Agr. Nat. Resour. 55 (2021) 7–14

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Efficiency of monoterpene compounds for control of rice pest *Pomacea canaliculata*

Cheewarat Printrakoon†, Vasakorn Bullangpoti*,†

Department of Zoology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand

Article Info

Article history:
Received 1 October 2019
Revised 16 June 2020
Accepted 14 July 2020
Available online 31 January 2021

Keywords:
Detoxification enzyme,
Golden apple snail,
Plant essential oil,
Plant molluscicides,
Pomacea canaliculata

Abstract

Pomacea canaliculata (golden apple snail) is one of the most destructive and invasive rice pests in Southeast Asia and Thailand. This work focused on the molluscicidal efficiency of thymol, eucalyptol, and linalool against *P. canaliculata* under laboratory conditions. All essential oils made the snail motionless, closed its operculum shell, turned the soft body at the spire reddish and typically caused death within 24 hr. The toxicity levels of *P. canaliculata* after being exposed to the three essential oil compounds were significantly ($p < 0.05$) different at both 24 hr and 48 hr, with thymol being the most effective control (dose required for 50% mortality after 24 hr = 20.7 mg/L). Snails treated with thymol seemed to relax before death, with some typically recovering within 48 hr. The percentage of egg hatching in the treatment group (28.89–48.84%) was significantly ($p < 0.005$) reduced compared to the control (87-88%), with thymol being the most effective inhibitor (percentage of egg hatching = 28.89%). Glutathione-*s*-transferase (GST) and acetylcholinesterase (AChE) activities were reduced after snails were exposed to all three compounds ($p < 0.05$; correlation factor approximately1.25–1.50-fold and 1.64–2.14-fold for GST and AChE, respectively). However, carboxylesterase reduced only linalool and thymol. The findings indicated that all three essential oil compounds were viable alternatives to chemicals for controlling *P. canaliculata*.

Introduction

The introduced golden apple snail, *Pomacea canaliculata* (Lamarck, 1822) has been found naturally in Thailand since 1984 (Keawjam and Upatham, 1990) and its spread has affected the freshwater ecology of Thailand. Rice is an essential crop grown worldwide and is one of the most important export products of Thailand (Joshi, 2007). The golden apple snail has become one of the most serious rice pests in Thailand, as it has in other Asian countries (Chang, 1985; Rejesus et al., 1990; Mochida, 1991 Anderson, 1993; Naylor 1996; Hayes et al., 2008). Because of their numerous eggs per cluster, high hatching success rate, and more frequent reproduction over their lifetime, golden apple snail populations are large and widespread in agriculture settings (Teo, 2004; Horn et al., 2008). Golden apple snails are highly invasive and difficult to eliminate or control, and they pose a threat to young seedlings of both transplanted and direct-seeded rice (Naylor, 1996).

Infestations by golden apple snails are responsible for an annual economic loss of approximately USD 1.47 billion in Southeast Asian rice production (Joshi, 2007). In Thailand, 141–257 ha have been seriously affected by this snail since 2001 (Joshi, 2007). According to Brito and Joshi (2016), seedlings aged more than more 30 d are more tolerant to snail damage than younger seedlings. These authors suggested that the most effective method of snail control relates to crop establishment;

† Equal contribution.
* Corresponding author.
E-mail address: fscivkb@ku.ac.th (V. Bullangpoti)

https://doi.org/10.34044/j.anres.2021.55.1.02

transplanted 20-day-old seedlings are better able to withstand snail damage than 13-day-old transplanted seedlings or direct-seeded rice.

Past studies have reviewed efforts to control the golden apple snail in rice paddies of Asia, including biological controls such as predator animals (Teo, 2001; Yusa et al., 2006; Dreon et al., 2014), but these efforts have proven to be of limited effectiveness in the field. Some farmers have attempted to remove or destroy the golden apple snail with synthetic molluscicides, especially niclosamide and metaldehyde (Coloso et al., 1998; Liu et al., 2006; Schnorbach et al., 2006), an approach that has been favored due to its immediate results (Cheng and Kao, 2006; Joshi, 2007; Joshi et al., 2008). However, these residues can persist in fields, causing health and environmental problems (Coloso et al., 1998; Liu et al., 2006; Schnorbach et al., 2006 Cheng and Kao, 2006; Joshi, 2007; Joshi et al., 2008).

Research efforts have focused on plant essential oil compounds for pest control. For example, eugenol (Gomes et al., 2011; Padori et al., 2012) and linalool (Heldwein et al., 2012; Fujiwara et al., 2017) are effective at anesthetizing some aquatic organisms.

Monoterpenes are secondary metabolites found in the plant kingdom and are the primary contributors to the organoleptic properties associated with various herbs, spices, citrus, conifers and most flowers and fruits (Davis, 2010). These 10-carbon, short-chain terpenes, often in combination with sesquiterpenes (C15) and diterpenes (C20), exhibit broad-spectrum antimicrobial, allelopathic, herbivore deterrent, and pollinator-attractant properties (Davis, 2010).

The current research investigated three monoterpene compounds—linalool, eucalyptol and thymol—which all have been reported to have insecticidal properties (Hummelbrunner and Isman 2001; Kumrungsee et al., 2014; Pavela 2014; Yotavong et al., 2015). All three compounds are phenolic monoterpenes found as a major component in many essential oil-bearing plants such as *Ocimum basilicum*, *Alpinia galanga* and *Thymus vulgaris*, respectively (Ruttanaphan, 2018; Tharamak et al., 2020). All are generally recognized as safe (GRAS) by the FDA (Ruttanaphan, 2018; Tharamak et al., 2020). Although there many researchers have reported insecticide efficiency (Hummelbrunner et al., 2001; Kumrungsee et al., 2014; Pavela 2014), there have been few studies on using plant essential oil compounds for the control of golden apple snails.

