THREE INDIGENOUS THAI MEDICINAL PLANTS FOR CONTROL OF *AEDES AEGYPTI* AND *CULEX QUINQUEFASCIATUS*

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Abstract. The potential larvicidal activity and insect growth regulator (IGR) properties of three selected indigenous medicinal Thai plants were tested against two species of mosquito with special reference to the late 3rd and early 4th instar larvae (L_3 and L_4, respectively). In case of larvicidal activity, *Thevetia peruviana* was the most potent, followed by *Pueraria mirifica*, and *Butea superba* was the least effective. In all cases, the late 3rd instar was more susceptible than the early 4th instar larvae, and the 48-hours exposure yielded more potent larvicidal activity than 24-hours exposure. However, at sublethal dosages, both *P. mirifica* and *B. superba* showed some dispersed effects interfering with ecdysis. A variety of toxic effects were observed and recorded in eight categories according to the stage of metamorphosis when death occurred. *P. mirifica* rendered the main deleterious effects in the pupa-adult period in both instar of *Ae. aegypti* and *Cx. quinquefasciatus*, whereas *B. superba* showed highest effect in black-pupa period of the late 3rd instar larval stage. The results were reversed for the early 4th instar larvae of both species of mosquito as the main effect appeared in the pupa-adult category. The overall results indicated that *T. peruviana* did not show any IGR properties; whereas, *P. mirifica* and *B. superba* seemed to exhibit the juvenile hormone type activity which resulted in abnormal death at various stages of development. *B superba* was more promising than *P. mirifica*, and *Ae. aegypti* was about 2 times more susceptible than *Cx. quinquefasciatus*. In addition, L_3 was always more susceptible than L_4 with both mosquito species.

INTRODUCTION

Mosquito-borne diseases remain a major problem in the world, particularly in tropical and subtropical regions. These diseases contribute significantly to disease burden, death, poverty, and social debility in tropical countries (Young-Su *et al*, 2002). Although vector control programs have been established for a long time, the main method for control of the vectors is the use of chemical insecticides. However, the effectiveness of vector control has declined due to the development of resistance in mosquitoes against currently used insecticides (Chandre *et al*, 1998; Penilla *et al*, 1998). Furthermore, there are many serious drawbacks with the use of chemical insecticides, for example, the increasing cost of new insecticides and annual importation expenditures, effects on non-target populations especially on humans, environmental pollution, such as entering the food chain, and the development of insecticide resistance and the emergence of refractory vector behavior.

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One approach to this problem has been to search for new and effective compounds that do not have any ill effects on non-target populations, are easily degradable, safe, and easily available at low cost. Thus, plants may be an alternative source of mosquito-control agents because they constitute a rich source of bioactive chemicals (Arnason *et al*, 1989; Sukumar *et al*, 1991; Wink, 1993). They are not only effective, but also greatly reduce the risk of potentially adverse ecological effects, do not have any ill effects on non-target populations, are degradable, safe, and easily available at low cost. They may prevent the possibility of the resistance that synthetic chemical insecticides typically bring about after prolonged use (Monzon *et al*, 1994). Overall, the search for such compounds has been directed extensively towards the plant kingdom (Sujatha *et al*, 1988; Mohsen *et al*, 1989; Sukumar *et al*, 1991).

Medicinal plants are commonly found throughout tropical and subtropical countries. A lot of research work on medicinal plants has been carried out on agricultural pests with promising results, and some have been produced at industrial level. However, very few have been reported for control of mosquito vectors, especially in Thailand. Some crude extracts from medicinal plants are currently being used as they are potentially economical, safe, and practical for control measures. Being "environmentally friendly," it is more practical and safe for the environment to use botanical

insecticides to control mosquitoes. Therefore, this study was designed to focus on three indigenous Thai medicinal plants and their potential specific effects on both *Ae. aegypti* and *Cx. quinquefasciatus*.

MATERIAL AND METHODS

Mosquitoes and plants

In this study, *Aedes aegypti* (Suan Pheung strain) and *Cx. quinquefasciatus* (Nakhon Pathom strain) were used. Both species of mosquito were colonized in the insectarium of the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University.

