Jurnal Kejuruteraan 38(4) 2026: 1991-2004
https://doi.org/10.17576/jkukm-2026-38(4)-34

Larvicidal Activity of Essential Oil from *Kaempferia galanga Linn* and its Toxicity Effect on Non-Target Organism (*Epipremnum aureum*)

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Received 8 November 2025, Received in revised form 11 March 2026
Accepted 11 April 2026, Available online 30 May 2026

ABSTRACT

Kaempferia galanga Linn. (KGL) is an herbaceous plant renowned for its antimicrobial, anti-inflammatory, antioxidant, nematicidal, and larvicidal properties. However, research on its mosquitocidal potential is limited. With the increasing global mosquito population, there is an urgent need for effective and eco-friendly mosquito control methods. This study aims to evaluate the larvicidal activity of KGL essential oil against Aedes aegypti larvae and its toxicity effect on the non-target organism Epipremnum aureum. The essential oil was extracted using solvent-free microwave extraction (SFME) at 450 W for 120 minutes and analyzed using Gas Chromatography-Mass Spectrometry (GC-MS), identifying ethyl p-methoxycinnamate as the major constituent. Toxicity assessment on Aedes aegypti larvae revealed LC_{50} and LC_{90} values of 53.97 ppm and 90.50 ppm, respectively, at 24 hours of exposure. The 50 ppm concentration provides a balanced effect, supporting significant stem elongation and moderate leaf area growth of Epipremnum aureum. These findings highlight the effectiveness of KGL essential oil as a natural larvicide and raise concerns regarding its impact on non-target plant species. Thus, while KGL essential oil shows promise as an alternative to synthetic pesticides, future studies should optimize the concentration for targeted applications, minimize non-target effects, and evaluate its environmental sustainability for large-scale implementation.

Keywords: Larvicidal activity; essential oil; kaempferia galanga linn; epipremnum aureum; aedes aegypti

INTRODUCTION

Kaempferia galanga Linn (KGL), also known as Kencur, Cekur, Sand Ginger, Cutcherry, or aromatic ginger, is an herbaceous plant belonging to the *Zingiberaceae* family, primarily grown in Southeast Asian countries, including Malaysia, Indonesia, Thailand, China, Taiwan, and India (Wang et al. 2021). This plant is valued for its versatile use in condiments, spices, cosmetics, and medicine. Its primary chemical constituent, *ethyl p-methoxycinnamate*, shows promise as a larvicidal agent, although further evidence is required to confirm its toxicity (Wang et al. 2023). This is particularly relevant given the global rise in mosquito populations. As of 2023, mosquito-borne infections present a significant public health challenge, especially in dengue-endemic regions like South Asia, Southeast Asia, and Latin America, where cases and economic impacts have steadily increased (Malavige et al. 2023). Recent studies have underscored the potential of natural products in mosquito control, emphasizing the need for continued research into environmentally friendly larvicides (Lu et al. 2023).

To control mosquito-borne diseases, various approaches have been applied, such as the usage of synthetic chemical pesticides (SPC) (AlSalhi et al. 2020) and *Bacillus thuringiensis israelensis* (BTi) (Nair et al. 2020). However, both larvicide possesses adverse effects on their own. The usage of SPCs is undoubtedly effective towards larvicidal activities. However, it contains carcinogenic properties that can be harmful to Non-Target Organism (NTOs) (Li et al. 2021). Even though BTi is often considered a safer and more environmentally

sustainable larvicide, it has been associated with food-borne infections, specifically diarrheal illness resulting from Bacillus thuringiensis (BTi) residues in food. These residues can produce enterotoxins within the human intestine (Biggel et al. 2022). The rapid and comprehensive usage of this bioinsecticide is concerning as the usage may harm NTOs in various ways.

As an alternative to previous larvicides, KGL essential oil is studied as eco-friendly for mosquito control (Abanit et al. 2022; AlSalhi et al. 2020). Chemical constituents of KGL, including phenol and phenolics, play a significant role in larvicidal activity as trans-ethyl-p-methoxycinnamate and trans-ethyl cinnamate exhibit potent larvicidal and repellent effects (Wang et al. 2021). The component is proven effective against larvicidal activity as AlSalhi et al. (2020), measured the LC50 and LC90 values against *Aedes vittatus* at 39.22 ppm and 80.90 ppm, respectively. Additionally, these products meet current regulatory standards, such as those set by the European Union's peer reviews conducted by the European Food Safety Authority (EFSA). These standards and regulations ensure the products' eco-friendliness for aquatic life, plants, and economic viability (Biggel et al., 2022).

Several extraction methods can be used to derive compounds from KGL, for example, hydrodistillation (HD) has been traditionally used to extract essential oil from plants (Srivastava et al. 2019). However, this method is tedious, time-consuming, and operates at high temperatures, which may cause degradation of thermolabile compounds. Abd Aziz et al. (2017) have studied the comparison of different extraction methods, which are HD, microwave-assisted extraction (MAE), and solvent-free microwave extraction (SFME), and the study resulted in the prominent advantages of using SFME. Another method of extraction noted is maceration, yet the method is far more time-consuming (Abanit et al. 2022) than other extraction methods. Another alternative method, supercritical fluid extraction (SFE), has received attention for extracting plant oil (Abd Manaf et al. 2013); however, SFE is not preferable for extracting essential oil with low oil content. High operational costs and maintenance make it not applicable for trace extract content. To date, there is no reported work on the study of the extraction of KGL extracts using the SFME method in the literature. SFME is a new approach that has been found to produce the highest extracts yield in a shorter time (Nurhaslina et al. 2022).

The essential oil larvicide is commonly tested on third-instar larvae to observe their toxicity level (World Health Organization, 2005). The efficiency of KGL essential oil against mosquito larvae has been the subject of several studies (AlSalhi et al. 2020; Luz et al. 2020), but none of them employed SFME to extract the essential oil. In addition, limited studies have been reported on the impact of essential oil on aquatic plants. Since aquatic plants and mosquito larvae depend on water for survival, this study aims to investigate the toxicity level of the KGL extracts to both mosquito larvae and plants that inhabit the same body of water.

