

Dept. of Animal Medicine,
Faculty of Vet. Med., Assiut University,
Head of Dept. Prof. Dr. I.S. Abdallah.

**ACUTE TOXICITY STUDIES OF THE MOULLUSCICIDE COPPER
SULPHATE (CuSO₄) ON SOME NILE FISH**
(With 6 Tables & 6 Figs.)

By
A.SH. SEDDEK
(Received at 28/10/1989)

دراسات السمية الحادة لمبيد القواقع (كبريتات النحاس) على بعض الأسماك
النيلية

عبد اللطيف شاکر صديق

يعتبر تلوث البيئة واحد من أهم وأخطر مشاكل العصر والتي لم تقتصر حدوثها على الإيجباري منها بل إمتدت أبدي البشر لتكون وسيلة فعالة في زيادتها والتوسع في أخطارها ولما كانت كبريتات النحاس واحده من المواد الكيماوية التي شاع إستخدامها كمبيد للقواقع في نهر النيل علازة على تعدد إستخداماته في علاج العديد من أمراض الأسماك والمتسببة من الطحالب والبروتوزوا والفطريات مهملين التأثيرات السمية له على الأسماك التي تعيش فيه ، رغم أن البعض منها كالذى إستحدث تربيته يستخدم في مقاومة الناموس (أسماك الناموس) والأخرى كغذاء أساسي للإنسان (البلطي والبيس) ومن هذا المنطلق كان إجراء دراسات التأثيرات السمية لكبريتات النحاس على هذه الأنواع الثلاث من الأسماك النيلية الزريعة لها أهميتها ودلالاتها . بالإضافة إلى ذلك فإنه قد تم دراسة تأثير درجة عسر المياه على حدة التسمم . وقد دلت النتائج أن الجرعة الحادة المميته المتوسطة (ج ح م . ه) بطريقتة ليتشفيلد وولكسون (١٩٤٩) قد بلغت ٣١٩ ، ١٧٢ ، ٢٣٩ جزء في المليون بالنسبة لأسماك البلطي والبيس وسك البعوض على التوالي وذلك في المياه الطبيعية تراوحت درجة العسر فيها ٥٤٤ - ٢٤٢ ملليجرام كربونات كالسيوم / لتر . أما الجرعة متوسطة السمية في الماء المقطر (٢٢٢ - ٢٢) ملليجرام / لتر من كربونات الكالسيوم) فقد بلغت ٢٢٢ ، ٢٠٥ ، ٨٤ جزء في المليون على التوالي . وقد تمثلت الأعراض بصفة أساسية على التأثير السمي المباشر على الجهاز التنفسي فبدت الخياشيم وقد إحتوت على غطاء جيلاتيني قد يكون سبباً مباشراً لفعول عمليات التبادل الغازي والموت نتيجة الاسفكسيا . ومما سبق يتضح مدى خطورة إستخدام كبريتات النحاس في الجرعات العادية (٢٠ جزء في المليون) خاصة على أسماك البعوض والتي تؤدي بحياتها جيمعاً لننتقل من مشكلة إلى أحداث أخرى وهكذا ، بالإضافة إلى التأثيرات السمية القاتلة على نسبة كبيرة من اللبيس والبلطي وهي من أهم مصادر الغذاء للإنسان .

SUMMARY

The present study was carried out to investigate the toxic effect of copper sulphate on three species of Nile fish, *Tilapia Nilotica*, *Labeo Nilotica*; and *Gambusia affinis*. LC₅₀ of copper sulphate using tap water media having hardness of 44.5-54.3 mg/L as calcium carbonate were

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31.9, 17.5 and 0.39 ppm in *Tilapia Nilotica*, *Labeo Nilotica* and *Gambusia affinis*, respectively. The use of distilled water media containing 3.2-4.3 mg/L CaCO_3 , revealed that LC_{50} of the same fish were 0.22, 0.205 and 0.84 ppm, respectively. The clinical signs were recorded.

