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Research article

Screening of natural product extracts for fly repellent and larvicide

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Abstract

Fly is one of the vectors of foodborne pathogenic vectors and causes myiasis in humans and animals. To prevent the contamination of food and myiasis, various chemical products are commonly used. However, eco-friendly is now a trend all over the world so natural products are the alternative ways to reduce the chemical residue in the environment. The present study was screened the 12 natural products in 4 groups; the essential oil plants, the alkaloid plants, the cyanogenic plant, and the inorganic compounds which use a simple extraction on the fly repelling and larvicide. The fly repelling efficacy with 2 criteria consist of the time of the first swarm and percent repelling (PR) in the first 15 min. The larvicidal efficacy was determined by the mortality rate within 24 h. The Kaffir lime peels aqueous crude extract exhibited the best result on fly repelling from both criteria with no significant difference. The bamboo shoots aqueous crude extract gave the best result on larvicide with significantly different from other groups ($P < 0.001$) which was faster than the positive control group (Coumaphos & Propoxur). Moreover, the high mortality rate reached 73.33 ± 15.28% within 24 h, higher than the positive control group. The present study indicates the power of common natural products on fly repellent and larvicide that may apply for fly repellant to reduce the food contamination especially fresh materials and prevention or treatment of animal and human myiasis in rural areas.

Keywords: Fly repellent, Larvicide, Myiasis, Natural product extract

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INTRODUCTION

Fly is a major vector cause of microbial pathogens contaminated food and human and animal myiasis in worldwide especially underdeveloped and developing countries (Sukontason et al., 2007; Chansang et al., 2010; Blazar et al., 2011; Thomas et al., 2013; Onyido et al., 2014). Each year, microbial pathogens cause millions of foodborne disease cases. Pathogens causing the most foodborne illnesses, hospitalizations, and deaths each year. In the United States reported low awareness of major microbial pathogens (*Salmonella*, *Campylobacter*, *Listeria*, *Shigella* spp. and *E. coli*) (CDC, 2011; Scallan et al., 2011; Bintsis et al., 2017).

Fly repelling is the first choice for reducing the pathogens contaminated food and also myiasis. So far, many repellents and insecticides in chemical and natural products, have been used such as permethrin is both an insecticide and a repellent, N, N-diethyl-m-toluamide or DEET is the most effective against a broad spectrum, N, N-diethyl phenyl acetamide or DEPA, 1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-1-methylpropylester or KBR 3023 and for natural products such as neem oil, citronella oil, and eucalyptus oil (Brown and Hebert., 1997; Peterson and Coats, 2001). However, myiasis will be appeared in lack of hygienic and neglect wounds leads to tissue damage in a wide area caused by fly larvae. The clinical signs vary depending on fly species and the affected organ or area. However, myiasis cases can cause economic loss in animal husbandries like reducing milk yield, hide quality, weight loss, and infertility (Rahman et al., 2009; Francesconi and Lupi, 2012). In pet animals, myiasis can occur in untreated or neglect wounds. Likewise, in humans, myiasis is most commonly found in the very old, the very young, or others who are unable or unwilling to maintain wound cleanliness as patients with abundant suppuration, low vision, or mental illness (Anderson and Huitson, 2004; Francesconi and Lupi, 2017).

The distribution of human myiasis is worldwide, with more species and mostly found in low socioeconomic regions of tropical and subtropical countries (Francesconi and Lupi, 2012). Similarly, companion animals, all over the world were also reported (Coronado and Kowalski, 2009; McGarry et al., 2012; Johnson et al., 2016; Han et al., 2018; Pezzi et al., 2019).

The most important treatment for myiasis in humans and animals is to wash and clean the wound. To reduce infection and other complications. The oral or topical ivermectin can be added to increase the effectiveness of treatment in humans (Osorio et al., 2006; Puthran et al., 2012; Singh et al., 2017; Tay et al., 2018). Moreover, in animals, the drug that has been widely used for a long time is ivermectin injection (Rahman et al.,2009). At present, there are many commercials antiparasitic drugs that have been studied their efficacy on the maggot killing or fly larvicides such as afoxolaner, spinosad, milbemycin, spinosad plus milbemycin, and nitenpyram (Correia et al., 2010; Han et al., 2017; Han et al., 2018) with good efficacy but expensive. Whereas the livestock animal cases focused on wound management using ivermectin and doramectin injection for the drugs of choice (Anziani et al., 2000; Rahman et al.,2009) however, it may residue in animal products such as meat and milk, which affect consumers.