In addition, the KGL essential oil is examined on aquatic plants for its effect on ecological system. Several species have been considered as plantlets: (*Chlorophytum comosum, Syngonium podophyllum, Tradescantia pallida, and Epipremnum aureum*). Based on its perennial growth, durability, fibrous roots, ease of propagation, ability to thrive with little nutrients and light, and potential for phytoremediation from the air and water media (Mishraet et al. 2021) *Epipremnum aureum* is regarded as a viable option as a plantlet. *Epipremnum aureum*, commonly known as Devil's ivy is a common houseplant that is either propagated in potted soil mix, water, and up a sphagnum moss pole (Boonnorat et al. 2021; Mahr; Yadav et al. 2021) have performed a study on the effect of different wastewater streams on the plant's nutrient removal. The plants cultivated with domestic wastewater showed minimal or no signs of nutrient deficit.

This work evaluates the larvicidal activity of essential oil extracted from KGL using the SFME method. Furthermore, the potential toxicity of this essential oil on the non-target organism *Epipremnum aureum* is also examined to assess the effectiveness of KGL essential oil as a natural larvicide against Aedes aegypti larvae while ensuring it complies with current regulatory standards for being environmentally friendly to aquatic life and plants and remains cost-effective.

METHODOLOGY

PLANT MATERIAL AND REAGENTS

KGL rhizomes were purchased at a local market in Klang, Selangor, Malaysia. Rhizome was washed, peeled, chopped into thin slices, and dried in a Taisite blast drying oven (model: FC0-30D) at 40 °C for 24 hours to achieve 21-22% moisture content. The dried rhizome was powdered by a Panasonic MX-337 conventional countertop grinder and sealed in an airtight glass container at room temperature. The sample is kept for a maximum of three months to ensure consistency in the extraction results. The amount of active chemicals in the sample may gradually decline due to plant degradation (Nurhaslina et al., 2022). Meanwhile, *Epipremnum aureum* plantlet was purchased at a local nursery in Selangor, Malaysia. The plant is used as a plantlet to test extracts' toxicity toward NTOs. 99.99%

Dimethyl Sulfoxide (DMSO) was purchased from Friendemann Schmidt Sdn. Bhd., Malaysia. DMSO is used as a stock solution solvent.

TARGET INSECT

Aedes aegypti larvae were purchased from the Institute for Medical Research (IMR), Setia Alam, Selangor, Malaysia. Mosquito larvae were reared in IMR laboratory following WHO (2005) guidelines where room temperature (25 ± 2 °C) with a light-dark cycle of 10:14 hours is maintained throughout the rearing period. Water was kept at its ideal temperature, and the larvae were given daily larval food for development. Third-instar larvae were collected for larvicidal analysis.

SOLVENT-FREE MICROWAVE EXTRACTION (SFME)

SFME extracted essential oil from KGL following Nurhaslina et al. (2022) and Oliveros-Diaz et al. (2022) methods with minor modifications. The extraction was conducted in a modified Samsung microwave oven (Model S28F303TFK/SM) with a flat-bottomed flask attached to a Clevenger apparatus and linked with a water circulation system. The microwave operates at 2450 MHz and can supply a maximum power of 1000 W. The inside dimension of the oven is 33 x 22 x 32 cm. Before extraction, 100 g of dried powdered sample was immersed in 200 mL distilled water at room temperature for 1 hour. Excess water was drained, and the sample was placed in the flat-bottomed flask. Extraction was executed for 120 mins at 450 W. The distillate obtained upon extraction was evaporated using a rotary evaporator (Model: Heidolph Laborota 400, Germany) until the essential oil was obtained. The essential oil was stored in amber glass vials and kept at -16°C until further analysis. The percentage of essential oil can be calculated using the formula Equation (1):

$$\text{EO Yield (\%w/w)} = \frac{\text{(Mass of EO extracted)}}{\text{(Mass of dried sample)}} \times 100\% \quad (1)$$

GAS CHROMATOGRAPHY-MASS SPEC

TROMETRY ANALYSIS (GC-MS)

GC-MS analysis was conducted following Yingngam et al. (2021) with the brand Varian originated from the USA. A computerized Hewlett-Packard system with a model of 450-gas chromatograph and a 240-mass spectrometer, fused-silica-capillary column polar stationary phase SLB®-5ms (film thickness of 30m × 0.25 mm × 0.25 µm), was used to analyze the essential oil stock solution. The chromatographic conditions were as follows: Helium gas carrier at a flow rate of 1.1 mL/min, injection volume value of 0.1 L at the temperature of 250 °C. The column temperature was maintained at 80 °C for 5 min, then raised to 150 °C at a rate of 6 °C/min and held for 5 min before being maintained at 220 °C at a rate of 3 °C/min for 5 min. The electron impact ionization was 70eV.

TOXICITY TEST OF KGL ESSENTIAL OIL TO MOSQUITO LARVAE

Larvicidal activity and toxicity were assessed on third-instar larvae of *Aedes aegypti* following the method from WHO (2005) with some amendments according to AlSalhi et al. (2020) and Yingngam et al. (2022). 200 mg of essential oil was diluted in 20 ml of DMSO followed by a ten-fold dilution method with distilled water to obtain several concentrations of 20, 40, 60, 80, and 100 ppm. DMSO blank control was diluted in 100 mL of distilled water without the KGL essential oil. In every sample, about 200 mL of the KGL essential oil diluted solution is introduced to 20 third-instar mosquito larvae. Every test was run in triplicate. Three duplicate containers were utilized in the blank control group. The test containers are held at 25–28 °C and preferably a photoperiod of 12 h light followed by 12 h dark (12L:12D). After 24 and 48 hours, larval mortality, LC_{50}, LC_{90}, lower and upper confidence limit (these are the lower and upper confidence limits for the LC_{50} and LC_{90} values) were documented. LC_{50} and LC_{90} values indicate the concentration of the essential oil required to kill 50% and 90% of the mosquito larvae, respectively. The mortality of larvae can be calculated using the formula Equation (2):

$$\text{Mortality (\%)} = (X-Y)/X \times 100\% \quad (2)$$

where X is the percentage of larvae survival in the untreated sample (control), and Y is the percentage of survival in the treated sample.