The present study investigated the control of the golden apple snail using integrated pest management strategies involving essential oils (Eos). These substances promise to create fewer environmental problems because they are more quickly degradable than synthetic pesticides (Hummelbrunner and Isman 2001; Kumrungsee et al., 2014; Pavela 2014; Yotavong et al., 2015). Thus, this study aimed to evaluate the molluscicidal effects of linalool, eucalyptol and thymol on the juvenile and egg stages of *P. canaliculata*. In addition, *P. canaliculata* detoxification enzyme activities were analyzed.

Materials and Methods

Collection of P. canaliculata

From a pond located on the Kasetsart University Bangkhen campus in Bangkok, Thailand, 50 mating pairs of male and female *P. canaliculata* were collected in June–August 2018. The mollusks were placed in a box (30 cm × 30 cm × 20 cm) containing dechlorinated tap water and were treated to mimicked natural pond conditions (temperature, 29.50 ± 0.02°C; relative humidity 75%; pH 6.87 ± 0.05; dissolved oxygen, 5.44 ± 0.14%; and total dissolved solids, 0.41 ± 0.01 g/L). Prepared rocks, dry grass and a tree branch were included to mimic the natural environment. The snails were left to mate. After the female snails had laid eggs above water, the egg masses (4–5 cm diameter) were collected 2 d after laying. Each egg mass contained approximately 400–1,000 eggs. Twenty egg masses were placed on netting (20 cm×25 cm) in the laboratory for the molluscicidal test of essential oils. Thirty egg masses from the same egg cluster were used in the hatching test.

Essential oil compounds

Linalool, eucalyptol and thymol were tested in these studies. All compounds were obtained commercially (97–99% purity) from Sigma-Aldrich (Singapore) and were evaluated individually to determine their efficacy levels against the juvenile stage and eggs of *P. canaliculata*. The compounds were dissolved in acetone (AR grade) for use in the molluscicidal efficiency tests.

Evaluation of the molluscicidal efficiency of essential oil compounds

The evaluation of the molluscicidal activity of essential oils against the juvenile snails of *P. canaliculata* was performed as per the World Health Organization guidelines (World Health Organization, 1965). Different concentrations of individual compounds were prepared to test against the snails. A stock solution of each essential oil compound was prepared by dissolving it in acetone at 30,000 mg/L. From this stock solution, the various concentrations were prepared by dilution with acetone in the range 0–20,000 mg/L.

The deep pink-red eggs of *P. canaliculata* became whitish-pink before hatching, which was used to time the essential oil treatments. Individual juvenile snails on the 1st day after hatching from the egg cluster were placed in plastic trays with 1,000 mL of different concentrations of each essential oil. Five different concentrations of each test solution of the compounds were tested, each with three replicates of 30 snails. Three replicates with 10 mg/L acetone were used as negative controls. The control experiments were performed using dechlorinated tap water.

All molluscicidal evaluations were carried out on the same day at room temperature (25 ± 1°C) under normal diurnal lighting. Snails exposed to different concentrations of the essential oil compounds were left for median lethal mortality (LC_{50}) observation for 24 hr. and 48 hr. The snails were not fed during the exposure and recovery periods. Upon observation, the dead snails were removed from the containers. The mortality of snails was calculated based on probit analysis using the StatPlus Program (Version for Mac; Analysesoft; Canada). The juvenile apple snails of the control group and treated groups at 24 hr. and 48 hr. were evaluated by comparing photos (taken with a Stereomicroscope, Model Nikon SMZ 800N NIS element D4.13.0064 bit).

Efficiency of essential oils: Egg hatching rate and hatching time

The different concentrations of individual compounds were prepared to test against the eggs of snails. A stock solution of individual essential oil compounds was prepared by dissolving in acetone, with concentrations in the range 0–20,000 mg/L.

Five different concentrations of 10 mL of each compound were tested by spray (Potter Spray Tower Model BURKARD) on the eggs with three replicates. Acetone was used as a negative control. Control experiments were performed using dechlorinated tap water alone. All experiments were carried out at room temperature (25 ± 1°C) under normal diurnal lighting. Snail eggs exposed to different concentrations of the essential oil compounds were counted to determine the egg hatching percentage and for hatching period observation.

Morphological analysis

After being treated with thymol, the juvenile apple snails were tested at 24 hr. and 48 hr.; some had died, and some recovered within 48 hr. The control group and treated groups were evaluated by comparing photos (taken using a Nikon stereomicroscope, Model SMZ 800N NIS element D4.13.0064 bit).

Mode of action study

The extraction method was modified from Phankaen et al. (2017). *P. canaliculata* surviving beyond 24 hr of treatment were dissected to inspect the liver which was then ground in a homogenized buffer and centrifuged at 4°C and 10,000×g for 10 min. The supernatant was transferred to a new tube for carboxylesterase (CE) and glutathione-*s*-transferase (GST) and acetylcholinesterase (AChE) analyses.

The CE activity was analyzed using *p*-nitrophenyl acetate (*p*NPA) assay modified from Phankaen et al. (2017) with measurements taken using a microplate reader (Biotek PowerWave XS microplate spectrophotometer) with the kinetic mode at λ_{max} = 410 nm at 37°C. The extinction coefficient for *p*NPA is 176.4705. Three biological replicates per treatment were tested.

The method for determining GST activity was that of Oppenoorth (1979) using 150 mM 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. The optical density was recorded using the microplate reader at intervals of 30 s for 3 min at 37°C and 340 nm. The extinction coefficient is 0.0096 for CDNB. Three biological replicates per treatment were tested.

