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POSSIBLE UTILIZATION OF METALLIC COPPER TO INHIBIT *AEDES ALBOPICTUS* (SKUSE) LARVAL DEVELOPMENT

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ABSTRACT. The effect of metallic copper on development of *Aedes albopictus* was studied in the laboratory. Multiwire electric cable was used as a source of metallic copper in flower saucers colonized by *Ae. albopictus.* A linear regression coefficient of 0.68 was obtained between copper concentration in the water during larval development and the relative production of adults. Larval mortality was higher in earlier instars with less evident effect on 4th-instar larvae and pupae. The effect of copper on larval development time and adult weight in both sexes was also observed. The strong algicidal action is presumed to only partially explain the effect of metallic copper on *Ae. albopictus* larvae. A direct toxic effect also may be involved. The use of metallic copper is suggested as a practical alternative method for preventing development of *Ae. albopictus* in small containers such as flower saucers found in urban areas.

KEY WORDS *Aedes albopictus*, mosquito control, development time, flower saucer, multiwire cable, adult weight, egg hatching

INTRODUCTION

Since September 1990, when its presence was first recorded in Genoa (Sabatini et al. 1990), *Aedes albopictus* (Skuse) has spread rapidly in northern and central Italy, proving that the climatic conditions in this area are conducive to the achievement of high population densities (Celli et al. 1994, Knudsen et al. 1996). This species achieves particularly high population densities in urban and suburban areas, where it colonizes man-made containers of various shapes and sizes, provided that these are in the shade and are capable of collecting and holding water for a sufficiently long time.

Because of the large number of these man-made breeding sites, controlling the species by means of traditional methods has proven to be very difficult and costly. Therefore, finding a control method that can offer long-term effectiveness for those small containers, which cannot be removed or eliminated, would be advantageous.

Copper, in the form of sulfate, is normally used as an algicide in rice fields and also shows toxic effects on mosquito larvae (Mulla and Chaney 1994). O'Meara et al. (1992a, 1992b), observed the toxic effects of copper released from bronze vases on *Ae. albopictus* larvae. In the laboratory, Della Torre et al. (1993) verified the larvicidal properties of metallic copper against *Ae. albopictus.*

In aquatic environments copper is distributed among the components of the ecosystem. The half-life of the copper ion in water is very short (1–7 days) and its disappearance is linked to adsorption phenomena of sediments, which act as copper reserves (Reinert and Rodgers 1987).

Aquatic macroinvertebrates seem to be sensitive to high concentrations of copper. Substrata contaminated with 900–4,500 mg/kg (very high concentration) of copper have caused the deformation of buccal organs in *Chironomus decorus* (Johannsen) and the prolongation of its larval stage (Kosalwat and Knight 1987).

The present study was conducted to examine the effects of metallic copper on larval development of *Ae. albopictus* in flower saucers, which are among the most common man-made containers colonized by this species in Italy (Bellini et al. 1994, 1995, 1996).

MATERIALS AND METHODS

Tests were carried out from December 2, 1994, to April 28, 1995, in a climatic chamber at 28 ± 1°C, 90% relative humidity, and with a photoperiod of 16-h light and 8-h dark. Plastic flower saucers (20 cm in diameter) containing 500 ml of water, and earthenware pots (with a base diameter of 10 cm) filled with peat moss were used. Previously dechlorinated tap water was added daily to compensate for evaporation.

Two doses of metallic copper (multiwire electric cable) were used: 2 g/liter and 8 g/liter. The test was repeated 3 times in succession using the same protocol and without changing any part of the experimental apparatus to verify the persistence of metallic copper action. Each dose was tested in 8 flower saucers and an additional 4 were used as a control group.

For each dose in 4 of the flower saucers the water level was kept constant during the entire experimental period, whereas in the other 4, no water was added between tests, allowing for desiccation to occur. This was done to determine the effect of periodic desiccation, which often occurred in the field, on the dissolution of copper when water was added again.