The indigenous Thai plants, *P. mirifica* and *B. superba* were collected from Chiang Khong district, in Chiang Rai Province, whereas *T. peruviana* was collected from Queen Sirikit Park. Dr Somran Suddee, of the Forest Herbarium National Park Wildlife and Plant Conservation Department, Thailand, authenticated the three indigenous Thai medical plants used in this study.

Larvicidal activity test

The three collected plants were washed in tap water, cut into small pieces, and dried in a hot air oven at 60° C. After the plants were completely dry, they were ground into powder, then macerated in 95% ethanol at room temperature for 3 days, and filtered. The combined filtrate was concentrated to dryness by rotary evaporation at 50° C and kept in a freezer at -20° C. In preparing test concentrations, each plant extract was volumetrically diluted in 95% ethanol.

The bioassay test was done according to World Health Organization guidelines (WHO, 1996). One ml of a standard, concentrated w/v in an organic solvent, was added to 99 ml of water. A group of 20 of the late 3rd or early 4th instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* were used per cup with 4-cup replications for each serial dilution concentration. The mortality rates were recorded after 24- and 48-hour exposures. Three subsequence sets of the experiment were carried out for each test.

Insect growth regulator (IGR) test

In the test for the natural products of plants that mimic the action of IGR, mortality delayed beyond the instar treated. For the accurate determination of the activities of the IGR and natural products, the mortality rates were recorded every day until all treated larvae died at various stages or all adult emerged. Due to the long duration of the test, the larvae were provided with food at intervals during the observation period (WHO, 1996). Regarding with the various dispersed effects that interfere with ecdysis, the characteristic toxic effects on mosquito larvae were judged and recorded according to the following 8 criteria:

1. L (death as larvae) This category represents death during the larval stage with no evident initiation of pupation.
2. L(P) (larval cuticle with pupa inside). Death in this category has occurred at an early stage of pupation. The pupal abdomen can be seen to be withdrawn from the terminal part of the abdomen and the pupal respiratory trumpets are visible.
3. L-P (larvae with pupae partly emerged). At this stage the larval skin has been ruptured and the pupal body has partly emerged from the thoracic split. The abdomen has retracted to at least halfway along the larval abdominal skin and has adopted the characteristic pupal shape.
4. WP (white pupae). The pupae have completely escaped from the larval cuticle but have remained completely unmelanized except for eye pigment. The abdomen is held in an abnormally straight position.
5. BP (brown pupae). The pupae show some melanization.
6. P(A) (pupae with adult visible inside). In this categories, most of the adult anatomy can be distinguished, but the pupal skin has not split. Unlike the previous categories, the dead insect normally floats, presumably because the internal air bubble is preserved.
7. P-A (pupae with adult beginning emergence). The adults have begun to escape from the pupal skin but are unable to free themselves completely. Sometimes the head and thorax are freed, but the abdomen remains enclosed. Occasionally, the whole body is nearly free except for the legs.
8. DA (death adult). This category is reserved for adults which have freed themselves completely from the pupal skin, but cannot escape from the water film.

Data analysis

For the larvicidal activity of the plant products against the mosquito vector, the LC_{50} value of each plant extract that exhibited adverse effects on the mosquito larvae was calculated by probit analysis of Finney (1971). If the control mortality rates are between 5% and 20%, the percentage mortality should be corrected by Abbott's formula as follows:

$$\% \text{ corrected mortality} = \frac{\% \text{ observed mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

When mortality in the controls was over 20%, the tests were discarded.

RESULTS

Larvicidal activity

In order to gain some insight into each activity of the three tested plants, their effects were investigated on both the late 3rd instar and early 4th instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus*. The exposure time was also extended from 24 to 48 hours in order to obtain the highest activity.

Based on the 24-hour' exposure results obtained from *P. mirifica* acting on the late 3rd and early 4th instar larvae of *Ae. aegypti*, the LC_{50} values were 889.33 and 1,023.75 mg/l, respectively; while for *Cx. quinquefasciatus,* the results of 1,710.48 and 1,777.19 mg/l, respectively, were obtained (Table 1). By contrast, at 48-hour' exposure, the late 3rd and early 4th instar larvae of *Ae. aegypti* were more susceptible, with LC_{50} values of 317.63 and 369.64 mg/l, respectively. The LC_{50} values for *Cx quinquefasciatus,* for the late 3rd and early 4th instar larvae, were 572.34 and 662.10 mg/l, respectively (Table 1).

