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LARVICIDAL ACTIVITY OF CITRUS PEEL ESSENTIAL OILS AGAINST AEDES AEGYPTI

SOLENE LACOMBE[1]*, VALENTINA MARCHETTI[2], PABLO BERMEJO[3]

Affiliation [1]Department of Pharmacognosy, Jura Centre for Medicinal Chemistry, Lons-le-Saunier, France
[2]Department of Phytochemistry, Cilento Institute of Pharmaceutical Sciences, Salerno, Italy
[3]Department of Phytochemistry, Cantabrian Coast Centre for Drug Development, Torrelavega, Spain

*Corresponding author: Solene Lacombe, s.lacombe@jcmc.fr
ORCID iD: Solene Lacombe 0000-0001-3307-5664; Valentina Marchetti 0000-0001-1735-9456; Pablo Bermejo 0000-0001-2998-0127

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Abstract

Citrus peel is an abundant by-product of the juice industry, and its essential oil is a candidate larvicide against Aedes aegypti, the main vector of dengue, Zika and chikungunya. This review gathers published work on the chemistry and extraction of citrus peel oils, the larvicidal evidence in Aedes and related mosquitoes, proposed modes of action, standard bioassay methods, and questions of formulation, safety and environmental fate. Reported activity is consistent but variable, usually moderate compared with synthetic larvicides, and strongly affected by oil composition, test conditions and volatility. Few studies address field performance, non-target organisms or resistance. The article is a narrative review and reports no new data.

1. INTRODUCTION

Dengue is now one of the fastest-growing mosquito-borne diseases, with transmission in more than a hundred countries and a large number of infections that go unrecognised [1]. *Aedes aegypti* carries dengue, Zika, chikungunya and urban yellow fever, and it has spread with trade, urban growth and poor water storage [2, 3]. There is no specific antiviral for dengue, and vaccine coverage is limited, so control of the vector remains the practical front line. That control consists mostly of removing breeding containers and treating water with larvicides, which makes the larval stage an attractive target, since larvae are confined to small bodies of water and easy to reach [4, 5].

The weak point of this strategy is dependence on a short list of chemicals. Organophosphate larvicides such as temephos have been used for decades, and resistance in *Ae. aegypti* has

been documented across several continents, alongside resistance to pyrethroids used against adults [6, 7]. Concerns about residues in drinking water containers add to the pressure. Plant-derived products are therefore of interest, and essential oils in particular, because they are mixtures of volatile terpenoids that act on several targets, degrade quickly in the environment and are often regarded as low-risk [8, 9]. Whether that last assumption is justified is a question this review returns to later.

Citrus peel oils have an obvious appeal. Peel is left over in vast amounts after juice extraction, the oil is already a commercial flavour and fragrance raw material, and the dominant constituent, limonene, is well known to be insecticidal [10, 11, 12]. This review brings together published evidence on the larvicidal activity of citrus peel essential oils against *Ae. aegypti* and related species, and asks how far it supports practical use. It is a narrative synthesis of reported work and presents no new experimental data.

2. CHEMISTRY AND EXTRACTION OF CITRUS PEEL OILS

Citrus essential oils are collected from oil glands in the flavedo, the coloured outer layer of the peel. Cold pressing or expression is the traditional method for sweet orange, lemon, mandarin and grapefruit, and gives an oil that keeps the fresh aroma and a small fraction of non-volatile material, including waxes, coumarins and polymethoxylated flavones [10, 11]. Steam or hydrodistillation yields a lighter oil that lacks these heavier compounds and can show some thermal alteration of terpenes. Newer approaches, including microwave-assisted and solvent-free methods, aim at shorter times and better recovery [13]. The extraction route changes composition, so two oils described as 'orange peel oil' may behave differently in a bioassay.

