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The potential use of essential oils against mosquito larvae: a short review

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Abstract

The insecticidal activity of essential oils (EOs) on mosquito larvae has gained quite a lot of interest in recent scientific literature, and many articles envisage EOs as promising alternatives to conventional larvicides. However, the EOs described so far are not comparable in terms of efficacy with chemical or microbial larvicides. Moreover, for most EOs the low mammalian toxicity and the lack of environmental impacts have been claimed rather than tested. In industrialized countries, the availability of effective and environmental friendly microbial larvicides, will likely limit the EO-based products to very small market niches. Only in developing countries, where most aromatic plants are native, the full potential of botanical larvicides may be fully exploited. However, several research gaps remain to be filled before EOs could be considered as substitutes of conventional products.

Key words: botanical insecticides, biopesticides, ecotoxicology, integrated mosquito management, Culicidae.

Introduction

A key tool in the integrated management of most mosquito species (Diptera Culicidae) is larval control (Becker *et al*., 2010), and a number of insecticides have been used to eliminate mosquito larvae. The organophosphate temephos continues to be widely used in developing countries (George *et al*., 2015), whereas in Europe and USA larval control relies on microbial insecticides - *Bacillus thuringiensis* (Berliner) subsp. *israelensis* (de Barjac) (Bti) and to a lesser extent *Bacillus sphaericus* (Neide) -, juvenile hormone agonists - s-methoprene and pyriproxyfen - and chitin synthesis inhibitors - diflubenzuron (Belinato and Valle, 2015; Medlock *et al*., 2012).

Because of concerns about the detrimental impacts on the aquatic biocoenosis, all chemical larvicides have been undergoing strict revisions by the European Community that led to the withdrawal of temephos in the European Union. Moreover, due to the relatively small market and low financial return, most of the major agrochemical companies have strongly reduced their investments either to develop or to re-register active ingredients against public health pest insects (Matthews, 2011). As a result, some larvicidal products were lost in industrialized countries and several others will be lost or will be subjected to major restrictions on their possible fields of application. In general, the developing countries impose less restrictive regulatory constraints (Isman, 2008), but the relatively high cost of conventional larvicides and the difficulties in supplying such products hinder larval control programs. For these reasons, there is a great interest in the development of new larvicides not detrimentally affecting either human health or aquatic environments, and which should be easily available at affordable costs also in the developing countries.

In the last few decades, essential oils (EOs) have regained interest as potential low-risk insecticides (Isman, 2006; Regnault-Roger *et al*., 2012). EOs are obtained by distillation or other extraction methods from plants, which constitutively or inductively produce these chemicals as defense against phytophagous insects. In general, terpenoids and to a lesser extent phenylpropanoids are the main constituents of EOs (Bakkali *et al*., 2008).

Several studies extracting EOs from a wide array of plant species and reporting insecticidal activity on mosquito larvae by either the whole extract or by some purified constituents have been published (Shaalan *et al*., 2005; do Nascimento *et al*., 2013; Liu *et al*., 2015; Pavela, 2015). Besides efficacy, most of the studies claimed low impacts on human health and lack of environmental side effects by EOs. This short review focuses and critically evaluates the features of EOs that have been most widely stressed when supporting their potential role as mosquito larvicides.

Larvicidal activity

The eons-long struggle between plants and phytophagous insects led to the selection of a plethora of secondary metabolites with insecticidal activity in a myriad of plant species. Therefore, it is not surprising to find EOs showing acute toxic effects to mosquito larvae when added to water in controlled laboratory conditions. However, the median lethal concentrations (LC_{50}) of EOs are often one or more orders of magnitude higher than LC_{50} values for chemical or microbial larvicides. For example, Romi *et al*. (2003) reported LC_{50}s of temephos on larvae of Italian populations of *Aedes albopictus* Skuse (Diptera Culicidae) ranging from 0.0026 to 0.0085 ppm, while the LC_{50} of Bti for field collected strains of *Aedes aegypti* (L.) (Diptera Culicidae) ranged between 0.0889 and 0.1814 ppm (Loke *et al*., 2010). Very few EOs show LC_{50}s below 1 ppm, and several published studies reported that more than 50 ppm were required to kill 50% of the tested larvae (Shaalan *et al*., 2005; Dias and Moraes, 2013; Pavela, 2015). On the basis of WHO guidelines (WHO, 2006), such high concentrations are absolutely unpractical. Shaalan *et al*. (2005) suggested to discontinue studies on extracts causing less than 100% larval mortality in laboratory conditions at a concentration of 10 ppm. The same authors pointed out, "too much research effort has been wasted on in-depth investigation of products that should have been discarded at this stage" (i.e. not causing 100% mortality at 10 ppm).

Besides activity on mosquito larvae, questions on practicability need also to be considered. EO-based products should be effective under a wide range of field conditions (organic matter in the water, salinity, temperature, pH, etc.). Also different levels of susceptibility among mosquito species need to be considered.

Last but not least, many authors have shown that the concentration of EOs in plants vary with time of year, geographic location, history of pathogen or insect infestations, age of the plants, climatic stresses and agronomic practices. Articles that merely report the extraction of known amount of plant materials and comparing the activity of serial dilutions do not provide adequate indication of the concentrations of active ingredients, making impossible to replicate by other researchers. To be useful the studies should include chemical analyses that document the concentration of the active ingredients. This problem has been discussed in detail by Isman and Grieneisen (2014).

