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Effect of copper sulphate on *Aedes aegypti* larvae in the laboratory

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Prospective controlled laboratory trials were conducted to determine the effect of copper sulphate ($CuSO_4.5H_2O$, 95.4%) on *Aedes aegypti* larvae using seven different concentrations, starting from 1.25 mg/L rising double strength up to 80.0 mg/L in accordance with WHO instructions on larval susceptibility test. These trials were undertaken in Health and Disease Control Unit, Mingaladon in October 2001 and included six replicates for each stage of larvae. The results showed that the larvae were highly susceptible to copper sulphate and its LC_{50} value for both stages was 2.25 mg/L and LC_{90} values were 10 mg/L and 15 mg/L for early and late stages respectively. Mosquito populations were found not to be significantly heterogeneous. Regarding residual effect, copper sulphate stock solutions (2,500 mg/L) of shelf-life day 1, 2, 3, 7, 10, 14, 21 and 28 were used and its effect against larvae normally persisted for about three weeks. This compound was found to be effective against the target species, harmless to human and environment, locally available and cheap. Therefore the role of copper sulphate was very promising to be used in the fields by its application in some minor water-storage containers, holding non-potable water such as altar flower vases, spiritual pots and bowls and ant-traps to suppress *Ae. aegypti* larvae in controlling dengue haemorrhagic fever effectively.

INTRODUCTION

Mosquitoes were commonly found in all types of flower containers except bronze vases in cemeteries in Florida, USA. In the laboratory test on *Ae. aegypti* larval development using plastic cups and bronze vases, 98% of larvae in the former completed development to adult stage whereas none in the latter survived beyond the second instar due to inhibition of larval development. It was suspected that copper, a main component of bronze vases, is the primary factor responsible for preventing mosquito development. In field surveys at cemeteries to find out mosquito prevalence in stone vases without liner, with aluminium liner and with copper liner, it was found that percentages of mosquito-positive vases were 60%, 50% and 11.7 % respectively. Percentage from vases with copper liner was significantly smaller ($p<0.01$). Various copper compounds are also used as fungicides and algicides [1, 2]. Copper sulphate {copper (II) sulphate or cupric sulphate}, one of the copper compounds, is easily available at local markets in Myanmar and also produced from Chemical Engineering Production Co-operative Society Ltd, Yangon (Khin Myo Win, personal communication, 2003). The present study was carried out with the objectives of [1] to determine the larvicidal effect of copper sulphate on *Ae. aegypti* (L.) larvae in 24 hours and [2] to assess its residual effect to be used in the control of dengue infection.

MATERIALS AND METHODS

The study design was a prospective controlled laboratory trial. The study period was from October, 2001 to January, 2003 and the larval collection area was Ward 3 Yanpye, Thaketa Township, Yangon, Myanmar.

Ae. aegypti larvae were collected randomly from at least twenty houses in the study area for the representative purposes. Copper sulphate (cupric sulphate pentahydrate, $CuSO_4.5H_2O$) and disposable plastic cups were purchased from local markets. Copper sulphate was chemically analyzed by using isometric method at Plant Protection Division, Myanmar Agriculture Service to determine the technical grade of cupric sulphate pentahydrate and the grade was found to be 95.4%.

Larval susceptibility tests were conducted, in accordance with WHO instructions [3], against early (first and second instars) and late (third and fourth instars) larval stages of *Ae. aegypti* using copper sulphate for the first time in Myanmar in the laboratory of Health and Disease Control Unit, Directorate of Medical Services, Mingaladon, Yangon. Seven concentrations starting from 1.25 mg/L rising double strength up to 80 mg/L were used for six replicates.

The larvae were first collected from domestic water containers (e.g.metal drums) in the study area using a clean plastic bucket (diameter 24.7 cm and height 25.0 cm) and then kept, for adaptation purposes, in a plastic tray (34.5 cm x 24.5 cm x 6.0 cm) containing rain water in the laboratory for one day before carrying out the test.