The main component of most citrus peel oils is limonene, commonly the great majority of the volatile fraction in sweet orange, with myrcene, sabinene, linalool, β-pinene and γ-terpinene appearing in smaller proportions [10, 11]. Lemon oil carries more β-pinene and γ-terpinene, and bergamot is notable for linalool and linalyl acetate. Grapefruit oil contains a low level of nootkatone, a sesquiterpene with its own insecticidal reputation. Oxygenated monoterpenes, which are present in small amounts, are often more bioactive than hydrocarbons and may contribute disproportionately to toxicity [14, 15]. Composition is established by gas chromatography with mass spectrometry (GC-MS), matching retention indices to reference libraries [16].

Cold-pressed oils bring one practical complication. The non-volatile fraction of some species, notably bergamot and lime, contains furocoumarins such as bergapten, which are phototoxic to human skin, and this is the reason many perfumery standards restrict their use in leave-on products [10, 11]. Distilled oils lack them. A larvicide intended for open water that people might handle in sunlight would sensibly avoid such constituents, and choice of oil type may therefore be a safety decision as much as a performance one. Papers that test cold-pressed oil on larvae seldom comment on this point, even though the same oil could not be freely applied by hand.

Variation within a species is wide. Cultivar, ripeness, climate, storage of the fruit and processing of the peel all influence the profile [10, 12]. Industrial peel from juice plants is a mixture of cultivars and may be heated or stored before the oil is recovered. Papers frequently identify the oil only as 'orange' or 'lemon', give no cultivar or source, and report no chromatographic data. Without a profile, activity cannot be tied to limonene content or anything else, and different laboratories cannot confirm each other's results. A minimum report should give the botanical name, cultivar, origin, extraction method, yield and main constituents with their percentages [15, 16].

Peel is also the by-product of a very large industry. Waste from juice processing contains fibre, pectin, flavonoids and essential oil, and valorisation of these streams is an active area of food science [12, 13]. If a larvicide could be obtained from material otherwise discarded or composted, the economics of vector control in low-income settings would shift in its favour. That argument depends on oil yield, which is

typically around a few tenths of a percent to one or two percent of fresh peel weight, and on the cost of getting oil from remote processing sites to the places where mosquitoes breed [12].

3. LARVICIDAL EVIDENCE

Essential oils from many plant families have been screened against *Ae. aegypti* larvae, and reviews list hundreds of studies, with Myrtaceae, Lamiaceae, Apiaceae, Rutaceae and Piperaceae among the most productive sources [15, 17, 18]. Within this crowd citrus oils usually sit in the moderate range. Comparative studies of edible plant oils against pyrethroid-susceptible and -resistant strains of *Ae. aegypti* showed that several oils kept their activity against resistant larvae, which is expected for compounds with a different mode of action from the synthetic insecticides [19]. Findings relevant to this review are summarised in table 1.

Direct work on the genus *Citrus* includes the peel oil of bitter orange (*C. aurantium*), which was compared with grapefruit oil for larvicidal activity against a malaria vector, with composition reported alongside [20]. Citrus limonoids, the bitter triterpenoids of seed and peel, were assessed against *Ae. albopictus* larvae and showed toxicity [21]. Constituent studies add detail: monoterpenoids such as limonene and α-pinene have measurable larvicidal and oviposition-altering effects on *Ae. aegypti* [22], and several essential oil components from Myrtaceae were active against the same species while being evaluated for aquatic toxicity [23]. Together these reports support the idea that peel oil toxicity comes mostly from its terpene fraction.

Table 1: Published findings on citrus oils, constituents and methods relevant to larvicidal use

