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Effect of neem insecticide on *Aedes aegypti* larvae in the laboratory

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Prospective controlled laboratory trials were conducted to determine the effect of neem insecticide (a.i. *azadirachtin* 0.75% SC) on third and fourth instars of *Aedes aegypti* larvae using six different concentrations (*aza* 0.0002% to 0.0064%) in accordance with WHO instructions on larval susceptibility test. These trials were performed in Health and Disease Control Unit, Mingaladon in September, 2001 and included six replicates, each consisting of total number of 150 larvae. It was found that the larvae were highly susceptible to *aza* and LC_{50} and LC_{90} were 0.00038% and 0.00087% respectively. The residual effect of insecticide was calculated applying the insecticide stock solution (*aza* 0.15%) of shelf-life day 1, 2, 3, 7 and 10 and it normally persisted for three days. Optimal concentration 0.0016% had the efficacy for seven days. The local product neem insecticide was found to be effective against *Ae. aegypti*, non-toxic to human and environment, easily available and cheap. Therefore, neem insecticide was found to be very promising to be used effectively in the field in treatment of miscellaneous water containers such as discarded unused tires, batteries, tins, coconut shells etc. – well-breeding sources of the vectors *Ae. aegypti* responsible for occurrence of dengue haemorrhagic fever.

INTRODUCTION

Phytochemicals derived from various botanical sources have provided numerous beneficial uses ranging from pharmaceuticals to insecticides. Synthetic organic insecticides, although highly efficacious against target species such as mosquitoes, can be detrimental to a variety of animal life including man. In addition to adverse environmental effects from conventional insecticides, most major mosquito disease vectors and pest species have become physiologically resistant to many of these compounds. These factors have created the need for environmentally safe, degradable and target-specific insecticide against mosquitoes. The search for such compounds has been directed extensively to the plant kingdom [1]. The most prominent phytochemical pesticides in recent years are those derived from neem trees, which have been studied extensively in the field of entomology and phytochemistry, and have uses for medicinal and cosmetic purposes [2]. The neem tree was described as *Azadirachta indica,* belonging to family *Meliaceae* (mahogany family). Neem is called *Tamar* in Myanmar where the neem tree could have originated. Among active ingredients in neem tree *azadirachtin* (*aza*), a very complex tetranortriterpenoid ($C_{35}H_{44}O_{16}$) is the main component which is present in its seed kernel in highest concentration [3]. In Myanmar, neem insecticide (a.i *aza* 0.75% SC) is produced mainly for agricultural pests from Neem Insecticide Factory in Paleik, Mandalay Division.

The currently used insecticides to control *Aedes* are temephos (abate) as a larvicide and malathion and fenitrothion as adulticides. They are effective but not locally available and purchased from foreign countries at the large expense of foreign exchange. They also have chemical toxicity to man if not properly used though they have immediate lethal action on the larvae. The routine vector control methods are easy to carry out but there are many difficulties on consumers' side in treatment of *Aedes* breeding water containers out of which miscellaneous containers play an important role in producing *Aedes*. In that situation neem insecticide was focused to study with the objectives of : (a) to determine the 24 hours exposure effect and residual effect of neem insecticide on the *Ae. aegypti* (L) larvae and (b) to evaluate its potential use for the control of dengue infection.

MATERIALS AND METHODS

Study design was a prospective controlled laboratory trial conducted in Health and Disease Control Unit, Mingaladon from September, 2001 to November, 2002 and field sample collection site was Ward 3 Yanpye, Thaketa Township, Yangon Division.

Ae. aegypti larvae were collected randomly from at least thirty houses in the collection site for representative purposes. Neem insecticide (a.i. *aza* 0.75% SC) was purchased from Neem Insecticide Factory. Methanol 5% as control agent was purchased from Myanma Pharmaceutical Factory and disposable plastic cups from local markets.

Larval susceptibility tests were conducted against third and fourth instars of the larvae by using the insecticide. Six concentrations of insecticide commencing from 0.0002% rising double strength up to 0.0064% were used for six replicates. The tests were carried out according to the WHO instructions on larval susceptibility test. Larvae were collected from domestic water containers (e. g. metal drums) in collection sites using a clear plastic bucket (diameter 24.7 cm and height 25.0 cm) and they were kept, in the laboratory for one day for adaptation purposes, in plastic trays (34.5 cm x 24.5 cm x 6.0 cm) containing rain water before carrying out the test.

