O_80

Screening of Medical Plants Extracts for Larvicidal Activity against *Culex quinquefasciatus* (Diptera: Culicidae)

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Abstract

Introduction: *Culex quinquefasciatus* (F. Culicidae) is involved in transmission of the filarial nematode, *Wuchereria bancrofti*, which is a seriously problem in several tropical and subtropical countries. Plant extracts, especially essential oils have been used as minor natural resource of insec ticides. They constitute a goodsource of bioactive compounds that are biodegradable into nontoxic products. To evaluate the larvicidal effects of the different plant extracts, *Cx. quinquefasciatus* larval stage were tested in this study. **Methods:** Efficacy of 21 essential oils extracted from steam distillation was screened against filarial vector, *Cx. quinquefasciatus*, for their larvicidal activity at the concentra-tion of 1,000 ppm in a preliminary study. The 3rd-4th instar larvae of *Culex mosquito* were exposed to extracted oils at different concentrations (25, 50, 75 and 100 ppm). **Results:** Of these, nine oils viz. kaempfer oil, holy basil oil, chinese chives oil, galanga oil, turmeric oil, dark blue temu oil, citronella grass oil, kaffir lime oil and sweet basil oil exhibited 100%larvicidal activity at 1,000 ppm. These nine oils were screened further against *Cx. quinquefasciatus*, for their larvicial effect at different concentra tions (25, 50, 75 and 100 ppm). Percentage mortality was determined after 24 hours exposure. The highest potential was established from kaempfer, followed by turmeric, holy basil, chinese chives, galanga, dark blue temul, kaffir lime, citronella grass and sweet basil, with LC_{50} values of 13.29, 17.54, 18.74, 20.59, 25.18, 25.93, 63.22, 68.34 and 71.38 ppm, respectively. **Conclusion:** Regarding these results, these oils could be used to develop a new formulation to control mosquito-borne disease vectors.

Keywords: plant extracts, *Culex quinquefasciatus*, larvicidal activity, steam distillation, botanical insecticide

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1. Introduction

Mosquitos (Diptera: Culicidae) are the most efficient vectors which transmit pathogens causing human malaria, dengue, yellow fever, filariasis, viral encephalitis and other mortal diseases (Service, 1983). Interestingly, *Culex quinquefasciatus* Say, commonly known as the southern house mosquito, is extensively distributed in tropical and subtropical zones and closely related to human habitation because of its anthropophilic, endophilic blood feeding habits and breeding sites (Forattini et al., 1993). In tropical Africa and Southeast Asia,*Cx. quinque-fasciatus* play a vital role of transmission of the filarial nematode, *Wuchereriabancrofti* (Foster and Walker 2002). Moreover, previous environmental observation from Thailand reported that insufficient disposal of human waste together with waste water provide main *Cx. quinquefasciatus* breeding sites that are always found in a big city i.e. Phitsanulok province (Pumidonming et al., 2005).

Synthetic insecticides have been widely used for controlling mosquito populations over the past years. Nonetheless, resistance to insecti cides, lethal effects on non-target organisms, and human toxicity are some undesirable effects caused by these methods (Llinás et al., 2010; Prakash and Rao, 1997).The development of biologically active materials for mosquitocides that do not confer cross-resistance to present insec ticides is constantly needed. Therefore, the use of natural products represents an alternative pest control method. Plants and their derivatives have been recognized as an important natural resource of insecticides (Prakash and Rao, 1997). Extracts from plants, especially essential oils have been extensively used as minor natural resource of insecticides that can act as repellents,oviposition attractants,larvicides and insect growth regulators (Mathew et al., 2009). They constitute a rich source of bioactive compounds that are biodegradable and potentially suitable for use to control mosquitoes (Batallán et al., 2010; 2013). Botanical insecticides used for their larvicidal properties against mosquito-borne disease vectors are low-cost, ecofriendly and not harmful to other non-target organism (Kaushi and Saini, 2009). Moreover, its larvicidal agent can be applied anywhere, where breeding sites are well defined, limited in number and accessible. To evaluate the larvicidal effects of the different plant extracts, *Cx. quinquefasciatus* larval stage were tested in this study.

