

Active metabolites of the genus *Piper* against *Aedes aegypti*: natural alternative sources for dengue vector control

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Abstract

The mosquito, *Aedes aegypti*, is the principal vector of the viruses responsible for dengue and dengue hemorrhagic fevers. The mosquito is widespread throughout tropical and sub-tropical regions; its prevalence makes dengue one of the most important mosquito-borne viral diseases in the world occurring annually in more than 100 endemic countries. Because blood is essential to their development cycle, the *Aedes* species maintains a close association with humans and their dwellings. Fittingly, the most widely adopted strategy to decrease the incidence of these diseases is the control of the mosquito larvae population. The emergence of insecticide-resistant mosquitoes has amplified the interest in finding natural products effective against *Aedes aegypti* adults, as well as larvae. Plant-derived compounds have played an important role in the discovery of new active entities for vector management as they are safer and have lower toxicity to humans in comparison to conventional insecticides. This review assesses a naturally occurring plant matrix and pure compounds of the *Piper* species, which have been shown to be active against *Aedes aegypti*.

Keywords: dengue; *Aedes aegypti*; *Piper*; Piperaceae; larvicidal metabolites; tropical diseases.

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Introduction

Dengue: an emerging disease in the world

Dengue is transmitted to humans by the *Aedes aegypti* and *Aedes albopictus* mosquitoes (Ramasamy et al. 2011). *Aedes aegypti* is the principal mosquito vector of dengue viruses. Humans and their dwellings provide the vector with the blood and stored clean water necessary for its life cycle (Platt et al. 1997). *A. aegypti* mosquitoes have adapted to the environment and have become highly resilient; this makes their eradication very difficult. *A. aegypti* eggs can survive long latency periods without water and can resist desiccation for several months in the environment. Dengue occurs annually and is endemic in over 100



countries in tropical and subtropical regions. Large mosquito populations are associated with the rainy season when the conditions are optimal for breeding and larval development (WHO 2009). Dengue epidemics commonly occur when a high number of vector mosquitoes coexist with a large population of humans with no immunity to one of the four virus types (Gould & Solomon 2008). Only in the 1950s did dengue emerge as a global problem; currently, more than one-third of the world's population lives in dengue transmission risk areas. Limited travel possibilities kept dengue geographically restricted and relatively controlled until the middle of the 20th century; during the 19th century, dengue was a sporadic disease with epidemics that lasted long intervals. After the Second World War, the *Aedes* mosquitoes were disseminated globally, especially in cargo transport, which is thought to have played a crucial role in the spread of the viruses. It was not until the 1950's that Dengue Hemorrhagic Fever (DHF) was documented during epidemics of the disease in the Philippines and Thailand. Then, in 1981 high numbers of DHF cases began to appear in the Caribbean and Latin America. (Dick et al. 2012). Incidence has increased 30-fold in the last 50 years. The World Health Organization (WHO 2012) estimates that 50–100 million dengue infections occur annually in more than 100 endemic countries. An estimated 2.5 billion people live in areas tropical and subtropical areas where dengue viruses can be transmitted. After malaria, dengue fever is the most widespread tropical disease. In tropical countries, epidemics of dengue result in thousands of hospital admissions, human suffering and massive economic losses; early recognition and prompt treatment can lower the risk of developing this severe disease (Epelboin et al. 2013).

Although the need for dengue vaccines was recognized in the 1940s, pharmaceutical companies were unresponsive because of the limited potential markets. This continued throughout much of the 20th century. However, in 2012, outbreaks were reported in several continents of the world making dengue the most relevant mosquito borne viral disease in the world; the disease has become an imminent threat to the health and economy of most tropical populations (Murray et al. 2013). The emergence and spread of all four serotypes of the dengue virus throughout the

tropical regions of the world represents the threat of a global pandemic with alarming risks and losses for both human health and the economy (WHO 2012). The search for new vaccines still continues today, and the development of dengue vaccines has increased dramatically. Currently, there is no effective vaccine to prevent the dengue virus serotypes; as a result, the best way to prevent dengue fever is to eliminate the dengue vector and the multiplication focus of the larvae. Research into the development of dengue vaccines has produced various candidates in phase II and I clinical trials. In 2012, Sanofi-Pasteur developed the only vaccine candidate in phase III clinical trials (Simmons et al. 2012); however, because of some particularities of the virus, the search for an effective dengue vaccine is still a challenge. The exposure to just one serotype will cause only minor illness, but the subsequent exposure to a second dengue serotype will increase the probability of the illness progressing to the severe and sometimes fatal dengue hemorrhagic fever. Therefore, the focus is to produce a tetravalent vaccine that provides long-term protection against all virus serotypes (Thisyakorn & Thisyakorn 2013); an effective vaccine should be equally potent against all four serotypes as a deficient vaccine may place the individual at risk for the illness to become severe. Mainly the focus of research has been finding a combination of specific vaccines for each serotype; recently, Sanofi Pasteur researchers proposed a vaccine following this combination. Early testing of this vaccine in humans has shown an equal immune response to all four serotypes. More than 4000 children were vaccinated in Thailand; the results showed that the vaccine was effective in preventing illness caused by three serotypes. The vaccine was effective 55.6% for serotype 1, 75.3% for serotype 3, and 100% for serotype 4. The vaccine, however, showed no effect against dengue serotype 2. This serotype is the most prevalent and chiefly responsible for outbreaks of severe illness worldwide (Guy et al. 2010). The results of the first trial of the effectiveness of a possible vaccine for dengue have engendered both enthusiasm and disappointment. Controlling the mosquitoes that transmit dengue is necessary but not sufficient to combat the disease. The discovery of a safe and effective vaccine to prevent the global dengue epidemics is long overdue.

Dengue in Brazil: the endemic country with the highest dengue presence in the world

Dengue has emerged as one of the major public health problems in Brazil, in the last years. Approximately 2.5 million cases of the disease and 280.000 hospitalizations were recorded between 2007 and 2010 (Brazilian Health Ministry 2012). Despite the significant investments and the efforts by health and disease prevention authorities, the incidence of dengue is still high, and the methods of control are limited. Serotypes 1, 2, 3 viruses are prevalent in all regions of Brazil; however, in the second half of 2010, the dissemination of DENV4 has been evident from the northern region to the northeast and southeast regions (WHO 2012).

Cycles of high dengue transmission dengue are characteristic in Brazil; significant outbreaks were reported in 1998 (DENV1), 2002 (DENV3), 2008 (DENV2) and 2010 (DENV1). The highest number of outbreaks occurred in 2002, 2008 and 2010

(Pan American Health Organization 2010); in 2011, 764.032 cases of dengue reported in Brazil and 591.384 in 2012 (Brazilian Health Ministry 2012.). Since November 15th, 2013, more than 385.354 cases have been confirmed and reported to the WHO in Brazil (Figure 1). A total of 4.332.731 cases of dengue were reported from 2001 to 2007 in all the Americas; 64.6% of these cases were reported in Brazil, Argentina, Paraguay and Uruguay, 6733 of these were DHF and 500 fatal. Of these cases, 98.5% were reported in Brazil where serotypes (DEN-1, DEN-2, DEN-3, DEN-4) circulate making it the country with the highest number of fatal cases in this region (PAHO 2008). Reports of dengue in Brazil have occurred since 19th century; however, epidemics have occurred more frequently in the country, in the last decades. In 2008 alone, between January and April, more than 158.000 dengue cases, thousands of hospital admissions and hundreds of deaths were reported in Rio de Janeiro. The military was called upon to aid in the massive response to improve the health-care and vector control operations in Rio de Janeiro

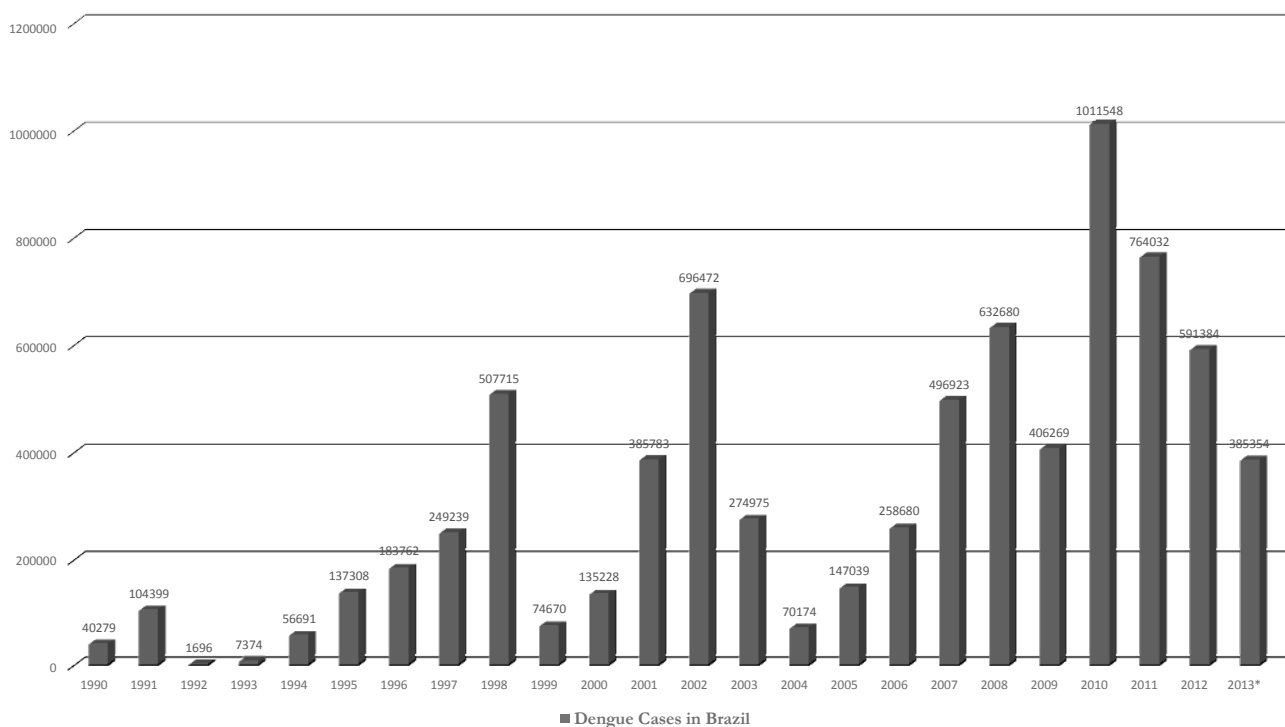


Fig. 1. Dengue cases reported by the Brazilian Health Ministry between 1990 and 2013* (Until November 15th 2013).