Nowadays, natural products or herbs are interesting for various purposes. Eco-friendly was the most considered point. Reducing the use of chemicals in humans and animals is much considered. Thus, various alternative fly repellant in reducing pathogens contaminated food and myiasis were focused such as neem (Siriwattanarungsee et al., 2008), tamarind seed coat extract (Soundararajan et al., 2017), and *Derris elliptica* (Sangmaneedet et al., 2005). Fly larvae expelling from fermented fish tank used fresh bamboo shoot (De Boer et. al, 2011). However, it is not practical uses because of complicated procedures and rare resource which is hard to apply in everyday life.

We, therefore, aimed to screen the efficacy of natural products which can find in Thailand's local market on the fly repellent and larvicide. Four groups; the essential oil plants, the alkaloid plants, the cyanogenic plant, and the inorganic compounds commonly found in Thailand with the simple technique were used. The time of the first swarm and percent repelling (PR) in the first 15 min, then the larval number and size were determined.

MATERIALS AND METHODS

Ethics approval

The Animal Ethics Committee of Khon Kaen University, Khon Kaen, Thailand (IACUC-KKU-5/63) approved this study.

Plant and chemical material preparations

The experimental groups were divided into 14 groups as follows 12 natural products experiments and 2 negative and positive control groups. Twelve natural products are mostly found in Thailand, consist of 4 groups; the essential oil plants, the alkaloid plants, the cyanogenic plant, and the inorganic compounds (Figure 1). The natural products were purchased from the local market in Khon Kaen (16°25'46.5"N 102°50'04.4"E). Fresh preparation of each treatment was used. In brief, each herb or natural product was individually mixed with distilled water in the ordinary electric blender in various concentrations showed in Table 1 and sieved with two layers of gauze. The solution of each natural product was ready to examine the fly repellent and larvicide except red lime and slaked lime which use only the supernatant. A positive control group was 3% w/w of Coumaphos & 2% w/w of Propoxur (Tanidil-T® , Bayer S.A, Brazil), purchased from the veterinary shop. The preparation was followed the drug instruction. The negative control was distilled water.

Table 1 The twelve natural products and the various ratio of each natural product mixed with distilled water

Group	Name of natural products	Concentration (% w/v)
Control (Negative)	Distilled water (DW)	
Control (Positive)	Coumaphos+Propoxur	10
Essential oil plant	Hoary basil (*Ocimum africanum*)	25
	Betel leaves (*Piper betle*)	25
	Kaffir lime leaves (*Citrus hystrix*)	25
	Kaffir lime peels (*Citrus hystrix*)	20
Alkaloid plant	Acacia leaves (*Leucaena leucocephala*)	25
	Yanang leaves (*Tiliacora triandra*)	20
	Cha-om (*Acacia pennata*)	25
	Tobacco (*Nicotiana tabacum*)	10
	Betel nut (*Areca catechu* Linn.)	25
Cyanogenic plant	Bamboo shoot (*Bambusa vulgaris*)	25
Inorganic compound	Red lime	20
	Slaked lime	20

