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Essential oils for the control of reduviid insects

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Abstract Chagas disease is an important vector-borne disease problem in South America, especially in rural areas where inhabitants are in contact with the reduviid insects that transmit the parasite *Trypanosoma cruzi*. Today, the main means of interrupting transmission of *T. cruzi* is to control the vector. Therefore, studies of new agents with activity against these vectors have a priority interest. This review covers recent studies on essential oils from plants that have demonstrated moderate to high activity against the main vectors of Chagas disease. Further, we investigate the constituents of essential oils of plants of the genera *Mentha*, *Thymus*, *Satureja* and *Artemisia* and their activity on *Rhodnius prolixus* using an excito-repellency test.

Keywords Activity · Chagas disease · Excito-repellency · *Rhodnius prolixus*

Abbreviations

EI-MS	Electron impact-mass spectrometry
EOs	Essential oils
GC–MS	Gas chromatography-mass spectrometry
RP	Repellency percentage
TIC	Total ion current
WHO	World health organization

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Introduction

Chagas disease is a parasitic infection caused by the protozoan *Trypanosoma cruzi* and recognized by WHO as one of the world's 13 neglected diseases (Hotez et al. 2007). One of the global public health challenges of this century is the control of vector-borne diseases. About 8 million people are infected by *T. cruzi* in Latin America and a big percentage of them will develop cardiomyopathy, digestive megasyndromes, or both (Rassi et al. 2010).

It can be transmitted by infected faeces of blood-sucking reduviid bugs of the subfamily Triatominae (Hemiptera, Heteroptera) through the insect sting, blood transfusion, congenital transmission, organ transplantation, laboratory accident and ingestion of food or liquid contaminated with *T. cruzi* (Rassi et al. 2010). But above all, the main route of transmission is through reduviid vectors.

Although many species of triatomine insects can be efficient vectors for *T. cruzi,* only three species are involved in the transmission of the disease to man. Among them, *Triatoma infestans* is the most important and widespread vector of Chagas disease in South America. These insects started to colonize intradomestic and peridomestic habitats becoming a species of epidemiological importance (Gaunt and Miles 2000). *Rhodnius prolixus* is normally associated with palm trees and recent works suggested that deforestation might have increased the need of these insects to colonize human's habitats (Abad-Franch et al. 2009). *R. prolixus* is found in Central American countries like Honduras and Nicaragua and also in Venezuela and Colombia. The last species is *Triatoma dimidiata* which has a similar distribution as *R. prolixus* but also occupies areas further north into Mexico (Galvao et al. 2003).

Triatominae are hemimetabolous insects, their life cycle is composed of eggs, five nymph stages and the male and female imagos. All nymph stages are blood-sucking and can get infected and then transmit the parasite (Noireau and Dujardin 2010).

As it is mentioned before these three species became important due to their ability to colonize domestic and peridomestic habitats. Chagas disease was originally restricted to poor and rural areas of South and Central America were houses are made of thatch roofs and mud walls. This type of houses offers the best living conditions to the insects, providing shelter and food (Campbell-Lendrum et al. 2007).

Due to human population growth, extensive deforestation, urbanization and other environmental alterations, the proliferation of many arthropod vectors have raised significantly during the last years, increasing the prevalence of vector-borne diseases; therefore one of the biggest challenges in public health is the improvement of their control (Labbé et al. 2011).

Another reason that is often thought to be the cause of the expansion of tropical vector-diseases is climate change, which can influence on vector distribution and transmission. However socioeconomic conditions are the main contribution to vector-borne disease prevalence. Tropical diseases are usually distributed in developing countries. In most cases there are not available vaccines, parasites are resistant to drugs and the access to these drugs is impossible for the population due to economic reasons, as happens with Chagas disease. Consequently vector control is the only realistic way to intervene.