(WHO 2012). **Figure 2** shows the fatal cases of DHF reported by the Brazilian Health Ministry between 1990 and 2012. At least 2.000 DHF related deaths have been reported in Brazil, in the last two decades;

Bolivia (16207), El Salvador (15207), Colombia (53303) and Peru (9917). Argentina has the lowest average with only 4501 dengue cases reported (WHO 2012) (**Figure 3**).

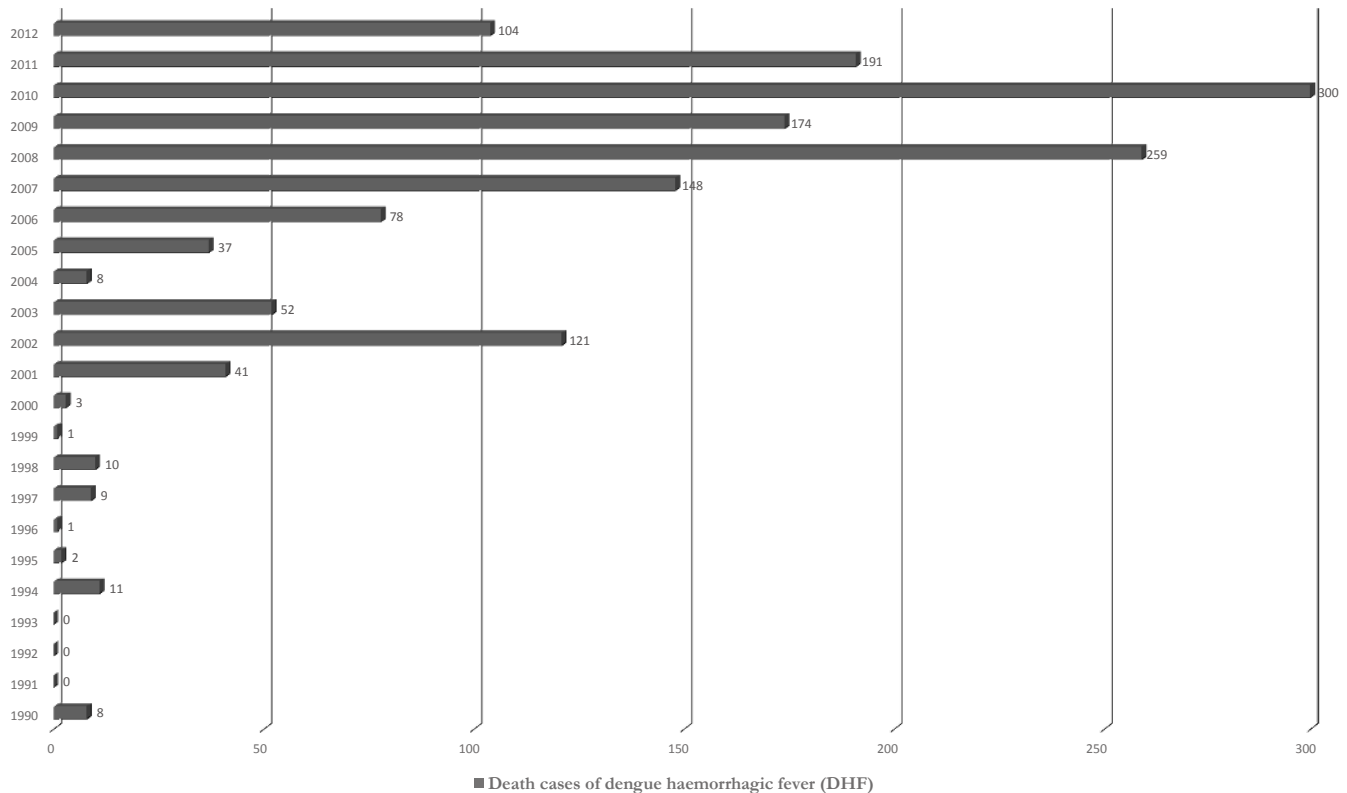


Fig. 2. DHF death cases reported by the Brazilian Health Ministry between 1990 and 2012.

more than 1470 deaths were confirmed from 2002-2012 alone. Overall, 5.350.176 cases of dengue were reported in Brazil, an average of 486.379,6 cases/year. Despite control efforts, more than one million cases of dengue were reported in Brazil in 2010; this reflects a deteriorating global situation in Brazil (Brazilian Health Ministry 2012). Fittingly, dengue has become a pressing issue within the current health system. From 2004-2010, more than half of the number of reported dengue cases in Latin America was issued by Brazil with an average number of cases of 447.446. For this same period, the ten most endemic countries in Latin America, after Brazil, are Honduras (25972), Costa Rica (18967), Mexico (75353), Venezuela (61612),

Natural Compounds as a potential alternative in the discovery of new active pesticides

Naturally occurring metabolites have a wide range of biological actions. Their chemical and biological diversity are the consequences of a long coevolutionary process involving a vast number of species in the environment (Duke et al. 2010). This biological potential is used in signing processes, mostly to protect the plant from a pathogen, herbivore, or competitor. Many plant products have insect repellents properties. These secondary metabolites act to influence the insect feeding behavior, growth and development cycle in order to protect the plant or in some cases to attract

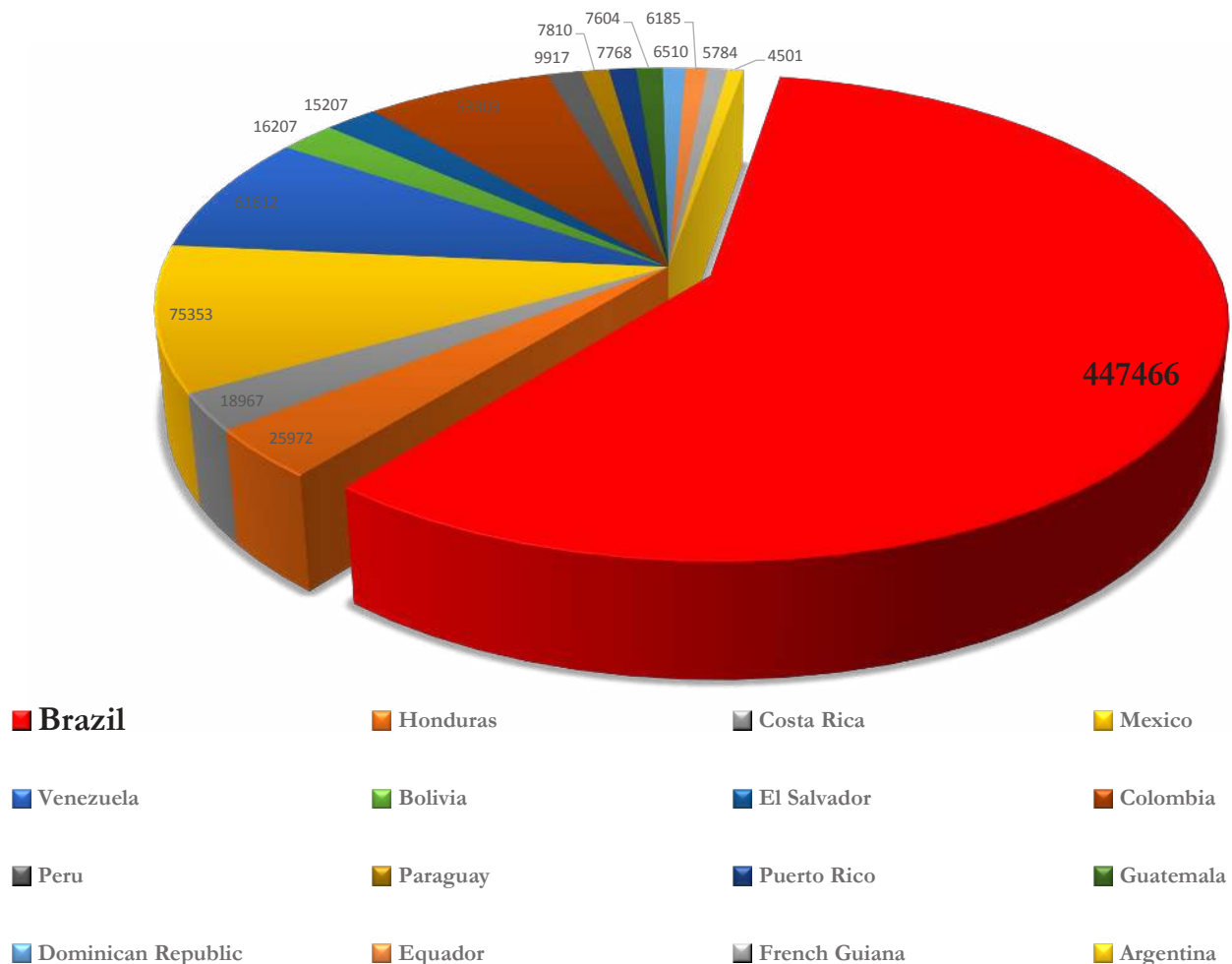


Fig. 3. Dengue average cases number reported cases in Latin America from 2004-2010.

pollinators as well (Heil & Bostock 2002). Many insect repellents are volatile compounds and can act as attractants in the pollination process. For example, geraniol, a common monoterpene in citric aromatic plants repels certain undesirable insects like houseflies but attracts insects like honeybees; geraniol is also produced by the scent gland of these bees (Duke 1990).