The AChE activity analysis method was modified from Ellman (1961) by measuring at λ_{max} = 412 nm in the kinetic mode. The enzyme activity was detected using the microplate reader. Three biological replicates per treatment were tested.

The data obtained in the evaluation of the molluscicidal efficiency of varied concentrations of the three essential oils were used to calculate the mortality rate (mean ± SD) among juvenile snails. The LC_{50} values at 24 hr. and 48 hr. were analyzed using the Stat Plus for Mac program (AnalystSoft Inc.).

Statistical analysis

Data of LC_{50} values and percentage hatching and number and date of hatching of the varied concentrations of essential oils were analyzed using analysis of variance in the SPSS Statistical Software version 16.0. (SPPS Inc.). Duncan's new multiple range test ($p < 0.05$) was used to compare means, with the mean LC_{50} of each oil compound compared between 24 hr and 48 hr using the Student's t–test. A statistical comparison of all enzyme activity was performed using Duncan's new multiple range test and Tukey's test in the Stat Plus for Mac program (AnalystSoft Inc.). The protein content of each fraction used as an enzyme source was determined using the Bradford method (Bradford, 1976) before measuring the enzyme activity for the total analyzed protein.

Results and Discussion

Molluscicidal activity of individual essential oil compounds in juvenile stage of P. canaliculata

The molluscicidal activities of each essential oil at different concentrations against *P. canaliculata* were evaluated. Neither death nor behavioral symptoms were noted in the control groups treated with water (Fig. 1A) or acetone (Fig. 1B), whereas the treated snails were motionless, the operculum shell was tightly closed, the soft body at the spire was reddish and snails typically died within 24 hr (Fig. 1C). After 24 hr, altered loss body pigment was observed and the pigment mostly disappeared within 48 hr after treatment (Fig. 1D). This indicated that the essential oils were responsible for the altered behavior and mortality of the snails.

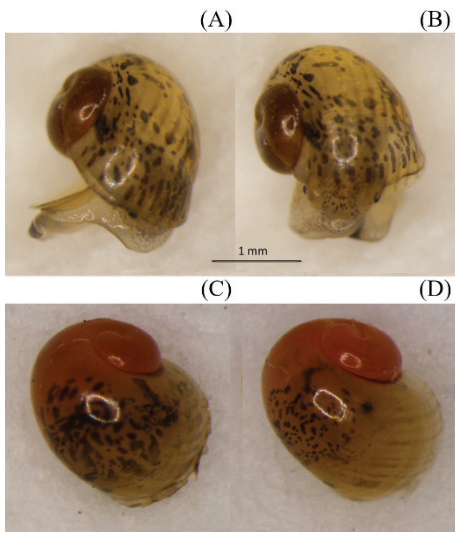

Fig. 1 Observation of hatched juvenile of *Pomacea analiculata* in different treated conditions: (A) active movement by foot and head extend in control with water and (B) motionless in control with acetone. Body pigments (black spot) of the dead snail mantle was decrease following exposure to essential oil compounds after (C) 24 hr and (D) after 48 hr.

An analysis of the molluscicidal activities of the three essential oil compounds on *P. canaliculata* showed that the mortality was dose-dependent. Thymol had the highest control efficiency, with LC_{50} values of 20.70 mg/L and 19.96 mg/L at 24 hr and 48 hr, respectively. Moderate snail control efficiency was observed using linalool, with LC_{50} values of 982.43 mg/L and 841.97 mg/L at 24 hr and 48 hr, respectively. In contrast, eucalyptol had the lowest control efficiency, with LC_{50} values of 3,618.19 mg/L and 3,469.38 mg/L at 24 hr and 48 hr, respectively. The toxicity levels of *P. canaliculata* after being exposed to the three essential oil compounds were significantly ($p < 0.05$; $p = 0.00$) different at both 24 hr and 48 hr, respectively. Following the same trend, higher efficiency of linalool and eucalyptol increased after longer exposure for 48 hr but there was no significant difference between the 24 hr and 48 hr exposure (Table 1). In addition, snails treated with thymol seemed to relax before death; some recovered within 48 hr and so they were not counted in the mortality analysis at 24 hr.

Evaluation of efficiency from essential oil compounds on egg hatching

The highest percentage of egg hatching was reported for undisturbed essential oil conditions. The maximum hatching was approximately 88% in the control (water) and 87% in the acetone treatment. All behavioral parameters and percentages of egg hatching were highly significantly ($p < 0.005$) different for the essential oil treatments compared to the water and acetone controls. Following the same trend, an increased concentration of essential oil decreased the egg hatching percentage. In the first group at the lowest concentration (2,500 mg/L) of three essential oils at 24 hr, the percentage of egg hatching significantly decreased, with thymol having the greatest decrease (70%), whereas at the 5,000 mg/L and 10,000 mg/L concentrations, there were no clear patterns (Fig. 2). At 20,000 mg/L, all three oil compounds were effective inhibitors of egg hatching; thymol was the most effective, with only 28.99% of eggs hatching under this condition, following by linalool (32.29%) and eucalyptol (48.84%). However, at the highest concentrations, there were no significant ($p = 0.12$) differences in the egg snail hatching rates among the three essential oils (Fig. 2).

In the untreated and essential oil conditions, the juvenile snails had completely hatched from the egg masses after averages of 11.63 ± 1.44 d and 11.88 ± 1.32 d, respectively from the natural control and acetone control. The efficiency of linalool and thymol on egg hatching followed the same pattern with more rapid hatching with an increased concentration of these essential oils (Fig. 3). The minimum days of hatching were significant ($p = 0.001$; $p = 0.008$) following treatment with the maximum concentration of 20,000 mg/L for 24 hr with thymol and linalool having means of 6.00 ± 2.16 day and 7.58 ± 2.07 day, respectively. However, there was no clear effect of eucalyptol on the hatching period, with this essential oils increasing the hatching period to its maximum at a concentration at 10,000 mg/L after an average of 16.66 ± 1.28 d that was longer than for the control conditions. However, eucalyptol had the lowest number of hatching days, with a mean 9.25 ± 1.33 d and this was significantly ($p = 0.011$) different from the others at a concentration of 2,500 mg/L for 24 hr.

