ASM Sc. J., 13, Special Issue 6, 2020 for InCOMR2019, 82-89

Molluscicidal Activity of Fresh Leaves from *Curcuma longa* and *Piper betle* Essential Oil against *Pomacea canaliculata*

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Pomacea canaliculata (GAS) can eliminate the young leaves and stem resulting in the death of damaged rice plants. Chemical pesticide was commonly used by farmers to control *P. canaliculata*. The research on natural source pesticide was important to reduce dependency on chemical pesticide due to hazard effect on environment and human. *Curcuma longa* and *Piper betle* has been used in controlling agriculture pest which interrupt insect behaviour and growth. However, only a few studies have focused on its potential for molluscicidal activities. This research was done to study the potential of *C. longa* and *P.betle* for molluscicidal activity towards *P. canaluiculata*. The phytochemical analysis was done using Thin Layer Chromatography (TLC) and Gas Chromatography Mass Spectrometry (GCMS) to analyse the active compounds of *C. longa* and *P. betle*. Meanwhile, mortality test was done to test the molluscicidal activity of selected essential oil towards *P.canaliculata*. Based on TLC result, citral and linalool compounds were present from *C. longa,* while eugenol and linalool were identified from *P. betle* essential oil. GCMS analysis revealed the major compound from *C. longa* essential oil was α-phellandrene (14.9%). While, major compound from *P. betle* essential oil was eugenol (15.6 %). For bioassay test, total of 10 treatments with five different concentrations each were carried out for 96 hours. The mortality test done indicated *P. betle* essential oils showed slightly higher *P. canaliculata* mortality (63.33%) compared to *C. longa* essential oil treatment (60%). This study provides an important foundation for future research on the potential value of *C. longa* and *P. betle* essential oil as molluscicides.

Keywords: essential oil; *Pomacea canaliculata;* molluscicidal; *Curcuma longa*; *Piper betle*

I. INTRODUCTION

Rice is the staple food which is mainly produced and consumed in Asian Region (Siwar *et al.*, 2014; Latip *et al.*, 2015) where 90% of crops were grown (GRiSP, 2013). More than 100,000 farmers in Malaysia were relying on rice production and related industry. The sustainable rice production was important for food security in manage destitution (Siwar *et al.*, 2014). Golden apple snail (GAS), *Pomacea canaliculata* was considered as major pest on rice production in Southeast Asia. *P. canaliculata* attack both transplanted seedlings and direct seeded rice (IRRI, 2011) and prefer to eat the base part of young rice seedlings which up to 15 days of transplanting (Zhao *et al.*, 2012; Latip *et al.*, 2016).

Chemical pesticide was commonly used as a control method practised by farmers because of rapid effect and

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effective (Joshi *et al*., 2008; Latip *et al*., 2016). The overuse of this chemical has caused a hazard to human and underground water (Latifah *et al*., 2011). Hence, the biological control through the use of botanical pesticide has become alternatives to reduce the dependency on chemical pesticide for controlling *P. canaliculata* (Latip *et al*., 2016). Botanical pesticide was environmentally friendly as it is naturally biodegradable, thus leaving no toxic residue after application (Raja, 2014). There was numerous potential indigenous plants in Malaysia that can be used as botanical pesticide. Neem, *Azadirachta indica* has been regarded as the most reliable source of eco-friendly botanical pesticide and was on the top of list for 2,400 plant species that were reported that can be used for controlling *P. canaliculata* (Latip *et al*., 2016). Plant secondary metabolites were functioned to protect plants from any harm in ecological environment (Stamp, 2003; Kabera *et al*., 2014). There were many secondary metabolites present in plants such as terpenoids, alkaloids, saponins, phenols, flavonoids, quinines, tannins, sterols and coumarins which involve in plant defences and react differently towards pest species (Raja, 2014). Among of these active compounds, terpene has contributed the biggest part in a repellent activity in plant essential oil compound (Paluch, 2009). The citronella essential oil, *Cymbopogon nardus*, and *Lemon eucalyptus* are the examples of the commercially established terpenes in market (Paluch, 2009).