MORPHOLOGICAL ASSESSMENT OF KGL BIOTOXICITY

A plantlet with three leaves is placed in a 500 mL plastic container. To prevent algae growth in all experimental conditions, cylindrical flasks made of opaque high-density polyethylene (HDPE) were utilized (Yadav et al. 2021). All containers were kept in identical surroundings with

constant ambient temperature (between 23–26 ºC during day and night) and light (16–8 hours of day and night periods) being constant. For every week, the growth of the plantlet is observed, and after 30 days of growth, harvesting is completed. The blank control group consisted of 500 mL of tap water, and every test was run in triplicate. The number of leaves, the weight of the plantlet (g), leaf area (cm^2), stem length (cm), and root length (cm) were assessed as morphological growth characteristics (Noor Asyraf et al. 2018). The leaf area of a plantlet was calculated using Equation (3) adapted from He et al. (2020).

$$\text{Area of leaf } (cm^2) = a \times L \times W \quad (2)$$

where L and W are the length and width of the leaf, respectively, and a is a constant with a value of 0.69.

TABLE 1. KGL essential oil yielded from different extraction method

Extraction Method	Extraction Time (min)	KGL Essential Oil Yield Percentage (%)
Hydrodistillation (HD)	240	1.6
Microwave-assisted extraction (MAE)	60	0.59
Solvent-Free Microwave Extraction (SFME)	120	1.33 ± 0.16

RESULTS AND DISCUSSION

ESSENTIAL OIL YIELD AND COMPOSITION

Table 1 presents an average oil yield of 1.33% after 120 minutes at microwave irradiation power of 450 W. In comparison, the extraction of rhizomes from the *Zingiberaceae* family, using HD and MAE methods as documented by AlSalhi et al. (2020) and Abd Aziz et al. (2017), resulted in yields of 1.6% at 240 minutes and 0.59% at 60 minutes, respectively. Despite the SFME running for only 120 minutes, it produced a substantial yield of 1.33%, highlighting the efficiency of the SFME process.

Figure 1 displays the gas chromatography-mass spectrometry (GC-MS) analysis of KGL essential oil. The chromatogram and associated mass spectra identify the predominant chemical constituents within the oil. The most abundant component identified is *ethyl p-methoxycinnamate*, which is known for its applications in various therapeutic and cosmetic formulations due to its UV-absorbing and anti-inflammatory properties. Following this, the second most abundant compound is (Z)-ethyl 3-(4-methoxyphenyl) acrylate, a derivative that contributes to the oil's fragrance and potential antioxidant benefits. The third major constituent is 2-propenoic acid, 3-phenyl-, ethyl ester, another essential compound that might play a role in the oil's overall effectiveness in skincare applications due to its possible antimicrobial and anti-inflammatory properties. These findings suggest a complex composition that underlines the multifunctional potential of KGL essential oil in various therapeutic and cosmetic contexts.

The GC-MS study yielded results consistent with earlier research (Alsalhi et al. 2020; Kumar, 2020; Wang et al. 2021). The same batch of KGL rhizomes was utilized to extract the essential oil using hydrodistillation, supercritical fluid, and steam distillation. The primary component, *ethyl p-methoxycinnamate*, is consistent in the rhizome essential oil despite the different extraction methods. This shows that the SFME reduces the extraction time, produces higher oil yield, and effectively preserves the quality of essential oils.

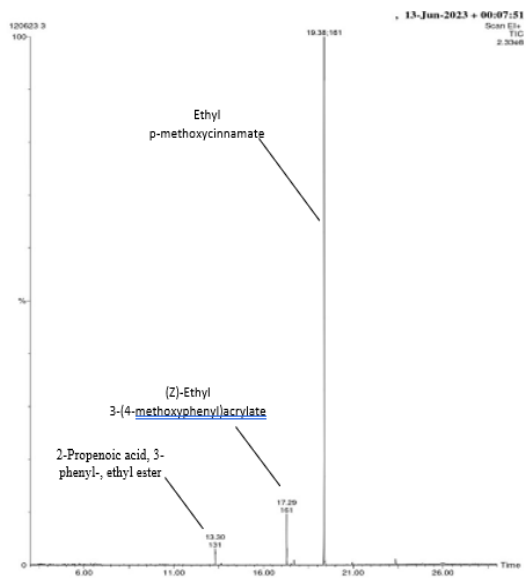


FIGURE 1. ADMIS GC-MS of KGL Essential Oil Analysis

TOXICITY OF ESSENTIAL OIL EXTRACTED FROM KGL TO AEDES AEGYPTI LARVAE

ESSENTIAL OIL TOXICITY TOWARD AEDES AEGYPTI LARVAE

The essential oil of KGL was investigated for its larvicidal activities against third-instar mosquito larvae. Table 2 and Figure 2 show that the essential oil of KGL is particularly efficient in inducing mosquito larval death, with LC50 and LC90 values of 53.97 ppm and 90.50 ppm, respectively,

for 24 hours, while 48.39 ppm and 74.33 ppm, respectively, for 48 hours. It is worth noting that the blank solution resulted in high survival rates, with 96.67% and 91.67% of the subjects surviving at 24 hours and 48 hours, respectively.

