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Original Article

Acute toxicity of essential oil compounds (thymol and 1,8-cineole) to insectivorous guppy, *Poecilia reticulata* Peters, 1859

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ABSTRACT

Thymol and 1,8-cineole are now known bioinsecticidal monoterpenes occurring in many essential oils. The present study determined their toxicity on insectivorous guppy fish, *Poecilia reticulata* Peters, 1859 known to feed on mosquito larvae. The toxicity was recorded post 24 h treatment in experimental aquaria. The estimated median lethal concentration (LC_{50}) values of thymol and 1,8-cineole for female fish were 12.51 and 3997.07 mg/L, respectively, and for males the concentration required was 10.99 and 1701.93 mg/L, respectively. There was significant inhibition of acetylcholinesterase (AChE) and carboxylesterase (CarE) in treated male fish after 1,8-cineole treatment, whereas thymol induced CarE in both sexes but induced only AChE in females. Overall, thymol and 1,8-cineole were moderately toxic to guppies compared to synthetic pesticides.

Introduction

For more than a decade now there has been substantial emphasis on the development of plant essential oil-based insect control products, as it is well known now that compounds from these oils control insect pests of agricultural crops and insect pests of public health and veterinary importance (Isman, 1999; Koul et al., 2008). Many of these compounds are known to interfere with the basic metabolic, physiological and behavioral functions of pests, and some are known to affect the growth, development, reproduction or survival of insects and vectors (Paruch et al., 2000; Hummelbrunner and Isman, 2001; Koschier and Sedy, 2001; Tripathi et al., 2003; Koul, 2005; Singh et al., 2008; Batish et al., 2008; Kulkarni et al., 2013).

Most essential oils are generally recognized as safe (GRAS) and approved by the US Food and Drug Administration for food and many appear in the food chemical codex. In Europe and Asia, the presumption of safe under conditions of use has been bestowed on essential oils based on similar considerations (Koul et al., 2008; Hüsnü and Buchbauer, 2010). The essential oil compounds have other substantial advantages as they occur in a variety of plant species worldwide and are simple compounds that could be easily isolated. In Thailand, thymol and 1,8-cineole occur in many plants which grow abundantly, such as greater galangal (*Alpinia galanga*), basil (*Ocimum basilicum*) and krervanh Thai (*Amomum krervanh*).

Earlier studies showed that thymol and 1-8 cineole were toxic to *Plutella xylostella* (Kumrungsee et al., 2014) and caused very moderate toxicity to its parasitoid, *Cotesia pultellae* (Yotavongse et al., 2015). Thymol and 1,8-cineole have also been reported as pesticidal against Varroa mote (*Varroa destructor*) (Jean-Luc et al., 2014), codling moth (*Cydia pomonella*) larvae (Durden et al., 2011), American cockroach (*Periplaneta americana*) (Sfara et al., 2009), and Northern house mosquito (*Culex pipiens*) larvae (Corbet et al., 1995).

This information suggests that both these compounds could be alternative insect control products for farmers but at the same time, there are challenges for non-targets as well; therefore, there is need to understand how these compounds influence other non-target organisms, especially aquatic organisms. The necessity for this is further substantiated by the fact that these two simple monoterpenes are lipophilic, and consequently a high rate of gill absorption in fish is possible and this in turn would be a contributing factor in the sensitivity of the fish to aqueous pesticide compounds exposure (Viran et al., 2003). Thus, the first objective of the present study was to determine the acute toxicity of thymol and 1-8, cineole, established as insecticidal compounds, to the insectivorous guppy *Poecilia reticulata*, the standard test species pursuant to American Public Health Association (APHA), the

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American Water Works Association (AWWA), and the Water Environment Federation (WEF). (1998) and Organisation for Economic Co-operation and Development (1993) under laboratory conditions.

In addition, fish also seem to be deficient in the enzyme system that hydrolyzes some pesticides (Cengiz, 2006; Kumar et al., 2009). The main reaction involved in the metabolism in fish is ester cleavage mainly due to the action of carboxylesterase and oxidative enzymes (Demoute, 1989). Accordingly, a study was conducted to determine if the candidate fish was able to detoxify the compounds used. Moreover, as many pesticides do inhibit acetylcholinesterase, the neurotransmitter enzymes, it was imperative to analyze acetylcholinesterase as well.