INTRODUCTION

Trace metals play an important role in the biochemical life processes of all aquatic plants and animals and their presence in trace amounts in the aquatic environment is essential. Although these metals are essential to aquatic organisms, high concentrations may be toxic. Copper can be found as a trace element in nearly all waters in addition to anthropogenic sources such as pollution from mining and plating industries and compounds applied as algicides and molluscicides. The effect of these metals on different aquatic organisms is often complex and difficult to interpret, BENNET (1980). Elevated levels of copper exposure to fish generally result in reduced growth, MOUNT (1968) and GOLLVIN (1984), Copper sulphate is widely used as antimycotic either for prophylaxis or treatment of some protozoal and fungal diseases such as Velvet disease, DUIJN (1973), readication of Gill rot caused by *Branchiomyces* fungi, REISHENBACH-KLINKE (1969).

The successive increasing use of copper sulphate in Egypt as molluscicide for controlling Bilharziasis in rivers canals and lakes initiated me to study its acute toxicity on three of the important Nile fish: *Tilapia Nilotica*, *Labeo Nilotica* and *Gambusia affinis*.

MATERIAL and METHODS

Copper sulphate was obtained from ADVIC laboratory chemicals, Cairo as pentahydrate powder, pure grade.

Tilapia Nilotica juveniles weighing from 5-10 gm *Labeo Nilotica* juveniles (1.5-3.0 gm) and *Gambusia affinis* (1.0-1.5 gm) were obtained from the River Nile and Elebrah-aemea canal at Assiut Governorate.

Fish were acclimatised to laboratory conditions at least two weeks before experimental testing. Water was aereated continuously to ensure oxygen saturation. Tetramine fish feed (Tetra, Dr. Baensch, Malle, West Germany) was twice daily and libidum and withhold three days prior to introduction to bioassay to empty the gut, according to United States Department of Interior Fish and Wildlife Service Report (1964).

LC_{50} determinations were carried out on two types of water, for every one the three species of Nile fish was investigated. The first test water was taken from tap with hardness of 44.5-54.3 mg/L as CaCO_3 , and pH value of 7.1-7.4 while the second distilled water had hardness of 3.2-4.3 mg/L as CaCO_3 , and pH value of 6.9-7.1. Number of 576 Nile fish were used for the 6 preliminary trials for determination of

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LC₅₀ of copper sulphate in the three species of fish for both types of water. 240 fish were used in the test proper for the 6 experiments. One group consisted of eight fish was used as control in each trial.

The LC₅₀ was determined by LITCHFIELD and WILCOXON method (1949).

RESULTS

The tests proper indicated that the LC₅₀ with 19/20 confidence limits were 31.9 (23.6-43.06), 17.5 (15.65-19.56) and 0.39 (0.27-0.55) ppm in tap water in *Tilapia Nilotica*, *Labeo Nilotica* and *Gambusia affinis*, respectively. While the values were 0.22 (0.192-0.252), 0.205 (0.175-0.243) and 0.084 (0.066-1.006) ppm, respectively, tables (1-6), fig. (1-6).

The clinical signs of copper sulphate acute toxicity in Nile fish were observed at the beginning as hurried respiration manifested by increased rate of gill cover movements. Rapid and irritable movements of fish was recorded 30 min. after dosing Gelatinous layer of a bluish red colour was detected covering the gill surface 2-6 h. from exposure. Before death, fish showed unbalanced movements, lying down in the bottom of aquarium with decreased respiratory movements.

DISCUSSION

Limited information is available on comparisons of different species of fishes under similar water quality conditions which would allow partitioning environmental from species differences in resistance to the toxins.

The results of LC₅₀ determination of copper sulphate (31.9, 17.5 and 0.39 ppm for *Tilapia Nilotica*, *Labeo Nilotica* and *Gambusia affinis* in tap water, respectively were higher in comparison to that obtained in case of using distilled water (0.22, 0.205 and 0.084 ppm for the same species, respectively). The variance between the use of distilled water and tap water in determination of LC₅₀ explained by BROWN (1968) who stated that heavy metals have been found to be uniformly less toxic as the hardness of water increased. The results of DURVE (1980) showed that LC₅₀ of copper sulphate on Teleost fish was 0.2 mg/L which agree the present investigation regardless the hardness of water. The increase of the LC₅₀ in *Tilapia* and *Labeo Nilotica* in tap water could be referred partially to increased calcium carbonate resulting in formation of copper carbonate which is considered less dangerous to fish rather than copper sulphate SHAW and BROWN (1973). TSAI and McKEE (1980) found that, LC₅₀ of copper sulphate to Goldfish reached 0.3 ppm in water hardness of 52 mg/L as calcium carbonate is parallel to the level recorded of LC₅₀ of copper sulphate in *Gambusia affinis* (0.39 ppm) in case of tap water (44.5-54.3 mg/L as CaCO₃) hardness. Regarding the hardness of the water media used by DURVE (1980), the level was 240 mg/L and the LC₅₀