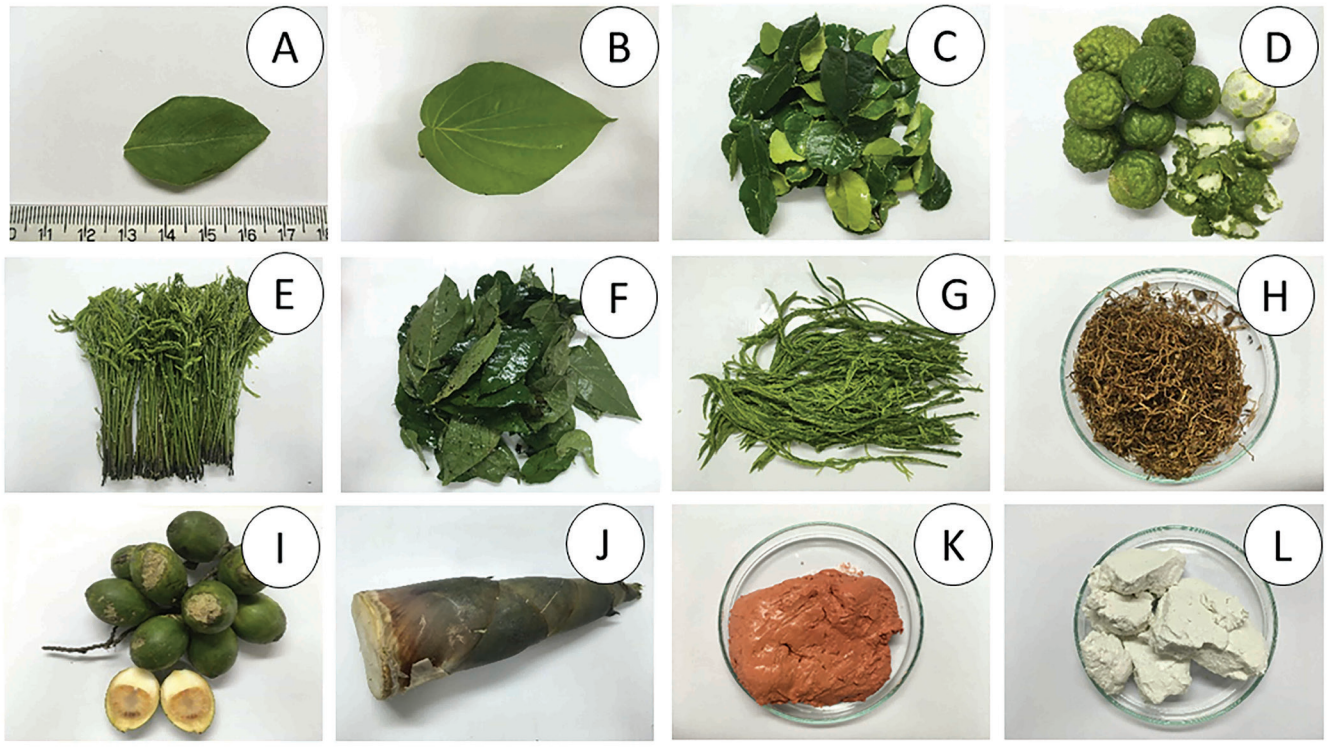

Figure 1 The natural products, A: Hoary basil (*Ocimum africanum*), B: Betel leaves (*Piper betle*), C: Kaffir lime leaves (*Citrus hystrix*), D: Kaffir lime peels (*Citrus hystrix*), E: Acacia leaves (*Leucaena leucocephala*), F: Yanang leaves (*Tiliacora triandra*), G: Cha-om (*Acacia pennata*), H: Tobacco (*Nicotiana tabacum*), I: Betel nut (*Areca catechu* Linn.), J: Bamboo shoot (*Bambusa vulgaris*), K: Red lime, L: Slake lime.

Bait preparation

Silver barb (*Barbonymus gonionotus*) fish carcasses were used as bait for observing swarm behavior of flies, purchased from the local market in Khon Kaen. Fish species was identified based on morphological keys as our previous study (Pumhirunroj et al., 2020). The freshness, size and species of fish were control factors. In brief, forty-two silver barb carcasses for three replications of each group were descaled, removed the intestines, and clean up with distilled water.

Repellent efficacy

Three silver barb carcasses for each group (12 natural products and 2 negative and positive control groups) were soaked with 15 ml of each group in the clear plastic bag for 1 min (modified from Muennu et al., 2009). Then soaked baits were placed on gauzes with solution's names-labeled papers and placed in the shade, air ventilation area, 1 meter high from the ground and kept out of pets or other animals which leads to interrupt the result, and control the relative humidity (39.67 ± 3.98%) and temperature (31.88 ± 1.50 °C).

The fly was identified based on morphological key (Bunchu, 2012). Fly repellent was evaluated by the 2 parameters: 1) the time of the first swarm, and 2) Percent Repelling (PR). The time of the first swarm of each treatment group was recording. Moreover, the swarming fly number was recorded in the data sheets every 1 min until the 15 min, and then the unusual or abnormal fly aspects were also recorded. After that, the average fly number in 15-time points of each group calculated the Percent Repelling (PR) to compare the repelling efficacy (Muennu et al., 2009). The video was also recorded for 15 min to recheck the fly number at each time point. The solution-soak baits were placed in that area to allow the fly can freely swarm for 24 h.