TABLE 1. Toxicity of KGL essential oil stock solution towards *Aedes aegypti* larvae

Residence time, t (hrs)	LC_{50} (ppm)	LCL (ppm)	UCL (ppm)	LC_{90} (ppm)	LCL (ppm)	UCL (ppm)	Linear Equation	R^2
24	53.97	41.67	64.00	90.50	81.00	100.00	y = 1.05x + 2.19	0.98
48	48.39	35.50	63.00	74.33	63.00	92.00	y = 0.99x – 1.65	0.99

LCL: Lower confidence limit
UCL: Upper confidence limit

This underscores the significant impact of KGL essential oil on the observed mortality rates. This study carefully controlled water quality and light intensity to ensure consistent conditions for all plant samples. While these factors are crucial for plant growth, the aim was to isolate the effects of the KGL essential oil treatment. It should be noted that maintaining constant water quality and light intensity does not imply they are irrelevant to growth; instead, it ensures that any differences in growth can be attributed to the essential oil treatment. The results indicate that the water condition combined with KGL essential oil may not have been optimal for plant growth. This suggests that further parameter adjustments might be necessary for improved outcomes.

In Figure 2, an inclined pattern illustrates the positive correlation between essential oil concentration and larvae mortality at varying exposure times. Extending the exposure to KGL essential oil increases larvae contact with the oil's active compounds, leading to more significant ingestion and absorption of these compounds, ultimately resulting in mortality. This consequently reduces the LC50 and LC90 values, as the required dose for mortality decreases with prolonged exposure. The same pattern is observed in Trinh et al. (2023) study of *Magnolia faveolate* leaves toward Aedes aegypti larvae, where LC50 and LC90 values dropped from 20.2 ppm and 29.6 ppm to 14.2 and 27.1 after 24 hours and 48 hours of exposure, respectively. The results indicate that the KGL essential oil effectively eliminates mosquito larvae, and prolonged exposure leads to higher mortality rates. Additionally, the study done by Anwar et al. (2022) supports our results by demonstrating that KGL extracts, particularly those derived from ethanol and water, exhibit high larvicidal activity. The study reported LC50 values of 1.563 ppm for ethanol and 1.33 ppm for water after 24 hours, further validating the potent larvicidal effects of KGL extract. These findings reinforce the potential of KGL as an effective, safe, and eco-friendly larvicide.

Larvae have a pair of foregut and midgut segments linked by a valve to form their digestive system. Digestion occurs in the midgut section, where food is metabolized, and nutrients are absorbed. When a larva dies from a larvicide, the midgut segment may rupture, giving the observed black appearance as seen in Figure 3 (a). The observed larvicidal effects of KGL essential oil can be primarily attributed to *ethyl p-methoxycinnamate*, a key bioactive compound. This conclusion is substantiated by comprehensive phytochemical

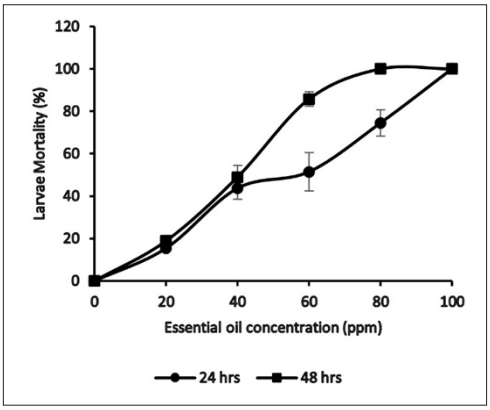
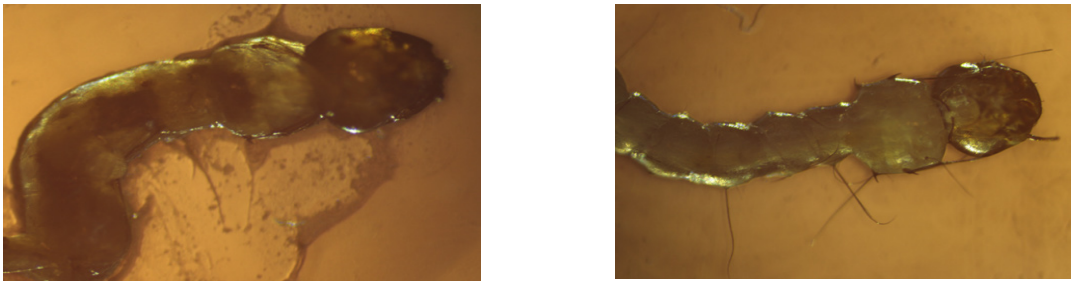


FIGURE 2. Larvae mortality at different essential oil concentrations at 24 and 48 hours

analyses and experimental studies and results are found in Figure 1. This high concentration of ethyl p-methoxycinnamate underscores its significant role in the biological activity of the extract. Building upon previous research by Yang et. al (2004), it was demonstrated that the methanol extract of KGL rhizome exhibited potent larvicidal activity, causing 100% mortality in mosquito larvae at a concentration of 100 ppm. This high efficacy

suggests that *ethyl p-methoxycinnamate*, a major constituent of K. galanga, is responsible for the observed mortality in the larvae. Meanwhile, the midgut of the larvae suffered the most harm due to the numerous functions that take place there, including digestion, nutrient absorption, ion transportation, and osmoregulation (Rohmah et al. 2020). On the other hand, Figure 3(b) observed an alive larva that shows no sign of darkening in the midgut area, suggesting that no damages are observed in the midgut section of the larvae.



(a) (b)

FIGURE 3. *Aedes aegypti* larvae observed using Olympus BX41 System Microscope in 10X magnification (a) Dead larvae subjected to essential oil exposure for 48 hours; (b) Alive larvae subjected to tap water exposure for 48 hours

TABLE 3. Plantlet weight when subjected to different essential oil concentrations for 5 weeks period

C[c] (ppm)	R[d]	Plant Weight by Week, W_p (g)				
		1	2	3	4	5
Blank	1	1.80	2.30	2.60	2.90	3.20
	2	2.50	3.70	4.00	4.10	4.20
	3	4.40	4.70	4.90	5.00	5.10
50 ppm	1	2.80	3.90	4.30	4.40	4.60
	2	2.70	3.30	3.30	3.70	3.70
	3	2.70	3.30	3.30	3.60	3.60
100 ppm	1	3.20	4.00	4.60	4.60	4.30
	2	2.80	3.10	3.30	3.30	3.30
	3	5.60	6.00	6.00	6.00	5.70