Mode of action

The GST activity of golden apple snails was reduced by all compounds ($p < 0.05$, Tukey's test). The correlation factor suggested a 1.25–1.50-fold decrease relative to the control groups. However, CE reduced only linalool and thymol. AChE was also reduced for all experiments, with a correlation factor of 1.64–2.14-fold. This mode of action would account for the mortality, which resulted from reduced neuro-enzyme levels and caused inhibition of both detoxification enzymes (Table 2).

Pomacea canaliculata can cause losses in agriculture products (Chang, 1985; Rejesus et al., 1990; Mochida, 1991; Anderson, 1993; Naylor, 1996; Sanico et al., 2002; Joshi, 2007), potentially causing economic disaster (Horgan et al., 2014). There have been many attempts to control the golden apple snail population, including biological control (Teo, 2001; Yusa et al., 2006; Dreon et al., 2014), but molluscicides have been favored due to the immediate results (Cheng and Kao, 2006; Joshi, 2007; Joshi et al., 2008). Essential oils are a viable option for molluscicide control. Although all three of the tested essential oil compounds cause mortality within 24 hr,

Table 1 The median lethal concentration (LC_{50}) [1] values of *Pomacea canaliculata* after exposure to different concentrations of each essential oil compounds for 24 hr and 48 hr

Compound	Exposure for 24 hr		Exposure for 48 hr		
	Slope[2] (SE)	LC_{50} (fiducial limits)	Slope (SE)	LC_{50} (fiducial limits)	t test (*p*-value)
Thymol	31.59	20.70	19.67	19.96	0.003
	(9.11)	(18.31–23.08)[b]	(3.01)	(18.93–20.57)[b]	
Linalool	6.37	982.43	5.36	841.97	0.314
	(0.73)	(895.94–064.35)[c]	(0.61)	(752.50–924.47)[c]	
Eucalyptol	15.73	3,618.19	15.14	3,469.38	0.092
	(1.5)	(3517.74–3718.99)[d]	(1.42)	(3369.01–3569.61)[d]	
p-value		0.00		0.00	

[1] LC_{50} values in a column with different lowercase superscripts are significantly ($p < 0.05$) different. Slope values is come from a probit equation of compound concentrations on death of *Pomacea canaliculata* exposed for 24 hr and 48 hr. Fiducial limts is underlying distribution on logistic growth of probit equation for mortality values analysis

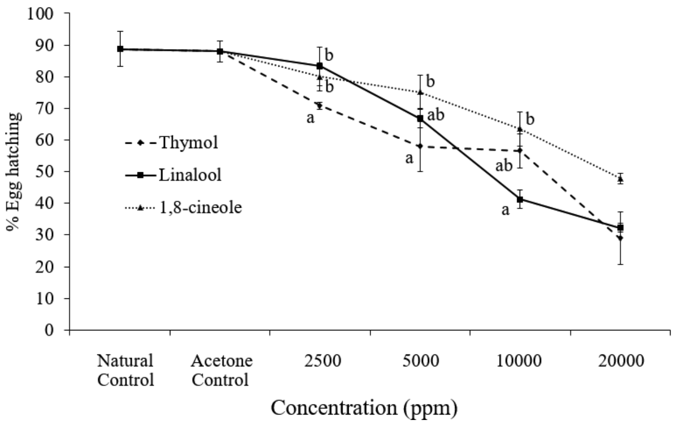

Fig. 2 Mean percentage ± SD of egg hatching rates of *Pomacea canaliculata* after exposure to controls and four different levels of three types of essential oil compounds at 24 hr. after treatment, where the same letters at each concentration indicate not significantly ($p > 0.05$) different among chemicals and ppm = parts per million.

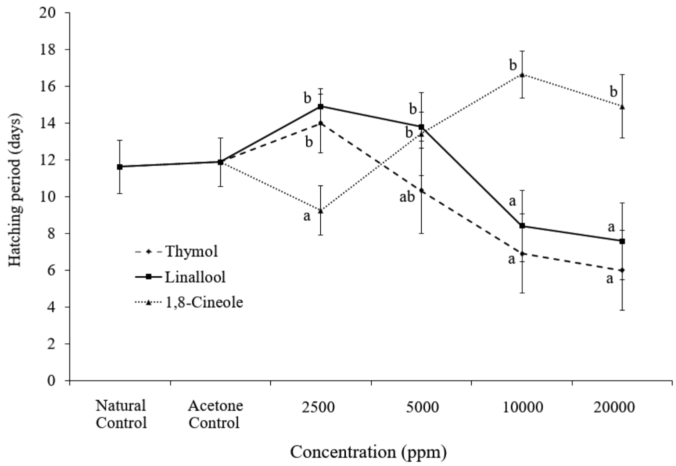

Fig. 3 Mean percentage ± SD of egg hatching period of *Pomacea canaliculata* after exposure to controls and four different levels of three types of essential oil compounds at 24 hr after treatment, where the same letters at each concentration indicate not significantly ($p > 0.05$) different among chemicals and ppm = parts per million.