Chagas control

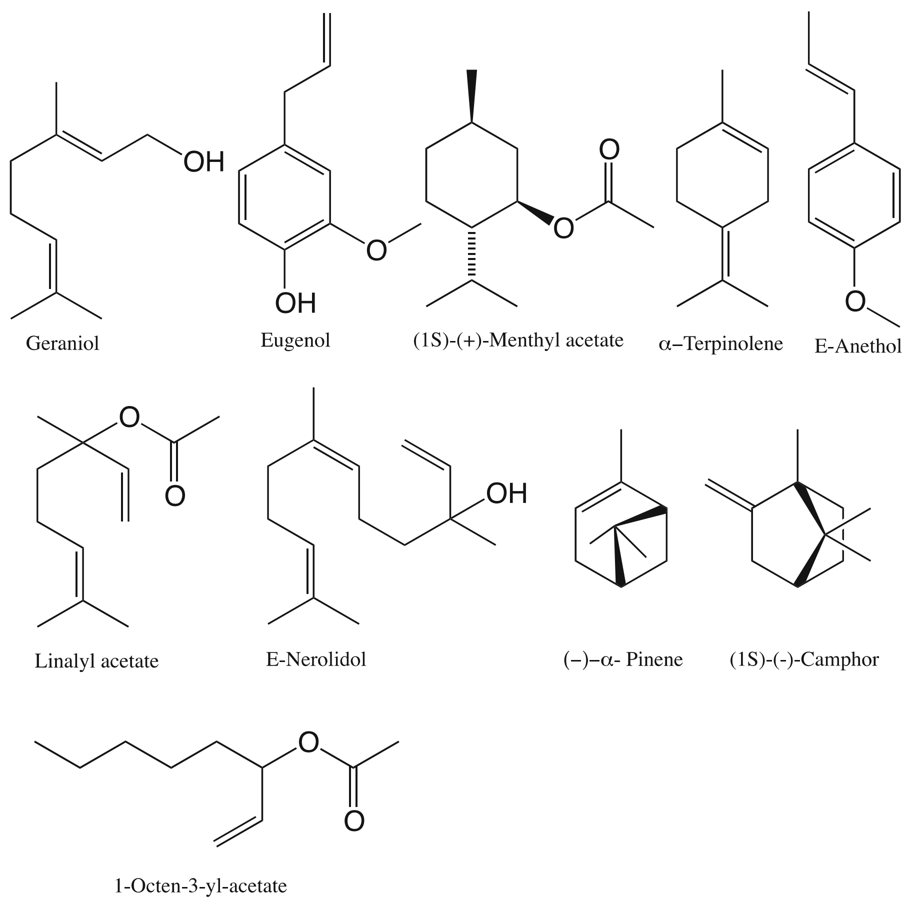
The results of previous surveys show that in 1980s 100 million people were estimated to be at risk of infection and 17 million were infected. Thanks to different campaigns and numerous trials performed in many countries, the main domiciliary vector was eradicated, as in the case of Uruguay in 1997, Chile in 1999 and some states of Brazil in 2006 (Dias 2007). This accomplishment was achieved because in 1991 six countries of Latin America decided to fight against this disease together. This vector control programme is known as Southern Cone Initiative to control/eliminate Chagas Disease. The main objective of this program was to eradicate the insect vector. Rural housing improvements, fumigation campaigns, community based monitoring and external evaluations were required, together with other actions such as screening of blood donors. Subsequently three other initiatives (Initiative of the Andean Pact, Central American Countries Initiative and the Amazon Countries Initiative) were added to the first, also with good results. However, immigration of people from endemic countries to other countries, like the European Union, USA and Canada is increasing the number of infected people in non-endemic countries which means that it is becoming an emerging health problem in these areas. Therefore we need to improve diagnosis and treatment of symptomatic individuals (Rassi et al. 2010).

Until now the most common way of triatomine control has been the use of synthetic insecticides. The use of these chemicals to control insects raises problems related to environment and human health and increases the selection of resistant strains. An alternative is the use of environmentally friendly natural products with activity against arthropods, which are biodegradable (Sfara et al. 2009). In this context, essential oils (EOs), which are volatile mixtures of different components with a variety of functional groups from a large number of plants, have a promising potential in insect control.