Materials and methods

Essential oil compounds

1,8-cineole (Fig. 1A) and thymol (Fig. 1B) were obtained commercially (97–99% purity) from Sigma- Aldrich (St. Louis, USA) and were evaluated individually to determine their efficacy levels against guppy. Compounds were dissolved in 0.5% triton X100 in acetone (AR grade). The dosing volume never exceeded 1 mL in the test aquarium (0–50 mg/L for thymol and 0–10,000 mg/L for 1,8-cineole).

Fish

Both male and female adult guppy, *Poecilia reticulata* (2 months old) with a mean weight of 0.20 g and a mean total length of 2.50 cm were obtained from a local breeder in Bangkok, Thailand and brought to the laboratory within 30 min in plastic bags with sufficient air. The plastic bags were placed in the maintenance aquarium for about 30–35 min for acclimatization. Then the bags were cut open and the fish were allowed to swim into the aquarium filled with dechlorinated tap water and allowed to acclimatize to laboratory conditions (26.0 ± 0.8 °C, 78 ± 2% RH, pH = 7.0). Test chambers were glass aquaria of 10 L capacity. Healthy fish were chosen for the experiments with the lengths and weights of the fish in the range 3.8–5.1 cm and 15–25 g, respectively.

Acute toxicity bioassay

A standard bioassay was followed (American Public Health Association (APHA), the American Water Works Association (AWWA), and the Water Environment Federation (WEF), 1998; Organisation for Economic Co-operation and Development (OECD), 1993). The procedure was performed with the approval of an appropriate Ethics Committee of Kasetsart University, Thailand under the reference number ACKU03858. Test chambers were filled with 5 L of non-chlorine water (drinking water). The water temperature was 26 ± 1 °C and the dissolved oxygen level was 7.2–7.9 mg/L. All determinations were repeated five times for each concentration. Groups of experimental animals, each consisting of 10 individuals, were selected at random and placed into aerated aquaria. After 24 h of adaptation, 1 mL of different concentrations of essential oil compounds were added to the experimental aquaria making total concentrations of 0–20 mg/L for thymol and 0–10,000 mg/L for 1,8-cineole. A control group was provided with 1 mL of 0.5% triton X100 in acetone (AR grade) in a test aquarium. The fish were not fed for the duration of the experiment. Mortality was recorded 24 h after the start of the tests. Dead individuals were removed immediately. Behavioral changes were followed closely. The median lethal concentration (LC_{50}) and 95% confidence limits were calculated using the Statplus for Mac software program (AnalystSoft Inc., Walnut, Canada).

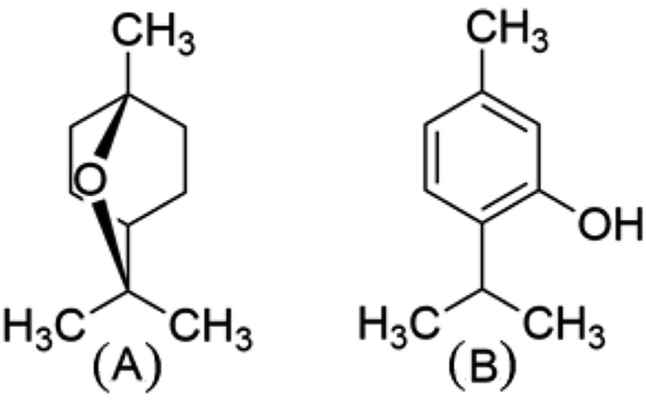

Fig. 1. Structure of (A) 1,8-cineole; (B) thymol.

Enzyme assays

Enzyme assays were carried out *in vivo* using a modified method of Bullangpoti et al. (2012). Twenty guppies were tested at the LC_{50} dose and control group (no compound added). Samples were prepared from 24 h surviving treated fish and replicated thrice. Each sample was homogenized in 0.5 mL buffer (100 mM phosphate buffer, pH 7.2 and 1% triton X100). The homogenate was centrifuged at 10,000 × *g* for 15 min at 4 °C and the supernatant was used as an enzyme source.