Stock solution of insecticide was made for one replicate containing six concentrations as follow: Five mililiter of insecticide was put into a clean disposable plastic bottle (diameter 7.5 cm and height 26.5 cm) containing 20 ml of rain water. Therefore the resultant stock solution was *aza* 0.15% solution. 0.33 ml of stock solution was taken and added into first clean disposable plastic cup (diameter 7.5 cm and height 11.5 cm) containing 224.67 ml of rain water and it was thoroughly stirred for 30 seconds with a glass-rod. Then 0.66 ml of stock solution was added into second cup containing 224.34 ml of rain water. Likewise double the amount of stock solution was added till the sixth cup was completed. Being a methanol neem seed extract, for control 0.5 ml of methanol 5% was added into the cup containing 224.5 ml of rain water. At the same time each batch of active and vigorous 25 larvae (13 third and 12 fourth instars) together with natural food from their habitats was transferred from plastic trays to 7 small clean disposable plastic cups (diameter 6.0 cm and height 4.8 cm) each containing 25 ml of rain water. Next each batch of 25 ml of rain water together with 25 larvae was poured

Table 1. Preparation of six neem insecticide concentrations (*aza* 0.75% SC)

No.	Initial rain water (ml)	Added stock solution (ml)	Added rain water (ml)	Resultant *aza* concentration (%)	Resultant *aza* concentration (ppm)
1	224.67	0.33	25	0.0002	2
2	224.34	0.66	25	0.0004	4
3	223.68	1.32	25	0.0008	8
4	222.36	2.64	25	0.0016	16
5	219.72	5.28	25	0.0032	32
6	214.4	10.6	25	0.0064	64

Aza concentration in stock solution of neem insecticide is 0.15%

into the six cups each containing 225 ml of insecticide solution and one control cup. The final *aza* concentrations in the cups were 0.0002%, 0.0004%, 0.0008%, 0.0016%, 0.0032% and 0.0064% (Table 1). Moribund and dead larvae were counted as dead after 24 hours exposure. The tests were done in six replicates.

For residual effect of insecticide, stock solution (*aza* 0.15%) was kept for one, two, three, seven and ten days and then tested against third and fourth instars (10 in numbers) separately using three replicates at each concentration. Then mortality percent-tages were calculated after 24 hours exposure and compared.

The temperature (21°C to 35°C) and relative humidity (64% to 92%) were recorded throughout the test period. Data analyses were done by using Epi Info Version 6.04 and S. Swaroop's statistical method for χ^2 test to determine correlation co-efficient and LC_{50} and LC_{90} values [4].

RESULTS

The test results showed that LC_{50} and LC_{90} values of *aza* for thrid and fourth instars of *Ae. aegypti* larvae were 0.00038% and 0.00087% respectively and 95% confidence upper and lower limits were described (Table 2). Correlation between dose and effect was in a strong degree but not statistically significant ($r = 0.66$, $p > 0.05$) due to a small sample size of concentration of insecticide ($n= 6$). The χ^2 test of goodness of fit of the regression line showed that the mosquito populations were significantly heterogeneous ($p< 0.01$). Regarding residual effect of insecticide, its effects were more or less the same up to 3 days in all concentrations except 0.0004% at which larval mortality gradually decreased. After 3 days mortality decreased in all concentrations up to seventh day except at the last two concentrations. After seven days, mortality increased again in first three concentrations, probably due to water pollution rather than the larvicidal action of insecticide, but decreased in 0.0016%. Cent per cent mortality was found at the last two concentrations in all days (Fig.1). Therefore neem insecticide is effective for three days

Table 2. Fitting a regression line and testing the goodness of fit [Data on suscep-tibility of *Ae. aegypti* larvae to neem insecticide (*aza* 0.75 % SC)]

No.	Aza concentra-tion (%)	Larvae dead/ tested	Observed mortality rate, (%) (corrected)	Expected mor-tality rate (%)	Observed minus expected rate	Contribution to χ^2
1	0.0002	12/150	8	4	4	0.04167
2	0.0004	83/150	55	54	1	0.00040
3	0.0008	111/150	74	88	-14	0.18561
4	0.0016	146/150	97	97.1	-0.1	0.00004
5	0.0032	150/150	100 (99.86)	99.4	0.46	0.00355
6	0.0064	150/150	100 (99.86)	99.84	0.02	0.00003
7	control	6/150	4	-	-	-
		Total				0.2313

LC_{50} = 0.00038 %
95 % confidence limit, lower = 0.00035 %
95 % confidence limit, upper = 0.00041 %
LC_{90} = 0.00087 %
95 % confidence limit, lower = 0.00077 %
95 % confidence limit, upper = 0.00098 %

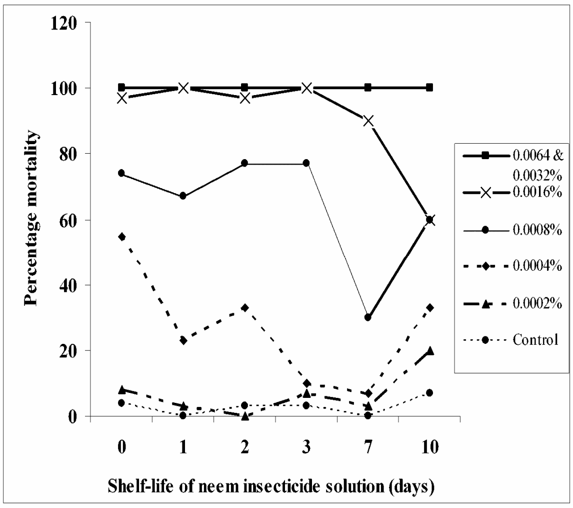

Fig. 1. Residual effect of six neem insecticide concentrations on third and fourth instars of *Ae. aegypti* larvae

in all concentrations. After three days, percent reduction of residual effect in terms of larval mortality was between 10% and 61%. Optimum concentration for seven days was 0.0016% to obtain 90% larval mortality.

