LARVICIDAL POTENTIAL OF FIVE PHILIPPINE PLANTS AGAINST *AEDES AEGYPTI* (LINNAEUS) AND *CULEX QUINQUEFASCIATUS* (SAY)

Monzon RB, Alvior JP, Luczon LLC, Morales AS and Mutuc FES

Department of Parasitology, College of Public Health, University of the Philippines Manila, Manila, Philippines

Abstract. Five species of Philippine plants, reported in the literature to have insecticidal properties, were selected for investigation, namely: *Anona squamosa* ("atis" or sugar apple), *Eucalyptus globulus* ("bagras" or olive gum eucalyptus), *Lansium domesticum* ("lansones"), *Azadirachta indica* ("neem") and *Codiaeum variegatum* ("San Francisco" or croton). These were screened and assayed for their larvicidal potential against *Aedes aegypti* (Linnaeus) and *Culex quinquefasciatus* (Say) by exposing 3rd-4th instar larvae to seven different concentrations (two-fold dilutions starting from 100 g% up to 1.5625 g%) of the crude aqueous extract derived from fresh leaves. Three trials were performed for each species of mosquito and for each of the five plants to determine the average mortality rate at various concentrations after 24 and 48 hours exposure. Probit analysis using the NCSS program was employed to determine the LD_{50} and LD_{90} values in order to compare the larvicidal potency of the five plants and to compare the susceptibility of *Ae. aegypti* and *Cx. quinquefasciatus*.

The extracts exerted maximum insecticidal activity after 48 hours exposure. Lansones and atis were the most effective against larvae of *Ae. aegypti* and *Cx. quinquefasciatus*, respectively. *Ae. aegypti* was more susceptible than *Cx. quinquefasciatus* with respect to lansones and neem but *Cx. quinquefasciatus* was more susceptible than *Ae. aegyti* with respect to eucalyptus, San Francisco and atis. These varying results are probably due to differences in levels of toxicity among the active insecticidal ingredients of each plant and in the physiological characteristics of the two mosquito species.

INTRODUCTION

The emergence of insecticide resistance and the growing concern for the ecological harm brought about by the use of chemical insecticides has led to the search for safer alternative control methods against mosquitos. Some plants have been found to contain natural toxins effective against mosquito larvae. Not only are they effective but they also greatly reduce the risk of potential adverse ecological effects and may prevent the possibility of resistance which synthetic chemical insecticides normally bring about after prolonged use.

Many studies on such plants have already been conducted around the world and a number of them are promising (Supavarn *et al*, 1975; Barber and Page, 1975; Qureshi *et al*, 1986; Lydon and Duke, 1989; Jackson *et al*, 1990; Kathiresan and Thangam, 1992). In the Philippines, few have attempted to identify such plants and to assess their larvicidal potential (Arzadon, 1991; Yap, 1991). The identification and eventual use of indigenous plants in the control of mosquito larvae may be very valuable for developing countries such as the Philippines and its Southeast Asian neighbors. Besides being more readily available, they are also more economical to use and the methods employed are usually simpler. Thus, this investigative study on the larvicidal potential of five selected Philippine plants is relevant since many plant species in the country have been reported in the literature to have medicinal and more specifically insecticidal properties. These claims need to be scientifically verified through controlled laboratory experimentation.

MATERIALS AND METHODS

Five plant species found in the Philippines and reported in the literature to have insecticidal properties were used in this study, namely: *Anona squamosa* (Linnaeus) or "atis", *Eucalyptus globulus* (Labill.) or "bagras", *Lansium domesticum* (Correa) or "lansones", *Azadirachta indica* (A. Juss) or "neem" and *Codiaeum variegatum* (Linnaeus) or "San Francisco. Only *A. indica* is not native to our country, being indigenous to India; it was introduced here in 1982.

