http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2021.756.764

Research Article
Anaesthetic Efficiency of *Cymbopogon citratus* Essential Oil and Clove Oil on *Macrobrachium rosenbergii*

[1,2]Wan Noazira Wan Adnan, [1]Nor Juneta Abu Seman, [1]Nurul Ulfah Karim, [1]Safiah Jasmani, [1]Nor Asma Husna Yusoff and [1,3]Marina Hassan

[1]Higher Institution Centre of Excellence (HICoE), Institute of Tropical Aquaculture and Fisheries, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia
[2]Department of Applied Sciences and Agriculture, Tunku Abdul Rahman University College, Jalan Segamat/Labis, 85000 Segamat, Johor, Malaysia
[3]STU-UMT Joint Shellfish Research Laboratory, Shantou University, Shantou 515063, China

Abstract

Background and Objective: Studies on plant herbs as alternatives to chemical anaesthetics in fish species are numerous, but little is known on crustaceans. A study was conducted to investigate the efficacy of *C. citratus* Essential Oil (EO) on the induction and recovery of *M. rosenbergii*. **Materials and Methods:** The *C. citratus* EO was obtained by hydrodistillation and analyzed using GC-MS. The prawns were exposed to *C. citratus* EO and clove oil in 100-1000 and 200-1000 μL L^{-1}, respectively. Different stages of induction and recovery times were recorded. **Results:** In GC-MS, citral (78.47%) was detected as a major compound in *C. citratus* EO. Prawns reached loss equilibrium at 500-1000 μL L^{-1} *C. citratus* EO within 15.55-6.52 min. Exposure of prawn to ≤500 μL L^{-1} *C. citratus* EO resulted in a high survival rate (100-94%). In clove oil, all tested concentrations caused significant induction in *M. rosenbergii* within 20.61-6.47 min. Recovery time and survival rate were significantly decreased with the increase of EO concentrations. The regression model showed the induction time in both anaesthetic agents was dependent on the concentration (R^2 = 0.86-0.96). The recovery time of *C. citratus* EO-exposed prawn was dependent on the concentrations (R^2 = 0.59). **Conclusion:** The study shows the potentiality of *C. citratus* EO as a natural anaesthetic in *M. rosenbergii*, although not as efficient as clove oil.

Key words: Anesthesia, clove oil, essential oil, lemongrass, *Macrobrachium rosenbergii*, sedative, stress

Citation: Adnan, W.N.W., N.J.A. Seman, N.U. Karim, S. Jasmani, N.A.H. Yusoff and M. Hassan, 2021. Anaesthetic efficiency of *Cymbopogon citratus* essential oil and clove oil on *Macrobrachium rosenbergii*. Pak. J. Biol. Sci., 24: 756-764.

Corresponding Author: Marina Hassan, Higher Institution Centre of Excellence (HICoE), Institute of Tropical Aquaculture and Fisheries, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia Tel: +609 6683949

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Anaesthetics are known to be effective in minimizing physical injuries and stress in prawns particularly during handling and transportation[1]. Tricaine methanesulfonate (MS-222) is a commonly used anaesthetic for aquatic organisms[2]. However, its usage is reported could cause retinopathy to the users, besides has a certain withdrawal period before product consumption[2]. Furthermore, this MS-222 also is not effective in many crustacean species[3]. Recently, the usage of natural plants as an anaesthetic in the aquaculture sector has attracted a lot of attention among researchers. Plant extracts such as Essential Oils (EO) and other active compounds are being reported to be more effective, less expensive and safer for aquatic life. Chilled sawdust has been reported efficiently in anaesthetizing *M. rosenbergii* during transportation[4]. A combination of eugenol and menthol effectively anaesthetize adult *M. rosenbergii* during the handling process[1].

Cymbopogon citratus is a plant from the family Gramineae and is widely cultivated in temperate and tropical regions including Indochina, Indonesia and Malaysia[5]. It is commonly known as lemongrass and popular in Malay, Thai and Vietnamese cuisine; frequently used in food processing as a food flavouring, perfume and cosmetic industry[5]. Recent studies have demonstrated that essential oil from the genus *Cymbopogon* has sedative effects on Silver Catfish (*Rhamdia quelen*) and Tambaqui (*Colossoma macropomum*)[6,7]. However, to date, there is still no information on the anaesthetic/sedative properties of *C. citratus* EO on crustaceans. As citral oil can produce anxiolytic, sedative and motor relaxant effects in mice[8], it was then hypothesized herein that *C. citratus* EO in appropriate concentrations, would promote an anaesthetic effect on giant freshwater prawn (*Macrobrachium rosenbergii*) as well.

Therefore, the present study aimed to determine the anaesthetic potential of the *C. citratus* EO through the evaluation of *M. rosenbergii* subjected to different concentrations of *C. citratus* EO and their recovery times. The survival rate after anaesthesia also was assessed.

MATERIALS AND METHODS

Study area: The study was carried out in the Institute of Aquaculture Tropical and Fisheries, Universiti Malaysia Terengganu (UMT), Terengganu, Malaysia from March, 2013-September, 2017.