Topic or material	Reported finding	Ref.
Citrus peel oil composition	Limonene is the main volatile; minor oxygenated terpenes vary with cultivar and method	[10, 11]
Cold-pressed versus distilled oil	Expression retains non-volatile coumarins and flavones that distillation leaves out	[10, 11]
Edible plant oils resistant Aedes	Several oils active against pyrethroid-resistant and susceptible strains	[19]
Bitter orange and grapefruit peel oil	Larvicidal activity against a malaria vector with composition reported	[20]
Citrus limonoids	Toxic to Aedes albopictus larvae in laboratory tests	[21]
Monoterpenoids Aedes aegypti	Larvicidal and oviposition-altering effects of single terpenes	[22]
Acetylcholinesterase	Monoterpenoids inhibit the enzyme with varied modes of inhibition	[24]
GABA and octopamine receptors	Terpenoid phenols and others act on insect receptor targets	[25, 26]
WHO larval test	Standard exposure of late third or fourth instar larvae for 24 hours	[5, 27]
Abbott correction and probit analysis	Control mortality correction and LC50 estimation	[28, 29]
Myrtaceae oils Daphnia magna	Acute toxicity to water fleas near larvicidal levels	[23]

Potency is the sticking point. Studies usually report LC_{50} values in the tens to hundreds of milligrams per litre, whereas temephos acts at a fraction of a milligram per litre, so a citrus oil is orders of magnitude less potent on a weight basis [5, 17]. Potency is not everything, because a cheap material used at several times the dose may still be worthwhile, but it affects cost, odour and the amount of oil that must be stored. Classification criteria help: a common view among natural-product entomologists treats essential oils with LC_{50} values below about 100 mg/L as promising and those above 1,000 mg/L as of little interest [15, 30].

The wider family of plant oils offers a useful yardstick. Eucalyptus oils from a dozen species were compared against *Ae. aegypti* larvae, and yield, composition and potency all varied widely between species, with 1,8-cineole-rich oils generally less toxic than those with other terpenes [31]. Oils from mint, sage and lemon balm were tested against *Culex pipiens*, the West Nile virus vector, and activity tracked the content of

particular oxygenated monoterpenes [32]. These studies show that mosquito genus and plant family both influence the outcome, so results for citrus in *Aedes* cannot be assumed to transfer to *Anopheles* or *Culex* without testing [15, 17, 20].

Commercial history provides a little context. Citrus peel oil and d-limonene are already used in some household insecticides and animal products, and botanical insecticide reviews mention citrus oil among the essential oils that have reached the market, usually as a contact insecticide for crawling insects rather than as a mosquito larvicide [8, 33]. This shows that regulatory pathways exist for citrus terpenes, but regulation of an agent used on food crops or pets is not the same as approval for application to water that people may drink from or that drains into streams [9, 30].

Counting the entries in table 1 by type of evidence shows the imbalance in the field (fig. 1). Compositional and larvicidal screening studies dominate, with fewer on mechanism and still fewer on formulation or non-target effects. That mirrors the wider literature on botanical larvicides, where reviewers repeatedly note large numbers of laboratory screens and a thin line of work that moves toward semi-field or field tests [17, 30]. The same reviewers point out that results from different laboratories are hard to compare, because larval stage, volume, solvent, exposure time and temperature vary from paper to paper [15, 18].

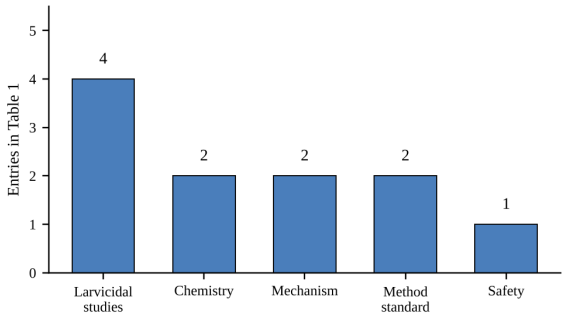


Fig. 1: Number of entries in table 1 by evidence type

4. PROPOSED MODES OF ACTION

The mechanisms of monoterpenoid toxicity to insects are not fully resolved, although three targets in the nervous system have received most attention. Some monoterpenoids inhibit acetylcholinesterase, with inhibition type and strength depending on the structure; López and Pascual-Villalobos described mixed and competitive modes for different compounds and pointed to the potential for pest control [24]. Others interact with octopamine receptors, which have no direct counterpart in vertebrates, a feature that would favour selective toxicity [26]. Thymol and related phenols modulate GABA-gated chloride channels in insects as well as in mammalian systems [25]. How closely citrus constituents follow these patterns is less clear, since limonene itself is a weak enzyme inhibitor [24, 34].