According to the results obtained with *B. superba* acting on the late 3rd and early 4th instar larvae of *Ae. aegypti* at 24-hour exposure, the LC_{50} values were 1,156.68 and 1,386.17 mg/l, respectively; whereas for *Cx. quinquefasciatus*, the LC_{50} values were 1,859.95 and 2,385.76 mg/l, respectively (Table 1). When the exposure time was increased to 48 hours**,** the LC_{50} values were 496.23 and 551.91 mg/l for the late 3rd and early 4th instar larvae of *Ae. aegypti*, respectively. Under the same conditions, the LC_{50} values were 794.33 and 1,026.65 mg/l for the late 3rd and early 4th instar larvae of *Cx. quinquefasciatus*, respectively (Table 1).

From the overall results of *P. mirifica* and *B. superba*, it was interesting to note that *Ae. aegypti* was more susceptible than *Cx. quinquefasciatus* (about 2 times), and the late 3rd instar was more susceptible than the early 4th instar larvae. In addition, it was very clear that the 48-hour exposure rendered more activity than the 24-hour exposure (about two times).

Apart from *T. peruviana* at 24-hour exposure with the late 3rd and early 4th instar larvae of *Ae. aegypti*, the LC_{50} values were 346.42 and 259.12 mg/l, respectively but for the same status, and *Cx. quinquefasciatus* had LC_{50} values of 211.37 and 255.07 mg/l, respectively (Table 1). With 48-hour' exposure of *T peruviana*, the LC_{50} values for the late 3rd and early 4th instar were 31.98 and 40.89 mg/l, respectively, for *Ae. aegypti;* whereas with *Cx. quinquefasciatus,* the values were 52.73 and 57.78 mg/l, respectively (Table 1). Again, the late 3rd instar of both species of mosquito were more susceptible than the early 4th instar larvae. It was curious that *Ae. aegypti* was more susceptible than *Cx. quinquefasciatus* in the response for the late 3rd instar, while both showed similar results for the early 4th instar.

On the whole, among the three indigenous Thai medicinal plants studied, *T. peruviana* was the most potent, followed by *P. mirifica*, while *B. superba* was the least effective. In all cases, *Ae. aegypti* was more susceptible (about 2 times) than *Cx. quinquefasciatus* to both plant species. As expected, the late 3rd instar was more susceptible than the early 4th instar larvae. In considering the length of exposure time, the 48-hour exposure of both species of mosquitoes against all tested plant extracts yielded more potent larvicidal activity than 24-hour exposure.

IGRs activity

According to the results obtained with *P. mirifica* acting on the late 3rd and early 4th instar larvae of *Ae.*

Table 1
LC_{50} values of three selected Thai plants on the late 3rd and early 4th instar larvae.

Plants		LC_{50} (mg/l) at 95% confidence			
		Ae. aegypti	*Cx. quinquefasciatus*	*Ae. aegypti*	*Cx. quinquefasciatus*
		24-hour exposure		48-hour exposure	
P. mirifica	L_3	889.33	1,710.48	317.63	572.34
	L_4	1,023.75	1,777.19	369.64	662.10
B. superba	L_3	1,156.68	1,859.95	496.23	794.33
	L_4	1,386.17	2,385.76	551.91	1,026.65
T. peruviana	L_3	346.42	211.37	31.98	52.73
	L_4	259.12	255.07	40.98	57.78

aegypti after continuous exposure, the LC_{50} values were 30.73 and 35.55 mg/l; while 58.99 and 65.14 mg/l were obtained for *Cx. quinquefasciatus*, respectively (Table 2).

The results obtained with *B. superba*, the LC_{50} values were 26.72 and 31.95 mg/l, respectively; whereas 53.66 and 60.59 mg/l were obtained for *Cx. quinquefasciatus*, respectively (Table 2). Based on the LC_{50} values, in all cases, *B. superba* seemed to have more activity than *P. mirifica*. Considering the different treated instar, the early 4th instar showed generally less effect than the late 3rd instar larvae.