Health impact

Many common EOs and their main constituents have been used for a long time as additives for foods and beverages, as fragrances for cosmetics and in medicinal products (Isman, 2000), therefore they have been well studied experimentally. In general, these EOs show low oral toxicity to mammals and other vertebrates (Trumble, 2002; Regnault-Roger *et al*., 2012). However, the biological activity of any given chemical is due to its structure rather than its origin (Coats, 1994). Low toxicity is not intrinsically connected with natural derivation of EOs nor should it be assumed without any experimental evidence for extracts not previously tested.

Besides contact dermatitis and allergic reactions that have been reported for the repeated skin applications of the oil of *Melaleuca alternifolia* (Maiden et Betche) Cheel and d-limonene, respectively, severe acute poisoning may results with some EOs such as those from *Artemisia absinthium* L., *Mentha pulegium* L. and *Hedeoma pulegioides* (L.) Pers. (Trumble, 2002). If promising larvicidal activity is demonstrated for an EO for which health hazards have not been previously established, specific assays should be carried out to determine mammalian toxicity. Such tests should be mandatory before starting any field trials.

Environmental impact

One of the most appealing features of EOs in comparison to conventional synthetic insecticides is their low persistence in the environment and the absence of accumulation along the food chains (Isman, 2006). Some investigations on side effects of EOs on aquatic biocoenosis have been published. However, most of these studies used fishes as non-target organisms (Govindarajan and Benelli, 2016; Pavela and Govindarajan, 2016), whereas arthropods were rarely considered.

In general, EOs act as contact insecticides with a neurotoxic mode-of-action targeting GABA and octopamine synapses and acetylcholinesterase (Regnault-Roger *et al*., 2012). Owing the presence of these molecular targets among all the arthropods, there are few chances to find high level of selectivity of EOs with respect to non-targeted arthropods. Similar LC_{50} values were reported for EOs of *Piper klotzschianum* (Kunth) C. DC. on both nauplii of *Artemia salina* L. (Anostraca Artemiidae) and larvae of *Ae. aegypti* (do Nascimento *et al*., 2013). Toxic effects of EOs and their constituents extracted from the Myrtaceae were also reported against *Daphnia magna* Straus (Cladocera Daphniidae) in laboratory assays (Park *et al*., 2011). On the contrary, EOs from *Pinus kesiya* Royle ex Gordon was relatively safe towards water bugs (Rhynchota Notonectidae and Belostomatidae) (Govindarajan *et al*., 2016). However, these extract displayed LC_{50} values higher than 50 ppm on tested mosquito larvae.

Further studies on the activity of EOs on other aquatic arthropod preying on mosquito larvae such as dragonfly nymphs and water beetles would be advisable. Also *Macrocyclops albidus* (Jurine) (Cyclopoida Cyclopidae) and other copepods that were recommended for biological control of mosquito larvae in water containers (Veronesi *et al*., 2105) should be considered.

With the exception of microbial larvicides (Lacey, 2007), detrimental impacts on non-target aquatic insects and crustaceans have been reported for all other types of insecticides used to control mosquito larvae (IGRs, diflubenzuron, temephos and pyrethroids) (Invest and Lucas, 2008; Abe *et al*., 2014). At the concentrations required to be effective on mosquito larvae, also EOs seem to lack selectivity. However, given their shorter environmental persistence, it is likely that the final impact of EOs on non-target arthropods under genuine field conditions will be lower in comparison with chemical larvicides.

Conclusions

Overly enthusiastic statements have often been made in a number of articles describing the potential utility of EOs as mosquito larvicides. In most cases the efficacy of mixtures of intact EOs or of some purified components has been overrated while any health and environmental hazards have been dismissed without experimental evidence. As demonstrated by the dearth of EOs that have been commercialized so far, a big disconnect exists between the laboratory trials reporting activity on mosquito larvae and the practice of mosquito larval control.

In industrialized countries, unless plant extracts or oils comparable in bioactivity to conventional larvicides can be found, the chance to go beyond very small market niches seems unlikely. For mosquito species breeding in natural areas and in farmlands EOs are likely to be outperformed by microbial larvicides, which are effective at lower concentrations and do not adversely affect non-target organisms. Regarding the control of mosquitoes breeding in urban areas, it is expected that insect growth regulators will continue to play a pivotal role because of the long residual activity in water containers where larvae develop.

As foreseen for control of crop and food pests (Isman and Paluch, 2011), the full exploitation of the potential of EOs may be achieved in tropical areas where most developing countries are located. The wide availability of native aromatic plants combined with the relatively high costs of chemical insecticides in South America, Sub-Saharan Africa and Southeast Asia, make EOs viable alternatives to conventional larvicides. However, even in the developing countries, the use of EOs on wide spatial scales to control mosquito larvae breeding in natural areas could be hindered by the high LC_{50} values displayed by most of the compounds tested so far. On the contrary, EOs may be relevant to control mosquito larvae breeding in water containers in households (such as *Ae. aegypti* and *Ae. albopictus*). However, research gaps concerning mammalian toxicity of many EOs suggested for this purpose remain to be filled.

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