Historically, plant essential oils were studied for stored-product pests and agricultural insects before mosquitoes, and early surveys established that many oils show contact, fumigant and antifeedant activity that depends on the species [33]. Limonene, because of its abundance and low cost, was among the first terpenes taken into practical products. Larvae of mosquitoes, living in water and breathing through a siphon, are exposed in a different way from a beetle in a grain store, so conclusions from fumigant tests may not carry over. The route of entry for an oil in water is probably cuticle, gut and tracheal system together [14, 34].

Physical effects probably add to neurotoxicity. Lipophilic oils spread over the water surface as a thin film and may hinder respiration through the siphon, the way mineral oil larvicides work, and they can penetrate the cuticle, disturbing water balance and gut epithelium [9, 14]. Histological studies in several oil-treated mosquito larvae describe damage to the midgut lining and swelling of the cells, signs that are non-specific but consistent with membrane disruption [15, 34]. The relative contribution of film effects and true toxicity is seldom separated, so a portion of reported larvicidal activity may be mechanical suffocation that would fail under flowing water or a covered container.

Synergy among constituents is often invoked to explain why whole oils sometimes outperform their main component. A minor oxygenated compound can raise penetration or block detoxification enzymes, and a

mixture may slow resistance because several targets are involved [9, 14]. The evidence in citrus oils is thin. Few studies test limonene alone, a reconstituted mixture of the main components and the natural oil side by side, which is the design required to demonstrate synergy. One could also ask whether larvae can adapt: metabolic resistance based on cytochrome P450 and esterase upregulation, already common in *Ae. aegypti*, could in principle detoxify terpenes, though documentation is scarce [6, 7].

5. BIOASSAY METHODS

The World Health Organization (WHO) has set out standard procedures for larvicide testing. Late third or early fourth instar larvae, in groups of about twenty to twenty-five, are placed in water in cups or beakers, exposed for twenty-four hours, and scored for mortality, with at least four replicates and an untreated control [5, 27]. If control mortality rises above about 5 per cent, mortality in treated groups is corrected by Abbott's formula, and tests with control mortality above about 20 per cent are discarded [28]. Concentration-response data are then analysed by probit methods to give LC_{50} and LC_{90} values with confidence intervals [29]. Following this scheme makes studies comparable, though many botanical papers depart from it quietly.

Delivery of the oil is the main technical difficulty. Oils do not dissolve in water, so most investigators dissolve them in ethanol, acetone or dimethyl sulfoxide (DMSO), or emulsify them with a surfactant such as polysorbate 80, before adding to the test water. Solvent and emulsifier can be toxic to larvae at higher levels and can also change oil availability, so a solvent-only control must always accompany the test, and the final percentage should be stated [5, 18]. Glass versus plastic containers, covers to limit evaporation, and stirring all matter, since limonene sticks to plastic and escapes through the surface. Nominal concentrations may be well above those that persist in the water.

Statistical reporting is worth a comment. Four replicates of twenty to twenty-five larvae per concentration is the usual minimum, and at least four or five concentrations should span mortalities from about 10 to 95 per cent so that the probit line is well defined [5, 29]. Papers that give an LC_{50} without confidence limits, slope or a goodness-of-fit statistic leave readers unable to judge precision. Overlapping confidence intervals show that two oils are not meaningfully different, which is relevant because many 'rankings' of oils rest on single-point differences. Replicating on different days with different larval cohorts is better than repeating within one cohort [28, 29].

Good practice also includes a reference larvicide as a positive control, a defined colony or field strain, constant temperature and light, and a check on larvae health before testing [5, 27]. Reporting the diet given and the age of the larvae in hours is valuable. For field relevance, semi-field trials in outdoor containers with natural light, organic load and temperature variation are the logical next step, and they remain rare in the citrus literature [17, 30]. Without such trials, laboratory numbers represent an upper bound on performance.