Table 2 Acetylcholinesterase (AChE, nM Acetylcholinesterase/min/mg protein), detoxification enzymes (CE = carboxylesterase, nM p-nitrophenol/min/mg protein; GST = glutathione-*s*-transferase, glutathione conjugated product/ min/ mg protein) activities on survival of *Pomacea canaliculata* after treatment with essential oil compounds for 24 hr

Treatment	AChE	CE	GST
Control	1.028 ± 0.05^{a}	190.76 ± 2.15^{a}	4.49 ± 0.54^{a}
Linalool	0.479 ± 0.01^{b}	120.65 ± 4.09^{b}	3.17 ± 0.53^{b}
Eucalyptol	0.483 ± 0.01^{b}	192.53 ± 4.63^{a}	2.99 ± 0.43^{c}
Thymol	0.627 ± 0.04^{c}	136.94 ± 9.57^{b}	3.58 ± 0.24^{d}

Values in the same column with different lowercase superscripts are significantly ($p < 0.05$) different using Tukey's test.
Control = treatment tested with acetone only; NF = no effect

thymol was most effective against snail larvae, which was consistent with reports in the literature (Franzios et al., 1997; Kumrungsee et al., 2014; Lee et al., 1997; Hummelbrunner and Isman, 2001). Based on a neurotoxic mode of action, some researchers noted that thymol in particular, was one of the most toxic essential oil compounds to insect larvae (Enan, 2001; Isman and Machial, 2006). The relaxation and recovery effects of thymol on juvenile apple snails before death seemed similar to the effects reported for eugenol and essential oils of leaves from *O. Majorana* and *O. americanum* on juvenile apple snails from Brazil (Bianchini et al., 2017). Bianchini et al. (2017) suggest that eugenol and the essential oil of *O. americanum* and *O. majorana* promoted muscle relaxation in *P. canaliculata*.

In addition, high concentrations of the three essential oils resulted in a decrease in golden apple snail egg hatching. In particular, the highest concentration in the laboratory (20,000 ppm treatment level) of all three oil compounds was the most effective, resulting in less than 50% of the eggs hatching. The highest tested concentration of thymol was the most effective treatment, resulting in an egg hatching rate of only 28.99%. Thymol at the lowest tested concentrations (2,500 mg/L) caused the egg hatching percentage to decrease by around 18%. After the linalool and eucalyptol treatments, the egg hatching percentages followed the same trend, and were significantly different from the control condition. (Singh et al., 2010) explained that the eggshell of *P. canaliculata* is mainly composed of calcium carbonate and attached organic matter. (Bianchini et al., 2017) demonstrated that many essential oils directly damage the *P. canaliculata* shell. In the current study, the decreased egg hatching due to the essential oils might be explained by these chemical substances suppressing the surface layer of the egg capsule by covering the surface (Wu et al., 2005) and possibly dissolving the organic matter on the eggshell that subsequently affected the embryo snail inside. Some authors (Turner, 1998; Pizani et al., 2005) have suggested that hatching failure can be caused by reduced oxygen availability to developing embryos, and essential oils that cover the surface layer of eggs are likely to prevent oxygen uptake. It was likely that a thin covering of oil evaporated; consequently, the percentage egg hatching of golden apple snails remained high when treated with low concentrations of essential oils. In terms of time to hatch, thymol and linalool decreased the egg hatching period of *P. canaliculata* from 11 d completed hatching by the control to one-half that period after both treatments. This was contrasted by eucalyptol that produced fluctuating results after treatment with no clear pattern of egg hatching time. The inhibitory effect of thymol and linalool on decreasing the period of hatching was not examined further.

Molluscicide evaluation to control golden apple snail had been of interest for a decade but with almost no effective success (Mochida, 1991) and some compounds tested were not safe for environmental and animal health (Anderson, 1993; Naylor, 1996; Cheng and Kao, 2006). The current study suggested that essential oils (such as thymol from Thai botanical extract) can be used for the management of larvae and eggs of golden apple snails in rice fields. Oil (thymol) from the herb thyme in the family Lamiaceae is among the best-known essential oils with bioactivity against insects and other pests; (Isman and Machial, 2006) reported that the thymol functional groups include alcohols and these compounds are usually responsible for the characteristic aromatic odors or flavors or both of the extracted plants. In addition, *Pomacea canaliculata* lays numerous eggs per egg mass (Teo, 2004, Horn et al., 2008). Thus, as shown in the current study, snail destruction in the egg stage before hatching or prolonging the time to hatch to allow extended physical destruction of the vulnerable egg masses could be an effective and practical method to decrease snail populations.

Animals use detoxification enzymes, including glutathione-*s*-transferase or carboxylesterases, to metabolize secondary plant metabolites and other toxins (Ramsey et al., 2010). The induction of detoxification enzymes by xenobiotic compounds is one mechanism by which pests can develop resistance to pesticides (Yu and Hsu, 1993, Li et al., 2007). The high toxicity of the pesticide results mostly from its high retention in the pests. The inhibitory effect can occur by competition between two or more compounds for the same detoxifying enzyme. Some compounds selectively inhibit only one detoxifying activity. For example, cimetidine can bind directly to the heme-iron of the cytochrome P450 reactive site to inhibit all cytochrome-dependent Phase I enzyme activities (Bailey et al., 1998). Furthermore, grapefruit juice, which contains high amounts of the flavonoid naringenin, inhibits the first-pass metabolism of many drugs that are detoxified through the Cyp3A4 enzyme-antiporter system in the human intestine (Bailey et al., 1998).

All tested essential oils in the current study reduced detoxification enzyme activity, suggesting that the mortality associated with GAS after exposure to essential oils was a result of a reduction in detoxification enzyme activity.