Preparation of experimental animals: *Macrobrachium rosenbergii* with an average body weight of 18-23 g were purchased from a local farmer at Jelebu, Negeri Sembilan and transported to the Institute of Tropical Aquaculture and Fisheries (AKUATROP) hatchery for acclimation. Animals were maintained in a continuously aerated fibreglass tank (size: 0.3×0.05×0.1 m) with controlled water and oxygen parameters; water temperature: 27±1°C, pH7.6±0.5, dissolved oxygen: 8.5±0.7 mg L^{-1} and total ammonia level: 0.5±0.5 mg L^{-1}. Dissolved oxygen was measured by YSI oxygen meter (Model Y556, USA) while a total ammonia level in the water was determined using the API Ammonia Test Kit (Mars® Fishcare) (MARS Fishcare, USA). Acclimatization was conducted for 7 days in a Semi-static system where half of the water volume in the tank was changed daily. Prawns were fed twice daily with commercial pellet and starved for 24 hrs before the experiments. Thirty healthy prawns were used in every treatment (n = 30), with ten per replicate and each treatment consisted of three replicates.

Plant collection: Fresh leaves of *Cymbopogon citratus* were collected from the local market at Gong Badak and Wakaf Tengah, Kuala Terengganu from September, 2012-March, 2013. Samples were cut and dried for 3-5 days in a ventilated drying oven (Memmert Model: SN30, Germany) at 25°C. Dried samples were ground into fine powder form by using a heavy-duty Waring blender (Fisher Scientific, USA) and kept in a sealed dark plastic bag at room temperature (27°C) until extraction started.

Plant extraction and analysis: The Essential Oil (EO) of *C. citratus* was extracted by hydrodistillation method using a Clevenger type apparatus for 3 hrs[9]. Collected EO yields were calculated w/w (%) before stored in amber glass bottles at -4°C until further analysis. Analysis of the EO was performed by Hewlett Packard 6890 Gas Chromatography coupled (GC) with Mass Spectrophotometer (MS) (Agilent Technologies, USA) equipped with DB-5 silica capillary column (Agilent Technologies, USA); (size: 30 m×0.25 mm×0.25 µm). The conditions were as follows: detector and injector temperature, 240°C; carrier gas, helium (99.99% purity) at 1.7 mL min^{-1}. The identification of the EO constituents was made through the comparison of substances mass spectrum with the database of the GC-MS (NIST. lib) (Agilent Technologies, USA), literature and retention time index[10].

Table 1: Induction stages and behaviour observation adopted by Vartak and Singh[11]

Stage	Behaviour
Normal	Prawns appeared normal but with a slight loss of reactivity
Light sedation	Prawns were stationary, remaining in one place. Increase movement appendages
Deep sedation	Prawns become agitated and swam faster, followed by limpness. The very rapid movement of the swimming appendages
Partial loss equilibrium	Prawns gradually lost control over walking legs and chelae. Prawns remained motionless at the bottom but a slight movement of antennae was seen
Total loss equilibrium/deep anaesthesia	Walking legs and chelae become tangled and stiff. Prawns become numb
Death	Prawns become rigid, colour changes to whitish and prawns died
Recovery	Prawns freely swimming

Anaesthesia induction and recovery: Clove oil and *C. citratus* EO were used in this experiment. Tested concentrations were as follow: clove oil (200, 300, 400, 500, 600, 700, 800, 900 and 1000 µL L^{-1}); *C. citratus* EO (100, 200, 300, 400, 500, 600,700, 800, 900 and 1000 µL L^{-1}). Firstly, the anaesthetic agent was dissolved in ethanol at a ratio of 1:10. The control treatment used solely ethanol following the highest concentration of anaesthetic agent. *M. rosenbergii* were caught using a dip net and introduced individually into the anaesthetic bath (size: 35×20×24 cm) containing 10 L of the treatment concentration. Once the prawns reached the last stage of anaesthesia (total loss equilibrium), they were then transferred to a tank with clean water to observe for recovery for a maximum of 30 min and the full recovery time was recorded. After recovery, prawns were grouped according to treatments and transferred to continuously-aerated 50 L tanks and reared for 24 hrs. Any mortality within these 24 hrs was recorded. The behaviours of *M. rosenbergii* were characterized according to the criteria evaluation of the stages of induction and recovery by Vartak and Singh[11], with slight modifications (Table 1). The time required for inducing anaesthesia and recovery was monitored. Similar methods and data observations were applied in both anaesthetics' agents.

Statistical analysis: All data were expressed as Mean±SEM. For anaesthesia induction and recovery, data normality was assessed by the Shapiro-Wilk test and the non-normal data was submitted to a square root transformation and analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's tests. Analysis was performed with MINITAB 16.0 software and $p<0.05$ was considered statistically significant.

RESULTS

Yield and GC/MS profiling for *C. citratus* EO: The hydrodistillation process of 880 g *C. citratus* yielded 5.61 g of EO which equally to 0.72% (w/w) of the total yield percentage. Forty-two volatile components were identified in *C. citratus* EO. Citral was found to be a major compound (78.47%), followed by 5-Hepten-1-yne-methyl (3.83%), β-myrcene (3.34%), β-ocimene (1.2%), Ethenyl-cyclohexane (1.63%) and Selina-6-en-4-ol (1.38%) (Table 2).