After allowing flies to swarm the solution-soak baits freely for 24 h, the solution-soak baits were moved into the boxes with 12 breathable holes on the box lid to avoid other flies lay more eggs. The solution-soak baits, fly eggs, maggots, gauzes, and the name-labeled papers were all included individual box in each treatment. Following this, the lids were closed then, let the fly eggs and maggots grew for one more day. The result was the number of fly eggs and maggots grew in the solution-soaked baits in each treatment, the picture of each treatment result was taken. In addition, the average maggot size was also recorded. These were the indirect criteria to evaluate the repelling efficacy of each treatment. The percentage repelling was calculated by

Percentage repelling = [(Number of landed flies in DW - Number of landed flies in Treatment group)/ Number of landed flies in DW] x100

Larvicidal activity

The larvicidal activity was determined by larval mortality rate after contacted with the natural products. The same batch of active third instar larvae was obtained from the negative control group then gently washed through the distilled water and kept in the wet condition for 1 h to observe larval motivation after that the active larvae were selected into the experiment groups. The experimental groups consist of 12 treatment groups and 2 negative and positive control groups. In each treatment consist of 3 replications, and 10 third instar larvae in each replication.

The selected larvae were dipped into the aqueous crude extract of each natural product for 3 sec, then placed the 10-selected larvae on the tissue paper size 9.5 × 5.5 inches soaked with 10 ml of the solution and covered with the 3 × 3 inches gauze soaked with 3 ml of treatment solution. Each replication is placed on the petri dish to observe the larvicidal activity of each group. The number of alive and dead larvae was recorded at 1, 15, 30, 45, 60, 75, 90, 105, and 120 min. After that, the larval mortality observation time points were every 60 min until 24 h. The No.0 paintbrushes were used to determine dead or alive larvae. The inactive larvae were gently touched with the paintbrush and observed under the stereomicroscope, the larvae absent of movement from any part of the body within 30 sec were signified as dead and, then analyzed by survival analysis. These protocol was modified from previous studies (De Boer et al., 2011; Khater et al., 2011; Khater et al., 2013; Suwannayod et al., 2018).

The mortality rate was calculated by

Mortality rate (%) = [(Number of all maggots - Number of alive maggots)/ Number of all maggot] x100

Statistical analysis

The fly repelling efficacy was represented with mean ± SD of the percentage repellent (PR) and the time of the first swarm was also reported. The differences were considered significant at $P < 0.05$. The mortality rate of the third-instar larvae was indicated the larvicidal efficacy which calculated and reported as mean ± SD then analyzed by survival analysis (Kaplan-Meier Estimate) and log-rank test to determine the differences in each group by STATA ($P < 0.05$). The percentage of larval mortality (mean ± SD) was compared with the negative and positive control group with the One-way ANOVA test. The tests for individuals differentiate with Tukey HSD at the 24^{th} h from all treatments.

RESULTS

Repellent efficacy

The fly repelling efficacy was observed in 2 periods; the 0 and 15 min using the 2 criteria, time of first swarm and percent repellent, and observation of larvae at 48 h post cultivation (number and size of maggot) for confirming the stability of natural products, summarized in Figure 2 and Table 2. Most of the fly is metallic fly, the early fly swarm was observed in bamboo shoots and acacia leaves groups within 5-sec subsequence to cha-om, betel leaves, red lime, Coumaphos & Propoxur, kaffir lime leaves, hoary basil, betel nut, tobacco, yanang leaves, distilled water, and slake lime within 60 sec and the late swarm was observed in kaffir lime peels group with the first swarm more than 5 min which was the best efficacy of fly repellant then the efficacy was reduced which may from the smelling reduction. However, in this study, we could observe the result of nicotine-affected mental signs of fly such as dizziness, laid down by their back for a few min was observed after contacted the solution within 15 min (Figure 3) which consistency to our indirect result in 48 h later.

Figure 2 Repelling efficacy of each group at 0 min (1) and 15 min (2), A: Distilled water, B: Coumaphos + Propoxur, C: Hoary basil (*Ocimum africanum*), D: Betel leaves (*Piper betle*), E: Kaffir lime leaves (*Citrus hystrix*), F: Kaffir lime peels (*Citrus hystrix*), G: Acacia leaves (*Leucaena leucocephala*), H: Yanang leaves (*Tiliacora triandra*), I: Cha-om (*Acacia pennata*), J: Tobacco (*Nicotiana tabacum*), K: Betel nut (*Areca catechu* Linn.), L: Bamboo shoot (*Bambusa vulgaris*), M: Red lime, N: Slake lime; the red arrow showed a cluster of flies (> 5 flies) landed on solution-soaked baits.