Curcuma longa has been used in controlling agriculture pest effectively due to the presence of many bioactive compounds, which interrupt insect behaviour and growth. *C. longa* has become a source of the active constituents for insect repellents and insecticidal agents (Damalas, 2011). *Piper betle* or known as betel has been identified as active antiprotozoal and antimalarial agents in many pharmacological studies (Abdullah *et al*., 2011). The fresh *P. betle* leaves resulted in antimicrobial, ringworm, antifungal, antiseptic and anthelminthic affects (Pradhan *et al*., 2014). The *P. betle* essential oil gives a better protection from mosquitoes *Anopheles stephensi* and *Culex fatigans* biting compared to the well-known mosquito repellent of citronella oil (Pal and Chandrashekar, 2010). New discovery in insect mode of action had provided new ideas for development of insect repellent activity which disturb insect behaviour without experience any restrictions of presently available insect repellent (Bohbot *et al*., 2014). In this study, both fresh leaves of *C. longa* and *P. betle* essential oils undergo a phytochemical screening with Thin Layer Chromatography (TLC), and Gas Chromatography Mass Spectrometry (GCMS) analysis to identify active compounds. The mortality test was conducted to evaluate the effectiveness of *C. longa* and *P. betle* essential oils as botanical pesticides for controlling *P. canaliculata*.

II. MATERIALS AND METHODS

A. Golden Apple Snail, *Pomacea canaliculata*

The adults of *P. canaliculata* were collected from rice field at *Tanjong Karang*, Selangor. The size of *P. canaliculata* used was in range of 20-35 mm height.

B. Essential Oil Extraction

The *C. longa* and *P.betle* leaves were sampled from herbs garden at *Uitm Pahang, Jengka* campus. Essential oils were extracted from fresh leaves of *C. longa* and *P. betle*, through the hydrodistillation extraction method by Zaibunnissa *et al.,* (2009) with some modification. Clevenger apparatus were set up for distillation process. The selected plant leaves sample were hydrodistillated for 6 hours until all the oil was extracted into the receiver flask.

C. Thin Layer Chromatography (TLC)

The TLC test was done using the method by Hassan (2009) for terpenoid screening. The standards were purchased for citral, eugenol, thymol, linalool and limonene as the targeted compound from the *C. longa* and *P. betle* essential oil. The mobile phase solvent of hexane and chloroform with the ratio 20:80 was used. The selected standards, *C. longa* and *P. betle* essential oil sample were spotted onto TLC plate and placed into solvent. Developed TLC plates were observed under short wavelength (UV 254 nm) and long wavelength (UV 366 nm). After that, TLC plates were sprayed with vanillin sulfuric acid reagent (5% sulfuric acid in ethanol and 1% vanillin in ethanol) and heated in the oven with 110°C temperature. The Rf value for each detected compound from essential oil were calculated.

D. Gas Chromatography Mass Spectrometry (GCMS)

The identification of active compounds from *C. longa* and *P. betle* essential oil was done with Gas Chromatography Mass Spectrometry (GCMS) analysis. Both essential oil samples were analysed using the method by Zaibunnisa *et al.,* (2009) with some modification. The column type used was HP-5MS capillary column, equipped with mass selective detector with electron impact mode (70eV). The temperature of column was set at 60°C to 325°C with holding time of 10°C/min. Helium gas used as carrier gas with flow rate of 1.0 ml/min in splitless mode. 1µl solution (0.1% essential oil in hexane) injected into GC by auto-sampler. The data from GC analysis compared with NIST library for identification of active compounds.

E. Mortality Test

The mortality test conducted adopted from the method used by Pinto *et al.* (2015) and Kijprayoon *et al.* (2014) with some modification. Five treatments concentration was prepared, which were 0.02, 0.04, 0.06, 0.08 and 0.10g/ml for *P. betle* essential oil while for *C. longa* essential oil were 0.04g/ml 0.044, 0.049, 0.054 and 0.06g/ml with 3 replicates each respectively. The niclosamide and distilled water were used as a control in this experiment. 10 ml of treatment solution was sprayed towards paddy seedlings before it was placed into the aquarium containing *P. canaliculata*. Data for *P. canaliculata* mortality were taken every 24 hours for four days. The Analysis of Variance (ANOVA) test was done for significant differences between means of treatment's concentrations. Meanwhile, probit analysis was done using POLO PLUS software to analyse the *P. canaliculata* mortality.

F. Statistical Analysis

The Analysis of Variance (ANOVA) following by Duncan's multiple tests were done using SAS for multiple comparisons where significant differences between means of treatment's concentrations were studied. The value of p less than 0.05 was considered statistically significant.