Although consumers show great interest in natural pesticides, they are not as interesting to industry. The synthesis of these natural components entails an increased financial burden because of their complex structures, sometimes, of multiple stereocenters. Additionally, altering their structure can often change

the efficacy of synthetic pesticides and intellectual property on these pesticides is difficult to defend and is easily obtained for altered compounds (Duke et al. 2010). There is a growing need to use environmentally friendly chemicals for pest management, but new active pesticide candidates should be efficient, and selectively toxic, highly toxic toward their intended targets and subtle toward non-target organisms, especially humans (Das 2013). Most pesticides are synthetic compounds. Some of them use natural toxins and a few synthetic versions of natural toxins. Due to the growing interest in safer and natural insecticides, there has been a significant rise in unmodified natural compounds. The acceptance of such products drive

research toward finding even better unmodified natural product insecticides (Dayan et al. 2009). Despite not being embraced by industry because of their natural compounds, from 1997 to 2010, 69.3% of all the registrations (277) made before the Environmental Protection Agency were of conventional and biopesticides. Five groups of insecticides, carbamates, neonicotinoids, pyrethroids, organophosphates, and natural products accounted for over three-quarters of worldwide sales. Three of these, the neonicotinoids, pyrethroids, and natural products are either entirely natural product based (unmodified) or derived from natural products. Combined worldwide sales percentage was 42.8%; pyrethroids accounted for 19.5%, neonicotinoids 15.7%, and natural products 7.6% (Cantrell et al. 2012).

The emergence of insecticide-resistant mosquitoes has bolstered the interest in finding new effective products against adult *Aedes aegypti* mosquitoes as well its larvae. The control of this insect pest remains an important issue in agriculture and medicine (Bisset et al. 2013, Vontas et al. 2012, Lima et al. 2011). Chemicals continue to lose their efficiency against *A. aegypti* compelling the use of larger quantities or different kinds of pesticides to eliminate the mosquito vector. Because no vaccine has been effective in preventing dengue, the best control measure to prevent infection is to eliminate adult mosquitoes, and their larvae. Many strategies have been used to control *A. aegypti* mosquitoes and larvae such as the use of bioinsecticides *Bacillus thuringiensis* H-14 (Bt), *Gambusia* and *Poecilia afinis* spp larvae-eating fish and chemical types of insecticides like pyrethroids, carbamates and organophosphates, as well as the improvement of basic sanitation (Garcez et al. 2013). However, the indiscriminate use of synthetic insecticides has led to the emergence of resistant strains of mosquitoes and prompted an uncontrolled increase in the mosquito population. This has also caused other undesirable effects, such as environmental pollution and toxicity to humans and other non-target organisms. As a result, finding new environmentally safe alternatives that are potentially more effective and suitable to use in programs to combat *A. aegypti* larvae is imperative. To this end, the current trend is the exploration of natural products of plant origin with larvicidal properties. Recent studies indicate that over 500 insect and mite

species are currently resistant to pesticides (Mann & Kaufman 2012). Plants have been the traditional source of natural pesticides as plants have adapted to increase their survival and reproduction by reducing the impact of herbivores. This work provides an overview of the use of the biologically active compounds found in the *Piper* species as an alternative against *Aedes aegypti* to control the dengue virus.

Larvicidal properties of crude extracts and sub-fractions of *Piper* species

With approximately 2000 species of shrubs, vines, small trees and herbaceous plants the Piperaceae family is widely distributed in tropical and sub-tropical regions throughout the world (Guimarães & Silvam 2009). The former genera *Ottonia* and *Pothomorphe* are now recognized as members of the genus *Piper* and are the most represented member of the Piperaceae family with approximately 1400 species. Several studies related to the phytochemistry of the *Piper* species have shown some bioactive metabolites including alkaloids, chromenes, amides, flavonoids and terpenoids. Many of these metabolites have economical and medicinal value (Lara Junior et al. 2012, Marques et al. 2010, 2013, Moraes et al. 2011, Parmar et al. 1997, Raimundo et al. 2009, Rebelo et al. 2012, Silva et al. 2010).

There are an estimated 700 species of the Piperaceae family in Brazil, 265 are related to genus *Piper* (Guimarães & Moneteiro 2006). Approximately 592 substances were isolated from the genus *Piper* in the chemical study published by Parmar et al. (1997). However, it is estimated that until 2000, worldwide, only 10% of all the *Piper* species had undergone phytochemical analysis (Myers et al. 2000). Ethnic groups in regions of northern Brazil (Amazon region) and South East Asia have traditionally used several species from this genus in medicine and religious activities (Chaveerach et al. 2006, Cunico et al. 2005). In recent years, the genus *Piper* has received considerable attention because of its chemical diversity and biologic properties. Biologically active compounds, such as amides, have been studied in the fruit of *Piper* plants; amides have been used as food-flavoring agents and have potent insecticidal properties (Ahmad et al. 2012). The insecticidal properties of several species of this genus have also been examined (Cunico et al. 2005). *Piper nigrum* and *Piper guineense* are used as insecticides

and molluscicides in different parts of Africa, while in India species like *Piper longum*, *Piper betle* and *Piper cubeba* have shown insecticidal activity against mosquitoes and flies (Lee 2005). In the Amazon region, ethnobotanical sources indicate that *Piper aduncum*, *P. amapaense*, *P. baccans*, *P. capitarianum*, *P. cyrtopodium*, *P. dilatatum*, *P. erectipillum*, *P. hostmannianum*, *P. tuberculatum* and *P. peltata* are traditionally used by indigenous people to prevent many conditions (Pohlit et al. 2004). The most recognized insecticidal compounds from the *Piper* species were isolated from *P. nigrum*, *P. guineense* and *P. tuberculatum*; their biological activities were studied in relation to a variety of insects with different mode actions, which include contact toxicity, synergism, repellent, and antifeedant properties (Scott et al. 2008). It is ethnobotanical studies that have guided the pursuit for insecticide active metabolites from the genus *Piper*. The traditional use of these active metabolites tends to stimulate phytochemical investigation; their long-established use frequently indicates the effectiveness of these natural compounds against parasites.

The search for new, natural insecticidal metabolites can be achieved by chromatographically separating them from complex natural matrixes. The effectiveness of phytochemicals against mosquito larvae can

vary significantly depending on the plant species, and especially, on the polarity of the solvent for extraction. The polarity of the solvents will determine which types of chemical compounds will be extracted from the complex natural matrixes (Ghosh et al. 2012). Many *Piper* extracts have been tested previously against different kinds of parasites and insects. Bio-guided isolation of secondary metabolites can be a useful tool in phytochemical research, once the biological activity is observed the isolation of target compounds can be accomplished with pharmacological accuracy (Lee 2005). This fractionation process can focus the active metabolites toward the pure compounds, limiting the wide range of possibilities of biological mechanism pathways. On the other hand, crude extracts or active sub-fractions could act by synergism processes, contributing to increasing the possibilities of effective mechanism actions by a variety of phytochemicals from different chemical classes. In literature sources, close to 1,200 plant species have been described that have potential insecticidal value; however, only 344 of these plant species exhibited mosquitocidal activity (Ghosh et al. 2012). According to Web of Science, 41 studies regarding *Aedes aegypti* have been carried out, 22 of them testing the larvicidal activity of the *Piper* species against *A. aegypti* (Figure 4). Most of all

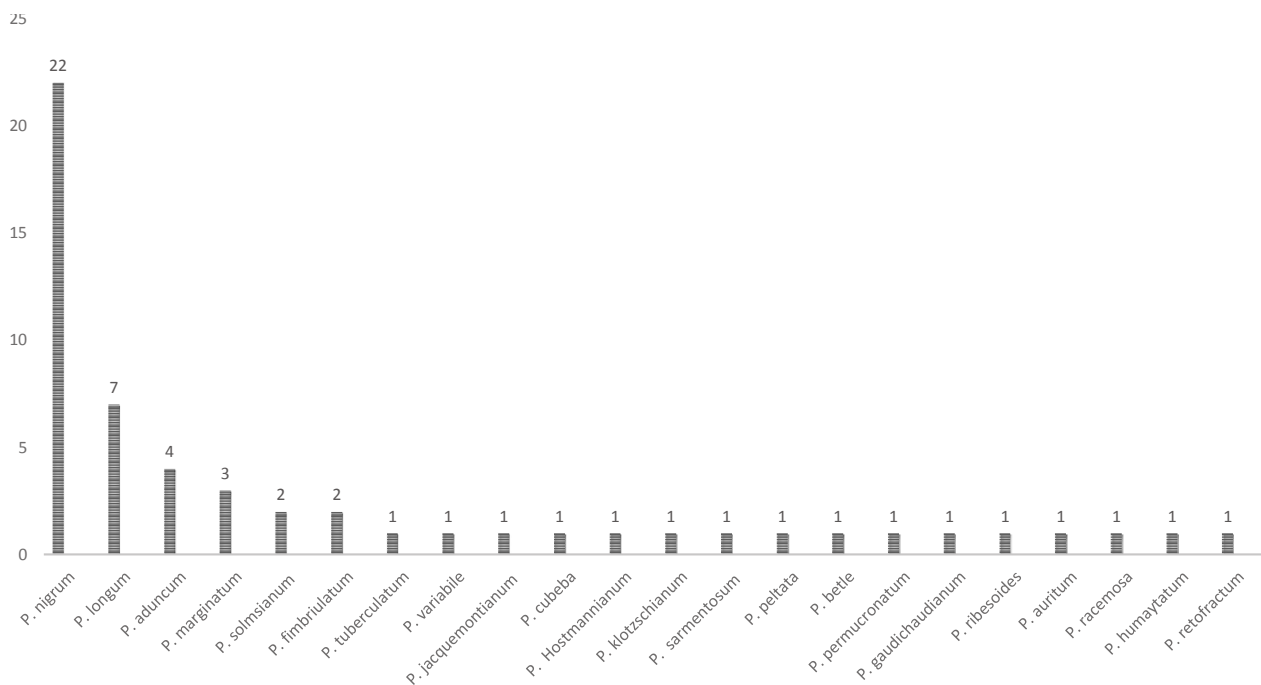


Fig. 4. *Piper* species with larvicidal activity against *A. aegypti* reported in Web of Science searching for topics (*Piper*) + (dengue) or (*Piper*) + (*Aedes aegypti*) until May 2013..

related works (22) are related to an active extract from *Piper nigrum*, essential oil and pure isolated secondary metabolites. Brazil is the country with the most works related to larvicidal activity of *Piper* derivatives and *A. aegypti* larvae (12) followed by Pakistan (11), India (4),

Panama (3), South Korea (3), Thailand (3) and others. In this context, the crude extracts and partitioned fractions of 13 *Piper* species were chemically studied and assayed against the dengue vector, *Aedes aegypti*, (Table 1). In most of the cases, the prior bio-guided

Table 1. *Piper* extracts and sub-fractions with larvicidal properties against *A. aegypti*.