Table 2 The effectiveness of 12 natural product extracts on the repellent efficacy

Group	Name of natural products	Time point of 1st swarm (sec)	The percentage repelling (PR)
Control (Negative)	Distilled water	40	-
Control (Positive)	Coumaphos + Propoxur	12	48.57 ± 28.70
Essential oil plant	Kaffir lime peels	313	98.08 ± 3.33
	Kaffir lime leaves	15	94.44 ± 5.46
	Hoary basil	16	34.44 ± 12.94
	Betel leaves	9	65.50 ± 4.09
Cyanogenic plant	Bamboo shoot	5	94.44 ± 5.46
Alkaloid plant	Betel nut	17	89.81 ± 9.85
	Tobacco	22	84.64 ± 4.38
	Cha-om	7	62.33 ± 16.18
	Yanang leaves	35	48.00 ± 10.31
	Acacia leaves	5	29.59 ± 7.29
Inorganic compound	Slaked lime	48	46.03 ± 28.70
	Red lime	11	20.37 ± 12.19

Figure 3 The blow fly (red circle) showed the nervous sign after contacted with tobacco solution-soaked baits.

Percent repellent in the top 5 groups was kaffir lime peel (98.08 ± 3.33) subsequence to kaffir lime leaves (94.44 ± 5.46), bamboo shoots (94.44 ± 5.46), betel nut (89.81 ± 9.85), and tobacco (84.64 ± 4.38) respectively as shown in Table 2. These groups also showed good repelling efficacy, the PR more than 80% while the time of the first swarm was different but no significant difference.

Larvicidal activity

The larvae which absent of vital signs, non-motile, or movement when stimulating with paintbrush under the stereomicroscope for 30 sec were identified as dead. Morphological abnormalities were pronounced in some treatment groups after treatment such as lengthening, soft, and mild swollen (Figure 4).

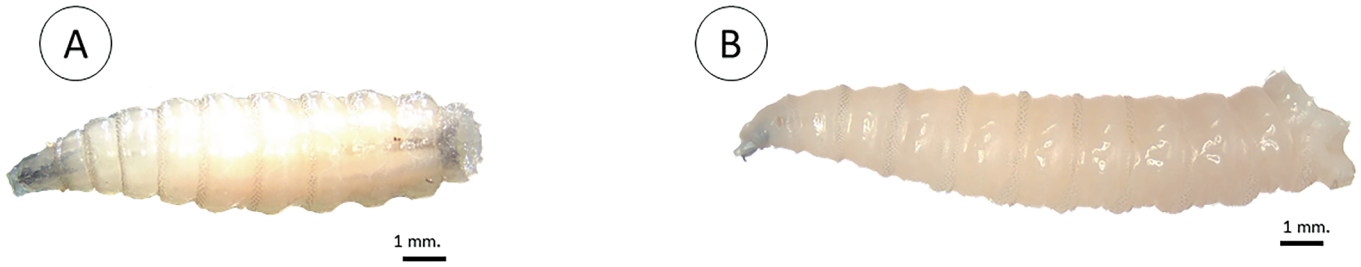

Figure 4 The representative of normal control or alive larvae (A) and dead larvae with lengthen and soft body (B) of larvae post treatment (1 h)

Larvae in the negative control group were still alive until the end of the observation time point at 24 h. The highest average larval mortality was found in the fresh bamboo aqueous crude extract group with significant differences from control groups ($P < 0.05$). The death of the first maggot was found within 60 min after observation and the highest mortality rate was found between 90-105 min while in the positive control, Coumaphos & Propoxur, was observed at 120 min subsequence to red lime, slaked lime, and kaffir lime leaves at the 3rd, 4th and 24th h respectively as shown in Figure 5.

