J.Sci.Soc.Thailand, 16 (1990) 133–139

RESEARCH ARTICLES

PHYTOCHEMICAL TOXICITY OF THE CRUDE EXTRACT FROM *DERRIS ELLIPTICA* BENTHAM ON MOSQUITO LARVAE

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(Received 10 July 1990)

ABSTRACT

A crude extract from the roots, leaves and creeping branches of Derris elliptica *Bentham (Papilioneae), and indigenous plant, kills mosquito larvae under laboratory conditions. Experiments were performed on second instar larvae of* Aedes egypti *Linnaeus,* Anopheles (cellia) minimus *Theobald, and* Culex quinquefasciatus *Say. The mortality rate was determined after exposure of larvae to various concentrations of crude extract at time intervals ranging from 1 to 96 hours. The results indicated that the extract from this plant had toxic effects on the development of larvae, and interfered with the formation of the cuticle and internal tissues, leading finally to total mortality. Further investigations of the active chemical constituents as well as field trials are planned.*

INTRODUCTION

Aedes aegypti Linnaeus, *Anopheles (cellia) minimus* Theobald, and *Culex quinquefasciatus* Say are important vectors of malaria, hemorrhagic fever, and *Bancroftian filariasis* in Thailand. Chemical insecticides are extensively used to control them. This method tends to cause serious environmental problems. Moreover, the regular use of chemicals helps to select for resistance in the insects themselves, which has been a major cause of failure in vector control campaigns. In order to overcome this problem many new and more powerful chemicals are being synthesized and used. but the problem persists. Search for safer chemicals which are effective as insecticides and pesticides, but less harmful to the ecosystem has also continued. It is believed that certain compounds extracted from medicinal plants might serve this purpose without causing pollution of the environment.

Among many known indigenous medicinal plants, hang-lai-dang (a local Thai name) was selected for this investigation. Its botanical name is *Derris elliptica* Bentham (Papilioneae).

It grows in abundance in humid forests or along river banks. Its root secretes a milky white resin which has been reported to contain an alkaloid. Local people make use of the plant by beating the whole part thoroughly and soaking it in water for some time; the water extract is then used to kill some insects, mites, ticks, lice, or wild fish before stocking shrimp ponds. Local traditional doctors also ferment the dry creeping part of the plant in alcohol (locally made whiskey) and use it as a drink to improve blood circulation or to cure some body disorders.

Because of its known toxic properties, we were interested to determine whether the extract could be used to kill mosquito larvae. If found effective, it could potentially become an alternative to toxic chemicals now used in mosquito eradication campaigns. Preliminary observations revealed high mortality rates of mosquito larvae of the three species mentioned earlier upon addition of the crude extract from this plant. We therefore decided to study its effects on mosquito larvae more thoroughly.

MATERIALS AND METHODS

The plant was collected from Roi-et and Kanchanaburi. It was cleaned in running water, followed by a 0.9% saline solution, and then chopped into small pieces. All of its parts were used, including the flowers. A one-kilogram quantity was extracted in a Soxhlet apparatus with 2 liters of methanol for 7 days. The solid part was filtered off and discarded. The solvent was removed in a rotary evaporator to leave a viscous residue. Distilled water (20 ml) was added and evaporated to give 20 grams of crude extract for use in the toxicity tests on mosquito larvae.

The solution was prepared by dissolving a weighed amount of the crude in distilled water to give a slightly colored solution from which no precipitation occurred upon standing throughout the investigation period. A 160 mg/l solution was prepared from 160 mg of the crude and one liter of distilled water. Less concentrated solutions were prepared from this stock solution by dilution with distilled water.

The subjects used in this investigation were the second instar larvae of *Aedes aegypti, Anopheles (cellia) minimus,* and *culex quinquefasciatus.* Twenty larvae of each species were used in every trial with a total of five replications along with the control which consisted only of water and larvae. Tap water aerated for 48 hours in plastic buckets was used in all treatments. The subjects were fed daily with a small amount of ground hamster food. Temperature were controlled at $27 \pm 4°C$ with relative humidity at $78 \pm 4\%$.

Mortality was recorded every hour during the first six hours of exposure and every six hours thereafter for a total period of 96 hours. The dead larvae were removed for morphological studies.

The percentage mortality was calculated by using the LC_{50} values of the crude extract solution as determined at 24, 48, 72 and 96 hours. Analysis of data was performed using probit analysis (Table 4).