The mortality may result from reduced acetylcholinesterase activities, causing paralysis and then knockdown. The current findings were consistent with those of previous studies reporting that natural plant products inhibit detoxification enzymes, such as extracts of *Melia amaranthus* and Derris in *Spodoptera exigua* (Rachokarn et al., 2008; Ruttanaphan, 2018), extracts of *Melia toosendan* Sieb. Et Zucc. Pron. in *S. litura* (F.) and *Melanoplus sanguinipes* (F.) (Feng et al., 1995) and extracts of *Alpinia galanga in Bactrocera dorsdalis* (Sukhirun et al., 2011).

In conclusion, the current study showed that essential oils are useful as botanical pesticides for controlling GAS, though the activity of the essential oils mostly decreased over time because of their high volatility. The persistence of the insecticidal activity of the essential oils was related to their chemical composition and the sensitivity of the target pest to the active compounds in the essential oils (Obeng-Ofori et al., 1997; Ngamo et al., 2007). Monoterpenes are the most active compounds that sustain essential oil insecticidal efficiency (Ngamo et al., 2007). Oils with a high content of hydrogenated compounds lost their activity more rapidly than those containing mainly oxygenated compounds (Huang and Ho, 1998). The speed of the oxidation of hydrogenated monoterpenes is greater for compounds such as sabinene and eucalyptol (Kim et al., 2003). This oxidation leads to the reduction of the insecticidal efficiency of the oil. Thus, before recommending use in the field, the persistence of the three compounds in the current research need to be investigated.

Acknowledgements

The authors thank the Department of Zoology, Faculty of Science, Kasetsart University, Bangkok, Thailand for research funding. International SciKu Branding (ISB) funding was received from the Faculty of Science, Kasetsart University.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

References

Anderson, B. 1993. The Philippines snail disaster. Ecologist 23: 70–72.

Bailey, D.G., Malcolm, J., Arnold, O., Spence, J.D. 1998. Grapefruit juice-drug interactions. Br. J. Clin. Pharmacol. 46: 101–110. doi.org/10.1046/j.1365-2125.1998.00764.x

Bianchini, A.E., Cunha, J.A., Brusque, I.C.M., Pinheiro, C.G., Schindler, B., Heinzmann, B.M., Baldisserotto, B. 2017. Relaxing effect of eugenol and essential oils in *Pomacea canaliculata*. Cienc. Rural. 47: e20170210. doi.org/10.1590/0103-8478cr20170210

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248–254. doi.org/10.1016/0003-2697(76)90527-3

Brito, F.C., Joshi, R.C. 2016. The golden apple snail *Pomacea canaliculata*: A review on invasion, dispersion and control. Outlooks Pest Manag. 27: 157–163. doi.org/10.1564/v27_aug_03

Chang, W. 1985. The ecological studies on the *Ampullaria* snail (Cyclophoracea: Ampullaridae). Bulletin of Malacology, Republic of China 11: 43–51.

Cheng, E.Y., Kao, C.H. 2006. Control of golden apple snail, *Pomacea canaliculata* (Lamarck), in Taiwan.. In: Joshi R.C., Sebastian, L.S. (Eds.). Global Advances in Ecology and Management of Golden Apple Snails. Philippine Rice Research Institute. Los Baños, the Philippines, pp. 155–167.

Coloso, R.M., Borlongan, I.G., Blum, R.A. 1998. Use of metaldehyde as a molluscicide in semi- commercial and commercial milk fish ponds. Crop Protect. 17: 669–674.

Dreon, M.S., Fernández, P.E., Gimeno, E.J., Heras, H. 2014. Insights into embryo defenses of the invasive apple snail *Pomacea canaliculata*: Egg mass ingestion affects rat intestine morphology and growth. PLoS Negl. Trop. Dis. 8: e2961. doi.org/10.1371/journal.pntd.0002961

Davis, E.M. 2010. Advances in the enzymology of monoterpene cyclization reactions. In: Liu H.W., Mander, L. (Eds.), Comprehensive Natural Products II. Chemistry and Biology. Elsevier Science. Oxford, UK, pp. 585–608. doi.org/10.1016/B978-008045382-8.00004-6

Ellman, G.L., Courtney, K.D., Andres, V., Featherstone, R.M. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7: 88–95. doi.org/10.1016/0006-2952(61)90145-9

Enan, E. 2001. Insecticidal activity of essential oils: Octopaminergic sites of action. Comp. Biochem. Phys. C. 130: 325–337. doi.org/10.1016/S1532-0456(01)00255-1

Feng, R., Chen, W., Isman, M.B. 1995. Synergism of malathion and inhibition of midgut esterase activities by an extract from *Melia toosendan* (Meliaceae). Pestic. Biochem. Phys. 53: 34–41. doi.org/10.1006/pest.1995.1052

Fujiwara, G.M., Annies, V., Oliviera, C.F., et al. 2017. Evaluation of larvicidal activity and ecotoxicity of linalool, methyl cinnamate and methyl cinnamate/linalool in combination against *Aedes aegypti*. Ecotoxicol. Environ. Saf. 139: 238–244. doi.org/10.1016/j.ecoenv.2017.01.046

Franzios, G., Mirotsou, M., Hatziapostolou, E., Kral, J., Scouras, Z.G., Mavragani-Tsipidou, P. 1997. Insecticidal and genotoxic activities of mint essential oils. J. Agric. Food Chem. 45: 2690–2694. doi.org/10.1021/jf960685f

Gomes, D.P., Chaves, B.W., Becker, A.G., Baldisserotto, B. 2011.Water parameters affect anesthesia induced by eugenol in silver catfish, *Rhamdia quelen*. Aquac. Res. 42: 878–886. doi.org/10.1111/j.1365-2109.2011.02864.x

Hayes, K.A., Joshi, R.C., Thiengo, S.C., Cowie, R.H. 2008. Out of South America: Multiple origins of non-native apple snails in Asia. Divers. Distrib. 14:701–712. doi.org/10.1111/j.1472-4642.2008.00483.x