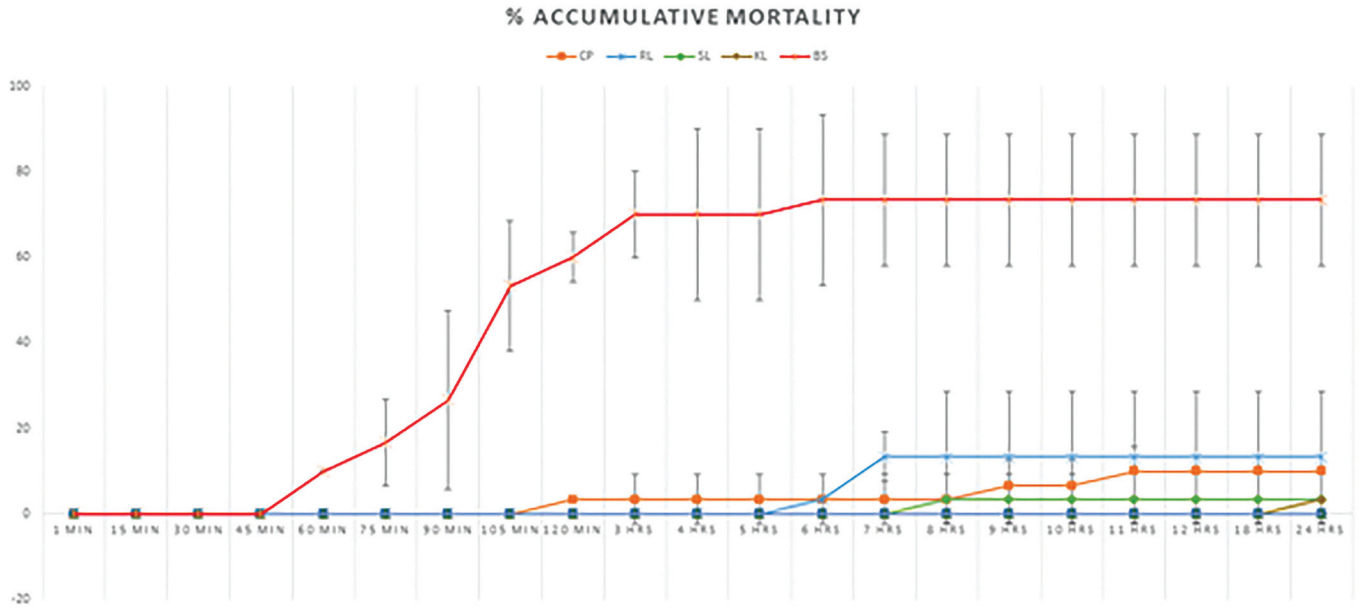

Figure 5 The percentage of accumulative larval mortality after contact with effective natural extract (Coumaphos + Propoxur; CP, Red lime; RL, Slake lime; SL; Kaffir lime leaves; KL, Bamboo shoot; BS) in each time point.

The end of observation time points at 24 h found the dead larvae from the bamboo shoot, red lime, Coumaphos & Propoxur, slake lime, and kaffir lime leaves aqueous crude extract groups, the average percentage of accumulative mortality were 73.33 ± 15.28, 13.33 ± 15.28, 10.00 ± 0.00, 3.33 ± 5.77, and 3.33 ± 5.77, respectively (Table 3). The bamboo shoots aqueous crude extract gave the best result on larvicide with significantly different from other groups ($P < 0.001$) which was faster than the positive control group (Coumaphos &Propoxur).

Table 3 The percentage of larval mortalities rate of third-instar maggots in each treatment group at 24th h

Group	Treatment	% Mortality* ± SD
Control (Negative)	Distilled water	0.00 ± 0.00
Control (Positive)	Coumaphos + Propoxur	10.00 ± 0.00
Essential oil plant	Kaffir lime peels	0.00 ± 0.00
	Kaffir lime leaves	3.33 ± 5.77
	Hoary basil	0.00 ± 0.00
	Betel leaves	0.00 ± 0.00
Cyanogenic plant	Bamboo shoot	73.33 ± 15.28*
	Betel nut	0.00 ± 0.00
	Tobacco	0.00 ± 0.00
Alkaloid plant	Cha-om	0.00 ± 0.00
	Yanang leaves	0.00 ± 0.00
	Acacia leaves	0.00 ± 0.00
Inorganic compound	Slaked lime	3.33 ± 5.77
	Red lime	13.33 ± 15.28

*The group showed the significantly difference ($P < 0.001$) compared to both control groups with Tukey HSD

In the statistical analysis of survival time, the bamboo shoot extract group was the only one group reached up the 25% and 50% (median survival) of survival time within 2 h with a significant difference from the negative and positive control group ($P < 0.001$) by log-rank test. Whereas the larvicidal ability from the red lime aqueous crude extract (lime water) group was no significant difference from both negative and positive control groups ($P > 0.05$) by log-rank test (Figure 6).