III. RESULTS AND DISCUSSION

A. Thin Layer Chromatography

Table 1 shows the TLC test for *C. longa* essential oil. The result revealed the presence of aromatic compound by black spot appearance under 254 nm UV light. The presence of terpenoid compound was identified as the visualized light blue, dark blue and purple colour on TLC plate after sprayed with vanillin/ H_2SO_4 reagent and heated at 110°C. The comparison of Rf value (0.37 and 0.48) from *C. longa* essential oil with the standard showed the presence of linalool (0.38) and citral (0.46). Meanwhile, for *P. betle* essential oil, there were clear blue, dark yellow and grey colours appeared on TLC plate after spraying with vanillin/H_2SO_4 reagent and heated at110°C. The presence of eugenol and linalool were identified by the dark yellow and dark blue colour present on TLC plate with Rf value of 0.48 and 0.39 which near to standard eugenol (0.51) and standard linalool (0.38).

Table 1. The TLC test result for *C. longa* and *P. betle* essential oil compared to selected standard

Essential oil	Rf Sample	Rf Standard	Standard	Color
C. longa	0.37	0.38	Linalool	Blue
	0.48	0.46	Citral	Dark blue
P. betle	0.39	0.38	Linalool	Blue
	0.48	0.51	Eugenol	Dark yellow

B. GCMS Analysis of C. longa and P. betle Essential Oil

Table 2 and Table 3 show the GCMS analysis result for compounds presence from both *C. longa* and *P. betle* extracted essential oil. The GCMS test was conducted to identify the active compounds in both essential oil samples by comparing the results with NIST library. The major active compound present in *C. longa* essential oil tested was α-Phellandrene (14.9%). There was only a small portion of minor active compound present, which were limonen-6-ol (3.2%), pivalate (3.2%), terpinolene (2.5%), turmerone (1%) and both cetene and β-Turmerone with 0.4%. Meanwhile, the active compound of eugenol was present in the *P. betle* essential oil sample which was equivalent to 15.6%. Minor active compounds present in the *P. betle* essential oil sample were chavicol (4.6%), chavicol acetate (4.7%), γ-Muurolene (1.1%) and others with small compositions.

Table 2. Percentage of Compounds from *C. longa* Essential Oil

Essential oil	Compounds	Area (%)
C. longa	α-Phellandrene	14.9
	Terpinolene	2.5
	Limonen-6-ol, pivalate	3.2
	(+)-Sabinol	0.4
	Cetene	0.4
	Turmerone	1
	β-Turmerone	0.4

Table 3. Percentage of Compounds from *P. betle* Essential Oil

Essential oil	Compounds	Area (%)
P. betle	Chavicol	4.6
	Chavicol acetate	4.7
	Eugenol	15.6
	Trans-Isoeugenol	0.2
	Methyleugenol	0.3
	γ-Muurolene	1.1
	Germacrene D	0.9
	Isogermacrene	0.2
	Caryophyllene	0.8
	Humulene	0.3
	α-Cadinol	0.2

C. Bioassay Test

The result from bioassay test conducted with *C. longa* essential oil was presented in Fig. 1. The *C. longa* essential oil treatment of TE5 (0.06g/ml) showed the highest mean percentage of GAS mortality after every 24 hours test was conducted which was 50% after 24 hours, 56.67% after 48 hours, 60% after 72 and 96 hours of exposure. Meanwhile, the treatment TE1 (0.04g/ml) showed the lowest mean percentage GAS mortality (20%) after 24- and 48-hours exposure, 23.33% after 72 hours and 26.67% after 96 hours of treatment exposure. The ANOVA test showed that the value was significantly different between treatments where p value is less than 0.05. Thus, the different treatment concentration gave a different level of mortality towards GAS.

The GCMS results in Table 2 in the analysis of *C. longa* essential oil showed the highest content of active compound α-phellandrene (14.9% area) which could be responsible for GAS mortality. Although the α-phellandrene content in the *C. longa* essential oil tested was small but still it contributed to the insecticidal activity of GAS as minor compounds could develop antagonistic effects towards essential oil activity (Pinto *et al.*, 2015; Botelho *et al.*, 2007). Phellandrene was found from the essential oil of bitter fennel (*Foeniculum vulgare*) possess molluscicides and larvacides properties towards freshwater snail and mosquitoes (Sousa *et al.*, 2015). *C. longa* leaves extract showed the presence of terpenes compound (Latip *et al.*, 2017). Terpenes compound can disrupt the normal GAS embryo development and give ovicidal effect on matured egg mass (Wu *et al.*, 2005; Latip *et al.*, 2017). The research done on *Zanthoxylum rhoifolium* showed there was an insecticidal activity from *C. longa* essential oil whereas their major compounds were β-Myrcene and β-phellandrene due to the synergistic effect of these compounds in the essential oil (Prieto *et al.*, 2011). Meanwhile, α-phellandrene and β-phellandrene were double-bond isomers and cyclic monoterpenes (NCBI, 2017).