Essential oils and chagas control

Essential oils are produced as secondary metabolites in aromatic plants. The main components of EOs are monoterpenes, which are lipophilic molecules (Figs. 1 and 2) able to go through the insect tegument (Sfara et al. 2009). There is a lot of research on the biological activity of essential oils on insects (Maistrello et al. 2001; Park et al. 2003; Choi et al. 2002; Gazim et al. 2011; Pohlit et al. 2011). However, there are few studies on Chagas disease vectors. In 1996 Fournet et al. studied the composition of the EOs from the Bolivian medicinal plants *Minthostachys andina* and *Hedomea mandonianum* by gas chromatography/mass spectrometry and their insecticidal activity against *R. prolixus* and *T. infestans*. Menthone, iso-menthone, linalool, pulegone, eucalyptol and limonene were some of the components found in the essential oils tested. The activity of essential oils obtained from aerial parts of *Hedeoma mandoniana* has been investigated on *Triatoma infestans* and *Rhodnius neglectus* (Vilaseca et al. 2004). The main components of these oils are pulegone (37–46 %), 1,8-cineole (10–14 %), linalool (7–12 %) and 1-octen-3-yl acetate (7–12 %). The assays, performed by topical application, showed a moderate activity (33.3 % of mortality) on *R. neglectus* exemplars. Laurent et al. (1997) tested the ovicidal and larvicidal activity on *T. infestans* of sixty-three EOs from twelve different families collected in different regions of Bolivia. Twenty of the sixty-three essential oils tested showed insecticidal activity when the impregnated paper was the type of application. *Satureja sp.*, *Pimpinella anisum* and *Chenopodium ambrosioides* had larvicidal activity while *Eryngium sp.*, *Tagetes minuta*, *Mynthostachys andina* and *M. mollis* showed ovicidal effects. EOs from *Tagetes pusilla*, *Mentha arvensis* and *Foeniculum vulgare* had both activities. Furthermore, they search the active components responsible of these two properties and they concluded that isomenthol, linalool,

Fig. 1 Some compounds present in essential oils with activity against reduviid insects