Forty 48-h-old eggs, obtained from a colony bred from a northern Italian population, were placed into

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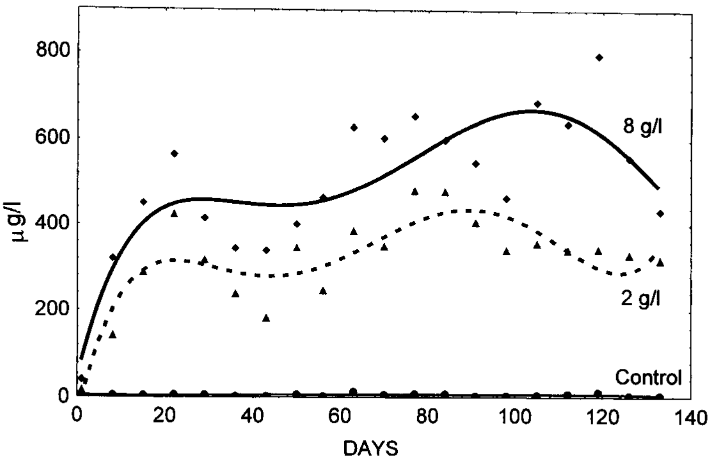

Fig. 1. Ionic copper concentration in water contained in flower saucers (weekly samples).

each flower saucer. The larvae were fed about 500 mg/flower saucer of cat food (Friskies®, Friskies Italia-NE.IT. S.p.a. Milan, Italy) containing 4 ppm of copper as reported on the label. Each flower saucer with pot was held in a gauze bag to prevent adult dispersion. Larvae were checked every 48 h and dead ones were removed; adults were collected every 24 h.

For each dose and control group, weekly samples of about 50 ml of water were taken in sterile containers for chemical and microbiological analysis. Samples were obtained by mixing small amounts of water taken from each flower saucer of the same treatment. The pH was measured immediately by a portable pH meter (Crison 507, Crison Strumenti S.p.a. Carpi, Italy). During the final phase of the study the sediments on the internal surface of the flower saucers were checked for possible copper accumulation.

To determine the bioaccumulation of copper, samples of 2nd-, 3rd-, and 4th-instar larvae (157 larvae), washed with double-distilled water, were dried in a stove at 105°C until a constant weight was achieved and weighed with an electronic microbalance (Sartorius XM 1000P, Sartorius S.p.a. Florence, Italy) (sensitivity 0.001 mg). The larvae were subsequently exposed to a wet etch test (HNO_3 suprapur, Merck, Frankfurt, Germany) employing a precipitating refrigerant. At the end of the mineralization process the samples were quantitatively retrieved and brought to a known volume of 25 ml with double-distilled water.

Ionic copper concentration was measured by inductively coupled plasma–atomic emission spectrometry, using a Jobin Yvon ICP sequential spectrometer (JY24 HR, Jobin Yvon, Lonjumeau, France) at a wavelength of 324.75 nm. Analytical accuracy was evaluated with reference to National Institute of Standards and Technology (NIST) standard 1643C. The concentration of algae was estimated by fluometrically measuring the quantity of chlorophyll *a* (Innamorati et al. 1990). Total microbial loads were calculated using Oxoid Standard Plate Count Agar (APHA, Unipatii Limited, Hampshire, United Kingdom) as a culture medium. The plates were placed in a thermostatically controlled chamber at 37 ± 1°C for 48 h. Adults produced in the 2nd test were dried at 50°C for 24 h and individually weighed to determine the possible effect of copper on adult size. The data were analyzed using 1- and 2-way analysis of variance, after arcsine transformation of the percentage values, followed by Tukey's test for the separation of the averages.

Table 1. Copper concentration in pools of 2nd-, 3rd-, and 4th-instar larvae collected during larval development.

Copper dose (g/liter)	No. pools	No. larvae	Copper concentration (μg/mg [±SD]) (dry weight)
Control	4	59	1.38 (±1.71)
2	3	34	2.49 (±2.63)
8	4	64	1.74 (±2.17)

RESULTS

The concentration of copper ion in the water increased progressively during the initial 3 wk, up to day 21 when 424 μg/liter and 565 μg/liter, for doses of 2 g/liter and 8 g/liter, respectively, were achieved. Concentration then fluctuated with a slight tendency to increase in the following weeks (Fig. 1). Copper concentration in the bottom and internal surface sediments was noticeably higher than in the water, with a range of values of 228–558 μg/g of dried matter. No relationship was evident between the metallic copper dose and copper

Table 2. Effect of metallic copper on concentration of algae and on total bacteria in the flower saucer water (total average of weekly data).

