
100	<i>epi</i> -zonarene	0.9	0.8		5.2			
	Number of identified metabolites	22	34	23	17	15	38	54

remarkable is azadirachtin (extracted from seeds of *Azadirachta indica* A. Juss. - Meliaceae), nicotine (abundant in tobacco plants *Nicotiana tabacum* L. - Solanaceae), pyrethrin [extracted from the flower of *Tanacetum cinerariifolium* (Trevir.) Sch.Bip. – Asteraceae], rotenone (obtained from species of *Derris*, *Lonchocarpus*, and *Tephrosia* - all Fabaceae genera) among others (Schmeltz 1971). These used to control a wide range of arthropod pests including

aphids, moths, beetles, fruit and vegetable mites, fire ants, mosquito larvae, lice, ticks and flies (Copping and Duke 2007). In developing countries, such products are traditionally used to control stored grain pests, such as coleopteran of the genus *Sitophilus* (De Oliveira et al. 2003), exhibiting low toxicity, easy biodegradation and species-specific selectivity (Borges et al. 2011, Vinturelle et al. 2017).

In our studies with metabolites obtained from 5 different plant genera of the Brazilian restinga vegetation, high biological activities were found involving increased mortality, inhibition of molting and metamorphosis, paralysis, body deformities, the appearance of overaged nymphs, extranumerary nymphs, adultoids and juvenoid, as well as partial or total inhibition of oviposition and egg hatching. These observations indicate the presence in obtained essential oils and purified fractions of chemical compounds capable of specifically interfering with the neuroendocrine system of arthropods by physiological routes related to the synthesis and release of vital hormones such as ecdysone (molt and metamorphosis inducing hormone) and juvenile hormone that controls, among others, of the reproductive and morphogenic processes. In some of the purified metabolites

(*i.e.*, α - and β -amyrin acetate) the presence of chitin synthesis inhibitory activity could also be inferred (Fernandes et al. 2013). Terpenes such as α - and β -amyrin acetate is usually associated with interference with the neuroendocrine system and inhibition of moulting in arthropods (Regnault-Roger 1997). Other terpenes present in some of the essential oils used (*i.e.* α -pinene and β -pinene) were previously recognized for their insecticidal activity (Viegas-Junior 2003). Similarly, insecticidal activity related to inhibition of anticholinesterase activity in insects (Ryan and Byrne 1988) can be observed in some of the compounds analyzed here (Oliveira et al. 2010, Lima et al. 2012, Gonzalez et al. 2014). However, the isolated action of the purified molecules rarely reproduces the variety of biological effects reported for the use of extracts, fractions and essential oils. In addition, as a rule,

TABLE II
Effects of extracts^I, essential oils^{II}, sample fractions^{III} and purified metabolites^{IV} obtained from plants of Brazilian restinga vegetation on mortality, behavior and development of arthropods of medical or agricultural interest. The number under brackets after the plants species name represents the number of secondary metabolites detected in each plant.

Studied Plant [Reference]	Activities and Alterations			
	Tested insect species			
	<i>Rhodnius prolixus</i>	<i>Oncopeltus fasciatus</i>	<i>Dysdercus peruvianus</i>	<i>Rhipicephalus microplus</i>
<i>Eugenia sulcata</i> (37) ^{II} (Gonzalez et al. 2014)	NT	Mortality	Mortality	NT
<i>Pilocarpus spicatus</i> (17) ^{II} (Mello et al. 2007)	Mortality Behavior ^{b,c} Development ^{d,e}	NT	NT	NT
<i>Manilkara subsericea</i> (2) ^{I,III,IV} (Fernandes et al. 2013)	NT	Mortality Development ^{d,e,f,g}	Mortality Development ^{d,e,f,g}	NT
<i>Myrciaria floribunda</i> (25) ^{II} (Tietbohl et al. 2014; Tietbohl et al - in press)	Mortality Development ^{f,g}	Mortality	Mortality	NT
<i>Zanthoxylum caribaeum</i> (54) ^{II} (Nogueira et al. 2014a; Nogueira et al. 2014b)	Mortality Behavior ^b Development ^g	NT	NT	Mortality of adult females Development ^h

NT.: Not tested; Behavioral Changes: Lethargy^a, Paralysis^b and Phagoinhibition^c.; Developmental Activity: Delay in the period of molting^d, Molting inhibition (permanent nymphs)^e, Metamorphosis inhibition (overaged nymphs)^f, Morphological deformations^g and reduction of oviposition and hatching^h.

the extracts and essential oils used show dose dependence biological effects, also indicative of a synergistic action between their different chemical constituents. From this perspective, the mode of action of the various secondary metabolites present in these compounds occurs as result of interactive mechanisms between them in the form of a vast network in which the metabolic pathways of insects and the molecules in the isolated fractions interact in a dynamic and flexible way. (Hummelbrunner and Isman 2001, Rattan 2010, Rizzati et al. 2016). In this way, the presence of various substances in these complex mixtures is likely to increase their effectiveness as insecticides/acaricides and reduce the evolution of resistance to these natural insecticides when compared to the purified molecules present in commercial insecticides, as reported for *Myzus persicae* – Hemiptera (Feng and Isman 1995, Isman 2006). These compounds are now being studied further in our laboratories to determine if they may, or may not, be useful in controlling insect populations and/or interfere with the life cycle and vector transmission of parasites.

CONCLUSIONS

For several of the compounds studied, the biological activities (insecticide and acaricide) found were surprisingly high and are undergoing further chemical and physiological studies to determine if these substances may or not be useful in controlling arthropods and vector transmission in the field. In addition, several of the collected botanical specimens are still the subject of further screening studies, which provide us with a perspective of long-term productive continuous supply of new information on the systems studied.

Crude extracts, secondary metabolites and essential oils obtained from plants in the Brazilian restinga vegetation, exhibit high activity as development regulators in arthropods and present viability for their use in integrated programs of

ecological control of populations of insects and ticks of interest in medicine and agriculture.

AUTHOR CONTRIBUTIONS

All the authors have contributed equally toward completion and contents of this review article. This statement is also to certify that all authors have seen and approved the manuscript. We warrant that the article is the Author's original work and declare no conflict of interest. This article does not contain any studies with human or vertebrate animal subjects.

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