A stock solution was prepared by adding 1,048 mg of copper sulphate into 400 ml of rain water in a glass beaker (dia. 9.0 cm and height 11.8 cm) to get the concentration of 2,500 mg $CuSO_4.5H_2O$ per litre of water. 0.125 ml of stock solution was taken and added into first clean disposable cup (diameter 7.5 cm and height 11.5 cm) containing 224.88 ml of rain water and it was thoroughly stirred for 30 seconds with a glass rod. Then 0.25 ml of stock solution was added again into second cup containing 224.75 ml of rain water. Likewise, double the amount of stock solution was added till the seventh cup was completed. For control 225 ml of rain water was filled in a separate cup. At the same time each batch of active and vigorous 20 early stages (10 first and 10 second instars) together with natural food from their habitats was transferred from plastic tray to 8 small clean disposable 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 20 larvae of early stages was introduced into seven test cups containing 225 ml of copper sulphate solution and into one control cup. The final concentrations of copper sulphate in test cups were 1.25 mg/L, 2.5 mg/L, 5.0 mg/L, 10.0 mg/L, 20.0 mg/L, 40.0 mg/L and 80.0 mg/L (Table 1). Moribund and dead larvae were counted as dead after 24 hours exposure. Mortality rates were recorded and plotted on a logarithmic-probability paper. The tests were done in six replicates. Similarly 20 larvae of late stages (10 third and 10 fourth instars) were tested in six replicates.

Table 1. Preparation of seven different concentrations of copper sulphate

No.	Initial rain water (ml)	Added stock solution (ml)	Added rain water (ml)	Resultant $CuSO_4.5H_2O$ concentration (mg/L)	Equivalent copper concentration (mg/L)
1	224.88	0.125	25	1.25	0.32
2	224.75	0.25	25	2.5	0.64
3	224.5	0.5	25	5	1.27
4	224	1	25	10	2.55
5	223	2	25	20	5.09
6	221	4	25	40	10.18
7	217	8	25	80	20.37

$CuSO_4.5H_2O$ concentration in copper sulphate stock solution is 2,500 mg/L.

For residual effect of copper sulphate, the stock solution (2,500 mg/L) was kept at room temperature for one, two, three, seven, ten, fourteen, twenty-one and twenty-eight days and then tested against ten larvae

(5 early and 5 late stages) in each concentration separately using three replicates. Then the mortality percentages were calculated after 24 hours exposure and they were compared. The temperature and relative humidity throughout the test period were 25 ± 7 ºC and 78 ± 8 % respectively. Data analyses were done 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} values of copper sulphate for both early and late stages was 2.25 mg/L and LC_{90} values for early and late stages were 10 mg and 15 mg/L respectively. The 95% confidence lower and upper limits were also described (Table 2, 3). There was a strong degree of correlation between dose and effect in both stages (r=0.66) but it was not statistically significant (p>0.05) due to small sample size of concentration of copper sulphate (n = 7). The χ^2 test for goodness of fit of the regression line showed that mosquito population were not found to be significantly heterogeneous in both early and late stages.

With regard to residual effect of copper sulphate it persisted for 21 days in first five concentrations. After 21 days it decreased gradually. Between 1st day and 28th day percentage reduction in larval mortality was from 9 % to 14 %. In the last two concentrations the residual effect did not reduce. Cent per cent mortality was found in the last concentration in all days up to 28th day (Fig.1).

DISCUSSION

Copper is widely used in cooking utensils, water distribution system, in making surgical instruments and intrauterine devices [5, 6]. In industry, it is used as an activator

Table 2. Fitting a regression line and testing the goodness of fit (Data on susceptibility of early larval stages of *Ae. aegypti* to copper sulphate)