<i>Piper</i> species	Plant Material	Extract	Lethal Conc.	Ref
<i>P. aduncum</i>	leaves	hexane extract	LC ₅₀ – 0.20mg/cm ²	Hidayatulfathi et al. 2004
<i>P. aduncum</i>	leaves	crude extract	active	Pohlit et al. 2004
<i>P. betle</i>	leaves	crude extract	LC ₅₀ – 236.73ppm	Tennyson et al. 2012
<i>P. cubeba</i>	fruits	acetone fraction	very low activity	Murthy & Rani 2009
<i>P. fimbriatum</i>	leaves	crude extract	LC ₁₀₀ < 30µg/mL	Calderón et al. 2006
<i>P. jacquemontianum</i>	Leaves	Crude extract	LC ₅₀ > 1000µg/mL	Cruz et al. 2011
<i>P. longum</i>	fruits	crude EtOH extract	LC ₅₀ – 2.23ppm	Chaithong et al. 2006
<i>P. longum</i>	fruits	crude EtOH extract	LC ₅₀ – 0.26µg/fem	Choochote et al. 2006
<i>P. longum</i>	fruits	crude MeOH extract	LC ₅₀ – 40ppm	Lee 2005
<i>P. longum</i>	fruits	crude hexane extract	LC ₁₀₀ – 10ppm	Lee 2005
<i>P. longum</i>	fruits	crude hexane extract	LC ₁₀₀ – 0.017ppm	Sarita et al. 2011
<i>P. nigrum</i>	fruits	crude MeOH extract	LC ₅₀ – 100ppm	Lee 2005
<i>P. nigrum</i>	fruits	crude EtOH extract	LC ₅₀ – 0.98ppm	Simas et al. 2007
<i>P. nigrum</i>	black fruits	crude EtOH extract	LC ₅₀ – 0.405ppm	Sarita et al. 2010
<i>P. nigrum</i>	white fruits	crude EtOH extract	LC ₅₀ – 0.356ppm	Sarita et al. 2010
<i>P. nigrum</i>	fruits	crude extract	LC ₅₀ – 1.84µg/mL	Grzybowski et al. 2012
<i>P. nigrum</i>	fruits	crude extract	LC ₅₀ – 2.26%	Nawaz et al. 2011
<i>P. nigrum</i>	roots	crude extract	LC ₅₀ – 1.66µg/mL	Ee et al. 2008
<i>P. nigrum</i>	roots	Ethyl acetate extract	LC ₅₀ – 3.96µg/mL	Ee et al. 2008
<i>P. nigrum</i>	roots	hexane extract	LC ₅₀ – 0.45µg/mL	Ee et al. 2008
<i>P. nigrum</i>	roots	choroform fraction	LC ₅₀ – 0.37µg/mL	Ee et al. 2008
<i>P. peltata</i>	roots	crude leaf extract	LC ₅₀ – 398µg/mL	Mongelli et al. 2002
<i>P. retrofractum</i>	fruits	crude extract	LC ₅₀ – 79ppm	Chansang et al. 2005
<i>P. ribesoides</i>	fruits	crude extract	LC ₅₀ – 8.13ppm	Chaithong et al. 2006
<i>P. ribesoides</i>	fruits	crude extract	LC ₅₀ – 0.15µg/fem	Choochote et al. 2006
<i>P. sarmentosum</i>	fruits	crude extract	LC ₅₀ – 0.14µg/fem	Choochote et al. 2006
<i>P. sarmentosum</i>	fruits	crude extract	LC ₅₀ – 4.06ppm	Chaithong et al. 2006
<i>P. tuberculatum</i>	leaves	crude extract	active	Pohlit et al. 2004
<i>P. variabile</i>	leaves	crude extract	LC ₅₀ > 1000µg/mL	Cruz et al. 2011

isolation of the pure active compounds was performed in order to obtain the pure compounds.

Piper nigrum is the most recognized and consumed *Piper* species in the world. In many countries, this species is considered the king of the spices; it is used in most of the food dishes because of its pungent principle, the amide, piperine. Most likely because of this, *P. nigrum* is also the most studied species in the Piperaceae family. Different parts of *P. nigrum* as well as its isolated metabolites have been used in active preparations with biological properties such as preservatives and insecticidal and larvicidal control agents (Ahmad et al. 2012).

At 100 ppm, the methanol extract of *P. nigrum* fruit showed strong larvicidal activity against *A. aegypti*. Further solvent fractionation also showed strong larvicidal activity in the hexane and chloroform fractions. Additional bioassay-guided fractionation of this extract exhibited four active components: pellitorine, guineensine, piperidine, and retrofractamide A (Lee 2005). Chansang et al. (2005) reported the larvicidal activity of the methanol crude extract of *P. retrofractum*; in this work, the LC₅₀ value was 79mg/L. Another study evaluated possible active combinations of different botanical sources including *Annona muricata* seed and *Piper nigrum* fruit ethanolic extracts; these extracts are rich in acetogenins and piperamides, respectively. Individual bioassays of *P. nigrum* extracts indicated an LC₅₀ value of 1.84 µg/mL against third instar larvae. The best combination was the *A. muricata*/*P. nigrum* extract combination at 9:1. The authors suggest that the synergism of this combination was responsible for the morphological effects that took place in the larvae's bodies that lead to their mortality (Grzybowski et al. 2012). Simas et al. (2007) reported on the fractionation of *P. nigrum* ethanol extract. Biological tests were carried out on pyrethroid-resistant *Aedes aegypti* larvae. The biomonitoring assays led to the isolation of the larvicidal amides piperolein-A (1.46ppm) and piperine (1.53ppm). LC₅₀ values of the ethanol crude extract were only 0.98 ppm, significantly lower than methanol extract. Sarita et al. (2011) evaluated the larvicidal potential of piper fruits. Three extracts of *Piper* species were studied; long pepper (*Piper longum* L.), and black and white pepper from *Piper nigrum* were tested against different instars of a field-collected Indian strain of *A. aegypti*. The three extracts were active against

A. aegypti; however, the ethanolic extracts of black and white *P. nigrum* were less toxic than the extracts of *P. longum*. Against early fourth instar larvae, lethal LC₅₀ values of ethanolic extracts of *P. longum*, white *P. nigrum* and black *P. nigrum* were 0.248, 0.356, and 0.405 ppm, respectively. Against third instar larvae; lethal LC₅₀ values recorded of the three extracts were 0.022, 0.015, and 0.016 ppm, respectively.

In Malaysia, a chemical and biological study of *P. nigrum* roots indicated that the crude root extracts and pure piperine showed significant activity against *A. aegypti* larvae. The crude hexane, chloroform, ethyl acetate and methanol extracts of *Piper nigrum* displayed high toxicity towards the larvae; LC₅₀ values were less than 4.0 µg/mL. The crude hexane extract yielded an LC₅₀ value of 0.45 µg/mL; the crude chloroform extract with an LC₅₀ value of 0.37 µg/mL, and the crude ethyl acetate an LC₅₀ value of 3.96 µg/mL while the methanol extract had an LC₅₀ value of 1.66 µg/mL. The pure isolated amide piperine also exhibited strong activity against the larvae with an LC₅₀ value of 4.46 µg/mL (Ee et al. 2008). Nonpolar fractions probably displayed a higher effectiveness effect in this work because of their ability to extract a higher number of active larvicidal metabolites such as *Piper amides* from the crude extract. This higher activity coincides with the higher concentration of greater diversity of active amides and in turn, with the low polarity of the fractions and their possible enrichment with active metabolites such as *Piper amides*. Nawaz et al. (2011) also reported that the extracts from *P. nigrum* were effective against *A. aegypti* with low LC₅₀ values (2.26% and 8.4%) after 24h and 48h of exposure respectively. In terms of LT₅₀, black pepper took 15h to kill 84% of 50% of the tested population of *A. aegypti*. As a result, *Piper nigrum* extracts can kill adult mosquitoes and could be used as an environment friendly alternative to control mosquitoes.

Crude extracts of *Piper longum* were also subjected to phytochemical and biological assessments. Fruit-derived materials *P. longum* were evaluated as larvicidal against fourth instar *A. aegypti* larvae. The crude methanol extract and the hexane fraction of *P. longum* fruit were active against the larvae; both extracts showed strong larvicidal activity and 100% mortality. Through additional chromatographic procedures, the biologically active component of *P. longum* fruits was

characterized as piperonaline with an LC_{50} value of 0.25mg/L (Yang et al. 2002). The methanol extract activity, which was also reported for *P. longum* fruits, was responsible for a strong mosquito larvicidal activity at 40 ppm. Further solvent fractionation showed strong larvicidal activity in the hexane fraction, which showed 100% mortality at 40, 20, and 10 ppm. Chloroform fraction showed 31%, and water fraction exhibited 15% mortality against *A. aegypti* at 40 ppm. Little activity was found in other organic solvent fractions (Lee 2005).