6. FORMULATION, SAFETY AND ENVIRONMENT

Essential oils are unstable in practice. Limonene oxidises on exposure to air, light and heat, forming carvone, limonene oxide and hydroperoxides, and the oxidation products are known skin sensitisers; storage in dark, full, cool containers and addition of antioxidants slow the change [35]. In open water the oil volatilises within hours to a few days, which gives a short residual effect, an advantage for environmental fate but a disadvantage for control programmes that cannot revisit containers daily [9, 30]. Formulation can change that profile. Emulsions and nanoemulsions, microencapsulation in polymers and cyclodextrin inclusion complexes slow release and improve dispersion in water [36].

Safety to non-target organisms is the other half of the claim to be low-risk. Essential oils are toxic to aquatic invertebrates and fish at moderate concentrations; tests on Myrtaceae oils reported acute toxicity to the water flea *Daphnia magna* at levels not far from those that kill larvae, which narrows the margin of

safety [23]. Reviews of botanical insecticides warn that 'natural' should not be confused with 'safe', and that regulatory approval requires data on toxicity, residues and effects on non-target species, as with any other pesticide [8, 9, 30]. Citrus oils have the advantage of long human use in food, but exposure by ingestion differs from release into water.

Practical use in households is realistic, but it raises its own questions. Containers used for drinking water cannot receive a product that alters taste and smell, which excludes a good proportion of the places in which *Ae. aegypti* breeds in homes. Sale of unlicensed 'natural' larvicides by informal vendors risks overdosing and misuse. Conversely, use in non-potable containers, ornamental ponds, tyre yards and drains is more plausible, and a slow-release floating formulation would suit these [4, 36]. Cost per treated container, shelf-life and ease of application should be compared with temephos and with biological agents such as *Bacillus thuringiensis israelensis* (Bti) before recommending any product.

Economics will in the end decide whether an oil is adopted. The market price of citrus oil is much higher than that of temephos on a per-treated-litre basis if the oil must be dosed at several tens of milligrams per litre, and only a local, low-cost supply of peel oil would change that calculation [12, 13]. Community-based programmes in which households prepare their own peel extracts have been proposed, but home preparations vary greatly and cannot be standardised, making dosing and safety control impossible [9, 30]. A central production model that supplies a stable, labelled, slow-release product is more consistent with public health practice, and with the regulatory evidence that would be required [8, 36].

7. LIMITATIONS AND FUTURE DIRECTIONS

Several limits apply to the literature. Oils are inadequately characterised, bioassay protocols deviate from standards, solvent controls are not always present, and potency is mostly moderate. Evidence from field-like conditions, resistant populations and non-target species is scarce [17, 19, 30]. Studies of different citrus species, cultivars and extraction methods are hard to line up, and the mixture of results leaves it unclear whether peel oil from a given species can be recommended over another. Bias towards publishing active oils is likely, as it is in other screening fields.

Future work could be organised around a short list of questions: how much does limonene alone explain, what formulation gives useful residual activity in water of realistic quality, how do oils perform against resistant field populations in outdoor containers, and what is the margin to non-target aquatic life [19, 23, 36]? Standard methods, composition data and exposure verification would let results from different laboratories be pooled [5, 16, 35]. Collaborations with juice processors could secure consistent, documented peel supplies and allow cost analysis [12].

A semi-field design would be simple to set up. Standardised containers of known volume, placed outdoors under shade, would be filled with water of defined quality, treated with a formulated oil, then seeded with larvae at intervals to measure residual activity over days to weeks. Mortality, emergence and oil concentration would be recorded together, and a reference larvicide run in parallel [5, 17, 30]. Such a trial would answer the question that laboratory data cannot, which is how long a volatile oil remains effective under tropical conditions.