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of Teleost of 0.2 ppm explain that hardness play but a small part in the toxicity of copper sulphate and the species variance play an important role, therefore, the variance in the present findings of LC_{50} determinations is attributed mainly to the species variance the prominent clinical signs recorded in this investigation as respiratory distress and accumulation of gelatinous layer covering the gill surface before death were explained by many authors. The uptake of copper by fish from surrounding waters can occur via 3 routes gills, body surface and the alimentary canal and also by temporary storage within tissues BRYAN (1971) and MURPHY, *et al.* (1978). It has been determined that gill surface area for most aquatic organisms is 2-10 times the general body surface. Gill tissue tends to concentrate copper from water STOKES (1979). However, in conditions of acute copper stress, the gill tissue secretes large quantities of mucus as an excretory mechanism. At times mucus production is so great that it coats the gill lamellae and death due to asphyxiation may result BRYAN (1971) and STOKES (1979). The same picture of the present investigation ensure the mucus secretion and the finding of CARPENTER (1927), WESTFALL (1945) and KLEIN (1962) who stated that copper coagulates mucus around the gills. Copper like other toxicants, its main effect may be on the oxygen - transport system or causes swelling and break down of the gill epithelium LLOYD (1961). The effect of copper sulphate on gills was a damage and decreased activity of gill Na^+ , K^+ -ATP ase from Coho Salmon exposed to Cu in fresh water, LOTZ and McPHERSON (1976). Copper and other metals are known to interact with ligands in proteins, particularly enzymes, thereby reducing their activities PASSOW, *et al.* (1961).

The Ministry of health in Egypt used copper sulphate at 20 ppm in the surface water as molluscicide. The results of the present LC_{50} ranged between 0.39 and 31.9 ppm must deemly warning about the great loss occurring in Nile fish which are considered one of the main sources of animal protein supplement in our food.

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Table(1) Solution of the dose response curve of CuSO_4 in tap water to *Tilapia Nilotica* (juveniles)

Dose ppm	Response	Observed %	Expected %	Observed minus expected	Contribution to $(\text{Chi})^2$
10.0	1/8	12.5	5	7.5	0.1100
22.5	2/8	25.0	24	1.0	0.0001
35.0	5/8	62.5	60	2.5	0.0001
47.5	7/8	87.5	89	1.5	0.0020
60.0	8/8	100(98.6)	96	2.6	0.0200

LC_{50} with 19/20 confidence limits: 31.9 (23.6 to 43.06) ppm

Table(2) Solution of the dose response curve of CuSO_4 in distilled water to *Tilapia Nilotica* (juveniles)

Dose ppm	Response	Observed %	Expected %	Observed minus expected	Contribution to $(\text{Chi})^2$
0.1	2/8	25.0	12.0	13.0	0.1400
0.2	4/8	50.0	42.0	8.0	0.0350
0.3	6/8	75.0	79.0	4.0	0.0100
0.4	7/8	87.5	96.0	8.5	0.1800
0.5	8/8	100(99.8)	99.9	0.1	0.0001

LC_{50} with 19/20 confidence limits: 0.22 (0.192 to 0.252) ppm

Table(3) Solution of the dose response curve of CuSO_4 in tap water to *Labeo Nilotica* (juveniles)

Dose ppm	Response	Observed %	Expected %	Observed minus expected	Contribution to $(\text{Chi})^2$
13.0	1/8	12.5	8.0	4.5	0.0255
16.0	3/8	37.5	31.0	6.5	0.0255
19.0	5/8	62.5	69.0	6.5	0.0240
22.0	7/8	87.5	94.0	6.5	0.0800
25.0	8/8	100(98.6)	99.2	0.6	0.0010