Short-term anaesthesia and recovery: Induction and recovery time of prawn tested with *C. citratus* EO and clove oil are presented in Table 3 and 4, respectively. Both anaesthetic agents tested in this study showed sedative and anaesthetic effects in *M. rosenbergii*. The application of sole ethanol did not produce any anaesthetic effect. Prawn exposed to *C. citratus* EO started to experience Light Sedation (LS) stage ranging from 300-400 µL L^{-1} after 20.68-12.56 min of exposure time. However, this concentration was unable to promote prawn into subsequent induction stages even after 30 min of observations. Increased of *C. citratus* EO to 500 µL L^{-1} led to 5.38 min of LS stage with totally loss equilibrium (deep anaesthetic) at 15.55 min and the subsequent increasing concentration reduced the induction time. The fastest induction was at 1000 µL L^{-1} with LS at 2.01 min and loss equilibrium at 6.52 min. The overall trend shows that the induction time was significantly dependent on the concentration of *C. citratus* EO ($R^2 = 0.86$-0.93) (Fig. 1). The reduction of induction time occurred with the increase of *C. citratus* EO concentration. Meanwhile, the opposite trend was identified in recovery time where the increase of *C. citratus* EO concentrations resulted in an elevation of the recovery time (Fig. 2). No mortality was observed within the range of 100-300 µL L^{-1} of *C. citratus* EO exposure. A 94% of survival rate was found at 500 µL L^{-1} of *C. citratus* EO treatment after 24 hrs of observations.

In clove oil, prawn exposed to all concentrations exhibited sedation and anaesthetic effects started at the lowest concentrations; 200 µL L^{-1} with 20.61 min to LS stage and 29.25 min to reach into totally loss equilibrium (deep anaesthetic) stage. Generally, clove oil concentrations significantly affected the induction and recovery time ($p<0.05$) in *M. rosenbergii* (Fig. 3-4). The increased concentrations of

Table 2: Chemical compositions of essential oil from *C. citratus* by GC-MS analysis

Constituent	Retention time (Rt)	Peak area (%)
α-Pinene	9.174	0.04
Camphene	9.634	0.07
5-Hepten-11-yne, 6-methyl	11.235	3.83
β-Myrcene	11.308	3.34
D-Limonene	12.335	0.06
Eucalyptol	12.397	0.09
β-Ocimene	12.756	1.23
Z-β-Ocimene	13.065	0.76
Linalool	14.694	0.52
Citronellal	16.300	0.33
Cyclohexane, ethenyl	17.215	1.63
Citral	20.449	78.47
2-Undecanone	20.713	0.05
Geranyl formate	20.871	0.18
Geranic acid	21.348	0.10
Neric acid	21.645	0.23
2-Butenoic acid, methyl ester	22.634	0.59
Geranic acid	22.791	0.36
Geranyl acetate	22.875	0.75
B-elemene	23.083	0.12
Caryophyllene	23.774	0.54
α Bergamotene	24.167	0.32
α Guaiene	24.245	0.06
Caryophyllene	24.352	0.10
β Farnesene	24.678	0.20
4, 11 Selinadiene	25.363	0.28
β Cadinene	25.542	0.10
2-Tridecanone	25.627	0.23
β Cadinene	25.761	0.10
β Bulnesene	25.907	0.17
α Amorphene	26.110	0.19
δ Cadinene	26.329	0.25
α Farnesene	26.514	0.06
α Gurjunene	26.666	0.06
Caryophyllene oxide	27.749	0.12
Selina-6-en-4-ol	28.625	1.38
Tau-Muurolol	29.097	0.14
α Cadinol	29.395	0.22
β Maaliene	29.496	0.20
Juniper camphor	30.310	0.12
Geranyl linalool	37.857	0.12
Farnesol	38.531	0.29

Table 3: Required time for the stages of induction and recovery from anaesthesia at different concentrations of *C. citratus* EO in *M. rosenbergii*

Concentration of *C. citratus*	Prawn behaviour					
EO (μL L^{-1})	LS (min)	DS (min)	PLE (min)	TLE (min)	Recovery (min)	Survival rate (%)
100	-	-	-	-	-	100
200	-	-	-	-	-	100
300	20.68 ± 6.20^{a}	-	-	-	-	100
400	12.56 ± 4.61^{b}	-	-	-	-	98
500	5.38 ± 1.78^{cA}	7.17 ± 3.14^{aA}	10.21 ± 3.97^{aB}	15.55 ± 6.33^{aC}	9.50 ± 3.17^{a}	94
600	5.04 ± 2.03^{cdA}	6.04 ± 1.34^{abA}	10.56 ± 3.64^{aB}	14.31 ± 5.24^{aC}	10.77 ± 2.89^{a}	89
700	4.28 ± 2.43^{cdA}	4.59 ± 1.92^{abA}	9.44 ± 2.60^{abB}	14.05 ± 2.44^{abC}	14.68 ± 2.16^{b}	88
800	3.44 ± 1.64^{cdA}	5.41 ± 2.31^{abB}	7.38 ± 2.57^{bC}	10.10 ± 2.28^{bD}	11.75 ± 2.07^{a}	82
900	3.57 ± 1.60^{cdA}	4.23 ± 2.41^{cA}	4.5 ± 2.35^{cA}	7.84 ± 0.99^{cB}	13.75 ± 3.56^{b}	78
1000	2.01 ± 1.53^{bF}	3.45 ± 2.23^{cI}	4.26 ± 2.12^{cI}	6.52 ± 0.95^{cF}	14.41 ± 1.61^{bC}	75