RESULTS AND DISCUSSION

Total mortality of *Aedes aegypti* occurred upon exposure to a 160 mg/l solution after 1 hour. Larvae of other species were found to be more sensitive. The solution of this concentration was therefore used as the highest concentration. Results are summarized in Tables 1, 2, and 3. Total mortality after 1 hour of exposure occurred at 80 ppm in *An. minimus* and at 120 ppm in *C. quinquefasciatus.* At lower concentrations living larvae developed into third (after 24 hours) and fourth instar larvae. At these stages the new larval cuticle was not fully developed and molting was unsuccessful. Incomplete pupae (pupal-adult intermediate forms) and malformed adults occurred, whereas the control larvae developed normally to adults. During the 96-hour period not more than 4% of the control larvae were found dead which was considered to be normal; the surviving larvae pupated and hatched into normal adults. Larvae in the treatment group surviving after 96-hour exposure were all deformed. Even at 2.5 mg/l concentration, adults could not emerge normally from the exuviae. However, they could feed themselves by sticking to the feeding sites. All died after 10 days of inactivity while adults from the controls were normal. Their progeny were collected and used for further experiments.

These results indicate that the crude extract from this plant has marked larvicidal effects. Inspection of the dead larvae revealed incomplete formation of the cuticle and internal tissues which rendered healthy development to later stages impossible. Certain compounds in this extract must play a major role in damaging these tissues which are essential for further development. Its high toxicity at low concentration (2.5 ppm) warrants further investigation in the field. If this is also successful, the plant may serve as an alternative to toxic chemicals used at present to eradicate mosquitoes. As far as the environment is concerned, no adverse effects are anticipated, especially at the concentrations employed. No toxicity on human beings have been reported when the plant is used as an internal "medicine". Laboratory experiments are now underway to determine its chemical constituents, especially those responsible for its larvicidal property. Its biodegradability will also be checked during field trials.

ACKNOWLEDGEMENTS

We wish to thank Drs. Sutharm Areekul, Apichai Dowrai, and Mr. Manus Kumpukul for identifying the species of medicinal plant; Dr. Warren Brockelman and Dr. Prapin Wilairat for reviewing the manuscript; and Mr. Narong Areyamuang for probit analysis of the data.

TABLE 1. *Effects on Aedes aegypti* Linnaeus

Extract Concentration (mg/l)	Total percentage mortality at various developmental stages (time in hours)									Mortality (after 10 days)
	1	6	12	24	48	72	96			
		L 2		L_3	L_4	L–P	P–A	I–A	A	
160	100	–	–	–	–	–	–	–	100	
80	72	22	6	–	–	–	–	–	–	100
40	27	30	22	15	6	–	–	–	–	100
20	10	12	15	12	13	15	10	12	1	100
10	8	11	14	10	8	9	9	10	15	100
5	5	9	10	9	6	7	6	12	15	100
2.5	3	7	9	6	4	6	5	10	14	100
Control	–	–	–	1	–	1	–	–	–	2

L_2 = second instar larvae; L_3 = third instar larvae; L_4 = fourth instar larvae; L–P = late puparia and incomplete puparia; P–A = pupal – adult intermediate form; I–A = incomplete adult; A : adult;

TABLE 2. Effect on *Anopheles (cellia) minimus* Theobald

Extract Concentration (mg/l)	Total percentage mortality at various developmental stages (time in hours)									Mortality (after 10 days)
	1	6	12	24	48	72	96			
		L 2		L_3	L_4	L–P	P–A	I–A	A	
80	100	–	–	–	–	–	–	–	–	100
40	68	29	3		–	–	–	–	–	100
20	31	21	29	17	2	–	–	–	–	100
10	10	15	16	20	18	2	10	9	–	100
5	6	5	8	11	14	3	8	15	15	100
2.5	4	7	6	7	4	4	7	13	14	100
1.25	2	5	2	3	2	3	3	9	7	100
Control	–	–	–	–	1	1	1	1	–	4

TABLE 3. Effects on *Culex quinquefasciaturs* Say

Extract Concentration (mg/l)	Total percentage mortality at various developmental stages (time in hours)									Mortality (after 10 days)
	1	6	12	24	48	72	96			
		L 2		L_3	L_4	L–P	P–A	I–A	A	
120	100	–	–	–	–	–	–	–	–	100
80	76	14	10	–	–	–	–	–	–	100
40	35	22	24	18	11	–	–	–	–	100
20	17	20	15	14	10	12	10	2	–	100
10	9	10	13	13	12	10	8	7	13	100
5	5	8	10	11	10	8	7	8	11	100
2.5	2	5	8	10	7	5	6	6	9	100
Control	–	–	–	–	–	1	2	–	–	3

TABLE 4. Summary of potencies of *Derris elliptica* Bentham crude extract when tested against second instar larvae of *Aedes argypti* Linnaeus, *Culex quinquefasciatus* Say, *Anopheles (cellia) minimus* Theobald.