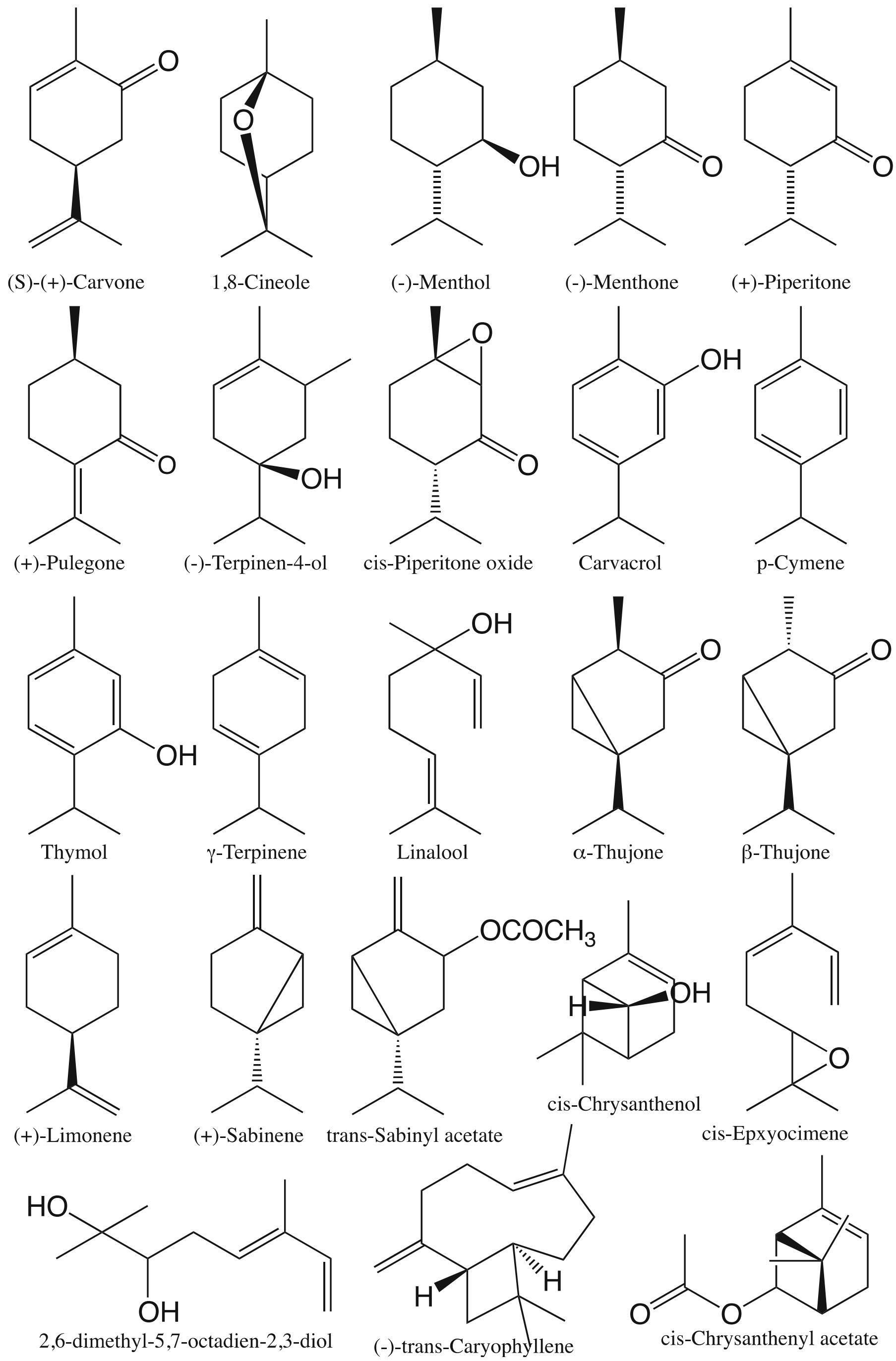


Fig. 2 Main components of the essential oils tested on *Rhodnius prolixus*

E-anethol and E-nerolidol where those with highest larvicidal activity, and L-menthol, isomenthol, piperitone, linalool and E-nerolidol with ovicidal activity.

Sfara et al. (2009) evaluated the fumigant and repellent activity of five essential oils from eucalyptus, geranium, lavender, mint, and orange oil and seven monoterpens (geraniol, eucalyptol, limonene, linalool, menthone, linalyl acetate and menthyl acetate) on first-instar nymphs of *R. prolixus*. They observed that eucalyptus essential oil and eucalyptol (1,8 cineole) were the most effective fumigants, while lavender and mint essential oils and the monoterpens geraniol, menthyl acetate and menthone had a repellent effect on nymphs.

Kurdelas et al. (2012) studied the chemical composition and anti-insect activity of *Baccharis darwinii* essential oil on *T. infestans*. They saw a good repellent activity and recognized the limonene, thymol and 4-terpinelol responsible of the anti-insect activity. Monoterpenes such as α-pinene, cineole, eugenol, limonene, terpinolene, camphor and thymol are usually the ones described as being the cause of the repellent activity against mosquitoes (Yang et al. 2004). Furthermore López et al. (2011) found that oils from two different species of *Tagetes* L. having the same components but in different percentages had different repellent activity. They studied the repellent properties of EOs on *T. infestans* from four species of *Tagetes* L. collected in Argentina and concluded that the relative proportion of each component can modify the anti-insect activity of an essential oil. Moreover, after 72 h of treatment the most repellent oils were from *T. minuta* and *T. filifolia*. These two plants have shown in previous experiments good mosquito repellency.

Lima et al. (2011) investigated the repellent activity of the essential oils of *Acantholippia seriphioides*, *Artemisia mendozana*, *Gymnophyton polycephalum*, *Satureja parvifolia*, *Tagetes mendocina*, and *Lippia integrifolia* from Argentina against *T. infestans*. The most repellent EOs in a 72 h treatment were *L. integrifolia* and *G. poycephalum* with a repellence percentage (RP) between 80 and 100 % at an EO concentration of 0,5 % (*w/v*), while *A. seriphioides* EO was not a good repellent but shown attractant properties at 72 h. The other three EOs had a RP between 60 and 80 %.

EOs can have different modes of action depending on their application. Mello et al. (2007) described the effects of *Pilocarpus spicatus* Saint-Hilaire (Rutaceae) EO on the development of fifth-instar nymphae of *R. prolixus*. The topical application of the EO caused paralysis plus high levels of toxicity whereas the moulting was barely inhibited. The ingestion of the EO resulted in high moulting inhibition and an increase in the intermoulting period. No toxicity was observed in this case.

Essential oils from Spanish cultivated plants and their effects on *Rhodnius prolixus*

Given the potential interest of EOs for the control of Chagas vectors, a series of aromatic plant species experimentally cultivated (CITA-Aragón, Spain) because of their medicinal and/or condiment value (Burillo and García-Vallejo 2003; Burillo 2009) were selected to test for the repellent effects of their EOs on *R. prolixus*. Aerial parts of *Artemisia absinthium*, *Mentha arvensis*, *M. spicata*, *M. longifolia*, *M. piperita*, *M. rotundifolia*, *Satureja montana, Thymus vulgaris,* and *T. zygis,* were collected at the flowering stage.