[c] C: Treatment Concentration
[d] R: Replicate

MORPHOLOGICAL GROWTH OF EPIPREMNUM AUREUM

ESSENTIAL OIL EFFECT ON PLANTLET WEIGHT

Table 3 represents the essential oil effect on plantlet weight. The amount of essential oil added to the water influences plant development significantly. In particular, all treatments demonstrated a rise in weight during the second week of the trial, with the 50 ppm treatment showing the most significant gain followed by blank and 100 ppm treatments. However, the increment shrank from the third to the fourth week, and by the fifth week, the weight of the plant receiving the 100 ppm treatment had dropped. Although the precise mechanism is unknown, higher essential oil concentrations can potentially interfere with the plant's metabolism, which might reduce growth and viability. This is in line with Dahiya et al. (2020), study where *Pogostemon benghalensis* essential oil causes oxidative damage in *Avena fatua L* and *Phalaris minor Retz*, thus inhibiting their growth. Therefore, a concentration range of 50 ppm is recommended for Kaempferia galanga essential oil. This dosage has been found to promote plant growth and offer protective benefits without causing phytotoxicity. However, higher concentrations, such as 100 ppm, may inhibit growth and cause stress to the plantlets.

It should be highlighted that the duration of exposure of plants to essential oil also can impact their growth. Although brief exposure to essential oils may not affect plant growth, prolonged exposure may result in the gradual

build-up of essential oils within the plant and interfere with regular biological functions, as shown in Figure 4. The findings indicate that the use of KGL essential oil significantly enhances plantlet growth with short-term exposure of up to 7 days. The data suggest that while KGL essential oil can initially promote plantlet growth at both 50 ppm and 100 ppm, prolonged exposure, especially at higher concentrations, may hinder growth and lead to degradation. The decline in weight increment at higher concentrations and longer exposure times could be due to the oil forming a hydrophobic barrier on the roots. This would reduce water uptake and impact the overall health and growth of the plantlets, necessitating careful control of essential oil concentrations and application duration. Thus, it is important to change the water medium weekly to support the excellent growth of the plantlets. It can be concluded that even though the studies on the antifungal and antioxidant properties of KGL essential oil have indicated the potential for bioactive properties, its application needs to be carefully controlled to avoid negative impacts on plant health. The formation of an oil layer can indeed reduce the root's ability to absorb water effectively, leading to stress and degradation over time.

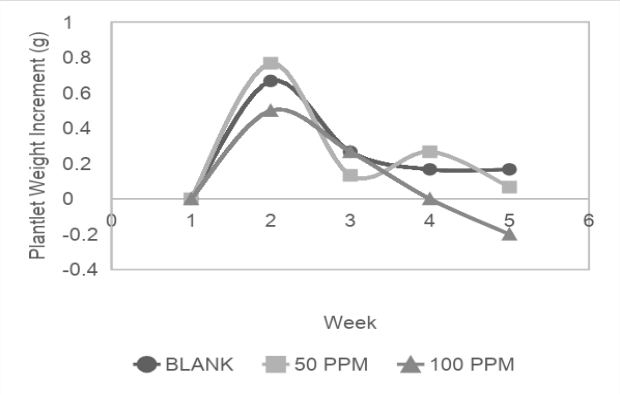


FIGURE 2. Plantlet weight increment when subjected to different EO concentrations for 5 weeks period

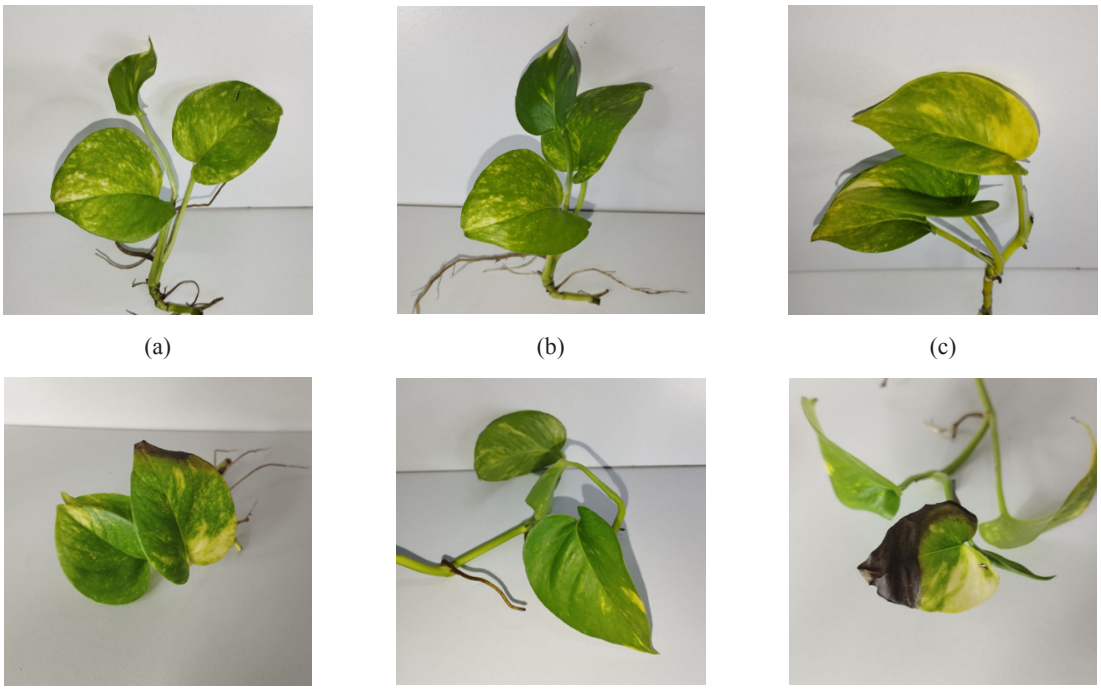
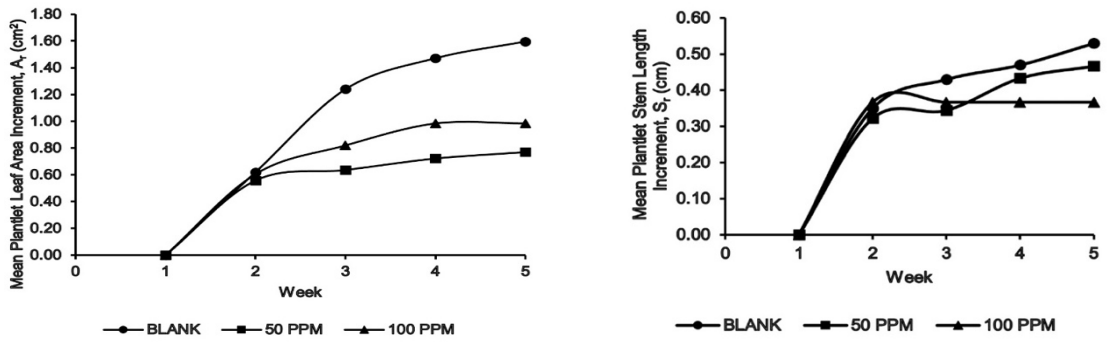