Copper dose (g/liter)	Algae		Bacteria	
	n	Means	*n*	Means
Control	8	43.30 (±35,95)	12	1,608.33 (±1,547,94)
2	8	0.05 (±0,10)	12	3,028.33 (±4,397,77)
8	8	0.07 (±0,14)	12	1,709.17 (±2,434,30)

accumulation in the sediments. Copper concentration in water was always very low in the control, with a maximum value of 13 μg/liter; the copper present probably came from the peat, from the cat food used to feed larvae, and from the water. Copper concentration in the larvae was not significantly different between the treated and control pools, nor between doses, showing no evident bioaccumulation phenomenon (Table 1).

Metallic copper had a strong inhibitory effect on algal growth at both doses, whereas no noticeable impact was detected on the number of bacterial colonies (Table 2). Therefore, copper does not seem to influence the microbial load. The algae present in the flower saucers were of the families Chlorophyceae and Cyanophyceae with *Scenedesmus crassus* as a predominant species.

The pH values were 8.03 (±0.12) at a dose of 2 g/liter, 8.09 (±0.12) at a dose of 8 g/liter, and 7.91 (±0.20) in the control. Values were significantly different between doses and between doses and the control.

The comparison of the percentages of larval mortality observed in the flower saucers in which the water level was kept constant and in those in which temporary desiccation occurred did not reveal significant differences ($P = 0.32$). Therefore, the overall data were considered in the subsequent statistical analysis.

No significant differences were observed ($F = 0.18$; df 2,17; $P < 0.83$) in the percentages of egg hatchings between the control group (160 eggs, hatching 55.6%) and the 2 g/liter dose (320 eggs, hatching 51.8%) and the 8 g/liter dose (320 eggs, hatching 59.2%).

The total adult production rate calculated with reference to the number of eggs tested was 61.7% in the control, 10.5% for the 2 g/liter dose, and 4.0% for the 8 g/liter dose. Significant differences were observed between treatments and control in all 3 replications. Differences between the 2 g/liter dose and the 8 g/liter dose were significant only in the first assay, although adult emergence was always lower at the higher dose (Table 3).

Although the range of copper concentration tested was narrow, the linear relation between average copper concentration in the water during single tests and the relative production of adults was significant ($R^2 = 0.68$, $P < 0.001$) (Fig. 2). The percentage of adults emerged fell in proportion to the increase in copper concentration, dropping to 0% in the test with the highest copper concentration (596 μg/liter).

Larval mortality was higher for both doses at the earlier stages. Although no significant difference was found between the mortality in the control group and in both doses in the 4th-instar and pupal stages, this may be attributed to the very low number of individuals surviving from earlier stages to be considered for data analysis (Table 4). Only in the first 2 stages is a linear relationship shown with copper concentration ($R^2 = 0.72$, $P < 0.001$). The regression lines relative to the different larval stages are also not parallel to each other (slope $F = 12.97$, $P < 0.001$).

Average development time, from egg immersion to adult emergence, was delayed by copper to 21.13 days at a dose of 2 g/liter and 26.69 days at a dose of 8 g/liter as compared to 13.9 days in the control group (Table 5).

Adult weight was also affected by the copper concentration experienced by the larvae during larval development with $R^2 = 0.78$, $P < 0.001$ for males, and $R^2 = 0.67$, $P < 0.001$ for females (Fig. 3). The parallelism test did not indicate significant differences in the regression coefficient ($F = 2.48$, $P = 0.12$), indicating that copper has a similar effect on the weight gain of adults of both sexes.