LC_{50} with 19/20 confidence limits: 17.5 (15.65 to 19.56) ppm

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Dose ppm	Response	Observed %	Expected %	Observed minus expected	Contribution to (Chi) ²
0.05	1/8	12.5	9.0	2.5	0.0030
0.15	3/8	37.5	30.0	7.5	0.0260
0.25	4/8	50.0	66.0	16.0	0.1000
0.35	7/8	87.5	92.0	5.5	0.0300
0.45	8/8	100(99.0)	99.7	0.7	0.0045

LC₅₀ with 19/20 confidence limits: 0.205 (0.172 to 0.243) ppm

Table(5) Solution of the dose response curve of CuSO₄ in tap water to *Gambosia affinis*

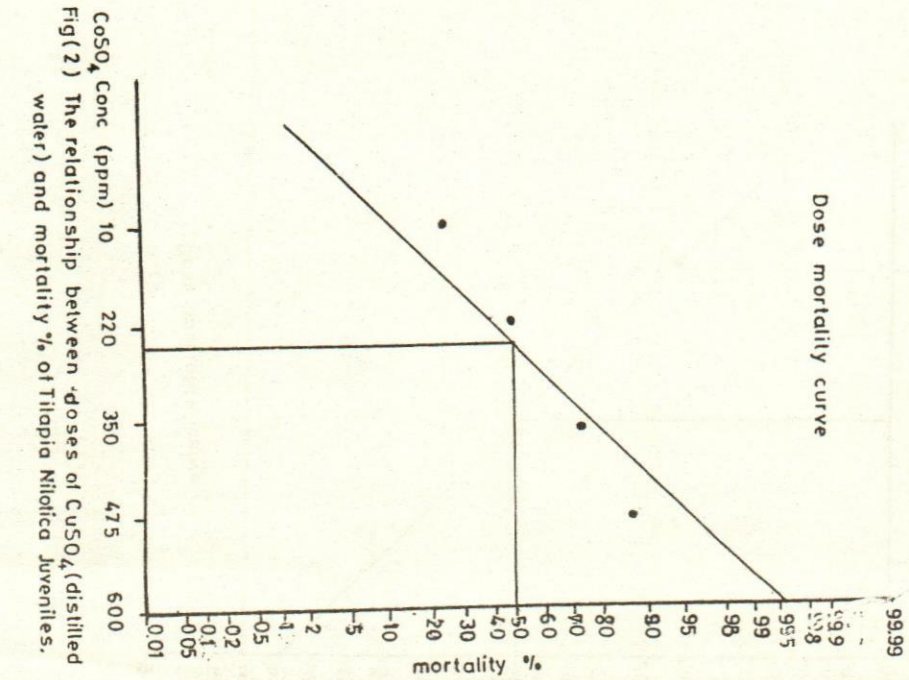
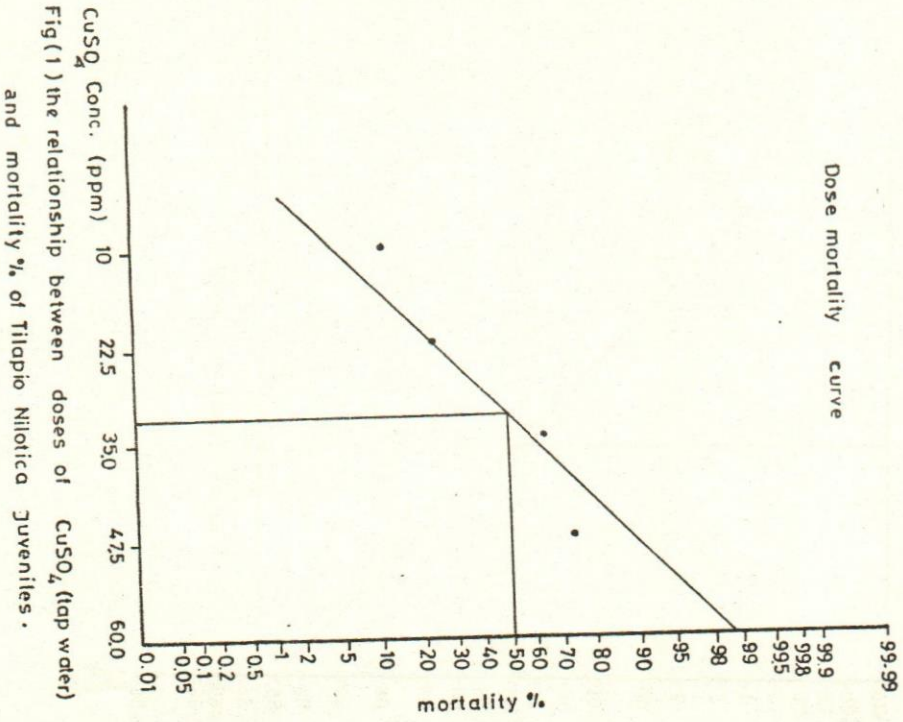
Dose ppm	Response	Observed %	Expected %	Observed minus expected	Contribution to (Chi) ²
0.1	1/8	12.5	7	5.5	0.0470
0.3	3/8	37.5	28	10.5	0.0560
0.5	5/8	62.5	64	1.5	0.0010
0.7	7/8	87.5	92	4.5	0.0180
0.9	8/8	100(99.0)	97	2.0	0.0140