It was clear that, all concentrations eventually produced a high kill at a characteristic point in larval stage, whereas the lower dose treatments gave more dispersed actions. As in all cases, the late 3rd instar was more susceptible than the early 4th instar. Therefore, the various category effects of late 3rd instar were selected as examples as shown in Table 3 and Figs 1-8. It caused harm to larvae and pupae during molting, especially at the time of metamorphosis. This strongly suggested that the action was a hormone mimic. However, from the experiments it is difficult to speculate on the actual mode of action of the each plant extract. At the high dose, death occurred most in the larval stage (Fig 1). Based on other assumptions, it may likely act as a larvicide. As the dose was reduced to a level resulting in overall mortality of 50% or less, the mortality rate in the larval instars declined sharply, but it had a delaying effect during pupation.

The results from Table 4 indicated that, *P. mirifica* showed main effect in the P-A period in both the late 3rd and early 4th instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* (Fig 7). *B. superba* had the highest effect in BP period in the late 3rd instar larvae of both species of mosquito (Fig 5). The results were reverse for the early 4th instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* whereas the main effect was appeared in the P-A period. Based on the overall results, it can be suggested that *P. mirifica* and *B. superba* appeared to exhibited a juvenile hormone type activity.

Table 2
LC_{50} values of two indigenous medicinal Thai plants on the late 3rd and early 4th instar larvae after continous exposure.

Plants		*Ae. aegypti*		*Cx. quinquefasciatus*	
		LC_{50} (mg/l)	Categories	LC_{50} (mg/l)	Categories
P. mirifica	L_3	30.73	P-A	58.99	P-A
	L_4	35.55	P-A	65.14	P-A
B. superba	L_3	26.72	BP	53.66	BP
	L_4	31.95	P-A	60.59	P-A

Table 3
The percent mortality values of *P. mirifica* and *B. superba* alcohol extract on the late 3rd instar larvae of *Cx. quinquefasciatus* after continuous exposure.

Plants	Conc (mg/l)	% Mortality (at 95% confidence)									LC_{50} (mg/l)
		L	L(P)	L-P	WP	BP	P(A)	P-A	DA	Total	
P. mirifica	5,500	100.00	-	-	-	-	-	-	-	100.00	58.99
	1,100	55.00	3.75	3.75	6.25	6.33	5.00	8.75	1.25	90.08	
	220	35.00	2.50	3.75	6.25	6.25	5.06	8.86	1.25	68.89	
	44	15.00	2.50	2.50	5.00	5.00	3.75	7.59	2.50	43.81	
	8.8	-	1.25	1.25	3.75	5.06	3.75	7.50	-	22.56	
B. *superba*	6,000	100.00	-	-	-	-	-	-	-	100.00	53.66
	1,200	51.25	2.5	3.75	8.86	11.39	6.25	7.50	2.50	93.95	
	240	31.25	3.75	3.75	8.75	12.50	3.75	8.86	1.25	73.84	
	48	8.75	2.50	2.5	6.33	10.00	6.25	7.59	-	43.92	
	9.6	-	-	2.5	5.06	8.75	3.75	5.00	-	25.06	

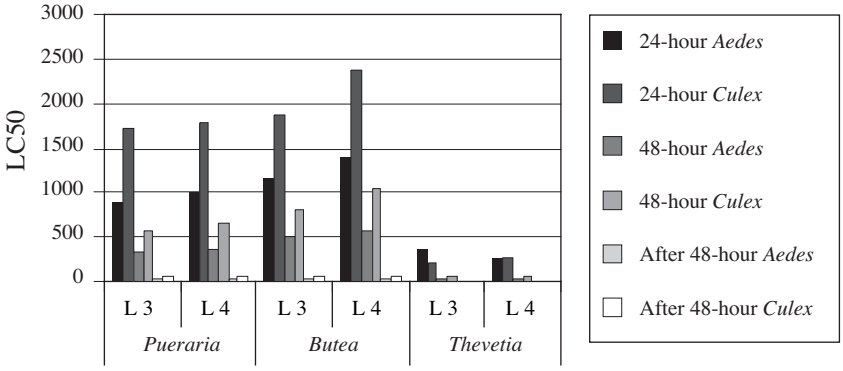

Fig 1- Histogram showing the LC_{50} values of three selected plants alcohol extract on the late 3rd and early 4th instar larvae of *Ae. aegypti* and *Cx. quinquefascitus* at 24-hour, 48-hour and after continuous exposure.