The plant material processing and essential oil extraction has been described elsewhere (Burillo and García-Vallejo 2003; Burillo 2009). For comparison purposes, a commercial *A. absinhium* essential oil sample was used (H2, waste oil fraction rich in thujones and terpenes; Hausmann Aromatic S.A., San Andrés de la Barca, Barcelona, Spain). Flower and leaf essential oils were analyzed by GC-MS (Burillo and García-Vallejo 2003; Bailén et al. 2012).

Rhodnius prolixus males and females from a laboratory colony (Facultad de Medicina UAM, Madrid, Spain) fed on living guinea pigs 1–5 days before the test were used for the bioassays. The tests were carried out in a two 250 ml Erlenmeyer flasks system connected by a glass tube (10 cm long, 1.5 cm in diameter). One treated (20 µl of 10 µg/µl solution of the EO) and one control (20 µl of solvent) paper disk (2 cm diameter) dried at room temperature were placed on each flask along with three *R. prolixus* adults. The flasks were covered (perforated parafilm) and maintained at 28 °C in darkness. The position of the insects was recorded every 5 for 30 min with the last observation at 60 min. This experiment was carried out in quadruplicate at different days. Data was transformed into repellency percentage (RP) as: RP = [1 − (%T/ %C)] x 100; where %T is the percentage of insects in the treatment flask and %C is the percentage of insects in the control flask. When %C = 0 (all insects are in the treatment flask) we consider RP = 0. Dimethyl phthalate was used as positive control.

The main components of the essential oils tested are shown in Table 1 and their chemical structures are in Fig. 2. The results of the repellent effect of the oils tested against *R. prolixus* are shown in Fig. 3.

All oils tested showed a significant repellent activity against *R. prolixus*. In the case of *Thymus* oils, there are differences in the potency of their activity, being highest in *T. zygis* and slightly lower in *T. vulgaris* (Fig. 3). Table 1 shows that the main

Table 1 Main components of essential oils with repellent activity against *R. prolixus*

Botanical name	Main component
Artemisia absinthium CE03Ae[a]	Cis-epoxiyocimene (60 %), chrysanthenol (13 %), 2,6-dimethyl-5,7-octadien-2,3-diol (6 %), linalool (4 %), chrysantenyl acetate (2 %), caryophyllene (2 %)
Artemisia absinthium H2[b]	β-thujone (57 %), α-thujone (22 %), limonene (5 %), sabinile acetate (4 %), sabinene (2.5 %)
Mentha arvensis	Carvone (75 %), 1,8-cineole (14 %), menthol (1 %)
Mentha spicata	Carvone (79 %), 1,8-cineole (12 %), menthol (2 %)
Mentha longifolia	Menthone (41 %), menthol (31 %), 1,8-cineole (13 %), iso-menthone (3 %), piperitone (2 %), pulegone (1 %), terpinen-4-ol (1 %)
Mentha piperita	Menthone (49 %), menthol (23 %), 1,8-cineole (12 %), iso-menthone (3 %), pulegone (2,5 %), piperitone (2 %), terpinen-4-ol (1 %)
Mentha rotundifolia	Piperitone oxide (25 %), M*(166). 67. 138. 41 (25 %), M*(122). 43. 112. 82 (17 %), terpinen-4-ol (8 %)
Satureja montana	Carvacrol (76 %), p-cymene (10 %)
Thymus vulgaris	Thymol (49 %), p-cymene (29 %), γ-terpinene (7 %), carvacrol (4 %), linalool (3 %)
Thymus zygis	Thymol (74 %), p-cymene (9 %), γ-terpinene (7 %), carvacrol (4 %), linalool (2 %)

[a] Essential oil of *A. absinthium* from field cultivation; [b] Commercial essential oil

compound in both cases is thymol; however, the proportion of thymol is higher in *T. zygis* than in *T. vulgaris*. This suggests that thymol may influence the activity of these oils, such as has been mentioned previously by other authors (Kurdelas et al. 2012; Nerio et al. 2010), although it is well known that the main component is not necessarily the main cause of the effect. It is also recognized that the relative proportion of each component can modify the anti-insect activity of an essential oil (López et al. 2011).