FIGURE 5. Leaf growth of *Epipremnum aureum* in different essential oil concentrations; (a): Plantlet in blank solution on 1st week; (b): Plantlet in blank solution on 5th week (c): Plantlet in 50 ppm solution on 1st week; (d): Plantlet in 50 ppm solution on 5th week; (e): Plantlet in 100 ppm solution on 1st week; (f): Plantlet in 100 ppm solution on 5th week

A study on the effects of rosemary essential oil on tomato seedlings by Souri and Bakhtiarizade (2019) shows similar patterns. In this rosemary study, it was observed that while lower concentrations of essential oil promoted plant growth, higher concentrations or prolonged exposure led to stress responses, such as reduced plant height and alterations in physiological traits like SPAD value (chlorophyll content). This is consistent with our observation that KGL essential oil is beneficial for plantlet growth in the short term but can lead to degradation with extended exposure. The study found that foliar application of 1000 ppm rosemary oil reduced plant height but increased other growth parameters such as root fresh weight and leaf SPAD value, indicating a complex interaction between the concentration of essential oil and plant growth responses. This supports our recommendation that KGL essential oil be used cautiously, with periodic changes to the water medium to avoid adverse effects from prolonged exposure.

ESSENTIAL OIL EFFECT ON LEAF AND STEM DEVELOPMENT

The plantlet exposed to blank solution develops healthily with a significantly deeper intensity of green color on its leaves after 5 weeks of exposure to the treated aquatic environment. On the other hand, plantlets exposed to treatments of 50 and 100 ppm appear with decayed leaves, with 100 ppm treated leaves showing more rotting than 50 ppm. The observation that plantlet thrives in the blank solution (Figure 5(b)), which has no essential oils, implies that other environmental factors, such as water quality and light intensity, do not affect the plant's ability for growth. As shown by the appearance of decayed leaves in Figure 5(d) and Figure 5(f), raising the dosage of KGL essential oil has a negative impact on the growth of *Epipremnum aureum*. This indicates that the symptoms reported, such as decaying leaves, could be attributed to a specific active ingredient found in essential oils that is detrimental to the plant. Essential oils are hydrophobic and tend to form a layer on the surface of the roots when applied excessively. This hydrophobic layer can prevent water from reaching the root surface, reducing the plant's ability to absorb water effectively. Reduced water uptake can impair the plant's physiological processes, including photosynthesis. Water is a crucial component in photosynthesis, and any hindrance in water absorption can lead to decreased photosynthetic efficiency and growth. Pavela and Benelli (2016) observed similar effects in their study, where they found that lower concentrations of essential oils could promote plant growth, while higher concentrations often resulted in phytotoxicity. For instance, their research showed that concentrations of essential oils below 0.1% significantly enhanced the growth of certain plant species. In contrast, concentrations above 0.5% caused notable growth inhibition and even plant death in some cases. This demonstrates the dual nature of essential oils as both growth promoters and inhibitors depending on the concentration used.



(a) (b)

FIGURE 6. Plantlet observation when subjected to different essential oil concentrations for 5 weeks period; (a) Leaf area increment; (b) Stem length increment

The amount of essential oil present significantly affects the plant leaves' growth and development. It is shown from Figure 6(a) that the plantlet in every treatment increased in leaf area in the second week of the trial, with the blank treatment showing the most significant growth, followed by the 100 ppm treatment and the 50 ppm treatment showing the smallest increase. However, the increase in leaf area declined steadily from the second to the fifth week.

Table 4 shows that as concentration increases, plantlet leaf area stops increasing in a shorter period, since both plantlets in 50 ppm and 100 ppm stop showing area increase at week 3 of observation. This shows that when essential

oil concentration rises, it may have long-term detrimental consequences on plant development, particularly in the leaf area. Similarly, the growth of stems parallels the area of the leaves observed. Higher concentrations of essential oil potentially interfere with the plant's metabolism, which might reduce growth and viability and can be explained by the phytotoxic effects of secondary metabolites in essential oils at high concentrations. Conversely, the positive influence of essential oils at lower concentrations on plantlet growth can be attributed to their role as growth regulators or protective agents, as noted by Verma et.al (2010).

Figures 6(a) and 6(b) present the effects of different essential oil concentrations on plantlet growth, revealing a complex relationship between phytotoxicity and tissue-specific stimulation. At 100 ppm, the essential oil promotes leaf expansion but inhibits stem elongation, indicating distinct sensitivities between leaf and stem tissues. Leaf tissues benefit from bioactive compounds, enhancing cell division, while stem tissues may experience growth inhibition due to oxidative stress or disruptions in cell wall synthesis. In contrast, the 50 ppm concentration offers a balanced effect, promoting notable stem elongation alongside some leaf area increase, though not as much as at 100 ppm. This concentration likely reduces harmful effects on overall plant growth while still harnessing the essential oil's benefits.