Carboxylesterases (CarE)

Enzyme solution (10 μL) was mixed with 10 mM *p*-nitrophenylacetate (pNPA) in DMSO (40 μL) and 50 mM phosphate buffer (pH 7.5, 190 μL). Enzyme activity was measured at 400 nm and 25 °C for 3 min using a microplane reader in the kinetic mode. The activity of enzyme was determined by using the extinction coefficient of 176.4705 for pNPA. Three replicates per treatment were estimated. The total protein content for each enzyme solution was determined using a Bradford kit (BioRad Laboratories, Hercules, CA, USA) with bovine serum albumin as the standard and analyzed using a spectrophotometer at 600 nm. Statistical comparison of CarE activity was done using analysis of variance and means were compared using Duncan's multiple range test in the Statplus for Mac software program (AnalystSoft Inc., Walnut, Canada).

Acetylcholinesterase (AChE)

The AChE activity was measured with acetylthiocholine (ASCh) (St. Louis, USA) as substrate using a spectrophotometric method (Ellman et al., 1961) where 50 μL of enzyme was pre-incubated for 30 min at 30 °C with 50 μL of 100 mM phosphate buffer (pH 7.2, 50 μL). Then, 50 μL TpS (100 mM DTNB in 0.1M phosphate buffer with 0.1M EDTA, 100 mM ASCh and 100 mM phosphate buffer, pH 7.2) were added. The change in absorbance at 412 nm was measured using a microplate reader, and the AChE activity was converted to nanomoles of acetylthiocholine hydrolyzed/min (e412 nm $= 1.36 \times 10^4$ $M^{-1}cm^{-1}$). Three replicates per treatment were estimated. Statistical comparison of AChE activities was done using analysis of variance and means were compared using Duncan's multiple range test in the Statplus for Mac software program (AnalystSoft Inc., Walnut, Canada).

Results

Toxicity of thymol and 1,8-cineole

The calculated 24-h acute LC_{50} values of thymol and 1,8-cineole for the female fish were 12.51 and 3997.07 mg/L, respectively, and for males were 10.99 and 1701.93 mg/L, respectively. (Table 1). No mortality occurred in the control group. The results showed that thymol was more toxic to fish than 1,8-cineole. The mortality was dose dependent. However, there was no significant ($p > 0.05$), sex-dependent difference in toxicity for both compounds.

Observations were made of the behavioral responses of the guppies during the acute toxicity tests. The control group showed normal behavior during the test period. The changes in behavioral response started after dosing with either essential oil compound, especially thymol, where paralytic, erratic swimming, rapid gill movement and motionless stature of fish were observed. Fish were seen adhering to the bottom of aquaria at all treatment concentrations. At a dose of more than 10 mg/L of thymol, the paralysis occurred within the first hour of treatment in aquaria.

Effect of thymol and 1,8-cineole on enzymes

The effects of the thymol and 1,8-cineole on the acetylcholinesterase activity and on the detoxification enzyme, carboxylesterase were studied *in vivo*. Only thymol-treated female fish showed induction of acetylcholinesterase activity, while all other treatments were inhibitory to the respective enzymes (Fig. 1). Thymol induced general esterase activity for both sexes, while 1,8- cineole was inhibitory for both sexes (Fig. 2).

Discussion

The present study on *Poecilia reticulata* was representative of insectivorous fish inhabiting a variety of water habitats and feeding voraciously on mosquito larvae. This fish is also a potential bio-indicator as a pollution marker like zebra fish because of its convenient breeding and important occurrence in metal-polluted waters (Widianarko et al., 2000).

Thymol and 1,8-cineole are now well known biopesticides from plant essential oils and have been shown to be toxic to many insects (Corbet et al., 1995; Sfara et al., 2009; Jean-Luc et al., 2014; Kumrungsee et al., 2014; Yotavongse et al., 2015). However, there are no reports that describe the toxicity of these two compounds to non-target animals like insectivorous fish. Therefore, it was interesting to study in-depth the acute toxicity to this economically important fish species. The 24 h LC_{50} values for females of thymol and 1,8-cineole for *P. reticulata* were 12.51 and 3997.07 mg/L, respectively; whereas for males they were 10.99 and 1701.93 mg/L, respectively. The results suggested that thymol was more toxic than 1,8-cineole to guppy fish. The present results also showed that although thymol induced carboxyl esterase activity, the toxicity was not impaired as expected and was still higher than 1,8-cineole. It is possible that thymol could inhibit other detoxification enzyme in fish. For example, lignans have a methyl moiety that is typical for the inhibition of piperonylbutoxide (the standard synergist and well-known cytochrome P450 inhibitor) or triphenyl phosphite (the standard synergist and well-known esterase inhibitor). In addition, the structure of 1,8-cineole is more sterically hindered than thymol, which apparently adhered to receptor sites more easily than 1,8-cineole to induce higher toxicity. Such a striking