8. CONCLUSION

Published studies show that citrus peel essential oils, and limonene in particular, are toxic to *Aedes* larvae in the laboratory, including strains resistant to conventional insecticides [19, 20, 22]. Potency is moderate, composition is variable and mechanisms are inferred from work on related terpenoids [24, 25]. Practical constraints, among them volatility, oxidation and toxicity to aquatic life, limit what can be claimed from small-scale tests [23, 35].

A realistic role for peel oil is in formulated, slow-release products for non-potable containers, supported by standard bioassays, verified exposure and semi-field trials [5, 36].

Reports should state the source and chemical profile of the oil, use solvent controls and compare results with a reference larvicide [16, 28]. With that discipline, a waste product of the juice industry could contribute to integrated management of dengue vectors without replacing existing tools [4, 9].

9. ABBREVIATIONS

GABA-Gamma-aminobutyric acid, LC50-Median lethal concentration, LC90-Concentration lethal to 90 per cent of larvae, WHO-World Health Organization, P450-Cytochrome P450 enzymes, Bti-Bacillus thuringiensis israelensis, GC-MS-Gas chromatography with mass spectrometry, DMSO-Dimethyl sulfoxide

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All the authors have contributed equally.

12. CONFLICT OF INTERESTS

Declared none

13. REFERENCES

1. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, *et al.* The global distribution and burden of dengue. Nature 2013;496:504–507.
2. Kraemer MUG, Sinka ME, Duda KA, Mylne AQN, Shearer FM, Barker CM, *et al.* The global distribution of the arbovirus vectors Aedes aegypti and Ae. albopictus. eLife 2015;4:e08347.
3. Weaver SC, Reisen WK. Present and future arboviral threats. Antiviral Res 2010;85:328–345.
4. World Health Organization. Dengue: guidelines for diagnosis, treatment, prevention and control. Geneva: WHO; 2009.
5. World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides. WHO/CDS/WHOPES/GCDPP/2005.13. Geneva: WHO; 2005.
6. Moyes CL, Vontas J, Martins AJ, Ng LC, Koou SY, Dusfour I, *et al.* Contemporary status of insecticide resistance in the major Aedes vectors of arboviruses infecting humans. PLoS Negl Trop Dis 2017;11:e0005625.
7. Vontas J, Kioulos E, Pavlidi N, Morou E, della Torre A, Ranson H. Insecticide resistance in the major dengue vectors Aedes albopictus and Aedes aegypti. Pestic Biochem Physiol 2012;104:126–131.
8. Isman MB. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu Rev Entomol 2006;51:45–66.
9. Regnault-Roger C, Vincent C, Arnason JT. Essential oils in insect control: low-risk products in a high-stakes world. Annu Rev Entomol 2012;57:405–424.
10. González-Mas MC, Rambla JL, López-Gresa MP, Blázquez MA, Granell A. Volatile compounds in citrus essential oils: a comprehensive review. Front Plant Sci 2019;10:12.
11. Sawamura M, editor. Citrus essential oils: flavor and fragrance. Hoboken: Wiley; 2010.
12. Marín FR, Soler-Rivas C, Benavente-García O, Castillo J, Pérez-Alvarez JA. By-products from different citrus processes as a source of customized functional fibres. Food Chem 2007;100:736–741.
13. Chemat F, Vian MA, Cravotto G. Green extraction of natural products: concept and principles. Int J Mol Sci 2012;13:8615–8627.
14. Koul O, Walia S, Dhaliwal GS. Essential oils as green pesticides: potential and constraints. Biopestic Int 2008;4:63–84.
15. Dias CN, Moraes DFC. Essential oils and their compounds as Aedes aegypti L. (Diptera: Culicidae) larvicides: review. Parasitol Res 2014;113:565–592.
16. Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. 4th ed. Carol Stream: Allured; 2007.
17. Pavela R. Essential oils for the development of eco-friendly mosquito larvicides: a review. Ind Crops Prod 2015;76:174–187.
18. Cavalcanti ESB, Morais SM, Lima MAA, Santana EWP. Larvicidal activity of essential oils from Brazilian plants against Aedes aegypti L. Mem Inst Oswaldo Cruz 2004;99:541–544.
19. Sutthanont N, Choochote W, Tuetun B, Junkum A, Jitpakdi A, Chaithong U, *et al.* Chemical composition and larvicidal activity of edible plant-derived essential oils against the pyrethroid-susceptible and -resistant strains of Aedes aegypti (Diptera: Culicidae). J Vector Ecol 2010;35:106–115.
20. Sanei-Dehkordi A, Sedaghat MM, Vatandoost H, Abai MR. Chemical compositions of the