ESSENTIAL OIL EFFECT ON ROOT DEVELOPMENT

According to Figure 7, it is observed that the concentration of essential oil has a notable impact on the elongation of plant roots. While the control group serves as the basis for comparison, it is important to focus on the specific effects of the KGL essential oil treatment on root growth. The observed differences in root growth among the treated groups suggest that the essential oil's application influenced plant development. The root length increment of the 50 ppm essential oil treatment was stable, implying that this essential oil concentration might have been the most favorable for root development.

TABLE 4. Leaf area when subjected to different essential oil concentrations for 5 weeks period

C^c (ppm)	R^d	Area by Week, A (cm^2)				
		1	2	3	4	5
Blank	1:L1	16.13	16.31	16.91	16.91	16.91
	1:L2	16.81	16.81	16.81	17.10	17.39
	1:L3	6.57	6.57	6.76	7.80	7.80
	2:L1	18.82	19.56	19.87	20.18	20.18
	2:L2	13.52	13.80	14.43	14.43	14.43
	2:L3	8.39	9.49	9.49	9.49	9.49
	2:L4	0.00	3.77	7.73	8.11	8.40
	3:L1	16.23	16.23	16.23	16.23	16.62
	3:L2	16.97	16.97	16.97	17.26	17.54
	3:L3	25.39	25.71	26.03	26.03	26.03
50 ppm	1:L1	19.13	20.34	20.34	20.34	20.34
	1:L2	7.78	9.11	9.49	9.49	9.49
	1:L3	20.18	20.18	20.18	20.18	20.18
	2:L1	18.39	18.95	18.95	18.95	18.95
	2:L2	14.30	14.55	14.55	14.55	14.55
	2:L3	10.76	11.12	11.12	11.55	11.55
	3:L1	9.00	9.40	9.73	9.73	9.73
	3:L2	10.01	10.35	10.35	10.70	11.12
	3:L3	15.73	16.28	16.28	16.28	16.28

continue...

...cont.

	1:L1	19.88	21.42	21.74	21.74	21.74
	1:L2	8.28	9.72	9.94	9.94	9.94
	1:L3	18.22	19.04	19.04	19.04	19.04
	2:L1	18.49	18.49	18.95	18.95	18.95
100 ppm	2:L2	10.70	10.70	11.03	11.50	11.50
	2:L3	3.59	4.00	4.00	4.14	4.14
	3:L1	29.19	29.19	29.19	29.19	29.19
	3:L2	14.71	15.94	15.94	16.81	16.81
	3:L3	28.54	28.54	29.15	29.15	29.15

[c] C: Treatment Concentration
[d] R: Replicate
1:L1: Replicate 1, Leaf Number 1
Bolded values indicate stagnant area increase

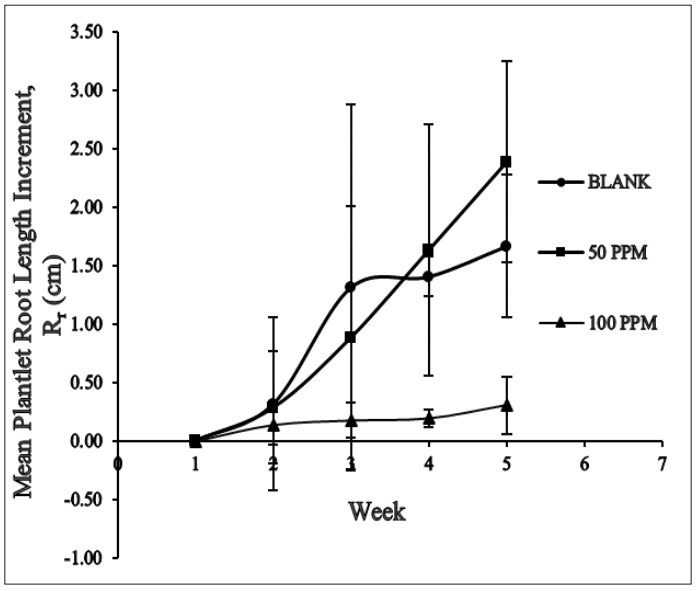


FIGURE 7. Root length increment when subjected to different essential oil concentrations for 5 week period

Nevertheless, the root length increment of the 100 ppm essential oil treatment was considerably reduced, indicating that elevated essential oil concentrations could potentially adversely affect root growth and progress. Excessive use of essential oils can disrupt the plant's physiological processes, including photosynthesis and nutrient uptake. Additionally, the hydrophobic nature of essential oils can form a barrier on the root surface, reducing water absorption and potentially hindering root growth. This highlights the importance of minimizing the essential oil concentrations to avoid adverse effects on plant growth.

According to the findings in Figure 8, both the blank and 50 ppm samples show significant root growth. Meanwhile, the roots exposed to 100 ppm of essential oil display signs of decay, such as a darkening color and a flaccid texture. In summary, these observations indicate that although essential oils may promote root growth, it is crucial to carefully consider the appropriate dosage and duration of exposure to prevent any negative impact on plant growth. This observation is supported by studies that have explored the effects of essential oils on plant root systems. For instance, Souri and Bakhtiarizade (2019) demonstrated that while essential oils like rosemary oil can stimulate root growth at lower concentrations, higher concentrations or extended exposure can induce stress responses, including reduced root vitality and overall plant health.