Table 1
Acute 24 h toxicity of thymol and 1-8, cineole to both sexes of guppy fish, *Poecilia reticulata*.

	Thymol		1-8 cineole	
	Female	Male	Female	Male
LC_{50} (mg/L)[a,b]	12.51[a]	10.99[a]	3997.07[b]	1701.93[b]
95% Confidence limits	6.86–19.02	5.70–13.94	2722.93–4280.53	1087.77–2766.35
LC_{90} (mg/L)[a,b]	23.11[a]	21.51[a]	7385.35[b]	8898.72[b]
95% Confidence limits	16.04–31.94	15.74–27.11	7022.53–8280.51	7087.93–10,766.02
Slope ± SE	4.81 ± 2.05	4.40 ± 1.85	4.81 ± 2.05	4.40 ± 1.85
Intercept ± SE	−0.27 ± 0.15	0.42 ± 0.15	−12.31 ± 0.15	−11.08 ± 0.16
Chi square	0.29	0.40	0.09	0.47

[a] No mortality in control group.
[b] Median lethal concentration (LC_{50}) and 90 percentage lethal concentration (LC_{90}) values followed by a common, lowercase, superscript letter in the same raw are not significantly different at the 5% level using Duncan's multiple range test.

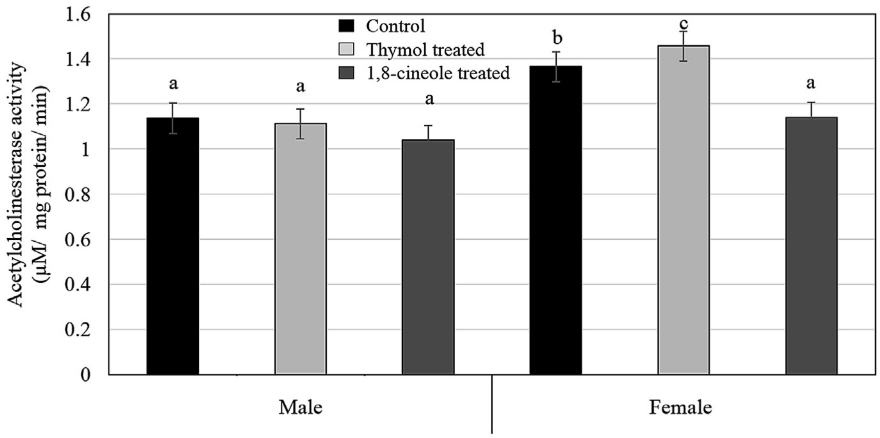

Fig. 2. Acetylcholinesterase activity in guppies after treatment with thymol and 1,8-cineole. (Activities followed by a common, lowercase, superscript letter in the same column for each sex are not significantly different at 5% level using Duncan's multiple range test).