The oil of *S.montana* also showed the maximum activity (Fig. 3). Only two compounds (carvacrol and p-cymene) were identified as the main components of this oil, carvacrol being the most abundant (75.95 %), suggesting that this compound is responsible for the high activity shown by the oil.

All the *Mentha* EOs tested showed significant repellent activity. *M. arvensis*, *M. spicata* and *M. rotundifolia* showed maximum activity whereas the activity of *M. longifolia* and *M. piperita* is somewhat lower. The composition of *M. rotundifolia* is completely different from the rest of the species in this study; therefore, its activity should have a different origin. The major components of the oil of this plant are piperitone oxide (25.15 %) and an unidentified compound of M*(166), 67,138, 41 (24.85 %). Additionally, 4-terpineol is also present in a large proportion (8.23 %) and this compound has been previously described as active against *T. infestans* (Kurdelas et al. 2012).

In the other four species of *Mentha*, 1.8-cineole is present in similar proportions (11.77–13.78 %). The insect repellent activity of cineole has been previously described by other authors (Nerio et al. 2010). The major component in the two most active oils (*M. arvensis* and *M. spicata*) is carvone (73.57 and 12.75 % respectively). While the major component of the two less active oils (*M. longifolia* and *M. piperita*) is menthone. These data may suggest that either carvone enhances the effect of 1.8-cineol, or the presence of menthone decreases the activity of 1.8-cineole.

Two oils from *A. absinthium* were tested (from a commercial fraction, H2 and from a experimentally cultivated population, CE03Ae), giving a maximum repellent activity of 93 % for H2 oil and 90 % for CE03Ae; nevertheless the potency is lower than the other essential oils tested. Thujones (β and α) are the major components of H2 oil (57 and 22 % respectively), a commercial waste oil fraction rich in thujones and terpenes, and can explain the repellent effect of this oil as it has been described in earlier studies against the sycamore lace bug *Corythucha ciliate* (Rojht et al. 2009) and the common tick *Ixodes ricinus* (Palsson et al., 2008). The major components for CE03Ae oil are cis-epoxyocimene, chrysanthemol, 2,6-dimethyl-5,7-octadien-2,3-diol and linalool (60, 13, 6 and 4 % respectively). Linalool have been reported to have repellent properties against *Tyrophagus putrescentiae* (Sánchez-Ramos and Castañera