LS: Light sedation, DS: Deep sedation, PLE: Partial loss equilibrium, TLE: Total loss equilibrium. Values are expressed as Mean±SD. Different lowercase superscripts in the same column indicate statistical difference among concentrations and different uppercase in the same row indicate statistical difference among stages after ANOVA and Tukey test ($n = 30$, $p<0.05$)

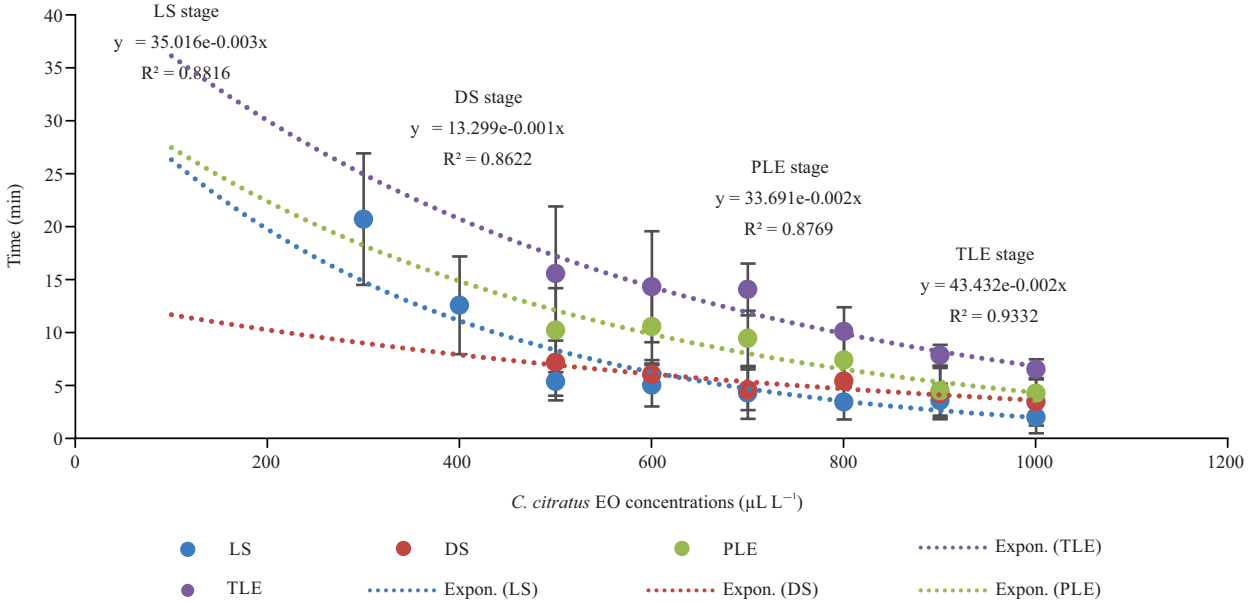

Fig. 1: Relationship between stages of induction time with *C. citratus* EO concentrations

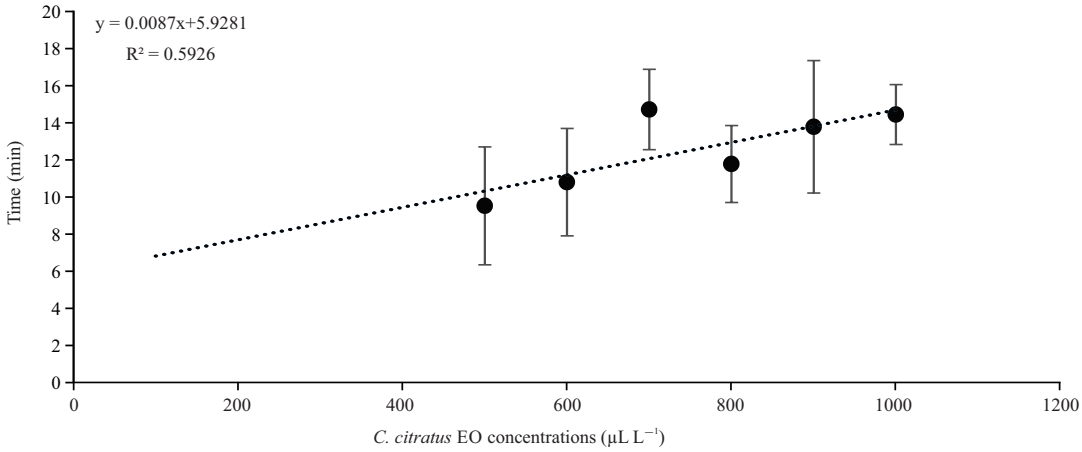

Fig. 2: Relationship of recovery time with *C. citratus* EO concentrations

Table 4: Required time for the stages of induction and recovery from anaesthesia at different concentrations of clove oil in *M. rosenbergii*