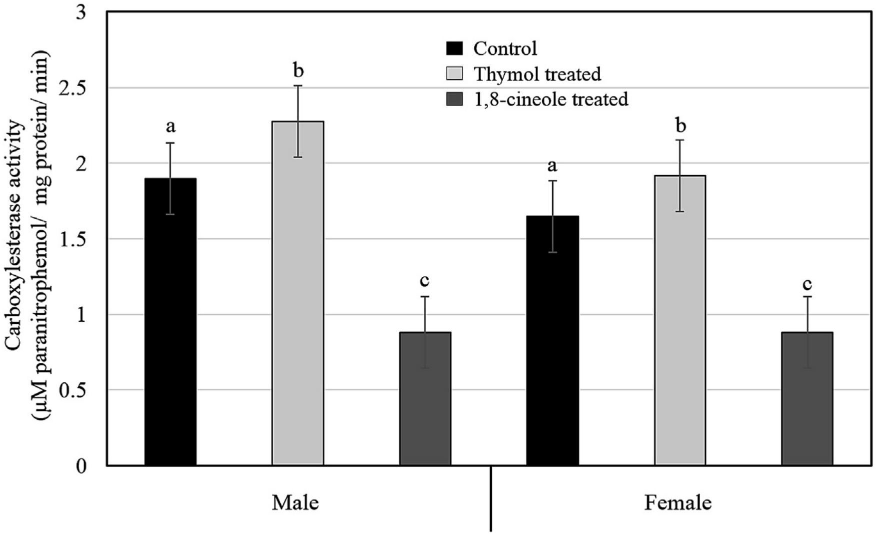

Fig. 3. Carboxylesterase activity in guppies after treatment with thymol and 1,8-cineole. (Activities followed by a common, lowercase, superscript letter in the same column of each sex are not significantly different at 5% level using Duncan's multiple range test).

effect is the same as was reported earlier by Zhang et al. (2016), describing the absence of steric effects and action directly related to the toxic potencies of the electrophiles.

Although thymol showed higher toxicity than 1-8-cineole, it was still less toxic to guppies compared to synthetic pesticides. Earlier reports suggested that the toxicity of synthetic pesticides to *P. reticulata* were much higher due to pyrethroids ($LC_{50} = 0.016$ mg/L), imidacloprid (over 80 mg/L) (Buffin, 2003), β-cypermethrin (LC_{50} = 21.37 μg/L) (Polat et al., 2002) or α-cypermethrin (LC_{50} = 9.43 μg/L) (Yilmaz et al., 2004). Thus, it can be easily suggested that thymol and 1-8-cineole were moderate in activity when compared to chemical insecticides. In comparison to other botanical insecticides, it appears that some extracts are much safer than pure compounds like mangosteen (*Garcinia mangostana*) extract (LC_{50} = 4.27 mg/L, Bullangpoti et al., 2007) or the extract of green amaranth (*Amaranthus viridis*) (LC_{50} = 947 mg/L, Arsirapoj et al., 2010). Visetson et al. (2005) found that water has an important role to play in the hydrolysis of many botanical insecticides such as selinadiene from nutgrass tuber (*Cyperus rotundus*) which degraded more than 80% in water within 12 h.

It was clear from the present study that only thymol-treated female guppy showed an induction of acetylcholinesterase activity, while in males the enzyme was inhibited (Fig. 1). This indicated a sex-dependent variable response perhaps because different sexes in vertebrates have different metabolisms and the chemicals could cause different actions. Fig. 3.

The present showed paralytic effects in fish, specifically due to thymol. The action could be related to the inhibition of acetylcholinesterase that may lead to quick action and paralytic symptoms. There are many reports that suggest an action on neuron enzymes from. For example, earlier studies suggested the inhibition of acetylcholinesterase activity due to 1,8-cineole compared to terpineol, limonene, alpha pinene and linalool (Miyazawa et al., 1998; Perry et al., 2002; Dohi et al., 2009). To date, it appears that the compounds used in the present study must be inhibiting neuroenzymes thereby causing paralytic, erratic swimming, rapid gill movement and the motionless stature of fish.

To the best of currently accessed knowledge, the present study is the only one to show the effect of thymol and 1,8-cineole on fish. Overall, it was clear that relationships among physicochemical properties, detoxification and neuroenzyme activities and acute toxicity endpoints of thymol and 1-8 cineole suggest that both compounds were moderately toxic compared to known data available for synthetic pesticides (Buffin, 2003; Yilmaz et al., 2004; Prusty et al., 2015). The present data will also be useful for generating a database for ecotoxicological studies specifically in aquatic media. Therefore, it is essential to generate such data for all botanical insecticides for ecological risk assessment.

Conflict of interest

None declared.

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References

American Public Health Association (APHA), the American Water Works Association (AWWA), and the Water Environment Federation (WEF), 1998. Standard Methods for the Examination of Water and Wastewater, Washington, DC., US.

Arsirapoj, C., Sudthonghong, C., Bullangpoti, V., 2010. Acute toxicity of *Amaranthus viridis* extract on guppies, *Poecilia reticulata*. Commun. Agric. Appl. Biol. Sci. 75, 199–202.