DISCUSSION

The effects of neem insecticide on insects are: phago- and oviposition-deterrent, repellent, antifeedant, growth retardant, molt-inhibitor and sterilant. Zebitz (1984) suggested that *aza* acts as an anti-ecdysteroid or affects the neuroendocrine control of ecdysteroids [1, 3]. Exposure of fourth instar larvae of *Ae. aegypti* to water extracts of neem resulted in conspicuous disruption of growth whereas exposure of first instar larvae caused a prolongation of the larval period and eventually about 90% mortality [5]. Aqueous extract of neem seed gave LC_{50} value of 78 mg/L when it was tested on first and fourth instars of *Ae. aegypti* larvae and LC_{50} value of methyl-tert-butyl-ether extract was 2 mg/L [3]. Effect of 'Margosan O' neem insecticide produced from USA (*aza* 0.3 %) on third and fourth instars of *Ae. aegypti* larvae showed that LC_{50} values were 12 ppm and 14 ppm respectively [6]. The results from the present study are much lower than those of above-mentioned study. The present study shows that effect of neem insecticide on *Ae. aegypti* larvae was very satisfactory. Residual effect also showed that the insecticide efficacy still persisted for three days. It is the same as the data in the study by IM Scott *et.al*, which showed that in the residue analysis, *aza* had a half-life of 36-48 hours in water exposed to normal sun light [7]. About 39% to 90% of third day efficacy remained till seventh day. Optimal concentration was 0.0016% for seven days.

Neem trees are widely and abundantly distributed in Myanmar where neem seed kernels were supposed to have highest concentration of *aza* (i.e. 6.1 ± 0.7 mg/g kernel) among many samples from 22 countries in the world [3]. The State has invested on a large scale in neem plantation in the country, for example in Shwe Pontaung region, Chauk Township, Magwe Division where nearly 0.5 million of neem trees are to be cultivated (300 neem trees per acre) from year 2000 to 2002 under the special project. In the country the existing accessible mature neem trees in central part alone can give about 766,000 bottles (500 ml) of neem insecticide annually [8]. At present, the factory produces 90,000 bottles (500 ml) annually. The local price is 500 kyats per bottle and four US $ per one liter bottle for export. Therefore, local price is cheap (Aye Ko, personal communication, 2001).

Dengue haemorrhagic fever (DHF) is one of the health problems in Myanmar especially in 5-15 years age group. It is endemic here with 3-4 years epidemic cycle. Reported cases are 1000-3000 every year in non-endemic year and 3000-15000 in epidemic year. Case fatality rate is 3.9 % [9]. At present, it is under 1 % probably due to the early referral of the cases to the hospitals. There are three categories of container type-major, minor and miscellaneous, which are all the breeding sites of *Ae. aegypti* (W. Tun Lin, unpublished data, 1995). Among many types in last category, tires may be key containers because they are used widely in various sizes from small to large since market-oriented economy is enacted and put into effect in our country. In a study by W. Tun Lin *et.al*., key container types, for example, tires and drums comprising 10-20 % of total containers were responsible for 82.6 - 99% of the *Aedes* being produced [10].

Regarding present vector control programme, the methods are simple but reportedly not carried out by local people regularly due to economic and administrative reasons, for example, going out for work, shortage of municipal workers to clean the solid waste and unwillingness of people to do. The insecticide has an unpleasant odour but the people normally accept it, if it is used in outdoor discarded materials like unused tires, batteries, jars, cisterns, plant containers and coconut husks and temporary small water pools when removal and destruction is not feasible. Neem insecticide alone does not complete but it will be complement for the programme. Even if the larvae are alive after being exposed to *aza* their growth will be delayed and male adults will become sterile. Such slow action of botanical insecticide is not well understood by most of the people who generally like synthetic insecticide, which gives immediate toxic effect on the insects. So the effect of botanical insecticide should be thoroughly explained to the consumers if it is used in the vector control programme. Dosage of neem insecticide (*aza* 0.75% SC) for discarded container is 1 ml per liter of water held to have 90% mortality of larvae. One millilitre of neem insecticide costs only one kyat. Conventional insecticides like fenitrothion and malathion are expensive. By using local insecticide the country will be able to save much foreign exchange.

Neem insecticide is effective, cheap and in accordance with State policy. So, this study would support the present vector control programme in the country to a certain extent. The findings in the present study are compatible with those in other studies and give useful basic research data for appropriate technology for DHF control in Myanmar. Therefore it is recommended that neem insecticide with extraordinary characteristics of being safe, effective, locally available, economical and non-toxic to human and environment should be used to control *Ae. aegypti* mosquito, the vector of DHF which affects thousands of children under fifteen in our country annually.

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