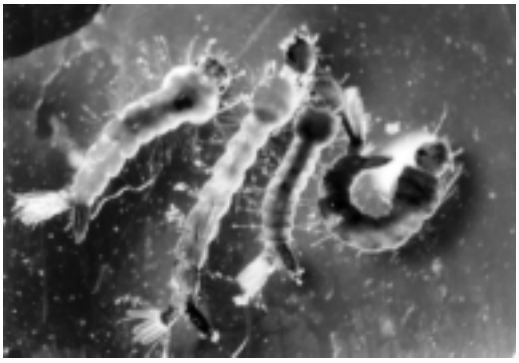

Fig 2- Death during the larval stage (L).

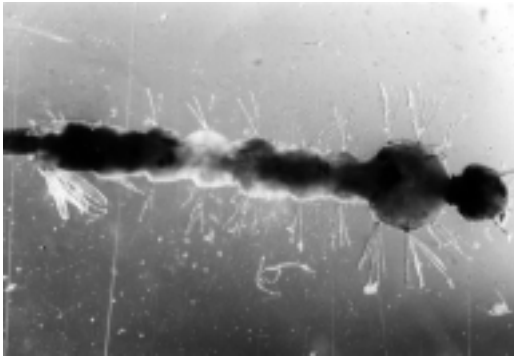

Fig 3- The larval cuticle with pupa inside L(P).

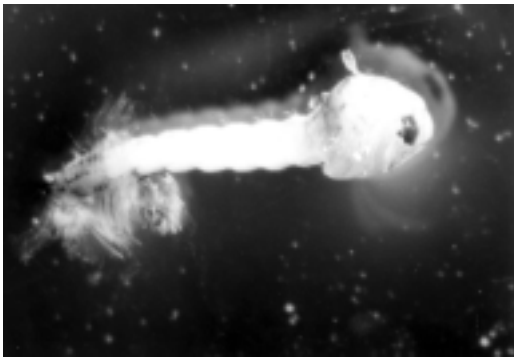

Fig 4- Death of pupa during development from larva to pupa (L-P).

Fig 5- Death of unmelanized pupa after molting from larva to pupa (WP).

DISCUSSION

The identification and eventual use of indigenous medicinal plants in the control of mosquito larvae may be very valuable for developing countries, such as Thailand and its Southeast Asian neighbors. In Thailand, some scientists have attempted to identify such plants and to assess their larvicidal potential (Chareonviriyaphap, 1987; Thengtriratana *et al,* 1990; Satoto, 1993; Pitasawat *et al,* 1998; Junjanwit, 1999; Nicharat, 2001).

In considering with larvicidal activity among the

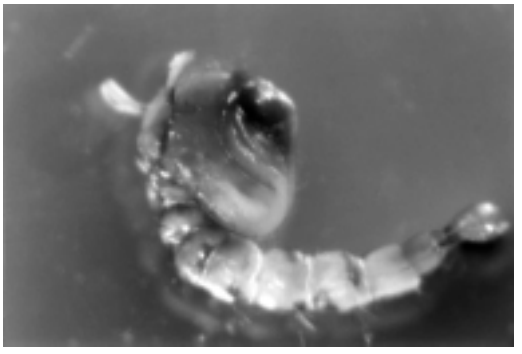

Fig 6- Death of pupa after larva molting to pupa, and the pigment was synthesized (BP).

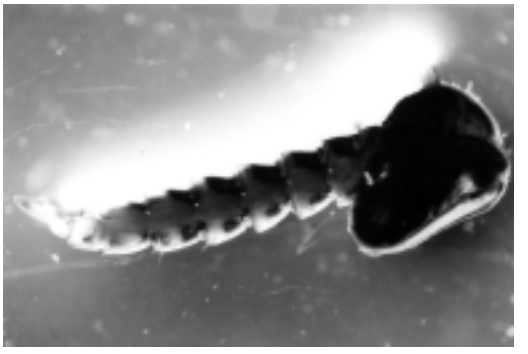

Fig 7- Death at P(A) stage of treated larvae showing visible adult within pupal exuvium.

Fig 8- Death of adults after complete molting from pupal skin, but some parts were attached to the pupa exuviae (P-A).