Batish, D.R., Singh, H.P., Kohli, R.K., Kaur, S., 2008. Eucalyptus essential oil as a natural pesticide. For. Ecol. Manag. 256, 2166–2174.

Buffin, D., 2003. Imidacloprid. Pesticide News, 30 January 2006. http://www.pan-uk.org/pestnews/Actives/imidaclo.htm.

Bullangpoti, V., Visetson, S., Milne, J., Milne, M., Sudthongkong, C., Pornbanlualap, S., 2007. Effects of alpha-mangostin from mangosteen pericarp extract and imidacloprid on *Nilaparvata lugens* (stal.) and non-target organisms: toxicity and detoxification mechanisms. Commun. Agric. Appl. Biol. Sci. 72, 431–441.

Bullangpoti, V., Wajnberg, E., Audant, P., Feyereisen, R., 2012. Antifeedant activity of *Jatropha gossypifolia* and *Melia azedarach* senescent leaf extracts on *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and their potential use as synergists. Pest Manag. Sci. 68, 1534–1540.

Cengiz, E.I., 2006. Gill and kidney histopathology in the freshwater fish *Cyprinus carpio* after acute exposure to deltamethrin. Environ. Toxicol. Pharmacol. 22, 200–204.

Corbet, S.A., Danahar, G.W., King, V., Chalmers, C.L., Tiley, C.F., 1995. Surfactant-enhanced essential oils as mosquito larvicide. Entomol. Exp. Appl. 75, 229–236.

Demoute, J.P., 1989. A brief review of the environmental fate and metabolism of pyrethroids. Pestic. Sci. 27, 375–385.

Durden, K., Sellars, S., Cowell, B., Brown, J.J., Pszczolkowski, M.A., 2011. *Artemisia annua* extracts, artemisinin and 1,8-cineole, prevent fruit infestation by a major, cosmopolitan pest of apples. Pharm. Biol. 49, 563–568.

Dohi, S., Terasaki, M., Makino, M., 2009. Acetylcholinesterase inhibitory activity and chemical composition of commercial essential oils. J. Agric. Food Chem. 57, 4313–4318.

Ellman, G.L., Courtney, K.D., Andres, V.J., Feather-Stone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88–95.

Hummelbrunner, A.L., Isman, M.B., 2001. Acute, sublethal, antifeedant and synergistic effects of monoterpenoid essential oil compounds on the tobacco cut worm (Lepidoptera: Noctuidae). J. Agric. Food Chem. 49, 715–720.

Hüsnü, C.B.K., Buchbauer, G., 2010. Handbook of Essential Oils: Science, Technology, and Application. CRC Press, New York.

Isman, M.B., 1999. Pesticides based on plant essential oils. Pestic. Outlook 68–72. April.

Jean-Luc, C., Téné, N., Bonnafé, E., Alayrangue, J., Hotier, S.L., Armengaud, C., Treilhou, M., 2014. Thymol as an alternative to pesticides: persistence and effects of Apilife Var on the phototactic behavior of the honeybee *Apis mellifera*. Environ. Sci. Pollut. Res. Int. 21, 4934–4939.

Koschier, E.H., Sedy, H.A., 2001. Effects of plant volatiles on the feeding and oviposition of *Thrips tabaci*. In: Marullo, R., Mound, L. (Eds.), Thrips and Tospoviruses, CSIRO, Canberra, pp. 185–187.

Koul, O., 2005. Insect Antifeedants. CRC Press, Boca Raton, FL, USA.

Koul, O., Walia, S., Dhaliwal, G.S., 2008. Essential oils as green pesticides: potential and constraints. Biopestic. Int 4, 63–84.

Kulkarni, R.R., Pawar, P.V., Joseph, M.P., Akulwad, A.K., Sen, A., Joshi, S.P., 2013. *Lavandula gibsoni* and *Plectranthus mollis* essential oils: chemical analysis and insect control activities against *Aedes aegypti, Anopheles sfttephensi* and *Culex quinquefasciatus*. J. Pest. Sci. 86, 713–718.