Chaithong et al. (2006) evaluated the larvicidal efficacy ethanol extracts of three *Piper* species; *P. longum*, *P. ribesoides* and *P. sarmentosum* against early fourth instar *A. aegypti* larvae. LC_{50} values of 8.13 ppm were found for *P. longum*, 4.06 for *P. sarmentosum* and 2.23 for *P. ribesoides*. In another study, acetone extracts from eight plant species collected in India were tested for their larvicidal activity against *A. aegypti* L. The buds of *Piper cubeba* L. were collected in India and chemically evaluated; the extracts exhibited toxicity against third instar larvae. The *P. cubeba* acetone extract displayed low activity and required a higher concentration to obtain 50% toxicity (Murthy & Rani 2009).

In Panama, one hundred fifty plant extracts representing 43 families, 73 genera, and 93 species were tested in a panel of antimalarial (*Plasmodium falciparum* W2), antileishmanial (*Leishmania mexicana*), antitrypanosomal (*Trypanosoma cruzi*), and larvicidal (*Aedes aegypti*) screens. Of these 150 plant extracts, only one (0.6%) *Piper fimbriatum* showed larvicidal activity with LC_{100} values < 30 $\mu\text{g}/\text{mL}$ (Calderón et al. 2006). In Guatemala, two native *Piper* species were chemically evaluated, and their dichloromethane and methanol extracts were tested for their biological activity. Dichloromethane extracts of *P. Jacquemontianum* and the methanol extract of *P. variable* were not active against *A. aegypti* larvae (Cruz et al. 2011). The adulticidal activity of methanol extracts of *Piper aduncum* from Malaysia was also tested against adult of *Aedes aegypti* (L.); the hexane fraction of its crude extract was effective as an insecticidal presenting LC_{50} and LC_{90} values of 0.20 mg/cm² and 5.32 mg/cm², respectively (Hidayatulfathi et al. 2004). Mongelli et al. (2002) evaluated the insecticidal activity of the leaves of *Piper peltata* on *A. aegypti* larvae and reported that the

larvicidal potential of roots crude extract presented an LC_{50} value of 398 $\mu\text{g}/\text{mL}$. Mongelli et al. (2002) also conducted the fractionation of the active methanol extract. The biomonitoring larvicidal fractionation isolated the catechol derivative, 4-nerolidylcatechol; this compound was effective against *A. aegypti* larvae with value of $LC_{50} = 9.1 \mu\text{g}/\text{mL}$. In India, the crude methanol leaf extract of three native plants were investigated. *Piper betle* crude extract was evaluated for *Aedes aegypti* larvicidal activity at concentrations varying from 62.5 to 1000 ppm, mortality was observed during 24 and 48 hours. The *Piper betle* was moderately active with LC_{50} values of 236.73 and 98.45ppm after 24 and 48 h, respectively (Tennyson et al. 2012).

Larvicidal potential of isolated metabolites from *Piper* species

Plant extracts and phytochemicals have been assessed as an alternative to toxic pesticides conventionally used for insect pests control. The biodegradability into nontoxic products of natural products make them appealing to find safer, effective and sustainable new insecticides that can be integrated into management programs, especially because of their commercial acceptance (Rimando & Duke 2006). Unfortunately, research of bioactive natural products is sometimes restricted to only a few institutes and universities.

There are certain types of secondary metabolites that have greater insecticidal properties than all the other biologically active substances found in nature. Among all potential bioactive metabolites there are many different structures and chemical classes such as amides, limonoids, stilbenes, coumarins, sesquiterpenes, and quinones, which has the highest number of bioactive compounds. It should also be noted that the low LC_{50} values observed for some of these compound classes could feature as an alternative product with greater potential for their use as an *A. aegypti* control agent (Garcez et al. 2013).

Amides, a chemical metabolite class very common in *Piper* species, were the most active natural compounds against *A. aegypti*. Numerous active amides assayed for *A. aegypti* larvae were obtained from extracts of black pepper originally from tropical countries. These amides are typically a long chain of unsaturated amides formed by an acyl derivative component and an amine

moiety. The long-chain unsaturated acyl component can be classified into two main structural types: (a) dienamines, derived from straight chain fatty acids and (b) a piperonal unit with chain extension by fatty acid (Rahman 2012). Some isolated metabolites from *Piper* species are shown in the (Figure 5). Amides (27) and lignans (3) are the most cited active chemical classes found in *Piper* species with larvicidal properties against dengue vector *A. aegypti* (Table 2). These are the chemicals most often found in *Piper* species, especially in *P. nigrum*, the most studied species from Piperaceae family (Rahman 2012, Siddiqui et al. 2003, 2004, 2005, Leite et al. 2012, Simas et al. 2004, 2007, Solis et al. 2005, Lee 2005, Da Silva et al. 2011).

frames: the piperidine alkaloid and N-isobutylamide alkaloids. The amide piperine is in the first group, with $LD_{50} = 5.10 \mu\text{g}/\text{mL}$ (Da Silva et al. 2011). This active compound was the first amide isolated from *Piper* species, especially from *P. longum* and *P. nigrum*, and underwent a wide range of biological activity studies (Parmar et al. 1997). On the other hand, are the amides pellitorine, guineensine, pipyahyine, pipnoodhine, pipercide, retrofractamide-A and refractoamide-D that come from N-isobutylamide alkaloids (Rahman 2012); all these isolated amides were tested against female adults of *A. aegypti*. In regards to the most toxic against female of *A. aegypti*, the insecticidal activity was more pronounced in pellitorine with ($LC_{50} = 0.92 \mu\text{g}/\text{mL}$

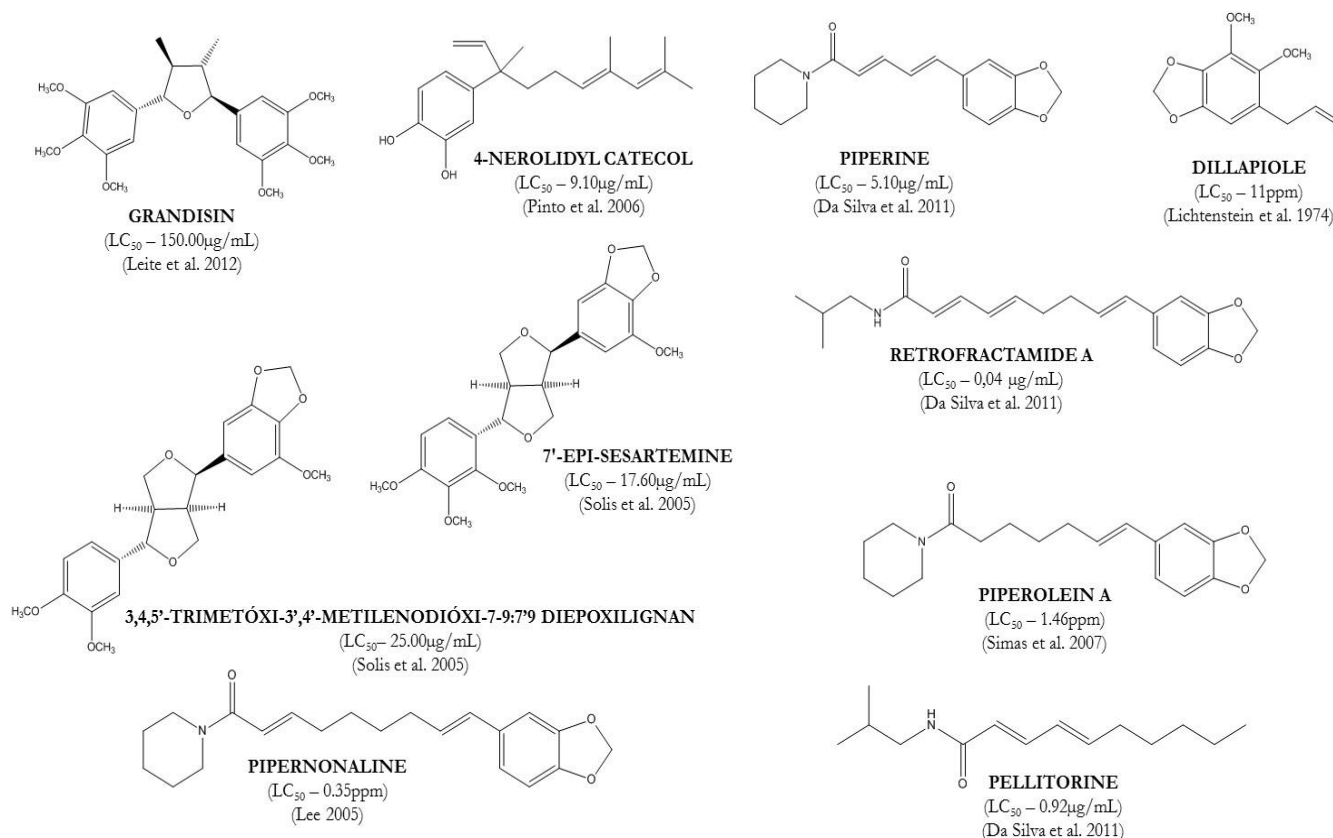


Fig. 5. Some isolated larvicidal metabolites from *Piper* species.