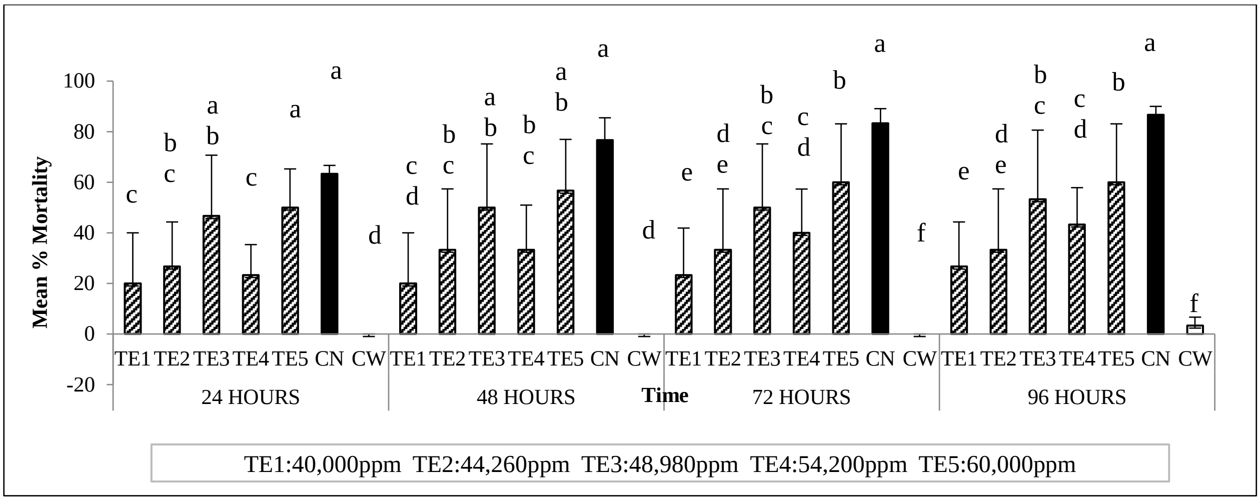

Figure 1. Mean Percentage Mortality of *P. canaliculata* after every 24 hours exposure with *C. longa* essential oil

*Means with the same letter above bars are not significantly different, p<0.05, Duncan's Multiple Range test

Fig. 2 shows the results from the bioassay test conducted on *P. betle* essential oil. After a 24 hours exposure, the *P. betle* essential oil treatment of SE3 (0.06g/ml) showed the highest mean percentage GAS mortality (26.67%). However, after 48 hours, the SE4 treatment (0.08g/ml) recorded the highest mean percentage GAS mortality (73.33%) and 90% after 72 and 96 hours of treatment exposure. There was no GAS mortality recorded by treatment SE1 (0.02g/ml) at the end of the test. The *P. betle* essential oil treatment of SE4 (0.08g/ml) recorded the highest mean percentage GAS mortality (90%) after 96 hours of treatment were conducted compared to negative control of niclosamide (86.67%) (commonly used mollucicides). The ANOVA test showed the value between treatment was significant as $p<0.05$.