Kumrungsee, N., Pluempanupat, W., Koul, O., Bullangpoti, V., 2014. Toxicity of essential oil compounds against diamondback moth, *Plutella xylostella*, and their impact on detoxification enzyme activities. J. Pest. Sci. 87, 721–729.

Kumar, A., Sharma, B., Pandey, R.S., 2009. Assessment of acute toxicity of λ-cyhalothrin to a freshwater catfish, Clarias batrachus. Environ. Chem. Lett. 9, 43–46.

Miyazawa, M., Wada, T., Kameoka, H., 1998. Biotransformation of (+)- and (−)-limonene by the larvae of common cutworm (*Spodoptera litura*). J. Agric. Food Chem. 46, 300–303.

Organisation for Economic Co-operation and Development, 1993. Guidelines for Testing of Chemicals. OECD, Paris, France.

Paruch, E., Ciunik, Z., Nawrot, J., Wawrzenczyk, C., 2000. Lactones.9. Synthesis of terpenoid lactones-active insect antifeedants. J. Agric. Food Chem. 48, 4973–4977.

Perry, N.S.L., Houghton, P.J., Jenner, K.A., Perry, E.K., 2002. *Salvia lavandulaefolia* essential oil inhibits cholinesterase *in vivo*. Phytomedicine 9, 48–51.

Prusty, A.K., Meena, D.K., Mohapatra, S., Panikkar, P., Das, P., Gupta, S.K., Behera, B.K., 2015. Synthetic pyrethroids (Type II) and freshwater fish culture: perils and mitigations. Int. Aquat. Res. 7, 163–191.

Polat, H., Erkoc, F.U., Viran, R., Kocak, O., 2002. Investigation of acute toxicity of beta-cypermethrin on guppies *Poecilia reticulata*. Chemosphere 49, 39–44.

Sfara, V., Zeebe, N., Alzogaray, R.A., 2009. Fumigant insecticidal activity and repellent effect of five essential oils and seven monoterperpenes on first instar nymphs of *Rhodnius prolixys*. J. Med. Entomol. 46, 511–515.

Singh, R., Rup, P.J., Koul, O., 2008. Bioefficacy of 1,8-cineole from *Eucalyptus camaldulensis* var. *obtuse* and linalool from *Luvanga scandans* against *Spodoptera litura* (Lepidoptera: Noctuidae) and combination effects with some other monoterpenoids. Biopest. Int. 4, 128–137.

Tripathi, A.K., Prajapati, V., Kumar, S., 2003. Bioactivity of l-carvone, d-carvone and dihydrocarvone towards three stored product beetles. J. Econ. Entomol. 96, 1594–1601.

Viran, R., Unlü Erkoç, F., Polat, H., Koçak, O., 2003. Investigation of acute toxicity of deltamethrin on guppies (*Poecilia reticulata*). Ecotoxicol. Environ. Saf. 55, 82–85.

Visetson, S., Milne, J., Milne, M., Bullangpoti, V., Rattanapan, A., 2005. Similarity and differences in toxicity and characteristic of monooxygenase activity in the diamond backmoth larvae and subterranean termite and mouse against some allelochemicals and conventional pesticides. In: The Seventh Nation Plant Protection Conference, Thailand.

Widianarko, B., Van Gestel, C.A.M., Verweij, R.A., Van Straalen, N.M., 2000. Associations between trace metals in sediment, water, and guppy, *Poecilia reticulata* (Peters), from urban streams of Semarang, Indonesia. Ecotoxicol. Environ. Saf. 46, 101–107.

Yilmaz, M., Gul, A., Erbasli, K., 2004. Acute toxicity of alpha-cypermethrin to guppy (*Poecilia reticulata*, Pallas, 1859). Chemosphere 56, 381–385.

Yotavongse, P., Boonsoong, B., Pluempanupat, W., Koul, O., Bullangpoti, V., 2015. Effects of the botanical insecticide thymol on biology of a braconid, *Cotesia plutellae* (Kurdjumov), parasitizing the diamondback moth, *Plutella xylostella* L. Int. J. Pest Manag. 61, 171–178.

Zhang, L., Geohagen, B.C., Gavin, T., LoPachin, R.M., 2016. Joint toxic effects of the type-2 alkene electrophiles. Chem. Biol. Interact. 254, 198–220.