Concentration of clove oil (µL L^{-1})	Prawn behaviour					
	LS (min)	DS (min)	PLE (min)	TLE (min)	Recovery (min)	Survival rate (%)
200	20.61±4.18^{aB}	23.68±4.09^{aB}	27.13±3.15^{aA}	29.75±2.42^{aA}	17.35±4.74^{aB}	85
300	23.84±3.27^{bA}	25.39±3.67^{bA}	26.84±4.04^{aA}	28.80±4.18^{aA}	18.56±3.05^{abAB}	74
400	17.66±2.23^{cC}	18.38±2.04^{cC}	22.98±2.23^{bB}	29.52±1.24^{aA}	20.58±4.31^{ba}	76
500	11.31±2.34^{dD}	12.6±2.32^{dD}	17.41±1.88^{cC}	24.51±2.49^{bB}	19.14±3.07^{abAB}	72
600	5.40±1.52^{eE}	6.59±1.73^{eEF}	10.68±2.80^{dD}	13.62±1.22^{cC}	10.41±3.56^{cD}	78
700	4.83±1.32^{efE}	7.41±1.74^{efE}	10.72±2.61^{dD}	13.98±2.57^{cC}	9.45±1.42^{cdD}	89
800	3.70±0.92^{fEF}	4.75±0.77^{gGHI}	6.52±1.01^{eFGH}	8.90±1.32^{dDE}	7.35 ± 1.33^{deE}	85
900	3.60±1.15^{fEF}	5.27±1.04^{fgFGH}	6.75±0.75^{eFG}	8.26±1.06^{dDEF}	7.25±1.10^{deE}	90
1000	3.35±1.00^{fEF}	4.21±1.04^{gHI}	5.24±1.31^{eGHI}	6.47±1.95^{eF}	6.08±1.65^{eE}	92

LS: Light sedation, DS: Deep sedation, PLE: Partial loss equilibrium, TLE: Total loss equilibrium. Values are expressed as Mean±SD. Different lowercase superscripts in the same column indicate statistical difference among concentrations and different uppercase in the same row indicate statistical difference among stages after ANOVA and Tukey test (n = 30, p<0.05)

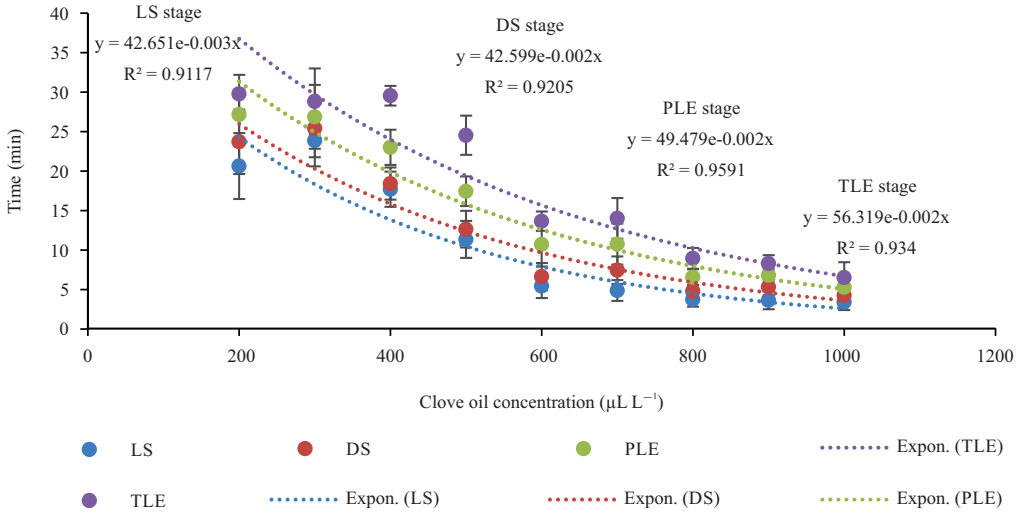

Fig. 3: Relationship between stages of induction time with clove oil concentrations

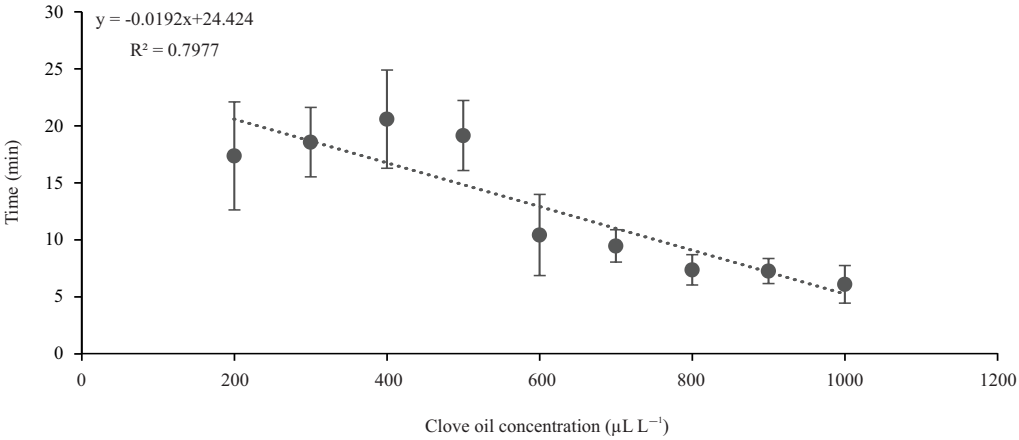

Fig. 4: Relationship of recovery time with clove oil concentrations

clove oil significantly decreased the time needed for sedation and anaesthetic (R^2 = 0.91-0.96). However, clove oil survival rate was contradicted with the *C. citratus* EO trend, wherein clove oil treatment the trend was increased with the increase of clove oil concentrations. The highest survival rate (92%) was observed at the highest treatment of clove oil (1000 µL L^{-1}).