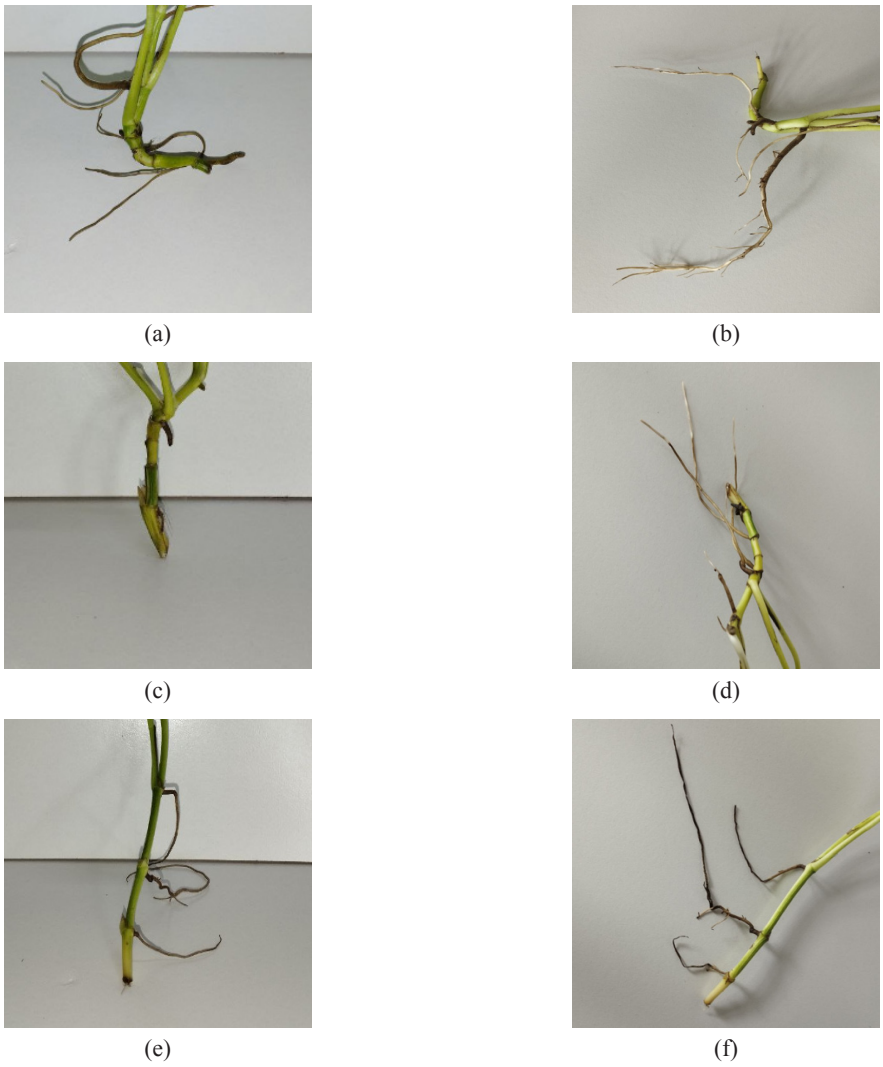


FIGURE 8. Root growth of *Epipremnum aureum* in different essential oil concentrations; (a): Plantlet in blank solution on 1st week; (b): Plantlet in blank solution on 5th week (c): Plantlet in 50 ppm solution on 1st week; (d): Plantlet in 50 ppm solution on 5th week; (e): Plantlet in 100 ppm solution on 1st week; (f): Plantlet in 100 ppm solution on 5th week

Similarly, Bakkali et al. (2008) highlighted the potential phytotoxic effects of essential oils, noting that their hydrophobic nature can form a barrier around roots, impeding water and nutrient absorption and leading to root desiccation and damage. Additionally, research on root system architecture under various biostimulant treatments, including essential oils, has shown that excessive or prolonged exposure can disrupt normal root development, corroborating the degraded root structures observed in the 100 ppm sample.

Table 5 shows the alternative hypothesis that there is a difference between the treatment and control in either direction is tested using the two-tailed test. If the p-value is lower than the alpha significance threshold, the test's result is regarded as significant. However, p-values of 0.05, 0.05, 0.05, and 0.04 from the weight of the plantlet in the blank solution, the weight of the plantlet in 50 ppm essential oil, the total most significant specific area of plantlet in 50 ppm, and the total most significant area on the blank solution are the only parameters that are significant to the study. Therefore, as the p-value of the rest is higher than 0.05, the essential oil is regarded to have an insignificant role in the growth of *Epipremnum aureum*. Upon observing plant growth, this two-tailed test is referred from Noor Asyraf et al. (2018).

TABLE 5. Mean comparison of growth parameters in various essential oil concentrations towards *Epipremnum aureum* growth after 5 weeks (Duncan test, α= 0.05)

C[c](ppm)	W_p [e] (g)	TBA[f] (cm^2)	TBSA[g] (cm^2/g)	RL[h] (cm)	SL[i] (cm)	RN[j]
Blank	0.05	0.04	0.11	0.35	0.28	0.42
50 ppm	0.05	0.08	0.05	0.20	0.28	0.13
100 ppm	0.24	0.30	0.92	0.18	0.20	0.42

[c] C: Treatment Concentration
[e] W_p: Plantlet Weight
[f] TBA: Total Biggest Area
[g] TBSA: Total Biggest Specific Area
[h] RL: Root Length
[I] SL: Stem Length
[j] RN: Root Number

CONCLUSION

This study aimed to assess the larvicidal efficacy of *Kaempferia galanga Linn* (KGL) essential oil and its impact on plant development. The findings indicate that KGL essential oil has the potential to be an effective natural larvicide, providing control over mosquito larvae populations without causing adverse effects on non-target species, as often seen with synthetic chemicals. However, the study also revealed that excessive use of the essential oil can harm plant development, particularly in root, leaf, and stem growth, as it forms a hydrophobic barrier on roots, leading to reduced water uptake and affecting overall plant nutrition. Optimizing the concentration and application methods of KGL essential oil is crucial to effectively manage mosquito populations while safeguarding plant health. This involves utilizing concentrations that can efficiently control larval populations while avoiding any negative impact on plant growth. Further research is essential to fine-tune these guidelines and explore the long-term effects on both target and non-target organisms. By balancing larvicidal efficacy with the need to protect plant health, KGL essential oil can be developed as a sustainable alternative to synthetic larvicides, thereby contributing to integrated pest management strategies that are effective and environmentally friendly.

ACKNOWLEDGEMENT

The authors are grateful to the Faculty of Chemical Engineering and Universiti Teknologi MARA (UiTM) for providing facilities for this project under GKP Grant (600-RMC/GPK 5/3 (114/2020.

DECLARATION OF COMPETING INTEREST

None.

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