Aedes aegypti larvae were collected from places with clean stagnant water and from artificial containers such as vases, drums, etc within the UP Manila school campus. On the other hand, *Culex quinquefasciatus* larvae were collected from polluted street canals in the same area. These were all brought back to the Department of Parasitology laboratory. Larvae were sorted out and only strong and healthy 3rd-4th instar larvae were selected for the assays.

For each of the five plants assayed, 3 kg of fresh leaves were weighed, cut into smaller pieces and then osterized with 3,000 ml of dechlorinated water to obtain a crude aqueous extract with an initial concentration of 100 g% (w/v). This concoction was then filtered using a strainer siever (# 60) to separate fibrous material and other large particles. The filtered crude aqueous extract which was used in the test proper was serially diluted, two-fold and six times, from the initial concentration of 100 g% to 50 g%, 25 g%, 12.5 g%, 6.25 g%, 3.125 g% and lastly 1.5625 g%.

Tests were performed in triplicate for each plant and species of mosquito. A total of 56 plastic cups were used for each assay consisting of 3 replicates (with corresponding negative controls) for each of the 7 different concentrations of the extract for both *Aedes aegypti* and *Culex quinquefasciatus*. Two hundred and fifty ml of each of the seven given concentrations were poured in each plastic cup except for the controls which contained only 250 ml dechlorinated water. After all the 56 cups were filled with the corresponding concentrations, ten healthy and active 3rd to 4th instar larvae which had previously been sorted out were transferred into each cup. A total of 560 larvae were used - 280 *Ae. aegypti* and 280 *Cx. quinquefasciatus*, for each plant assayed.

The experiments were performed at room temperature and exposed to the normal daylight hours. Observations were done after 24 and 48 hours to record the number of dead larvae in each plastic cup. Identical procedures were applied for all 5 plants.

Probit analysis using the Number Cruncher Statistical System (NCSS) program was employed on the results obtained to determine LD_{50} and LD_{90} values.

RESULTS

Table 1 presents the average percent mortality (based on 3 simultaneous trials) of *Aedes aegypti* and *Culex quinquefasciatus* 3rd - 4th instar larvae after 48 hours exposure to various concentrations of crude aqueous extracts from the 5 plants. At the highest concentration of 100g%, all plants produced 100% mortality with both species of mosquitos, except for eucalyptus which killed only 80.0% of *Ae. aegypti*. On the other hand, at the lowest concentration of 1.5625g%, at least 50% mortality was attained by atis and neem against *Ae. aegypti* and by eucalyptus against *Cx. quinquefasciatus*; however, San Francisco registered zero mortality for both species.

With the use of probit analysis, the LD_{50} and LD_{90} values of the crude extracts from each of the 5 plants after 24 hours and 48 hours exposure were determined and are shown on Tables 2 and 3, respectively. These values can be used to evaluate and compare the larvicidal potency of each plant against *Aedes aegypti* and *Culex quinquefasciatus*. The lower the values obtained then the more potent or effective is the plant's larvici-

Table 1

Average percent mortality of *Aedes aegypti* and *Culex quinquefasciatus* 3rd - 4th instar larvae after 48 hours exposure to various concentrations of crude aqueous extracts from 5 selected Philippine plants.

Concentration (g/100 ml)	Atis		Eucalyptus		Lansones		Neem		San Francisco	
	Aedes	*Culex*	*Aedes*	*Culex*	*Aedes*	*Culex*	*Aedes*	*Culex*	*Aedes*	*Culex*
100.0	100.0	100.0	80.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
50.0	93.3	100.0	50.0	70.0	100.0	90.0	100.0	60.0	86.7	100.0
25.0	70.0	90.0	20.0	96.7	93.3	83.3	83.3	50.0	13.3	76.6
12.5	73.3	80.0	6.7	96.7	93.3	70.0	90.0	36.7	26.7	50.0
6.25	50.0	70.0	3.3	96.7	66.7	70.0	70.0	26.7	3.3	60.0
3.125	70.0	40.0	0.0	83.3	43.3	46.7	76.6	33.3	0.0	6.7
1.5625	50.0	46.7	6.7	56.7	13.3	23.3	73.3	10.0	0.0	0.0
Control	2.8	11.4	4.3	21.4	2.8	5.7	5.7	4.2	1.4	6.1

Table 2

Larvicidal activity of the 5 plants on 3rd - 4th instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* after 24 hours [in g% (w/v) or g/100 ml].