Modelling of induction time versus anaesthetic concentration showed that induction time was strongly dependent on the anaesthetic concentrations in all stages of induction in both *C. citratus* (Fig. 1) and clove oil (Fig. 3); R^2 = 0.86-0.93, R^2 = 0.91-0.96, respectively. Meanwhile, modelling of recovery time versus concentrations in *C. citratus* EO (Fig. 2) can be used in predicting the recovery times in *M. rosenbergii* (R^2 = 0.59). However, clove oil recovery times does not dependent on the concentration of clove oil (R^2 = 0.7977) (Fig. 4).

DISCUSSION

In this present study, hydrodistillation of *C. citratus* yielded a total of 0.715% which is following Viana *et al.*[12], but contradicted with Jalal *et al.*[13]. Differences might be due to the different techniques applied in sample preparations, extraction methods and sample preservations as volatile compounds in plant cells are highly sensitive and easy to get release into the environment[9]. Meanwhile, citral was detected as a major compound in *C. citratus* EO in the percentage of 78.47%, followed by 5-hepten-yne-6 dimethyl (3.38%), β-myrcene (3.34%), ethnyl cyclohexane (1.63%), selina 6-en-4-ol (1.38%) and β-trans-ocimene (1.23%) (Table 1). The finding was similar to the previous study where they also found citral as a major compound in *C. citratus* EO but differs in percentage amounts[14-17]. Meanwhile, myrcene and geraniol

were reported to be dominant in other Cymbopogon species instead of citral[7,18]. Variations in extract compositions are influenced through several factors including geographical area, harvesting period and extraction methods[19,20]. Citral contained EO have documented attributes to various biological activities including antimicrobial, antifungal, antioxidant, anti-depressant, anti-inflammatory, sedation and/or anaesthetic effects[7,12,20-24].

The data of Table 3 and 4 represent the induction time, recovery and survival of both anaesthetic agents; *C. citratus* EO and clove oil in *M. rosenbergii*, as respectively. *C. citratus* EO as low as 200-300 μL L^{-1} induced *M. rosenbergii* to light sedation stage but no observation of deep anaesthesia even after 30 min of exposure. Although a low concentration of *C. citratus* EO did not determine deep anaesthesia, it is capable to promote sedation effect on *M. rosenbergii* with the shortest induction time at 12.56 min, 400 μL L^{-1}. This finding was in similar range with *C. nardus* EO for sedation effect in tambaqui fish[7]. However, a similar concentration of menthol (200 μL L^{-1}) promoted the latest sedation effect in adult *M. rosenbergii*, 202.3 min. This differential efficiency might be due to the chemical specificity of receptors[1]. Meanwhile, dos Santos *et al.*[6] also reported the sedation effect of silver catfish at a low concentration of *C. flexuosus* EO (25 μL L^{-1}). This sedation effect is important particularly during non-invasive procedures such as tagging, biometrics and transportation[25]. A total loss equilibrium/deep anaesthesia sometimes may not be desirable during transport, as overcrowding of sedated prawn at the bottom tank might lead to asphyxiation[26]. Besides, prawn with highly sedated may experience mechanical injury by hitting the tank walls during transportation[27].

Plotted graph (Fig. 1) shows a clear concentration-dependent trend in *C. citratus* EO towards the induction of *M. rosenbergii* to anaesthesia. The optimum concentration which exhibited rapid sedation and/or deep anaesthesia with fast recovery and high survival rate was at 500 μL L^{-1}. Even though the time for induction was exceeded the recommended standards of induction; 3-5 min[28], but the time for recovery was rapid (<10 min), with a high survival rate (94%), which met the standards criteria for commercial anaesthetics. This rapid recovery was presumably because of the fast release of drug uptakes via the gills[7].

In clove oil treatment, the result shows the ability of cove oil to cause total loss equilibrium (deep anaesthesia) in *M. rosenbergii* at 1000 μL L^{-1} within 6.47 min. Meanwhile, the light sedation stage was started even earlier at 200 μL L^{-1} after 20.61 min treatment. The induction time of clove oil in this study was shorter than reported by Vartak and Singh[11] in the same species of prawn. Differences might be due to the experimental conditions such as water prawn size, disease status, stress and other physicochemical properties at the moment of induction[29,30]. As reported by other previous research; fish weight is significantly influenced by the time needed for the induction[31].

Results showed that all stages in induction time are strongly predictable using concentrations of *C. citratus* EO and clove oil. However, recovery time in the clove oil exposed prawn is increased with the increasing of concentrations. The regression model is important for the determination of the interrelation of y-variables with -variables. However, further study on the respective compound which is involved in this anaesthetic/sedation effect might be an advantage if could be determined, which now become our limitation in this study. Hence, the treatment of the isolated single compounds in *M. rosenbergii* might be important for a thorough determination of the mechanisms occurring during anaesthesia in crustaceans.