The insecticidal activity of *P. nigrum* fruits has shown a wide variety of active amides. In most cases, the structures are derived from two main chemical

and $LC_{50} = 0.17 \mu\text{g}/\text{female}$), retrofractamide A ($LC_{50} = 0.04 \mu\text{g}/\text{mL}$ and $LC_{50} = 1.5 \mu\text{g}/\text{female}$), guineensine ($LC_{50} = 0.89 \mu\text{g}/\text{mL}$ and $LC_{50} = 1.7 \mu\text{g}/\text{female}$), and

Table 2. *Piper* extracts and sub-fractions with larvicidal properties against *A. aegypti*.

Active Metabolites	Plant Material	<i>Piper</i> species	Lethal Conc.	Ref
dillapiole	leaves	<i>P. aduncum</i>	LC ₅₀ – 11ppm	Lichtenstein et al. 1974
dillapiole	leaves	<i>P. aduncum</i>	LC ₅₀ – 0.381µg/cm ²	Pinto et al. 2012
7'-epi-sesartemine	leaves	<i>P. fimbriulatum</i>	LC ₁₀₀ – 17.60µg/mL	Solis et al. 2005
3,4,5'-trimetóxi-3',4'-metilenodióxi-7-9:7'9' diepoxilignan	leaves	<i>P. fimbriulatum</i>	LC ₁₀₀ – 25.00µg/mL	Solis et al. 2005
pipernonaline	fruits	<i>P. nigrum</i>	LC ₅₀ – 0.35ppm	Lee 2005
piperine	fruits	<i>P. nigrum</i>	LC ₅₀ – 5.10µg/mL	Da Silva et al. 2011
piperine	fruits	<i>P. nigrum</i>	LC ₅₀ – 4.46µg/mL	Ee et al. 2008
piperine	fruits	<i>P. nigrum</i>	LC ₅₀ – 1.53ppm	Simas et al. 2007
retrofractamide A	fruits	<i>P. nigrum</i>	LC ₅₀ – 0,04 µg/mL	Da Silva et al. 2011
retrofractamide A	fruits	<i>P. nigrum</i>	LC ₅₀ – 0.039ppm	Lee 2005
Refractoamide D	fruits	<i>P. nigrum</i>	LC ₅₀ – 25ppm	Rahman 2012
pipsacedine	fruits	<i>P. nigrum</i>	LC ₅₀ – 45ppm	Siddiqui et al. 2004
pipbinine	fruits	<i>P. nigrum</i>	LC ₅₀ – 40ppm	Siddiqui et al. 2004
pipericide	fruits	<i>P. nigrum</i>	LC ₅₀ – 0.10ppm	Lee 2005
guineensine	fruits	<i>P. nigrum</i>	LC ₅₀ – 0.89µg/mL	Da Silva et al. 2011
pipyahyine	fruits	<i>P. nigrum</i>	LC ₅₀ – 30.00µg/mL	Siddiqui et al. 2004
pellitorine	fruits	<i>P. nigrum</i>	LC ₅₀ – 0.92µg/mL	Da Silva et al. 2011
pipnoohine	fruits	<i>P. nigrum</i>	LC ₅₀ – 35.00µg/mL	Siddiqui et al. 2004
pipwaqarine	fruits	<i>P. nigrum</i>	LC ₅₀ – 30ppm	Siddiqui et al. 2005
tert-butyl dodecadienamide	fruits	<i>P. nigrum</i>	LC ₅₀ – 15ppm	Rahman 2012
tert-butyl hexadecadienamide	fruits	<i>P. nigrum</i>	LC ₅₀ – 25ppm	Rahman 2012
piperanine	fruits	<i>P. nigrum</i>	LC ₅₀ – 17ppm	Rahman 2012
methylenedioxyphenyl non atriénylpyrrolidine	fruits	<i>P. nigrum</i>	LC ₅₀ – 20ppm	Rahman 2012
methylene dioxyphenyl und ecatriényl piperidine	fruits	<i>P. nigrum</i>	LC ₅₀ – 25ppm	Rahman 2012
tert-butyl hexadecadienamide	fruits	<i>P. nigrum</i>	LC ₅₀ – 25ppm	Rahman 2012
[(2E,4E)-octadienoyl]-N-isobutylamide	fruits	<i>P. nigrum</i>	LC ₅₀ – 23ppm	Rahman 2012
(2E,4E)-eicosadienoyl-N-isobutylamide	fruits	<i>P. nigrum</i>	LC ₅₀ – 25ppm	Rahman 2012
[(2E)-hexadecanoyl]-pyrrolidine	fruits	<i>P. nigrum</i>	LC ₅₀ – 64ppm	Rahman 2012
[(2E,4E)-octadecadienoyl]-N-isobutylamide	fruits	<i>P. nigrum</i>	LC ₅₀ – 25ppm	Rahman 2012
piperolein-A	fruits	<i>P. nigrum</i>	LC ₅₀ – 1.46ppm	Simas et al. 2007
pipzorine	fruits	<i>P. nigrum</i>	LC ₁₀₀ – 70.00ppm	Rahman 2012
piptigrine	fruits	<i>P. nigrum</i>	LC ₅₀ – 15ppm	Rahman 2012
sarmentine	fruits	<i>P. nigrum</i>	LC ₁₀₀ – 27.00ppm	Rahman 2012
pipgularine	fruits	<i>P. nigrum</i>	LC ₁₀₀ – 6.00ppm	Siddiqui et al. 2003
4-nerolidyl catecol	fruits	<i>P. peltata</i>	LC ₅₀ – 9.10µg/mL	Pinto et al. 2006
grandisin	leaves	<i>P. solmsianum</i>	LC ₅₀ – 150.00µg/mL	Leite et al. 2012

pipericide ($LC_{50} = 0.1 \mu\text{g/mL}$ and $LC_{50} = 2.0 \mu\text{g/female}$) (Da Silva et al. 2011). Regrettably, despite the low LC_{50} of the natural amides, the potency of the reference chemical, chlorpyrifos, with an LC_{50} value of $0.0014 \mu\text{g/female}$ for 24h was very low. In the nonpolar petroleum ether extract of dried ground fruits of *Piper nigrum*, the isolated amides pipnoohine, and pipyahyine exhibited toxicity at 35.0 and 30.0 ppm, respectively, against fourth instar larvae of *Aedes aegypti* (Siddiqui et al. 2004). The biomonitored fractionation of *Piper nigrum* ethanol fruits extract collected in the Amazon region of Brazil, prompted the isolation of larvicidal amides piperolein-A and piperine; these compounds were tested against pyrethroid-resistant *Aedes aegypti*. Ethanol extract ($LC_{50} = 0.98 \text{ ppm}$) was the most toxic, followed by piperolein-A ($LC_{50} = 1.46 \text{ ppm}$) and piperine ($LC_{50} = 1.53 \text{ ppm}$) (Simas et al. 2007).

Amides can also be found in the roots of *Piper nigrum*. Chemical investigations revealed that the amides piperine, sylvamide and 2,4-tetradecadienoic acid iso-butyl amide are the main constituent amides isolated from the roots of *P. nigrum*. In this work, piperine exhibited LC_{50} values of $4.46 \mu\text{g/mL}$ against *A. aegypti* (Ee et al. 2008).

As previously discussed, the fruits of *Piper nigrum* have a high diversity of bioactive amides. The insecticidal amide, pipwaqarine was isolated from nonpolar fractions of petroleum ether and ethyl acetate fractions of dried ground fruits; it yielded LC_{50} values of 30 ppm against fourth instar *Aedes aegypti* larvae (Siddiqui et al. 2005). Ethanol extracts of fruits have shown potent insecticidal activity in recent studies; several compounds have been isolated including the amides pipkirine and wisanine. Piptigrine was isolated from the dried ground fruits of *P. nigrum*; it presented an LC_{50} value of 15.0 ppm against fourth instar larvae of *A. aegypti* (Rahman 2012). The methanol extract of dried and grounded *Piper nigrum* fruits yielded 14 compounds, 13 of which were amides. In total, two new isomeric insecticidal amides, pipsaecdine and pipbinine, and 11 known amides as well as piptaline were isolated. Pipsaecdine ($LC_{50} = 45.0 \text{ ppm}$) and pipbinine ($LC_{50} = 40.0 \text{ ppm}$) were the most toxic compounds against fourth instar *A. aegypti* larvae (Siddiqui et al. 2004). In 2003, the larvicidal pìpgulzarine, pìpzorine, and pìptahsine, was isolated from the dried fruits of *Piper nigrum* along

with four known amides, N-isobutyl-(2E,4E,14Z)-eicosatrienamamide, pellitorine, pipericide, piperine and sarmentine. Pìpgulzarine was very toxic to the larvae with an LC_{100} value of 6.00ppm, followed by sarmentine with $LC_{100} = 27.00\text{ppm}$; pìpzorine was 10 times less effective, with an LC_{100} value of 70.00ppm (Siddiqui et al. 2003). Siddiqui and colleagues also reported a myriad of amides with larvicidal toxicity isolated from fruits of *P. nigrum* purchased from the local market in Karachi. Of these amides, tert-butylododecadienamamide was found to be a larvicidal, with toxic effects in a concentration of 15ppm while piperanine was also toxic with an LC_{50} value of 17ppm. The following amides methylenedioxyphenylnonatrienoylpyrrolidine, [(2E,4E)-octadienoyl]-N-isobutylamide, tert-butylhexadecadienamamide and methylenedioxyphenylundecatrienoylpiperidine, and (2E,4E)-eicosadienoyl-N-isobutylamide showed a similar arvicidal activity of 20ppm, while the amide [(2E)-hexadecanoyl]-pyrrolidine was the less effective with an LC_{50} value of 64ppm (Rahman 2012).