No.	$CuSO_4.5H_2O$ concentration	Larvae dead/ tested	Observed mortality rate, (%) (adjusted)	Expected mortality rate (%) (from graph)	Observed minus expected rate	Contribution to χ^2
1	1.25	29/ 120	24	18	6	0.0244
2	2.5	60/ 120	50	56	-6	0.0146
3	5	85/ 20	71	78	-7	0.0286
4	10	106/ 120	88	89	-1	0.001
5	20	114/ 120	95	94	1	0.0018
6	40	118/ 120	98	97	1	0.0034
7	80	120/ 120	100 (99.38)	98.2	1.18	0.0079
8	Control	5/ 120	4		-	-
			Total			0.0817

LC_{50} = 2.25 mg/L,
95 % Confidence limit, lower = 1.95 mg/L
95 % Confidence limit, upper = 2.60 mg/L
LC_{90} = 10.0 mg/L,
95 % Confidence limit, lower = 7.92 mg/L
95 % Confidence limit, upper = 12.63 mg/L

Table 3. Fitting a regression line and testing the goodness of fit (Data on susceptibility of late larval stages of *Ae. aegypti* to copper sulphate)

No.	$CuSO_4.5H_2O$ concentration (mg/L)	Larvae dead/ tested	Observed mortality rate, (%) (adjusted)	Expected mortality rate (%) (from graph)	Observed minus expected rate	Contribution to χ^2
1	1.25	32/ 120	27	21	6	0.022
2	2.5	68/ 120	57	56	1	4E-04
3	5.0	93/ 120	78	76	2	0.002
4	10.0	104/ 120	87	87	0	0
5	20.0	106/ 120	88	92	-4	0.022
6	40.0	110/ 120	92	95.5	-4	0.029
7	80.0	120/ 120	100 (99.06)	97.2	1.9	0.013
8	Control	3/ 120	3	-	-	-
			Total			0.087

LC_{50} = 2.25 mg/L
95 % Confidence limit, lower = 1.86mg/L
95 % Confidence limit, upper = 2.72mg/L
LC_{90} = 15.0 mg/L
95 % Confidence limit, lower = 10.34mg/L
95 % Confidence limit, upper = 21.75 mg/L

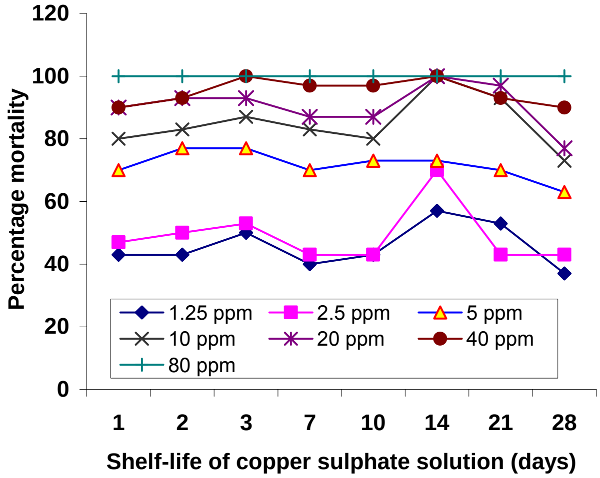

Fig.1. Residual effect of seven copper sulphate concentrations on *Ae. aegyti* larvae (both early and late stages)

in the froth flotation of sulphide ores, production of chromated copper arsenate wood preservative and others and in agriculture, it is used as a fungicide (Borde-aux mixture), pesticide and algicide, and nutritional supplements in animal feeds and growth promotors, as well as for disease control in livestock and poultry and as fertilizers. Latest approach to control of midge larvae in drinking water supplies is suppression of planktonic first stage larvae by using two disinfectants, chloramine and copper sulphate [7, 8].