CONCLUSION

In conclusion, *C. citratus* EO can be used for prawn anaesthesia, although it is not as efficacious as clove oil, the most commonly-used herbal anaesthetic. *C. citratus* EO at 300-400 μL L^{-1} can be used safely for inducing sedation in *M. rosenbergii*. These sedation effects are very useful, particularly during non-invasive procedures. Meanwhile, 500 μL L^{-1} of *C. citratus* EO is suitable in promoting the anaesthesia effect (5.38-15.55 min) with fast recovery (<10 min) and a high survival rate (92%). Thus, it can be recommended as a new potential plant anaesthetic in *M. rosenbergii* species.

SIGNIFICANCE STATEMENT

This study discovers the relationship between induction and recovery time at a varying concentration of *C. citratus* and clove oil in *M. rosenbergii* that can be beneficial for comparison between the newly found natural aesthetic with the current-accepted-anaesthetic clove oil. This study will help the researchers out there particularly people in aquatic fields to uncover the potentiality of *C. citratus* EO as an anaesthetic agent in freshwater prawns which is still rarely studied compared to fish. Thus, a new theory on the dose requirement for anaesthetic/sedative effect on *M. rosenbergii* may be arrived at.

ACKNOWLEDGMENTS

This research was funded by the Higher Institution Centre of Excellence (HICoE), Institute of Aquaculture Tropical and Fisheries (AKUATROP), UMT under the vote number of 66955. The author also would like to pay our respects to Dr. Safiah Jasmani who had passed away in September, 2017, for her contributions in initiating and planning this study.

REFERENCES

1. Saydmohammed, M. and A.K. Pal, 2009. Anesthetic effect of eugenol and menthol on handling stress in *Macrobrachium rosenbergii*. Aquaculture, 298: 162-167.
2. Popovic, N.T., I. Strunjak-Perovic, R. Coz-Rakovac, J. Barisic, M. Jadan, A.P. Berakovic and R.S. Klobucar, 2012. Tricaine methane-sulfonate (MS-222) application in fish anaesthesia. J. Applied Ichthyol., 28: 553-564.
3. Coyle, S.D., R.M. Durborow and J.H. Tidwell, 2004. Anesthetics in Aquaculture. SRAC Publication, Texas, pp: 6.
4. Salin, K.R., 2005. Live transportation of *Macrobrachium rosenbergii* (De Man) in chilled sawdust. Aquac. Res., 36: 300-310.
5. dos Santos, A.C., G.B. Junior, D.C. Zago, C.C. Zeppenfeld and D.T. da Silva *et al*., 2017. Anesthesia and anesthetic action mechanism of essential oils of *Aloysia triphylla* and *Cymbopogon flexuosus* in silver catfish (*Rhamdia quelen*). Vet. Anaesth. Analg., 44: 106-113.
6. Barbas, L.A.L., M. Hamoy, V.J. de Mello, R.P.M. Barbosa and H.S.T. de Lima *et al*., 2017. Essential oil of citronella modulates electrophysiological responses in tambaqui *Colossoma macropomum*: A new anaesthetic for use in fish. Aquaculture, 479: 60-68.
7. do Vale, T.G., E.C. Furtado, J.G. Santos and G.S.B. Viana, 2002. Central effects of citral, mycrene and limonene, constituents of essential oil chemotypes from *Lippia alba* (Mill.) N.E. Brown. Phytomedicine, 9: 709-714.
8. Kulkarni, S.B., M.Y. Kariduraganavar and T.M. Aminabhavi, 2003. Molecular migration of aromatic liquids into a commercial fluoroelastomeric membrane at 30, 40 and 50°C. J. Appl. Polym. Sci., 90: 3100-3106.
9. Kasali, A.A., A.O. Oyedeji and A.O. Ashilokun, 2001. Volatile leaf oil constituents of *Cymbopogon citratus* (DC) Stapf. Flavour Fragrance J., 16: 377-378.
10. da Cunha, M.A., F.M.C. de Barros, L. de Oliveira Garcia, A.P. de Lima Veeck and B.M. Heinzmann *et al*., 2010. Essential oil of *Lippia alba*: A new anesthetic for silver catfish, *Rhamdia quelen*. Aquaculture, 306: 403-406.
11. Vartak, V. and R.K. Singh, 2006. Anesthetic effects of clove oil during handling and transportation of the freshwater prawn, *Macrobrachium rosenbergii* (de man). Isr. J. Aquac., 58: 46-54.
12. Viana, G.S.B., T.G. Vale, R.S.N. Pinho and F.J.A. Matos, 2000. Antinociceptive effect of the essential oil from *Cymbopogan citratus* in mice. J. Ethnopharmacol., 70: 323-327.
13. Elyemni, M., B. Louaste, I. Nechad, T. Elkamli and A. Bouia *et al*., 2019. Extraction of essential oils of *Rosmarinus officinalis* L. by two different methods: Hydrodistillation and microwave assisted hydrodistillation. Scient. World J., Vol. 2019. 10.1155/2019/3659432.
14. Nakamura, Y., M. Miyamoto, A. Murakami, H. Ohigashi, T. Osawa and K. Uchida, 2003. A phase II detoxification enzyme inducer from lemongrass: Identification of citral and involvement of electrophilic reaction in the enzyme induction. Biochem. Biophys. Res. Commun., 302: 593-600.
15. Blanco, M.M., C.A.R.A. Costa, A.O. Freire, J.G. Santos Jr. and M. Costa, 2009. Neurobehavioral effect of essential oil of *Cymbopogon citratus* in mice. Phytomedicine, 16: 265-270.
16. Aiemsaard, J., S. Aiumlamai, C. Aromdee, S. Taweechaisupapong and W. Khunkitti, 2011. The effect of lemongrass oil and its major components on clinical isolate mastitis pathogens and their mechanisms of action on *Staphylococcus aureus* DMST 4745. Res. Vet. Sci., 91: e31-e37.
17. Costa, C.A.R.A., D.O. Kohn, V.M. Lima, A.C. Gargano, J.C. Florio and M. Costa, 2011. The GABAergic system contributes to the anxiolytic-like effect of the essential oil from *Cymbopogon citratus* (lemongrass). J. Ethnopharmacol., 137: 828-836.
18. Tajidin, N.E., S.H. Ahmad, A.B. Rosenani, H. Azimah and M. Munirah, 2012. Chemical composition and citral content in lemongrass (*Cymbopogon citratus*) essential oil at three maturity stages. Afr. J. Biotechnol., Vol. 11. 10.5897/ajb11.2939.
19. Tondolo, J.S.M., L.D.P. Amaral, L.N. Simões, Q.I. Garlet and B. Schindler *et al*., 2013. Anesthesia and transport of fat snook *Centropomus parallelus* with the essential oil of *Nectandra megapotamica* (Spreng.) Mez. Neotrop. Ichthyol., 11: 667-674.
20. Wang, Y.S., Z.Q. Wen, B.T. Li, H.B. Zhang and J.H. Yang, 2016. Ethnobotany, phytochemistry and pharmacology of the genus *Litsea*: An update. J. Ethnopharmacol., 181: 66-107.
21. Kamle, M., D.K. Mahato, K.E. Lee, V.K. Bajpai, P.R. Gajurel, K.S. Gu and P. Kumar, 2019. Ethnopharmacological properties and medicinal uses of *Litsea cubeba*. Plants, Vol. 8. 10.3390/plants8060150.
22. Villalobos, M.C., 2015. Antioxidant activity and citral content of different tea preparations of the above-ground parts of lemongrass (*Cymbopogon citratus* Stapf.). J. Agric. Food Chem., 46: 1111-1115.
23. Martins, H.B., N. das Neves Selis, C.L. Silva e Souza, F.S. Nascimento and S.P. de Carvalho *et al*., 2017. Anti-inflammatory activity of the essential oil citral in experimental infection with *Staphylococcus aureus* in a model air pouch. Evidence-Based Compl. Alt. Med., Vol. 2017. 10.1155/2017/2505610.