Table 3. Adult emergence ratio and the number of eggs tested for each assay.[1]

Copper dose (g/liter)	1st replication		2nd replication		3rd replication	
	No. flower saucers (eggs)	Adult emergence ratio (mean [±SD])	No. flower saucers (eggs)	Adult emergence ratio (mean [±SD])	No. flower saucers (eggs)	Adult emergence ratio (mean [±SD])
Control	4 (160)	0.75 (±0.05) a	4 (160)	0.50 (±0.11) a	4 (160)	0.60 (±0.03) a
2	4 (160)	0.31 (±0.19) b	8 (320)	0.09 (±0.20) b	8 (320)	0.10 (±0.02) b
8	8 (320)	0.07 (±0.10) c	8 (320)	0.04 (±0.08) b	8 (320)	0.00 (±0.00) b

[1] Within the columns, the values followed by different letters are significantly different when Tukey's test is used.

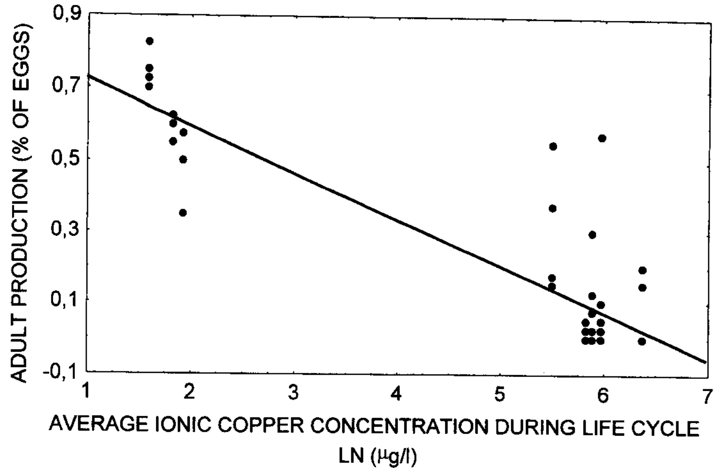

Fig. 2. Effect of ionic metallic copper concentration in the water on production of *Aedes albopictus* adults (percent of adults obtained from initial number of eggs).

DISCUSSION

Copper multiwire electric cable in water releases ions in a concentration that is dependent on the copper to water ratio being tested. Also, the surface of metallic copper used plays an important role in the quantity of ions released but this aspect was not pursued. In the experimental conditions (pH approximately 8) the copper ions are probably in the form of chelate compounds. The concentration of copper ions in the water is subject to fluctuation, probably as a result of adsorption and blockage phenomena of the sediments and suspended solid substances. In fact, the copper is easily adsorbed by clay and by humic substances. The presence of sediments in water can therefore influence the concentration of free copper. Moreover, the presence of sediments affects larval feeding, thus influencing the copper assimilation rate. This specific aspect was not investigated; we simply preferred to organize the study by reproducing the very common situation encountered in the field (plastic flower saucer with earthenware pot filled with peat).

In the flower saucers continuous release of ions by metallic copper in water made it possible to reach and maintain a copper concentration capable of exerting strong toxic action on *Ae. albopictus* larvae at both the doses tested. We must stress that the experimental conditions (pH of water approximately 8) were not particularly favorable to copper solubility; copper solubility increases in acid conditions.

Mosquito larvae feed on microorganisms present in the water. Our findings confirmed the observation of Walker et al. (1996) who, in a Florida cemetery, were not able to show any decrease in bacterial abundance in bronze vases (releasing copper ions) in comparison with nonbronze vases. On the contrary, the observed strong algicidal action of metallic copper may play an important role in delaying larval development and in producing smaller adults, but also direct toxic mechanisms must be involved to explain the high larval mortality recorded in the earlier instars. We must stress that the majority of the algae identified in the flower saucers belong to the genus *Scenedesmus,* which is not digested and cannot serve as a nutrient (Marten 1986, Laird 1988). The toxic action of copper has been documented for other aquatic organisms (Kosalwat and Knight 1987).