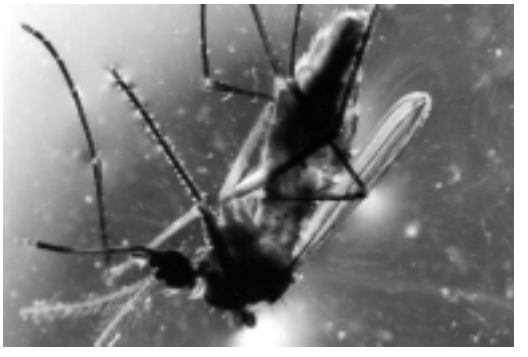

Fig 9 Adult having freed itself completely from the pupal skin but could not fly from the water, leading to death (DA).

three species of selected plants in this study, the seeds of *T. peruviana* demonstrated excellent insecticidal properties against the late 3rd and early 4th instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus*. These findings are supported by Evans and Kaleysa Raj (1988). They studied the crushed aqueous extracts of 24 Indian plants that could possibly act as a larvicide for *Cx. quinquefasciatus*. They found that only six were active against *Cx. quinquefasciatus* larvae (*Quassia amara, T. peruviana, Anacardium occidentale, Carica papaya, Hevea braziliensis* and *Nerium indicum)*. Finally, they concluded that *T. peruviana* was one of the three most active plants. In addition, this was supported by Satoto (1993) as *T. peruviana* was found to be toxic for 4th instar larvae of *Cx. tritaeniorhynchus* with the 24-hour LC_{50} values between 1 mg/l to 100 mg/l (LC_{50} = 32.01 mg/l). The author did not mention any effects on molting and metamorphosis. Our findings for *T. peruviana* was in agreement with these results; no previous IGR data has been reported.

However, from this study, *P. mirifica* and *B. superba* would not be economical for field use in larval control as the LC_{50} values after 24- and 48-hour exposures were greater than the recommended dosage, which should be less than 100 mg/l.

In certain circumstances, the same phytochemical toxin from a single plant species exhibits varying degrees of toxicity to different mosquito species. Minijas and Sarda (1986) demonstrated that crude extracts containing saponin from the fruit pods of *Swartzia madagascariensis* produced higher mortality rates in the larvae of *An. gambiae* than in larvae of *Ae. aegypti*; no mortality was induced in the larvae of *Cx. quinquefasciatus*. In addition, Rahuman *et al* (2000) reported that *Ae. aegypti* (LC_{50} = 57.23 mg/l) was more susceptible than *Cx. quinquefasciatus* (LC_{50} = 129.24 mg/l) when exposed to the acetone extract of *Feronia limonia* dried leaves.

From the results obtained in this study, it was apparent that, the 3 selected plants (*P. mirifica, B. superba* and *T. peruviana*) are more effective against

Ae. aegypti than *Cx. quinquefasciatus,* especially at 48-hour exposures. Thus, it may be conclude that *Ae. aegypti* is more susceptible than *Cx. quinquefasciatus*, but the conclusion only confirms that the plants differ in the chemical composition of their active components. Conversely, Pizarro and Oliviera Filho (1999) showed that *Cx. quinquefasciatus* (LC_{50} = 183 mg/l) was more susceptible than *Ae. aegypti* (LC_{50} = 322 mg/l) when exposed to different concentrations of the *Agave* (*Agave sisalana*) leaves extract. Another possibility to consider is the inherent physiological difference between the two species of mosquitoes.

Difference certain stages of mosquitoes are more susceptible to phytochemicals. A butanol extract of soapberry plant, *Phytolacca dodecandra*, was very toxic to 2nd and 3rd instar larvae of *Ae. aegypti*, *Cx. pipiens* and *An. quadrimaculatus*; but the eggs and pupae were unaffected, and adults died only after ingestion of the concentrated extract (Spielman and Lemma, 1973). Similarly, Pelah *et al* (2002) reported that the larvicidal activity of commercial bark saponin extract from *Quillaja saponaria*, was toxic to the 3rd and 4th instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus*, but did not effect egg hatching ability in either species. However, from the overall result from this study, *T. peruviana*, *P. mirifica* and *B. superba* were more toxic to the late 3rd instar compared with the early 4th instar larvae of both *Ae. aegypti* and *Cx. quinquefasciatus*.