LC₅₀ with 19/20 confidence limits: 0.39 (0.27 to 0.55) ppm

Table(6) Solution of the dose response curve of CuSO in distilled water to *Gambosia affinis*

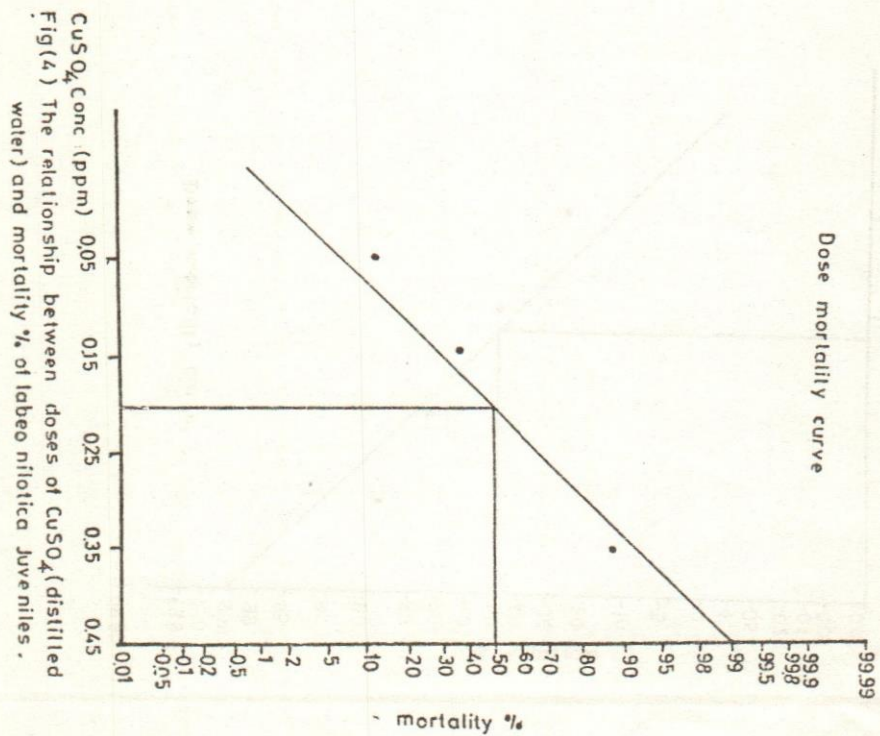
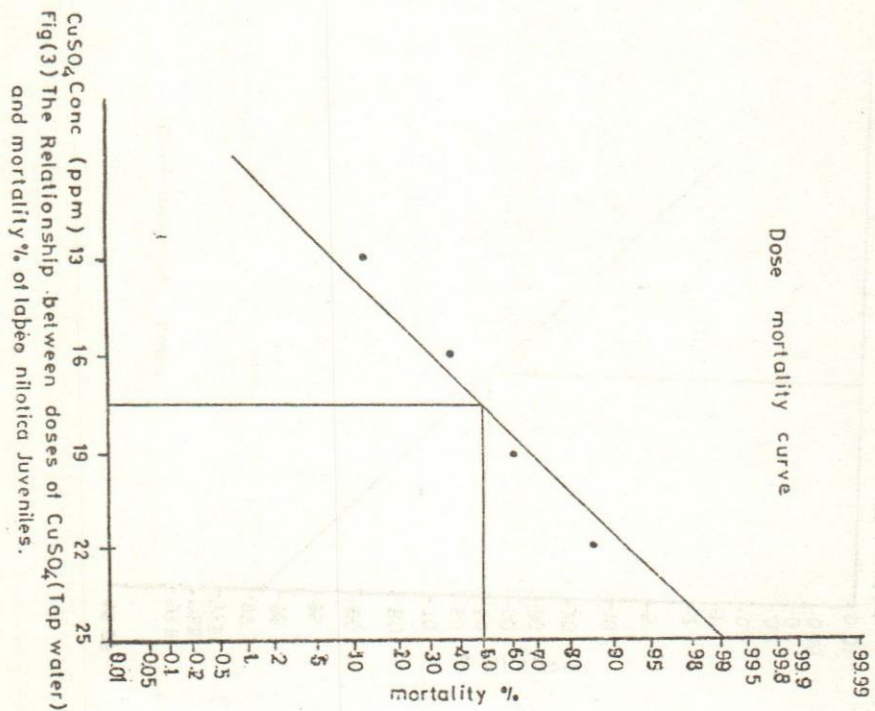
Dose ppm	Response	Observed %	Expected %	Observed minus expected	Contribution to (Chi) ²
0.04	2/8	25.0	13	12	0.1100
0.08	4/8	50.0	46	4	0.0054
0.12	6/8	75.0	80	5	0.0150
0.16	7/8	87.5	96	8.5	0.1800
0.20	8/8	100(99.7)	99	0.7	0.0050