With the analyses carried out on the concentration of copper in the 3rd- and 4th-instar larvae, we were not able to show any increase in the bioaccumulation of copper in the treated larvae in comparison with control larvae of the same age. More

Table 4. Distribution of mortality during preimaginal stages.[1]

Copper dose (g/liter)	Mortality (% [±SD])			
	Larvae			
	1st and 2nd instars	3rd instars	4th instars	Pupae
Control	0.00 (±0.00) a	0.00 a	1.9 (±3.1) a	5.6 (±4.8) a
2	70.5 (±30.1) b	33.7 (±31.0) b	14.2 (±19.5) a	16.3 (±17.6) a
8	84.9 (±21.9) b	32.5 (±39.5) b	16.5 (±18.6) a	27.2 (±41.3) a

[1] Within the columns, the values followed by different letters are significantly different when Tukey's test is used.

Table 5. Effect of metallic copper doses on development time (from egg immersion to adult emergence).[1]

Copper dose (g/liter)	*n*	Development time (days) (means [±SD])
Control	289	13.9 (±5.3) a
2	70	21.1 (±9.6) b
8	38	26.7 (±10.1) c

[1] Within the columns, the values followed by different letters are significantly different when Tukey's test is used.

definitive studies are necessary to define this aspect, particularly focusing on a copper accumulation trend during larval development.

Copper does not seem to have clear inhibitory effects on egg hatching, although the number of eggs hatching in the groups exposed to copper was slightly lower than that of the control group.

Earlier instar larvae demonstrated a higher sensitivity to the action of copper compared to older larvae. Larvae that are able to reach the 4th instar have a good probability of developing into adults. In addition to its larvicidal effect, metallic copper also significantly affects the reproduction capacity of the surviving individuals. At higher doses copper results in the doubling of development time, thereby affecting the number of generations that the species can produce during a season. Furthermore, the resulting reduced weight of the adults probably influences both their fertility and their flight ability.

In Italy the greatest difficulty encountered in controlling *Ae. albopictus* populations in urban areas is linked to the characteristic of this species to colonize not only street drains, but also different types of microhabitats in private areas, such as flower saucers, making it difficult to eliminate or control the species with conventional methods.

The experiment, conducted under conditions that can be regarded as very similar to those normally occurring in the field, showed that metallic copper has a strong toxic effect on larval development of *Ae. albopictus* in flower saucers. In consideration of the persistence of its action, the simplicity of its use, its wide availability, and its low risk for humans, metallic copper can be regarded as a possible alternative agent for the control of *Ae. albopictus* in small man-made containers. Moreover, metallic copper in a large-scale field pilot program conducted in northern Italy did not have a toxic effect on plants. Thus, metallic copper is much more convenient than copper sulfate, which is known to be phytotoxic.

The main advantage of metallic copper in comparison with other products is the persistence of action, probably lasting for several seasons, without need for replacement, even in the case of periodic dessication–submersion cycles.

When using copper in mosquito control, selection of the material to be utilized must be done very carefully because some copper objects (such as vases and drain pipes) are treated with protective substances that prevent dissolution in water. Electric wires seem to be the most effective choice because they have a good surface to volume contact coefficient, which allows for a rapid transfer of copper once they are immersed in water.

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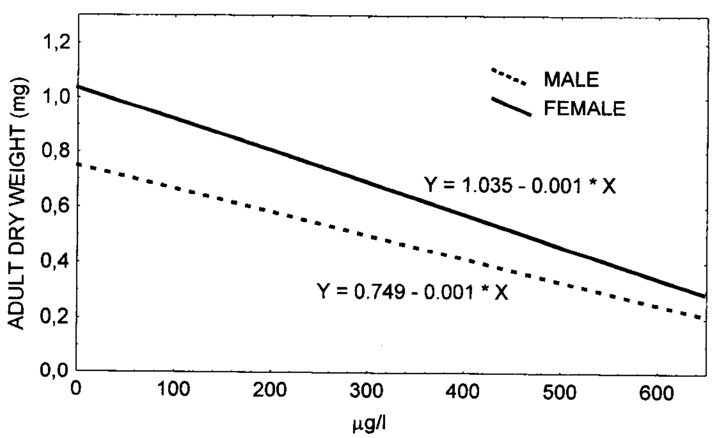

Fig. 3. Effect of ionic copper concentration in the water on adult weight (males, $n = 21$; females, $n = 24$).

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