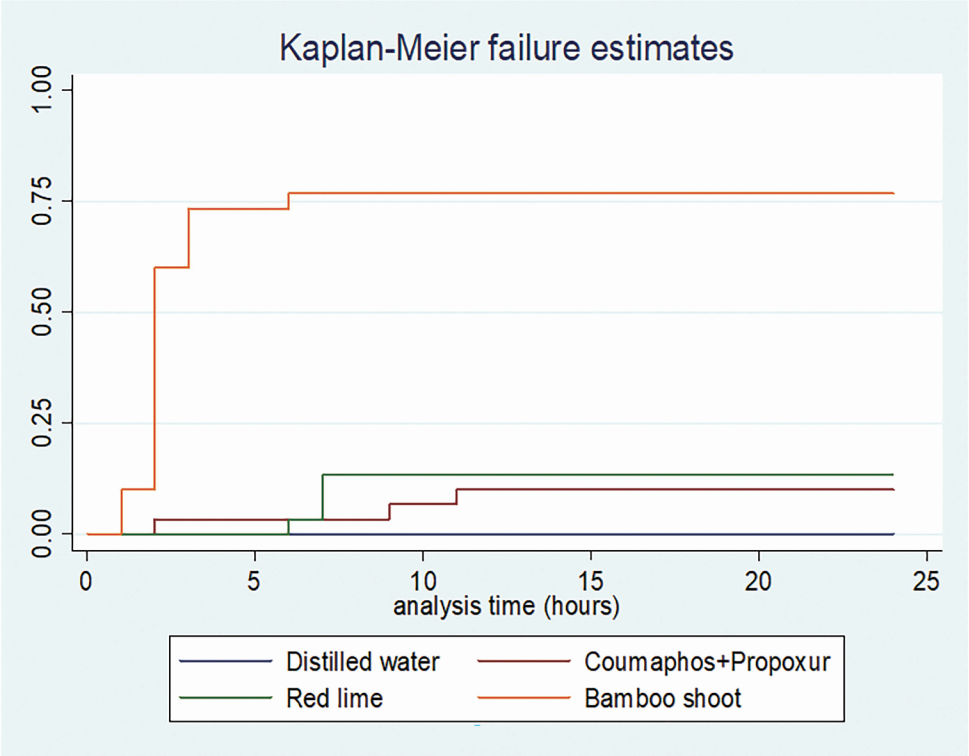

Figure 6 The accumulative maggot death within 24 h of Red lime and Bamboo shoot aqueous crude extract compared with control groups.

DISCUSSION

Our present study demonstrated the efficacy of twelve common natural products which are commonly found in tropical countries and a simple preparing process for fly repelling and larvicide. The fly repelling efficacy was found in the essential oil plants (kaffir lime peels, kaffir lime leaves), cyanogenic (bamboo shoot), and alkaloids (betel nut, tobacco) group with not significantly different from control groups. The bamboo shoot aqueous crude extract was highly effective on larvicide against the third-instar larvae.

The best result of repelling efficacy was found in the kaffir lime peels crude aqueous extract within 15 min and thereafter we could see the fly swarm and observed the larvae development. The kaffir lime leaves crude aqueous extract was exhibited the subordinate efficacy. The strong smell of both solutions from the essential oil group can cover the fishy smell which could not attract the flies (Agouillal et al., 2017). The kaffir lime peels could give the essential oil when used the ethanol extracts (Mya et al., 2017) or when extracted with a complicated procedure such as separated from the aqueous phase, dried over anhydrous sodium sulfate (Na_2SO_4) (Sutthanont et al., 2010), these complex procedures were also used with other essential oil plants include the kaffir lime leaves. Hence, the crude extract with distilled water used in this experiment was not found an effective yield.

The recovery maggot number and length in baits from this group were the smallest among other groups. This indirect criterion was indicated the prolonged larvicidal efficacy of the extract. The fly repellent efficacy in tobacco

leaves was accordingly in a previous study which was mostly found against the mosquito. The tobacco leaves from the previous report were extracted with fast pyrolysis (500 °C temperature) process. The nicotine was the highest yield from this bio-oil, its distinctive odor repelled the mosquito and interrupted to land on the surface (Jufri et al., 2016). But no other signs from mosquito was noted in any research. Another alkaloid plant that showed the fly repellent effect was betel nut (*Areca catechu* Linn.) aqueous crude extract which is the first reported. The previous studies reported the medicinal use of betel nut with various properties, helminthicide, mosquito larvicidal, antimicrobial, anti-venom, and antioxidant (Ahuja and Ahuja, 2011; Jaiswal et al., 2011; Tennyson et al., 2012; Arulpriya et al., 2013; Sari et al., 2017; Sarpangala et al., 2017; Mading et al., 2018; Yamson et al., 2019).