Based on the results of a number of animal studies involving oral and intraperitoneal exposure to various copper compounds, it is generally agreed that copper and its salts are not animal carcinogens [9]. In prospective studies to determine associations between serum copper level generally greater than 1.25 mg/L and either total or breast cancer, there is no convincing evidence of dose-response trend. Moreover there has been no association between intake of copper and cancer in those few analytical epidemio-logical studies in which it has been investigated. There is also little convincing evidence that copper plays an aetiological role in the development of cancer in humans. The International Agency for Research on Cancer concluded in 1987 that there are no data on the carcinogenicity of copper 8-hydroxyquinoline in humans and insufficient data in animals. In connection with dermal exposure, it is non-toxic and though copper algicides are used in the treatment of water in swimming pools and reservoirs, there are no reports of toxicity from these application. No occupational studies were also found to indicate that copper exposure resulted in reproductive or developmental effects. On available data on human exposure worldwide but particularly in Europe and the Americas, there is a greater risk of health effect from deficiency of copper intake than from excess copper intake. A provisional tolerable daily intake (PTDI) of copper (Cu) from all sources was established as 0.5 mg/kg body weight [10]. As regard with content in water, copper should not exceed 3.0 ppm and sulphate ($SO^{=}_4$) should not exceed 250 ppm in natural or treated water [5]. WHO proposed that provisional guideline value of copper in drinking water was 2 mg / L [10].

In one of the laboratory tests the larvicidal properties of metallic copper against *Ae. albopictus* showed that development time from larva to adult was delayed and the resultant reduced weight of the adults probably influences both their fertility and flight ability [11]. The present study showed that the effect of copper sulphate on *Ae. aegypti* larvae was very satisfactory and correlation was strong but not statistically significant due to small sample size of copper sulphate concentrations. Its residual effect normally persisted for three weeks. Beyond three weeks it should be further tested in future. The optimal concentration was 15 mg/L and it was also the LC_{90} value for late larval stages as well as the dose not causing phytotoxicity in most plants and flowers in the vases (Htin-Zaw-Soe, unpublished data 2003).

Among the three categories of container type-breeding sites of *Ae. aegypti* - indoor and outdoor flower vases, plant pot saucers, spiritual pots and/or bowls (Nat-sin-ou) and ant-traps fall under the minor category.They are man-made and water is usually held in them for a long time in the shade creating the well-breeding and well-producing sites of *Aedes* mosquitoes. Traditionally spiritual pots and bowls cannot be removed and eliminated. Among them some hold small messy plants like "Kanyut" (*Asparagus officinalis*) and "Myezar" or Bermuda grass (*Cynodon dactylon*) and householders were found to rarely empty them and change the plants, but frequently pour water on them. It was done like that due to their traditional belief which said that "The more the leaves of the plants sprout, the better is the household economy". In such condition copper sulphate should be used. To prevent larval breeding and emergence routine vector control method of changing water weekly should be done. But most of the people do not follow it though the method is simple and easy due to various reasons like state of being busy with daily works, scarcity of water and unwillingness to do so. Even if they follow they only change water in vases but do not thoroughly wash or scrub the roots or stems of the flowers or plants or inner wall of the vases. Therefore the eggs and larvae would be probably stuck to the roots and stems of the plants or to the inner wall of the vases thereby the remaining eggs and larvae may develop and finally the adults may emerge. Moreover, the flower vases and plant containers are usually placed indoors and mosquito-positive ones would make man-vector contact more easily than other outdoor containers. So attention must be paid to them though they are of minor category. In these conditions copper sulphate should be considered to be used. The dosage of copper sulphate is 15 mg/L every three weeks to have 90 % larval mortality. For a flower vase containing one litre of water it requires 15 mg of copper sulphate. The cost is only 0.03 kyat and it is very cheap. Metallic copper is also effective but its initial investment is larger than that of copper sulphate. Conventional larvicides like temephos (abate) are effective but expensive and imported from foreign countries at the large expense of foreign exchange. By using locally available copper sulphate, the country will prevent waste of foreign exchange.

It is concluded that the present study provides useful basic research data for appropriate technology for *Aedes* control and it could complement the present vector control programme. Therefore copper sulphate, a larvicide of being cheap, effective against target species, locally available, applicable and harmless to human and environment is recommended to be tested under field conditions and to be used in future in treatment especially of spiritual pots and bowls to control *Ae. aegypti* in our country where thousands of children under 15 years are stricken with dengue haemorrhagic fever annually.

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