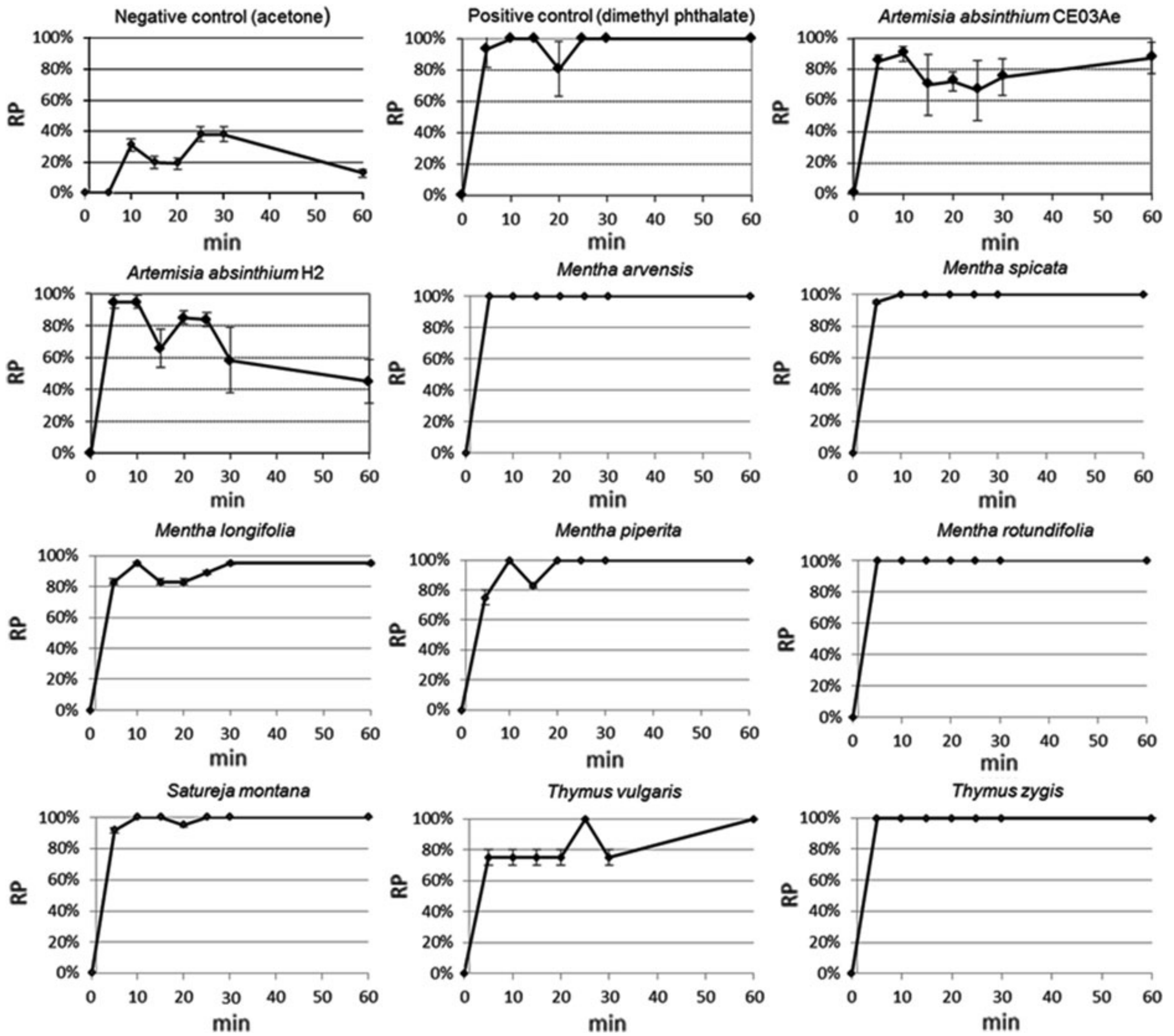


Fig. 3 Repellent activity against *Rhodnius prolixus* of essential oils (20 µl of a 10 µg/µl solution) from *Artemisia absinthium*, *Mentha arvensis*, *M. spicata*, *M. longifolia*, *M. piperita*, *M. rotundifolia*, *Satureja montana*, *Thymus vulgaris* and *T. zygis*. The graphs show the percentages of repellency at different time intervals between 5 and 60 min and 95 % confidence limits

2000), *Culex pipiens* (Choi et al. 2002), *Thrips tabaco* (Koschier et al. 2002), *Tribolium castaneum* and *Rhyzopertha dominica* (Ukeh and Umoetok 2011), and also *Myzus persicae* (Rodilla et al. 2008).

It should be pointed out that the activity of an EO can be modulated by minor components and that it can also be dependent on the target, life cycle stage, etc. (Kurdelas et al. 2012). Synergism or antagonism between the components can be an important factor modulating the biological effects of an EO. This phenomenon may result in a higher activity in the EO compared to the activity of the isolated components (Martin et al. 2011). However, further research should be conducted on the activity of pure terpene components.

As noted in the introduction of this review the different campaigns and numerous trials for vector control, helped to eradicate the main domiciliary vector, but the truth is that some countries are suffering reinvasion of the vector in treated houses due to the existence of peridomestic populations (Abad-Franch et al. 2009). This problem of reinvasion is rather difficult to eliminate but it would be great to be able to control it with a product that can last for long periods of time and be environmentally friendly. EOs are environmentally friendly, however we have to improve the efficiency developing different formulations with fixative materials competent in order to increase the protection times (Nerio et al. 2010).

Studies on different types of formulations have already been made thus obtaining creams, polymer mixtures and microcapsules to improve repellency duration (Sharma and Ansari 1994; Dua et al. 1996; Nentwig 2003; Chang et al. 2006). It has also been studied the use of additives such as paraffin (Oyedele et al. 2002) or combinations of EOs and other repellent extracts like coconut oils (Das et al. 1999).

The insights and studies described and analyzed show the need of more investigation and experimentation in this area to progress on Chagas disease prevention.

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