Huang, Y., Ho, S.H. 1998.Toxicity and antifeedant activities of cinnamaldhyde against grain storage insects, *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. J. Stored Prod. Res. 34: 11–17. doi.org/10.1016/S0022-474X(97)00038-6

Heldwein, C.G., Silva, L.L., Reckziegel, P., Barros, et al. 2012. Participation of the GABAergic system in the anesthetic effect of *Lippia alba* (Mill.) N.E. Brown essential oil. Brazilian J. Med. Biol. Res. 45: 436-443

Horgan, F.G., Stuart, A.M., Kudavidanage, E.P. 2014. Impact of invasive apple snails on the functioning and services of natural and managed wetlands. Acta Oecol. 54: 90–100. doi.org/10.1016/j.actao.2012.10.002

Horn, K.C., Johnson, S.D., Boles, K.M., Moore, A., Siemann, E., Gabler, C.A. 2008. Factors affecting hatching success of golden apple snail eggs: Effects of water immersion and cannibalism. Wetlands 28: 544–549. doi.org/10.1672/07-11.1

Hummelbrunner, L.A., Isman, M.B. 2001. Acute, sublethal, antifeedant and synergistic effects of monoterpenoid essential oil compounds on the tobacco cutworm, *Spodoptera litura* (Lep., Noctuidae). J. Agric. Food Chem. 49: 715–720. doi.org/10.1021/jf000749t

Isman, M.B., Machial, C.M. 2006. Pesticides based on plant essential oils from traditional practice to commercialization. In: Rai, M., Carpinella, M.C. (Eds.). Naturally Occurring Bioactive Compounds. Elsevier BV. Amsterdam, the Netherlands, pp. 29–44.

Joshi, R.C. 2007. Problems with the management of the golden apple snail *Pomacea canaliculata*: An important exotic pest of rice in Asia. In: Vreysen, M.J.B., et al. (Eds.). Area-Wide Control of Insect Pests. Springer. Heidelberg, Germany, pp. 257–264.

Joshi, R.C., Martín, R.S., Saez-Navarrete, C., Alarcon, J., Sainz, J., Antolin, M.M., Martin, A.R., Sebastian, L.S. 2008. Efficacy of quinoa (*Chenopodium quinoa*) saponins against golden apple snail (*Pomacea canaliculata*) in the Philippines under laboratory conditions. Crop Prot. 27: 553–557. doi.org/10.1016/j.cropro.2007.08.010

Keawjam, R.S., Upatham, E.S. 1990. Shell morphology, reproductive anatomy and genetic patterns of three species of apple snails of the genus *Pomacea* in Thailand. J. Med. Appl. Malacol. 2: 45–57.

Kim, S.I., Roh, J.Y., Kim, D.H., Lee, H.S., Ahn, Y.J. 2003. Insecticidal activities of aromatic plant extract and essential oils against *Sitophilus oryzae* and *Callosobruchus chinensis*. J. Stored Prod. Res. 39: 293–303. doi.org/10.1016/S0022-474X(02)00017-6

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. doi.org/10.1007/s10340-014-0602-6

Lee, S., Tsao, R., Peterson, C., Coats, J.R. 1997. Insecticidal activity of monoterpenoids to western corn rootworm (Coleoptera: Chrysomelidae), two spotted spider mite (Acari: Tetranychidae), and house fly (Diptera: Muscidae). J. Econ. Entomol. 90: 883–892. doi.org/10.1093/jee/90.4.883

Li, X.C., Schuler, M.A., Berenbaum, M.R. 2007. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. Annu. Rev. Entomol. 52: 231–253. doi.org/10.1146/annurev.ento.51.110104.151104

Liu, W.H., Chio, Y.W., Huang, D.J., Liu, M.Y., Lee, C.C., Liu, L.L. 2006. Imposex in the golden apple snail (*Pomacea canaliculata*) in Taiwan. Sci. Total Environ. 371: 138–143.

Mochida, O. 1991. Spread of freshwater *Pomecea* snails (Pilidae Mollusca) from Argentina to Asia. Micronesica 3: 51–62.

Naylor, R.1996. Invasion in agriculture: Assessing the cost of the golden apple snail in Asia. Ambio. 25: 443–448.

Ngamo, T.S.L., Ngatanko, I., Ngassoun, M.B., Mapongmestsem, P.M., Hance, T. 2007. Persistence of insecticidal activities of crude essential oils of three aromatic plants towards four major stored product insect pests. Afr. J. Agric. Res. 2: 173–177.

Obeng-Ofori, D., Reichmuth, C., Bekele, J., Hassanali, A. 1997. Biological activity of 1,8-cineole, a major component of essential oil of *Ocimum kenyense* (Ayobangira) against stored products beetles. J. Appl. Entomol. 121: 237–243. doi.org/10.1111/j.1439-0418.1997.tb01399.x

Oppenoorth, F.J., Vanderpas, L.J.T., Houx, N.W.H. 1979. Glutathione *s*-transferase and hydrolytic activity in a tetrachlorvinphos-resistant strain of housefly and their influence on resistance. Pest. Biochem. Phys. 11: 176–188. doi.org/10.1016/0048-3575(79)90057-9

Parodi T.V., Cunha M.A., Heldwein C.G., et al. 2012. The anesthetic efficacy of eugenol and the essential oils of *Lippia alba* and *Aloysia triphylla* in post-larvae and sub-adults of *Litopenaeus vannamei* (Crustacea, Penaeidae). Comp. Biochem. Phys. C. Toxicol. Pharmacol. 155: 462–468.