Plant	*Aedes aegypti*		*Culex quinquefasciatus*	
	LD_{50}	LD_{90}	LD_{50}	LD_{90}
Atis	2.4196	201.5054	4.7693	50.7992
Eucalyptus	92.0123	810.6377	0.0030	E H V
Lansones	5.0134	24.8273	8.3409	53.3052
Neem	4.8336	60.4570	44.4121	554.2487
San Francisco	37.6191	100.3039	12.2811	50.2234

Note: EHV = extremely high value

Table 3

Larvicidal activity of the 5 plants on 3rd 4th instar larvae of *Aedes aegypti* and *Culex quinquefasciaus* after 48 hours [in g% (w/v) or g/100 ml].

Plant	*Aedes aegypti*		*Culex quinquefasciatus*	
	LD_{50}	LD_{90}	LD_{50}	LD_{90}
Atis	2.0276	66.8678	2.7740	23.7285
Eucalyptus	51.8570	302.4222	0.0816	27.9742
Lansones	4.0289	16.3316	4.0847	37.7165
Neem	0.3976	21.5592	15.3266	186.3480
San Francisco	27.5710	84.2266	9.4214	34.9225

dal potential.

It will be noted first of all from comparing Tables 2 and 3 to each other that the LD_{50} and LD_{90} values for the 48 hour readings are lower than those from the 24 hour readings. This signifies that all plants are more potent or effective after 48 hours exposure.

From Table 3 it can be seen that for *Aedes aegypti*, lansones is the most effective larvicide (LD_{90} = 16.3316 g%) while the least effective is eucalyptus (LD_{90} = 302.4222 g%). For *Culex quinquefasciatus* however, atis was the most potent larvicide (LD_{90} = 23.7285 g%) while the least potent was neem (LD_{90} = 186.3480 g%).

The susceptibility of *Aedes aegypti* and *Culex quinquefasciatus* to the 5 plants after 48 hours can also be compared based on Table 3. No generalization can be made as to which species was more susceptible to the 5 plant larvicides assayed in this study. *Ae. aegypti* was more susceptible than *Cx. quinquefasciatus* with respect to lanzones and neem while the reverse is true with respect to eucalyptus, San Francisco and atis leaves. Comparison of the LD_{90} values after 48 hours between the two species and among the 5 plants is graphically presented in Fig 1.

It is therefore difficult to rank the 5 plants according to their larvicidal potency since their effectiveness varies between the two species of mosquitos. However, if combined mortality rates of the two mosquito species are considered, a new set of LD_{50} and LD_{90} values can be derived after 48 hrs exposure and these are shown in Table 4. Based on these new values, it can be generalized that the most potent plant in terms of overall larvicidal activity was lansones, followed by atis, San Francisco, neem and lastly eucalyptus. This conclusion is graphically presented in Fig 2.

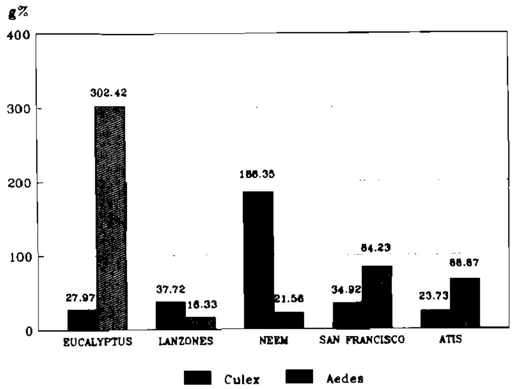

Fig 1–Comparison of LD_{90} values of *Aedes aegypti* and *Culex quinquefasciatus* 3rd - 4th instar larvae among each of the 5 plant species assayed.