2. Materials and Methods

Extraction of essential oil

For plant materials, 21 medical plant species obtained from local area were screened (Table 1). All plant oils were extracted in laboratory of Chemistry program, PSRU by using steam distillation. Afterwards, those plant oils were evaporated to dryness under reduced pressure. The crude extract obtained was kept in dark glass bottles covered tightly with aluminum foil, labeled then stored in a refrigerator till needed for experiments. An adequate of selected oils was dissolved in distilled water using 2 ml of 95% ethanol to produce a stock solution at 1,000ppm and later this solution was applied to prepare the serial dilutions oftarget oilthrough dilution of thestock solution with distilled water for bioassay.

Larvicidalbioassy

Culex quinquefasciatus were collected from Pibulsongkram Rajabhat University (PSRU), Phitsanulok, Thailand and maintained at Biology program, Faculty of Science and Technology, PSRU. Culex larvae were morphologically identified using the key of Rattanarithikul (2005). The 3^{rd}-4^{th} instar larvae of *Cx. quinquefasciatus* were used for larvicidal activity. Three replicates were done with 10 larvae per replicate for each concentration (WHO, 1981). In addition to three replicates,the control contained 0 ppm of oil, 2ml of 95% ethanol and distilled water. For preliminary screening 1,000 ppm of the respective oils were used against *Cx. quinquefasciatus*; the selected active oils were screened at 25, 50, 75 and 100 ppm against this mosquito species (Table 2).

Statistical analysis

The mortality data were subjected to log probit regression analysis (Finney, 1971) to determine the median lethal concentrations (LC_{50}) and 90% lethal concentration (LC_{90}). The percentage of larval mortality was calculated and when control mortality ranged from 5-20% it was corrected using Abbott's formula (Abbott, 1925). LC_{50} and LC_{90} values were calculated by Probit regression (SPSS 17.0).

3. Results and Discussion

In the preliminary screening 21 essential oils were screened against *Cx. quinquefasciatus* at the concentration of 1,000 ppm in order to select effective oils on the basis of larvicidal property for further study. The results showed that larvicidal bioassay increased with increased dosage in all trials. However, it was observed that nine oils viz. kaempfer oil, holy basil oil, chinese chives oil, galanga oil, turmeric oil, dark blue temu oil, citronella grass oil, kaffir lime (peel) oil and sweet basil oil showed 100% larvicidal activity. Besides these, eucalyptus oils showed 83.33% larvicidal activity. Among the 21 oils, peanut oil exhibited the least larvicidal effect (Table 1).

Among the oils tested against Cx. quinquefasciatus, the most promising oils were kaempfer, turmeric and holy basil oils which recorded low LC_{50} and LC_{90} values of 13.29, 17.54, 18.74and 31.20, 38.26, 33.26 ppm, respe ctively with 95% confidence lower limits of 0.76, 7.35 and 5.61 ppm and upper limits of 20.68, 23.72 and23.68, respectively for larvicidal activity. Sweet basil oilshowed the least larvicidal activity with LC_{50} and LC_{90} values of 71.38 and 339.04ppm, respectively (Table 2).Applying essential oils as botanical insecticides to control mosquito vectors is a better and environmentally safe choice than the use of synthetic chemical insecticides. Tikar et al (2008) stated that the development of pesticide resistance in Cx. *quinquefasciatus* population against temephos, fenthion, cyper methrin, a-cypermethrin and l-cyhalothrin indicat ing the need of search for safe and effective alternative safe control measures. Besides development of insecticides resistance, they are toxic to non-target organisms (Suman et al., 2010).