Time (hours)	d.f.	X^2	Variance	LC_{50} Value (mg/l)	Regression line equation (Y=a+bx)	Probability heterogeneity accept
Aedes aegypti Linnaeus.						
1	4	27.23	0.03	30.70	Y=2.2 +1.88X	6.81
24	3	26.34	0.02	5.91	Y=3.32+2.18X	8.78
48	2	0.01	0.04	4.79	Y=3.66+2.03X	0.01
72	2	0.60	0.04	4.33	Y=3.76+1.59X	0.30
96	1	0.67	0.14	5.51	Y=3.92+1.45X	0.67
Culex quinquefasciatus Say.						
1	4	13.43	0.03	48.78	Y=1.78+1.91X	3.36
24	3	6.76	0.02	9.10	Y=3.57+1.49X	2.54
48	2	0.78	0.04	6.31	Y=3.98+1.27X	0.39
72	2	1.87	0.04	4.43	Y=3.97+1.58X	0.94
96	1	0.66	0.14	2.12	Y=4.25+2.3 X	0.66
Anopheles (cellia) minimus Theobald.						
1	4	16.82	0.03	30.7	Y=2.2 +1.88X	4.21
24	3	25.00	0.03	5.91	Y=3.32+2.18X	8.33
48	2	3.94	0.05	4.79	Y=3.61+2.03X	1.97
72	2	4.28	0.04	4.33	Y=3.76+1.95X	2.14
96	1	0.08	0.10	5.51	Y=3.92+1.45X	0.08

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บทคัดย่อ

สารสกัดจากส่วนราก เครือ และใบของพืชสมุนไพรพื้นบ้าน หางไหลแดง *(Derris elliptica* Bentham : Papilioneae) เมื่อนำมาทำเป็นสารละลายเจือจางในน้ำ ปรากฏว่ามีคุณสมบัติกำจัดลูกน้ำยุงได้ในสภาวะห้องทดลอง ลูกน้ำยุงที่ใช้อยู่ในระยะการเติบโตที่ 2 ของยุง 3 ชนิด คือ ยุงลาย *(Aedes egypti* Linnaeus) ยุงก้นปล่อง *(Anopheles (cellia) minimus* Theobald) และ ยุงรำคาญ *(Culex quinquefasciatus* Say) การเฝ้าสังเกตการตายของลูกน้ำยุงในสารละลายที่มีความเข้มข้นต่าง ๆ กัน ทำเป็นช่วงเวลาต่อเนื่องกันไปตั้งแต่ชั่วโมงแรกจนถึง 96 ชั่วโมง พบว่าหลังการทดลอง 1 ชั่วโมง ลูกน้ำยุงบ้านตายหมดที่ความเข้มข้น 160 ส่วนในล้านส่วน ลูกน้ำยุงก้นปล่องตายหมดที่ 80 ส่วนในล้านส่วน และยุงรำคาญ 120 ส่วนในล้านส่วน เมื่อใช้สารละลายที่เจือจางลงมา มีลูกน้ำยุงเหลืออยู่หลัง 24 ชั่วโมง และเติบโตเข้าสู่ระยะที่ 3 และ 4 ได้ แต่ผนังลำตัวของมันไม่สามารถเจริญได้สมบูรณ์ เนื่องจากลอกคราบไม่ได้ เข้าดักแด้ผนังตัวโม่งไม่สมบูรณ์ และตัวเต็มวัยผิดปกติ ส่วนลูกน้ำยุงในกลุ่มควบคุมเจริญเติบโตได้ตามปกติ การทดลองนี้บ่งให้เห็นว่า สารสกัดจากพืชสมุนไพรมีผลทางพิษวิทยาต่อการเติบโตของลูกน้ำ โดยเฉพาะการสร้างเนื้อเยื่อใหม่ของตัวลูกน้ำและตัวโม่ง ทำให้ไม่สามารถเจริญเติบโตไปเป็นยุงที่สมบูรณ์ได้ จึงตายหมด การทดลองขั้นต่อไป คือ การนำสารพิษในพืชนี้มาประยุกต์ใช้ควบคุมกำจัดลูกน้ำยุงในภาคสนามตามแหล่งน้ำทั่วไป