The lignan, 3,4,5'-trimethoxy-3',4'-methylenedioxy-7,9':7',9 diepoxy lignan together with 7'-epi-sesartemin and diayangambin and the flavonoid 5-hydroxy-7,4'-dimethoxyflavone, were isolated from the leaves of *Piper fimbriatum*. The most active compounds assayed against *A. aegypti* larvae was the lignan 7'-Epi-Sesartemin that showed pronounced larvicidal activity against *Aedes aegypti* ($LC_{100} = 17.6 \mu\text{g/mL}$), followed by the 3,4,5'-trimethoxy-3',4'-methylenedioxy-7,9':7',9 diepoxy lignan with $LC_{100} = 25.00 \mu\text{g/mL}$ (Solis et al. 2005). The lignan, grandisin isolated from leaves of *Piper solmsianum* collected in Brazil was reported as a larvicidal compound; this is the lignan of this species most frequently reported. Grandisin caused third instar *A. aegypti* larvae mortality with LC_{50} value of $150 \mu\text{g/mL}$. Larvae treated with grandisin showed an LC_{50} value of $50 \mu\text{g/mL}$ and underwent morphological body changes, with intense tissue destruction and cell disorganization (Leite et al. 2012). The treatment of L1 larvae of *A. aegypti* with grandisin presented 100% larval mortality at a concentration of $100 \mu\text{g/mL}$ (Cabral et al. 2009).

Bioactive amides were also found in *Piper longum*. The larvicidal amides piperidine, pipernonaline, piperoctadecalidine, pellitorine, guineensine, pipericide, and retrofractamidine had previously been isolated

from this species. The highest larvicidal activities were presented by piperide and retrofractamide A with LC₅₀ values 0.10 and 0.039 ppm against *A. aegypti*, respectively while piperonaline was less effective with an LC₅₀ value of 0.35ppm (Lee 2005). In another study using *P. longum*, piperonaline showed an LC₅₀ value of 0.25 mg/L. In this report, no larvicidal activity was observed with the amides piperettine, piperine, or piperlongumine (Yang et al. 2002).

The insecticidal activity of the leaves of *Piper peltata* (Pothomorphe peltata) was evaluated on *A. aegypti* larvae. The methanol extract was chemically fractionated. The larvicidal biomonitoring led to the isolation of a catechol derivative: 4-nerolidylcatechol. This compound showed LC₅₀ values of 9.1µg/mL against *Aedes aegypti* larvae (Mongelli et al. 2002). Another natural occurring compound, dillapiole, a phenylpropanoid isolated from essential oil from leaves of *Piper aduncum*, was assayed in vivo against larvae and pupae of *A. aegypti*. The pure active metabolite was reported as a natural insecticide compound with LC₅₀ = 11ppm (Lichtenstein et al. 1974) and LC₅₀ of 0.381µg/cm² (Pinto et al. 2012) against *A. aegypti*. The mortality, oviposition, chromosome breakage, and anaphase bridges were significantly greater in the extract treatments in concentrations of 200 and 400 µg/mL than in the controls. The genotoxic effects and larvicidal activity of dillapiole described here suggest that this natural product may be a useful alternative for the control of *A. aegypti* (Rafael et al. 2008). Based upon these results and earlier findings, bioactive compounds derived from *Piper* may be useful to develop a lead product for potentially safer fungicidal, insecticidal, and mosquito larvicidal agents.

The use of larvicidal essential oils from the *Piper* species against *A. aegypti*

Literature sources on the genus *Piper* shows high variability in the chemical composition of species with active essential oils. In this study, a total of 14 species were potentially toxic against *A. aegypti*. The species featured in the literature sources are: *P. gaudichaudianum*, *P. betle*, *P. tuberculatum*, *P. longum*, *P. nigrum*, *P. hostmannianum*, *P. aduncum*, *P. klotzschianum*, *P. humayatum*, *P. racemosum*, *P. auritum*, *P. marginatum*, *P. sarmentosum* and *P. permucronatum*. The most common components found in the *Piper* species studied are camphor, apiole,

myristicin, safrole, sarisan, dillapiole, linalool, nerolidol, α and β-pinene, α-humulene, β-caryophyllene, as well as propenylphenols. The larvicidal activity of the essential oils of *Piper* species is shown in **Table 3**.

Piper aduncum is the most studied *Piper* specie with larvicidal property against *A. aegypti* larvae. In Brazil, the essential oils from specimens of *Piper aduncum* can be found in various chemotypes depending on the region (Lara Junior et al. 2012). In the Brazilian Amazon region and North Brazil, the essential oil of *P. aduncum* leaves is rich in dillapiole (35-90%), a phenylpropene derivative, while in Southeastern Brazil, in the Atlantic Forest they contain no dillapiole only terpenes such as (E)-nerolidol and linalool. In this study, dillapiole rich oil (95.0-98.9%) was tested against *A. aegypti* larvae and adult insects. The results showed mortality of 100% of larvae after 48 h at a concentration of 100 ppm, and a mortality of 100% of the adult insects after 30 min at a concentration of 600 ppm (Almeida et al. 2009). Isomeric isodillapiole showed no significant activity. The pure active metabolite proved a natural insecticide compound with LC₅₀ = 11ppm against *A. aegypti* (Lichtenstein et al. 1974). Literature sources reported that dillapiole, usually isolated from essential oils from the leaves of *P. aduncum*, has insecticidal, fungicidal and antimicrobial activities. This compound and its more stable reduced derivative dihydrodillapiole are used individually or in combination insecticidal formulations. It was observed that in treatments using 200 and 400 µg/mL of dillapiole in larvae samples of *A. aegypti* produced nuclear abnormalities in cells' pupae; several cellular abnormalities were observed (Rafael et al. 2008). The bio-efficacy of *P. aduncum* L. essential oil formulated in aerosol was also evaluated against *A. aegypti*. An aerosol spray test was carried out, and the knockdown effect was assessed within 20 minutes of exposure. The results showed that the essential oil induced a significantly higher mortality in *A. aegypti* (80%) while the commercial aerosol spray composed by 0.09% prallethrin and 0.05% d-phenothrin caused a 97.7% mortality of *A. aegypti* (Norashiqin et al. 2011). Based on this data, *P. aduncum* essential oil has the potential to be used as an aerosol spray.

Dillapiole is also the major component of *P. permucronatum* (54.70%). The main components of *P. permucronatum* are myristicin (25.61%), dillapiole (54.70%), asaricin (8.55%) and elemicin (9.92%). This

Table 3. *Piper* crude essential oils with larvicidal activity against *A. aegypti*.

<i>Piper</i> species	Plant Material	Active sample	Lethal Conc.	Ref
<i>P. aduncum</i>	leaves	essential oil	LC ₅₀ – 100ppm	Almeida et al. 2009
<i>P. aduncum</i>	leaves	essential oil	LC ₅₀ – 0.0057%	Leyva et al. 2009
<i>P. auritum</i>	leaves	essential oil	LC ₅₀ – 0.0017%	Leyva et al. 2009
<i>P. betle</i>	leaves	essential oil	LC ₅₀ – 48ppm	Nagori et al. 2011
<i>P. gaudichaudianum</i>	leaves	essential oil	LC ₅₀ – 121.00 µg/mL	Morais et al. 2007
<i>P. hostmannianum</i>	leaves	essential oil	LC ₅₀ – 54.00µg/mL	Morais et al. 2007
<i>P. humaytatum</i>	leaves	essential oil	LC ₅₀ – 156.00 µg/mL	Morais et al. 2007
<i>P. klotzschianum</i>	seeds	essential oil	LC ₅₀ – 13.27 µg/mL	Nascimento et al. 2013
<i>P. klotzschianum</i>	roots	essential oil	LC ₅₀ – 10.00 µg/mL	Nascimento et al. 2013
<i>P. longum</i>	fruits	essential oil	LC ₅₀ – 6.35µg/female	Chaiyasit et al. 2006
<i>P. marginatum</i>	fruit	essential oil	LC ₅₀ – 20ppm	Autran et al. 2009
<i>P. marginatum</i>	fruit	essential oil	LC ₅₀ – 8,29 µg/mL	Costa et al. 2010
<i>P. nigrum</i>	fruits	essential oil	LC ₅₀ – 9.1ppm	Massebo et al. 2009
<i>P. nigrum</i>	fruits	essential oil	LC ₅₀ – 2.26%	Nawaz et al. 2011
<i>P. permucronatum</i>	leaves	essential oil	LC ₅₀ – 36.00µg/mL	Morais et al. 2007
<i>P. racemosa</i>	leaves	essential oil	LC ₅₀ – 0.0027%	Leyva et al. 2009
<i>P. sarmentosum</i>	leaves	essential oil	LC ₅₀ – 16.03ppm	Intirach et al. 2012
<i>P. tuberculatum</i>	leaves	essential oil	LC ₅₀ – 106.3 µg/mL	Lavor et al. 2012

essential oil proved to be potent with LC₅₀ = 36 µg/mL against the parasite larvae. In *P. hostmanianum*, dillapiole is the third major constituent found in 7.66%. The main volatile components of this species are asaricin (27.37%), myristicin (20.26%), and dillapiole (7.66%). *P. hostmanianum* also has larvicidal properties against the dengue vector with LC₅₀ = 54 µg/mL (Morais et al. 2007). Both species are widely distributed in the in the Amazon Forest, in North Brazil.