Table 4

Larvicidal activity of the 5 plants on 3rd - 4th instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* combined after 48 hours [in g% (w/v) or g/100 ml].

Plant	LD_{50}	LD_{90}
Atic	2.4730	35.4145
Eucalyptus	8.0850	550.8987
Lansones	4.0764	23.5144
Neem	3.6530	112.4111
San Francisco	16.1087	60.5852

DISCUSSION

The results show that the degree of potency of the 5 selected plants against the two species of mosquitos differs greatly. It can thus be postulated that the active larvicidal components present vary from one plant species to another. Since identification of these active ingredients is beyond the scope of the study, this hypothesis cannot be further investigated.

For *Aedes aegypti*, the plant with the lowest LD_{90} value (16.3 g/100 ml) or the most potent among the five selected species of plants was *Lansium domesticum* or "lansones". In contrast, for *Culex quinquefasciatus*, lansones ranked fourth in relative potency with an LD_{90} value of 37.7 g/100 ml. This however does not mean that it is not effective against *Culex quinquefasciatus* since its LD_{90} value was still relatively low, only a few units away from the dose required for *Aedes aegypti*. This verifies the presence of chemically active ingredients in lansones as reported both in the literature and folklore. The outer skin of the fruit is bitter and very rich in tannins. The Peninsular Malays use the juice of the fruit for treating sore eyes and concoctions of its bark and leaves for treating dysentery. In the Philippines, the rind is dried and burned inside the house to drive away mosquitos by means of its smoke which emits a pleasant odor. It is also believed that inhalation of this smoke has a soothing effect on tuberculous persons. Thus, although only the fruit peelings have been reported as having insecticidal properties, the leaves have also been shown in this study to contain similar components.

The second most potent plant against *Aedes aegypti* was *Azadirachta indica* or "neem" with an LD_{90} value of 21.6 g/100 ml. However, it was shown to be the least potent against *Culex quinquefasciatus*. Although

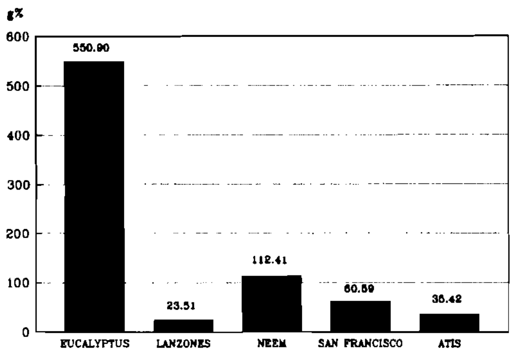

Fig 2–Comparison of LD_{90} values for all 3rd - 4th instar larvae COMBINED among each of the 5 plant species assayed.

ranked second most potent against *Ae. aegypti*, its LD_{90} value was still lower compared to that of atis, which at 23.7 g/100 ml was the most potent against *Cx. quinquefasciatus*. This implies that the ranking of the plants according to their potency is relative and variable, depending on the mosquito species concerned. The seeds of this plant are a more potent source of azadirachtin, a compound claimed to have insect repellant and growth disrupting properties. Neem extracts can also be used as dye, fodder, pharmaceutical preparations, malaria treatment, lubrication, cake and wood.

Anona squamosa or "atis", the third most effective plant against *Aedes aegypti*, was the most potent against *Culex quinquefasciatus*. Its potential as a larvicidal plant is further supported by the results of a recent study conducted in Thailand by Satoto (1993) which revealed that atis seeds are one of the most effective larvicides against *Cx. tritaeniorhynchus*, a ricefield breeder. In addition to insecticidal properties, the plant has been reported to serve as relief for sinusitis and dizziness and as cough expectorant.

The fourth most potent plant against *Aedes aegypti* is *Codiaeum variegatum* or "San Francisco" which proved to be the third most potent larvicide against *Culex quinquefasciatus*. Although never shown before to be effective against mosquito larvae, this plant's leaves have been shown by Garcia (1990) to have molluscicidal properties against *Oncomelania hupensis quadrasi* and therefore this strongly suggests the presence of an inherent pesticidal component in San Francisco leaves.