Regarding to the differences in exposure times, the activities at 48-hour exposure are lower than those at 24 hours. This can signify that all tested plants are more potent or effective after 48 hours exposure. Mwangi and Rembold (1988) obtained a yield of 0.59% for the most active acetone extract of *Melia volkensii* fruits;the 24-hour LC_{50} of the active fraction was reported for second instar *Ae. aegypti* larvae (78 mg/l) to be far higher than the 48-hour LC_{50} values. Their results strongly support the finding of this study. Concerning hormonal mimic activity, a parallel between the mode of action of *P. mirifica* and *B. superba* on juvenile hormone mimics can be suggested. The results indicated that the tuberous root of *P. mirifica* and *B. superba* demonstrated potent IGR properties. It was indicated that *P. mirifica* produced its main effect in the P-A period in both the late 3rd instar and early 4th larvae of *Ae. aegypti* and *Cx. quinquefasciatus.* In the case of *B. superba*, the results indicated that the highest effect was in the BP period, in the late 3rd instar larvae of both *Ae. aegypti* or *Cx. quinquefasciatus*. The results were reversed for the early 4th instar larvae of both species, whereas the main effect appeared in the P-A period. However, it was not remarkable because death occurred in the BP category that also involved metamorphosis.

It is well established that the compounds of *P. mirifica* are deoxymiroestrol and miroestrol, which is structurally similar to estradiol. Many research studies have been performed on *P. mirifica*, such as biological and chemical studies of its estrogenic effects (Schoeller *et al*, 1940; Kashemsanta *et al*, 1961; Sawatdipong, 1979). Pharmacological reports in Thailand during the period 1981-1998 concluded that dried powder extracts from *P. mirifica* had female estrogenic effects on various tested animals and insects. Similarly, Thengtriratana *et al* (1990) studied the effects of substances in the tuberous root of *P. mirifica* on *An. dirus*. The results indicated that the body length, the percentage of the mortality rate, and the length of time from newly hatched larva to pupa in the treatment group were significantly different from those in the control group. Moreover, the results on the abnormal development of cockroach (Radomsuk and Smitasiri, 1994) reported the effects on the virgin female American cockroaches when fed with ethanolic and aqueous extracts from *P. mirifica* tuber mixed with food or water for 15 days. The results indicated that the ovum size of the *P. mirifica*-treated group was significantly smaller than the controls; abnormal ovaries were usually found. In addition, when *P. mirifica* extracts were fed to both sexes of cockroaches reared together for 30 days, it was found that the number of ootheca and non-hatching ootheca were quite high in most of the *P. mirifica*-treated groups. After cessation of feeding with *P. mirifica,* the result was followed up for 15 days. It was observed that the number of ootheca of some in the *P. mirifica*-treated group increased to normal, and these ootheca were always normal.

Regarding to *B. superba,* no previous studies on its larvicidal or insect growth regulator activity could be found in the literature. However, active ingredient in *B. superba* including flavonoids was reported as secondary plant chemicals with juvenile hormone activity (Frenkel, 1959; Bowers, 1983; Morgan and Mandawa, 1985; Sukumar *et al*, 1991).

Overall, the imbalance between growth stimulating and growth-inhibiting hormones caused by the plant extracts may prolong the larval, pupal and developmental periods (Novak, 1966). Prolongation of larval and pupal periods may be due to the inhibition of the molting process caused by an increased titer of juvenile hormones in the insect body. Larval and pupal mortality during the respective stages, and molting could either be due to the presence of toxic ingredients

in the extract or the imbalances between growth-stimulating and growth-inhibiting hormones. Further studies are needed to establish a more exact explanation for the cause of death.

The effects of chemicals derived from plant extracts, *P. mirifica* and *B. superba*, resemble known insect hormones; and definitely affect the mosquito larvae at the time of metamorphosis. It is not clear whether this is due to mimicking of the juvenile hormone, to a blocking hormone degradation, or to some other physiological interference at these vital points. The definite mode of action may for the moment, be left unspecified. Various functions occurring during molting and metamorphosis may be affected, such as cuticle tanning and hardening. To discover the actual mode of action, it is likely to prove a highly difficult biochemical research. At this stage, what was attempted was to distinguished the types of toxic actions of the alcohol extract from both *P. mirifica* and *B. superba* on the basis of visible effects produced.

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