Many biological activities have been reported involving *Piper nigrum* fruits. Its essential oil is composed by caryophyllene (54.92%), caryophyllene oxide (13.26%), α-caryophyene (3.97%), copaene (2.90%), cadina-1 (10), 4-diene (2.61%), tumerone (2.38%). Larvicidal property was reported about this oil with LC₅₀ = 9.1ppm (Massebo et al. 2009). Another study assessed the repellency potential of

these volatile fractions. A high degree of repellency was obtained with *P. nigrum* (2.3h), with a 3-minute exposure period to *A. aegypti* while *P. betle* was (1.3h). The repellency and ovipositional deterrent activity of essential oils and standard repellents were studied for gravid *A. aegypti*. The oviposition deterrent effects of essential oils showed a moderate degree of deterrence for *P. nigrum* (82%) and *P. betle* (78.9%) essential oils (Tawatsin et al. 2006). The volatile oils from fresh roots, stems, leaves and seeds of *P. klotzschianum* were chemically studied and tested against fourth instar *A. aegypti* larvae. The main chemical constituent identified from different parts of this plant species was 1-butyl-3,4-methylenedioxybenzene, followed by the 2,4,5-trimethoxy-1-propenylbenzene found in the root and also 1-butyl-3,4-methylenedioxybenzene found in the stems and leaves. The main compounds found in the seeds were 1-butyl-3,4-methylenedioxybenzene,

limonene and α -phellandrene. The activity against fourth instar *A. aegypti* larvae was observed in the essential oils from the seeds ($LC_{50} = 13.27 \mu\text{g/mL}$) and roots ($LC_{50} = 10.0 \mu\text{g/mL}$) of this plant (Nascimento et al. 2013). The larvicidal activity of essential oils from the leaves of *Piper tuberculatum* was evaluated using third instar larvae of *A. aegypti* showing $LC_{50} = 106.3 \mu\text{g/mL}$. Its essential oil was mainly composed by monoterpenes such as α - and β -pinene, trans-cimene and the sesquiterpenes, β -caryophyllene and β -farnesene (Lavor et al. 2012). Sesquiterpenes were also found in great quantity in the essential oils from *P. gaudichaudianum*: viridiflorol (27.50%), aromadendrene (15.55%), β -selinene (10.50%), and selin-11-en-4- α -ol (8.48%) as well as in *P. humaytanum* are β -selinene (15.77%), caryophyllene oxide (16.63%), spathulenol (6.33%), and β -oplophenone (6.02%). The essential oils of both species were also tested against *A. aegypti* larvae. The results of larvicidal tests for *P. gaudichaudianum* and *P. humaytanum* showed LC_{50} values of 121 $\mu\text{g/mL}$ and 156 $\mu\text{g/mL}$, respectively (Morais et al. 2007). The insecticidal activity of *P. longum* essential oils was tested using topical test application on two populations of *Aedes aegypti* in pyrethroid-susceptibility bioassays. The results revealed that this essential oil displayed a promising efficacy against both laboratory and natural field strains of *A. aegypti*. The highest potential was established from long pepper with an LC_{50} value of 6.21 $\mu\text{g/mg}$ female for the laboratory strain and 6.35 $\mu\text{g/mg}$ female for the field strain, respectively. The major compound identified in this species was sesquiterpene β -caryophyllene, followed by 3-carene, eugenol and limonene (Chaiyasit et al. 2006). In Brazil, the essential oils of leaves, stems and inflorescences of *P. marginatum*, harvested in the Atlantic forest, were analyzed, and they revealed the presence of major compounds asarone and patchouli. The essential oils from fruits exhibited potent activity against fourth instar larvae of *A. aegypti* with LC_{10} and LC_{50} values of 13.8 and 20.0 ppm, respectively. However, the essential oil of the fruit had no effect on the oviposition at a concentration of 50 ppm (Autran et al. 2009). The larvicidal potency of the essential oils of *P. auritum* and *P. racemosum* collected in Cuba were assessed. The essential oils from the leaves of *P. auritum* and *P. racemosum* were active, in low concentrations, against third instar larvae of *A. aegypti* with $LC_{50} = 0.0017\%$ and $LC_{50} = 0.0027\%$ respectively. *P. auritum* essential oil

is mainly composed of safrole (93.24%) and miristicine (4.34%), while the major compounds of *P. racemosum* are 4-terpineol (20.7%), 1,8-cineol (20.4%), eugenol (10.7%) and α -terpineol (10.0%) (Leyva et al. 2009).

Many isolated components from essential oils have been tested and displayed distinct larvicidal activity. In the essential oil mixture, the combination of these terpenes and arylpropanoids can act, in some cases, in synergism increasing the toxic effect against the parasites. Chantraine et al. (1998) and Simas et al. (2004) reported the larvicidal activity for E-nerolidol in *A. aegypti*, with a value of LC_{50} (9.0 ppm) and ($LC_{50} = 17.0$ ppm), respectively. Nerolidol is usually found in the essential oils of the *Piper* species and may be used in many topical formulations can increase the absorption of lipophilic drugs into membrane cells (El-Katan et al. 2001). The sesquiterpene farnesol showed larvicidal activity with $LC_{50} = 13$ ppm, and monoterpene geraniol was found to be less active ($LC_{50} = 81.6$ ppm), while linalool was scarcely active with $LC_{50} > 100$ as well as menthol. The phenylpropanoids safrole, eugenol and cinnamic aldehyde were active with LC_{50} of 49.0, 44.5 and 24.4 ppm, respectively. Other monoterpenes were also tested such as β -pinene ($LC_{50} = 42.5$ ppm), α -pinene ($LC_{50} = 74.3$ ppm) and carvone ($LC_{50} = 43.8$ ppm) (Simas et al. 2004). The essential oils proved to be an efficient alternative to control and eradicate mosquito vectors. Volatile aromatic compounds are useful to repel and eradicate *A. aegypti* adult mosquitoes indoors. Although the reported activity of these oils is considered high, when compared to synthetic larvicides like temephos with LC_{50} and LC_{90} values 0.0177 and 0.0559 $\mu\text{g/mL}$, respectively, these essential oils show much lower activity, (Becker 2010). In this context, essential oils could be used as an adjuvant in repellent formulations to increase their efficacy and decrease their toxicity to humans.

Conclusions

Bio-insecticides have a great potential to find low-risk options, from an ecological point-of-view, to control insects. Existing literature presents several examples of effective, natural metabolites that could be used in pest control plans. Finding an environmentally friendly mosquito-control management plan using phytocompounds will not completely replace synthetic insecticides. Natural compounds are perishable, and

higher concentration doses are needed to effectively eliminate insects. The best alternative is to use these compounds as a supplement to optimize and increase the sustainability of current integrated pest control strategies. Moreover, the composition of many of these extracts and essential oils is very complex making it more difficult for the pest to develop resistance in comparison with synthetic insecticides. The risks to environmental biodiversity must be considered when using plant material as a source of bioactive compounds; endangered species must be avoided and biomass must be limited. An ideal insecticide plant would be perennial, widely distributed and prevalent in large amounts in nature or easily cultivated, using renewable plant parts. The species *Piper nigrum*, *Piper aduncum* and *Piper longum*, may be a feasible alternative source of larvicidal metabolites to control dengue mosquitoes. These species are distributed widely in tropical countries and are well adapted even in cultivated areas. In total, 32 studies were reported involving the biological activity of these species. Once these species reach arboreal size from 4-5 meters in height, *P. nigrum*, *P. aduncum* and *P. longum* are of particular interest. All of the compounds tested that showed promise were obtained from aerial parts of the plant, especially from the fruits. Active amides and lignans from *Piper* species were abundant in these species and could be applied in the future used as a supplement to synthetic insecticides and also as a model for new active larvicidal compounds in the synthesis and studies of molecular improvement.

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Conflicts of interests

The authors declare no conflicts of interests.

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Metabolitos activos del género *Piper* contra *Aedes aegypti*: fuentes alternativas naturales para el control de vectores de dengue

Resumen. El mosquito, *Aedes aegypti*, es el principal vector del dengue y de la fiebre hemorrágica. El mosquito está diseminado en regiones tropicales y subtropicales. La prevalencia del dengue lo hace una de las enfermedades virales, transmitidas por mosquitos, más importantes en el mundo, ocurriendo anualmente en más de 100 países endémicos. Dado que la sangre es esencial para su ciclo de desarrollo, la especie *Aedes* mantiene una estrecha relación con los seres humanos y sus viviendas. La estrategia más ampliamente adoptada para disminuir la incidencia de estas enfermedades es el control de las larvas de los mosquitos. La aparición de mosquitos resistentes a los insecticidas ha amplificado el interés en la búsqueda de productos naturales, eficaces contra *Aedes aegypti* adultos y larvas. Los compuestos derivados de plantas han jugado un papel importante en el descubrimiento de nuevas entidades activas para el control del vector; estos son más seguros y menos tóxicos para los seres humanos en comparación con los insecticidas convencionales. Esta reseña evalúa matrices vegetales de origen natural y compuestos puros de especies *Piper*, que han demostrado ser activos contra *Aedes aegypti*.

Palabras clave: dengue; *Aedes aegypti*; *Piper*; Piperaceae; metabolitos larvicidas; enfermedades tropicales.

Metabólitos secundarios do gênero *Piper* contra *Aedes aegypti*: fontes naturais alternativas para o controle do vector da dengue

Resumo. O mosquito *Aedes aegypti* é o principal vetor dos vírus responsáveis pela dengue e pelas febres hemorrágicas de dengue. O mosquito está generalizado em todas as regiões tropicais e sub-tropicais; a sua prevalência torna a dengue uma das doenças virais mais importantes transmitidas por mosquitos no mundo que ocorrem anualmente em mais de 100 países endêmicos. Como o sangue é essencial para o seu ciclo de desenvolvimento, a espécie *Aedes* mantém uma estreita associação com os seres humanos e suas habitações. Apropriadamente, a estratégia mais adotada para diminuir a incidência dessas doenças é o controle da população de larvas de mosquito. O surgimento de mosquitos resistentes aos inseticidas tem ampliado o interesse em encontrar produtos naturais eficazes contra *Aedes aegypti* adultos, bem como larvas. Compostos derivados de plantas, têm desempenhado um papel importante na descoberta de novas entidades ativas para gestão de vetores em que são mais seguros e possuem baixa toxicidade para os seres humanos, em comparação com os inseticidas convencionais. Esta avaliação avalia a matriz de plantas que ocorrem naturalmente e compostos puros das espécies de *Piper*, que têm se mostrado ativa contra *Aedes aegypti*.

Palavras-chave: dengue; *Aedes aegypti*; *Piper*; Piperaceae; metabólitos larvicidas; doenças tropicais..