Pavela, R. 2014.Acute, synergistic and antagonistic effects of some aromatic compounds on the *Spodoptera littoralis* Boisd. (Lep., Noctuidae) larvae. Ind. Crop. Prod.60: 247–258. doi.org/10.1016/j.indcrop.2014.06.030

Phankaen, Y., Manaprasertsak, A., Pluempanupat, W., Koul, O., Kainoh, Y., Bullangpoti, V. 2017. Toxicity and repellent action of *Coffea arabica* against *Tribolium castaneum* (Herbst) adults under laboratory conditions. J. Stor. Prod. Res. 71: 112–118.

Pizani, N.V., Estebenet, A.L., Martin, P.R. 2005. Effects of submersion and aerial exposure on clutches and hatchlings of *Pomacea canaliculata* (Gastropoda: Ampullariidae). Am. Malacol. Bull. 20: 55–63.

Rachokarn, S., Piyasaengthong, N., Bullangpoti, V. 2008. Impact of botanical extracts derived from leaf extracts *Melia azedarach* L. (Meliaceae) and *Amaranthus viridis* L. (Amaranthaceae) on populations of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) and detoxification enzyme activities. Commun. Agric. Appl. Biol. Sci. 73: 451–457.

Ramsey, J.S., Rider, D.S., Walsh, T.K., De Vos, M., Gordon, K.H., Ponnala, L., Macmil, S.L., Roe, B.A., Jander, G. 2010. Comparative analysis of detoxification enzymes in *Acyrthosiphon pisum* and *Myzus persicae*. Insect Mol. Biol. 19: 155–164. doi.org/10.1111/j.1365-2583.2009.00973.x

Ruttanaphan, T., Pluempanupat, W., Bullangpoti, V. 2018. Cypermethrin resistance in *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) from three locations in Thailand and detoxification enzyme activities. Agr. Nat. Resour. 52: 484–488. doi.org/10.1016/j.anres.2018.10.014

Rejesus, B.M., Saysboc, A.S., Joshi, R.C. 1990.The distribution and control of the introduced golden apple snail (*Pomacea* sp.) in the Philippines. In: Introduction of Germplasm and Quarantine Procedure. Planti. Kuala Lumpur, Malaysia, pp. 213–224.

Sanico, A.L., Peng, S., Laza, R.C., Visperas, R.M. 2002. Effect of seedling age and seedling number per hill on snail damage in irrigated rice. Crop Prot. 21: 137–143. doi.org/10.1016/S0261-2194(01)00075-8

Schnorbach, H.J., Rauen, H.W., Bieri, M. 2006. Chemical control of the golden apple snail, *Pomacea canaliculata*. In: Joshi, R.C., Sebastian, L.S. (Eds.). Global Advances in Ecology and Management of Golden Apple Snails. Philippine Rice Research Institute. Muñoz, Philippines, pp. 419–438.

Sukhirun, N., Pluempanupat, W., Bullangpoti, V., Koul, O. 2011. Bioefficacy of *Alpinia galanga* (Zingiberaceae) rhizome extracts, (E)-p-acetoxycinnamyl alcohol, and (E)-p- coumaryl alcohol ethyl ether against *Bactrocera dorsalis* (Diptera:Tephritidae) and the impact on detoxification enzyme activities. J. Econ. Entomol. 104: 1534–1540. doi.org/10.1603/EC11080

Singh, S.K., Yadav, R.P., Singh, A. 2010. Molluscicides from some common medicinal plants of eastern Uttar Pradesh, India. J. Appl. Toxicol. 30: 1–7. doi.org/10.1002/jat.1498

Teo, S.S. 2001. Evaluation of different duck varies for the control of the golden apple snail *Pomacea canaliculata* in transplanted and direct seed rice. Crop Prot. 20: 599–604. doi.org/10.1016/S0261-2194(01)00029-1

Teo, S.S. 2004. Biology of the golden apple snail, *Pomacea canaliculata* (Lamarck, 1822), with emphasis on responses to certain environmental conditions in Sabah, Malaysia. Molluscan Res. 24: 139–148.

Tharamak, S., Yooboon, T., Pengsook, A., Ratwatthananon, A., Kumrungsee, N., Bullangpoti, V., Pluempanupat, W. 2020. Synthesis of thymyl esters and their insecticidal activity against *Spodoptera litura* (Lepidopter: Noctuidae). Pest Manag. Sci. 76: 928–935. doi.org/10.1002/ps.5598

Turner, R.L. 1998. Effects of submergence on embryonic survival and developmental rate of the Florida apple snail, *Pomacea paludosa*: Implications for egg predation and marsh management. Florida Scientist 61: 118–129.

World Health Organization. 1965. Snail control in the prevention of bilharziasis. World Health Organization. https://apps.who.int/iris/handle/10665/41772, 14 December 2020.

Wu, D.C., Yu, J.Z., Chen, B.H., Lin, C.Y., Ko, W.H. 2005. Inhibition of egg hatching with apple wax solvent as a novel method for controlling golden apple snail (*Pomacea canaliculata*). Crop Prot. 24: 483–486. doi.org/10.1016/j.cropro.2004.08.010

Yotavong, 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. doi.org/10.1080/09670874.2015.1030001

Yu, S.J., Hsu, E.L. 1993. Induction of detoxification enzymes in phytophagous insects: roles of insecticide synergists larval age, and species. Arch. Insect Biochem. Physiol. 24: 21–32. doi.org/10.1002/arch.940240103

Yusa, Y., Sugiura, N., Wada, T. 2006. Predatory potential of freshwater animals on an invasive agricultural pest, the apple snail *Pomacea canaliculata* (Gastropoda: Ampullariidae), in southern Japan. Biol. Invasions. 8: 137–147. doi.org/10.1007/s10530-004-1790-4