Eucalyptus globulus leaves were found to be the least effective against *Aedes aegypti* (LD_{90} = 302.4 g/100 ml) but the second most potent against *Culex quinquefasciatus*. It was therefore concluded to be a poor larvicidal agent against *Ae. aegypti* contrary to what was assumed at the start of the study. However the promising results against *Cx. quinquefasciatus* (LD_{90} = 28.0 g/100 ml) paralleled those obtained from the study of Satoto (1993) which showed eucalyptus to be quite effective against *Cx. tritaeniorhynchus*. The seeds should have a higher insecticidal potential since the oil which is extensively used in medicine is derived from this part of the plant.

Using the combined mortality values of *Aedes aegypti* and *Culex quinquefasciatus* larvae as shown in Table 4, it is significant to note that lansones is the most potent among the 5 plants. Lansones is one of the most popular fruit-bearing plants in the Philippines known to have insecticidal properties, although the fruit peeling is the only part of the plant reported to be an effective mosquito repellant. However, being a fruit-bearing tree, obtaining large quantities of lansones leaves could be detrimental to fruit production which is the greater economic value of this species. The second most effective larvicidal plant based on combined results is atis. Similar to lansones, atis is also a fruit-bearing tree and thus its extensive use as a plant larvicide poses similar problems.

The third most potent larvicide among the 5 plants is San Francisco. This is a common inedible ornamental shrub usually grown in large groups and are therefore more preferred than the two plants mentioned earlier. As for neem and eucalyptus, they may be the least potent based on combined mortality values but they may be of great value in controlling particular species of mosquitos. For example, neem has a respectable LD_{90} of 21.5592 g% for *Aedes aegypti* while the LD_{90} of eucalyptus for *Culex quinquefasciatus* is 27.9742 g%. Other studies have already confirmed the efficacy of these two species of plants against other species of mosquitos. For example, Yap (1991) reported the efficacy of neem bitter seeds against *Anopheles litoralis* while Satoto (1993) proved that eucalyptus leaves were effective against *Cx. tritaeniorhynchus*.

Jueco *et al* (1984) showed that *Aedes aegypti* is more susceptible than *Culex quinquefasciatus* with respect to *Bacillus thuringiensis israelensis* serotype H-14. Furthermore, *Cx. quinquefasciatus* larvae prefer a habitat more exposed to pollution (*eg* canals), unlike *Ae. aegypti* larvae which live in cleaner stagnant waters, suggesting that *Cx. quinquefasciatus* is a stronger and more tolerant species. Thus, it was assumed at the onset of the study, that *Ae. aegypti* is generally more susceptible. But the results showed no definite pattern and therefore no generalizations can be made with regards to susceptibility.

It is interesting to note that among the 5 selected plants, atis, San Francisco and eucalyptus are more effective against *Culex quinquefasciatus* while lansones and neem are more effective against *Aedes aegypti*. This does not allow us to conclude that *Cx. quinquefasciatus* is more susceptible than *Ae. aegypti* but only confirms that the plants differ in the chemical composition of their active components. Another possibility to consider is the inherent physiological differences between the two species of mosquitos.

Fluctuations in the mortality curves are probably due to the varying strengths of the larvae collected for the experiments. Heterogeneous populations were sometimes utilized when there was difficulty in obtaining sufficient number of larvae from a single source. This may be the reason for some of the deaths observed among the *Culex* controls. Nevertheless, the average mortality among controls did not exceed 10%.

Only the leaves of the five plants were used in this study and they were shown to contain certain components that can effectively kill mosquito larvae, although in different dosages or concentrations for each of the target species. It is recommended that other parts of the plants (seeds, stems, fruits, etc.) be investigated as these may carry the active larvicidal ingredients in a more concentrated form. The more promising species can be tested further in the future.

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