The cyanogenic plant (bamboo shoot) and the alkaloid plant (betel nut) were also effective on the fly repelling within 15 min. The previous study results showed the repelling efficacy of fresh bamboo shoot slice on the maggot repelling and did not mention the result on the adult fly (De Boer et al., 2011). Although the betel nut crude aqueous extract showed the efficacy against other fungi such as *Mucor* spp., *Cladosporium* spp., and *Aspergillus niger* or inhibited the development of aflatoxin producing fungus, *Aspergillus flavus* (Anthikat and Michael, 2009) but no previous report on the fly repelling.

The repelling efficacy was absent in both inorganic compounds; red lime and slaked lime, in the present solution concentration. The lowest PR was found in the red lime group, whereas the slaked lime group showed PR lower than 50%. Also, the maggot number and length were indicated the less repelling efficacy. The maggot number of the red lime group was higher than the negative control group with a significantly different ($P < 0.05$), the slake lime was also showed the higher maggot number with a not significant difference. Moreover, the maggot length was pointed in the same way that found the larger size of maggot in both groups. Similar results were found in hoary basil, betel leaves, cha-om, yanang leaves, and acacia leaves aqueous crude extract groups. At 30 min, most of the natural products were observed a lot of flies except betel nut subsequence to bamboo shoots and kaffir lime leaves, it may cause of each stability of natural products in this experiment which was fresh preparation and used aqueous extract (Thakur et al., 2011; Wang et al., 2019).

The dead maggots from this group were straight and enlengthen with no color changed (Figure 4). Not all larvae were killed due to some of these larvae escaped from the solution-soaked tissue (bamboo shoots aqueous crude extract). Similar results were observed in a previous study using bamboo shoot on repel and larvicide of myiasis-causative fly maggots, the fresh peeled and sliced bamboo exhibited significant repellence and larvicidal activity against blowfly larvae (De Boer et al., 2011). The main active compound in fresh bamboo is cyanide, the mechanism is an antifeeding properties of insect. The toxicity can be found in a high dose which uncommon in a food plant, and the concentration in this experiment did not reach the lethal dose (Jones, 1998). This demonstrated efficacy is beneficial for application in myiasis wounds due to the ease to remove these escaped maggots. Which can be indicated both larval repelling and larvicidal efficiency of fresh bamboo shoots. Moreover, the bamboo shoot was also used in Chinese medicine for a long time for cleaning wounds and healing infections (Ambika and Rajagopal, 2017; Choudhury et al., 2012) from this reason, the fresh bamboo aqueous crude extract was safe for the wound.

The dead larvae were also found in the red lime, slaked lime, and kaffir lime crude aqueous extract groups, the average mortality percentage was very low compared with the negative control group. The results in these groups were varying due to the stability of the active substance which should further study. No dead larvae were found in kaffir lime peels, hoary basil, betel leaves, betel nut, tobacco, cha-om, yanang leaves, and acacia leaves. Despite their antiparasitics or mosquito larvicidal efficacies were found from previous studies (Sutthanont et al., 2010; Tennyson et al., 2012; Pisutthanan and Pisutthanan, 2013; Singthong et al., 2014; Nouri et al., 2016; Mya et al., 2017; Lusiyana et al., 2018; Raveen et al., 2019; Weber et al., 2019; Yamson et al., 2019), the fly larvicidal efficacy was absent in this study. Although the lack of fly larvicidal efficacy in crude aqueous extract in these herbs, the higher concentration or the different process probably found the active substance which could effective. Whereas, inactive maggots were found in the positive control group however the vital sign has existed.

CONCLUSIONS

The natural products which easily find in the local market in Thailand and other tropical countries can be simple prepared and used for the fly repellent and larvicide. Moreover, the effective herbs on repelling or larvicidal efficacy should find out the suitable concentration which can give the best effect to be able to bring knowledge for further dissemination or to produce various forms of innovative products which easier to use.

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AUTHOR CONTRIBUTIONS

Data curation, investigation, methodology and formal analysis; KB, SA, AA, SB, OP, and TB. Conceptualization and project administration; KB, TB. Supervision and visualization; TB. Writing-original draft and writing-review & editing; KB, OP, and TB.

CONFLICT OF INTEREST

No conflict of interest

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