However, the screening of larvicidal activity was limited to only one mosquito vector, Cx. *quinquefasciatus* in the current study, the present results provided nine additional botanical agents which were comparable with the earlier and above mentioned screening works and these nine plants might be applied in purified form as alternative control strategies. Further tests are needed to elucidate this activity against a wide range of all stages of mosquito species and also the active ingredients of the extract responsible for larvicidal property should be identified.

Table 1 : Preliminary screening of selected essential oils against *Culex quinquefasciatus*.

Common Name	Part Used	Botanical Name	Larvicidal Activity of 1,000 ppm at 24 h (%)
1. Kaempfer	Roots	*Boesenbergia rotunda*	100
2. Holy basil	Leaves	*Ocimum sanctum*	100
3. Chinese chives	Leaves	*Allium tuberosum*	100
4. Galanga	Roots	*Alpinia galanga*	100
5. Turmeric	Roots	*Curcuma longa*	100
6. Dark blue temu	Roots	*Curcuma aeruginosa*	100
7. Lemongrass	Stems	*Cymbopogon citratus*	46.67
8. Citronella grass	Stems	*Cymbopogon nardus*	100
9. Kaffir lime	Leaves	*Citrus hystrix*	76.67
	Peels		100
10. Lime	Peels	*Citrus aurantifolia*	56.67
11. Holy basil	Leaves	*Ocimum basilicum*	66.67
12. Eucalyptus	Leaves	*Eucalyptus globulus*	83.33
13. Kitchen mint	Leaves	*Mentha cordifolia*	36.67
14. Sweet basil	Leaves	*Ocimum basilicum*	100
15. Sesame	Seeds I	*Sesamum indicum*	40
	Seeds II		33.33
16. Kusom	Seeds	*Schleichera oleosa*	26.67
17. Peanut	Seeds	*Arachis hypogaea*	20
18. Coconut	Seeds	*Cocos nucifera*	46.67
19. Avocado	Pericarps	*Persea americana*	43.33
20. Green bean	Seeds	*Vigna radiata*	66.67
21. Betel nut	Seeds	*Areca catechu*	23.33

Mean of 3 replicates with 10 larvae per replicate (n = 30). I = white sesame. II = black sesame.

Table 2: Larvicidal activity of the selected plant oils against Culex quinquefasciatus

Plant Oils	LC_{50} (ppm)	95% Confidence limit		LC_{90} (ppm)
		Lower	Upper	
1. Kaempfer	13.29	0.76	20.68	31.20
2. Turmaric oil	17.54	7.35	23.72	38.26
3. Holy basil oil	18.74	5.61	23.68	33.26
4. Chinese chives oil	20.58	11.72	25.36	37.17
5. Galanga oil	25.18	19.27	29.59	43.46
6. Dark blue temu oil	25.93	12.63	35.51	152.55
7. Citronella grass oil	63.22	44.74	73.80	192.55
8. Kaffir lime oil	68.34	28.17	320.10	467.48
9. Sweet basil oil	71.38	53.60	116.43	339.04

Each experiment is replicated with three times with 10 larvae for each replicate (n = 30). Probit analysis was employed for larvicidal activity.LC_{50} = Lethal concentration 50% or median lethal concentration. LC_{90} = Lethal concentration 90%

4. Conclusion

A larvicidal bioassay was carried to evaluate the larvicidal activity of medical plant extracts against *Culex quinquefasciatus*. Of all 21 essential oils, nine plant oils showed good larvicidal property with the LC_{50} ranging from of 13.29-71.38 ppm. Based on the mortality rate, the concentration of 100 ppm of all plant oils had the highest insecticidal efficacy on mosquito larvae with 100% after 24 hours of exposure. Larvicidal activity increased with increased dosage in all trials. The results reported here open the possibility of further investigations of efficacy on its larvicidal property of natural product extract. In order to achieve the best yield with good quality and quantity, the most suitable procedure should be determined.

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