peel essential oil of Citrus aurantium and its natural larvicidal activity against the malaria vector Anopheles stephensi (Diptera: Culicidae) in comparison with Citrus paradisi. J Arthropod Borne Dis 2016;10:577–585.

21. Hafeez F, Akram W, Shaalan EAS. Mosquito larvicidal activity of citrus limonoids against Aedes albopictus. Parasitol Res 2011;109:221–229.
22. Waliwitiya R, Kennedy CJ, Lowenberger CA. Larvicidal and oviposition-altering activity of monoterpenoids, trans-anethole and rosemary oil to the yellow fever mosquito Aedes aegypti (Diptera: Culicidae). Pest Manag Sci 2009;65:241–248.
23. Park HM, Kim J, Chang KS, Kim BS, Yang YJ, Kim GH, *et al.* Larvicidal activity of Myrtaceae essential oils and their components against Aedes aegypti, acute toxicity on Daphnia magna, and aqueous residue. J Med Entomol 2011;48:405–410.
24. López MD, Pascual-Villalobos MJ. Mode of inhibition of acetylcholinesterase by monoterpenoids and implications for pest control. Ind Crops Prod 2010;31:284–288.
25. Priestley CM, Williamson EM, Wafford KA, Sattelle DB. Thymol, a constituent of thyme essential oil, is a positive allosteric modulator of human GABA-A receptors and a homo-oligomeric GABA receptor from Drosophila melanogaster. Br J Pharmacol 2003;140:1363–1372.
26. Enan E. Insecticidal activity of essential oils: octopaminergic sites of action. Comp Biochem Physiol C Toxicol Pharmacol 2001;130:325–337.
27. World Health Organization. Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. WHO/VBC/81.807. Geneva: WHO; 1981.
28. Abbott WS. A method of computing the effectiveness of an insecticide. J Econ Entomol 1925;18:265–267.
29. Finney DJ. Probit analysis. 3rd ed. Cambridge: Cambridge University Press; 1971.
30. Pavela R, Benelli G. Essential oils as ecofriendly biopesticides? Challenges and constraints. Trends Plant Sci 2016;21:1000–1007.
31. Lucia A, Licastro S, Zerba E, Masuh H. Yield, chemical composition, and bioactivity of essential oils from 12 species of Eucalyptus on Aedes aegypti larvae. Entomol Exp Appl 2008;129:107–114.
32. Koliopoulos G, Pitarokili D, Kioulos E, Michaelakis A, Tzakou O. Chemical composition and larvicidal evaluation of Mentha, Salvia, and Melissa essential oils against the West Nile virus mosquito Culex pipiens. Parasitol Res 2010;107:327–335.
33. Isman MB. Plant essential oils for pest and disease management. Crop Prot 2000;19:603–608.
34. Rattan RS. Mechanism of action of insecticidal secondary metabolites of plant origin. Crop Prot 2010;29:913–920.
35. Turek C, Stintzing FC. Stability of essential oils: a review. Compr Rev Food Sci Food Saf 2013;12:40–53.
36. Moretti MDL, Sanna-Passino G, Demontis S, Bazzoni E. Essential oil formulations useful as a new tool for insect pest control. AAPS PharmSciTech 2002;3:E13.