The GCMS analysis results (Table 3) for *P. betle* essential oil showed a high content of active compound for eugenol which is 15.6%. Eugenol becomes a source for natural herbicides as stated by Mukhopadhyay (2000). The main active compound eugenol in clove essential oil becomes the main reason for high *Zabrotessub fasciatus* mortality in the test conducted (Paranhos *et al.*, 2006). The research had been conducted by Jairoce *et al.*, (2016) for insecticidal activity of clove essential oil towards *Acanthoscelides obtectus* and *Sitophilus zeamais* showed the maximum concentration of 35μL g-1 had resulted in 100% mortality for both tested *P. canaliculata* after 48 hours of exposure. The compound which was responsible for this insecticidal activity of clove essential oil was eugenol. It was supported by the GCMS analysis for clove essential oil where the eugenol was identified as major compound with 62.72% concentration (Jairoce *et al.*, 2016). The potential use of clove essential oil as repellence, contact or fumigations were proved by research done with clove oil where clove oil had caused mortality for *Acanthoscelides obtectus* and *Sitophilus zeamais* thus become alternatives for chemical insecticides (Jairoce *et al.*, 2016). Clove bud oil treatment with concentration of 0.116% had caused 100% mortality for *Cornu aspersum* eggs and juveniles in potted plants after 24 hours treatment. The treatment resulted with LC_{50} values which are 22 times more toxic than commercial molluscicides tested (McDonnell *et al.*, 2016). Eugenol and showed synergistic effect when used together with commercial diatomaceous earth-based insecticides where the treatment showed higher LD_{50} value compared with DE alone against *Sitophilus oryzae* (Islam *et al.*, 2010).

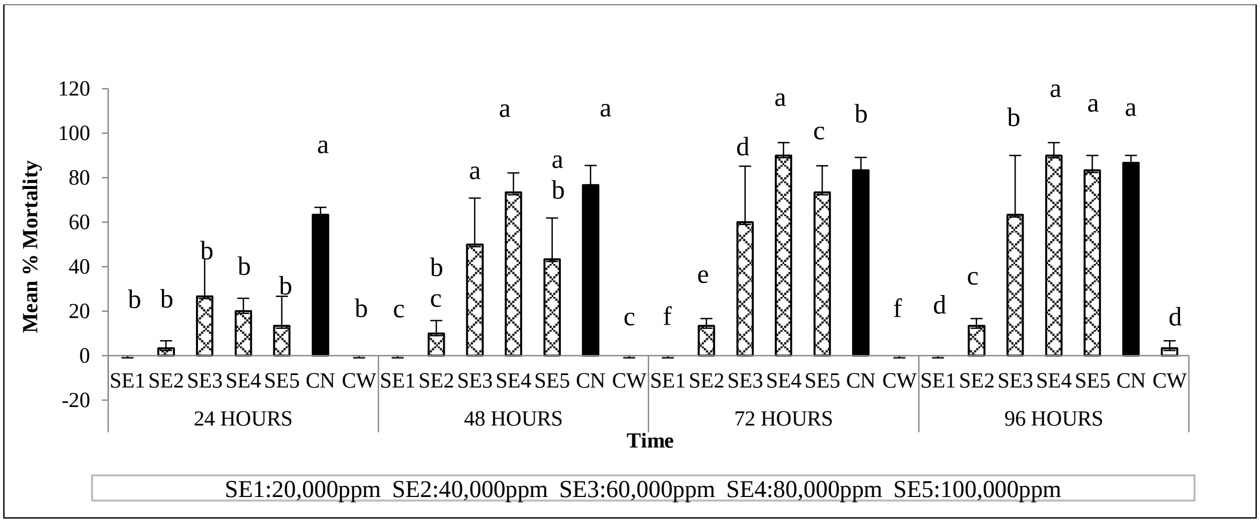

Figure 2. Mean Percentage Mortality of GAS after every 24 hours exposure with *P. betle* essential oil.

*Means with the same letter above bars are not significantly different, p<0.05, Duncan's Multiple Range test

IV. CONCLUSIONS

The comparison between *C. longa* and *P. betle* essential oil treatment after 96 hours of exposure with the same concentration (0.06g/ml) of TE5 and SE3 showed *P. betle* essential oil treatment resulted in a slightly higher *P. canaliculata* mortality which is 63.33% compared to turmeric essential oil treatment, which was 60% mean percentage *P. canaliculata* mortality.

From this comparison, *P. betle* essential oil treatment showed good results for mortality test. However, the *C. longa* essential oil treatment also showed a similar result in relation to *P. canaliculata* mortality. These findings indicated that both plants should be studied more extensively to reveal other potential values as a natural source for botanical pesticide in controlling golden apple snail, *P. canaliculata*.

V. ACKNOWLEDGEMENTS

This work was supported by internal funds from the Universiti Technology MARA, Malaysia through Research Entity Initiative (REI) grant, 600-IRMI/REI/5/3 (005/2018). We also acknowledge the laboratory technical department from Faculty Plantation and Agrotechnology, UiTM Shah Alam, UiTM Jengka campus and UiTM Puncak Alam campus for allowing us to use their laboratory facilities.

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