24. Sneddon, L.U., 2012. Clinical anesthesia and analgesia in fish. J. Exot. Pet Med., 21: 32-43.
25. Coyle, S.D., S. Dasgupta, J.H. Tidwell, T. Beavers, L.A. Bright and D.K. Yasharian, 2005. Comparative efficacy of anaesthetics for the freshwater prawn *Macrobrachium rosenbergii*. J. World Aquacult. Soc., 36: 282-290.
26. Wagner, G.N., T.D. Singer and R.S. McKinley, 2003. The ability of clove oil and MS-222 to minimize handling stress in rainbow trout (*Oncorhynchus mykiss* walbaum). Aquac. Res., 34: 1139-1146.
27. Gilderhus, P.A. and L.L. Marking, 1987. Comparative efficacy of 16 anesthetic chemicals on rainbow trout. North Am. J. Fish. Manage., 7: 288-292.
28. Gomes, D.P., B.W. Chaves, A.G. Becker and B. Baldisserotto, 2011. Water parameters affect anaesthesia induced by eugenol in silver catfish, *Rhamdia quelen*. Aquacult. Res., 42: 878-886.
29. Bowker, J.D., J.T. Trushenski, D.C. Glover, D.G. Carty and N. Wandelear, 2015. Sedative options for fish research: A brief review with new data on sedation of warm-, cool- and coldwater fishes and recommendations for the drug approval process. Rev. Fish Biol. Fish., 25: 147-163.
30. Tsantilas, H., A.D. Galatos, F. Athanassopoulou, N.N. Prassinos and K. Kousoulaki, 2006. Efficacy of 2-phenoxyethanol as an anaesthetic for two size classes of white sea bream, *Diplodus sargus* L. and sharp snout sea bream, *Diplodus puntazzo* C. Aquaculture, 253: 64-70.
31. Adnan, W.N.W., N.U. Karim, N.A.H. Yusoff, M.I. Zakariah and M. Hassan, 2021. Effect of *Cymbopogon citratus* essential oil (EO) on handling stress in giant freshwater prawn (*Macrobrachium rosenbergii*). Pak. J. Bio. Sci., 24: 13-18.