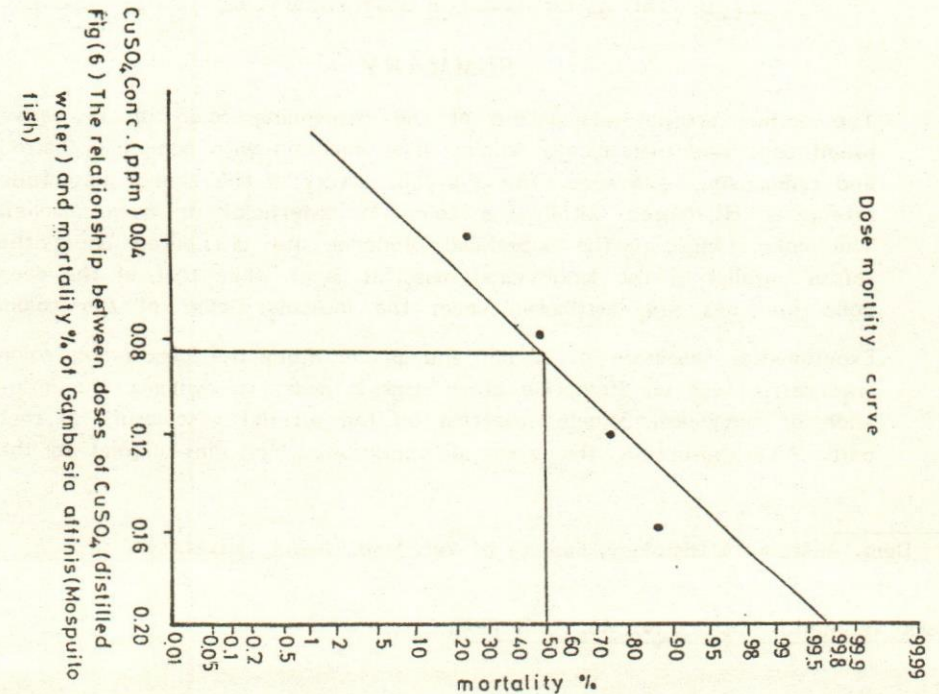
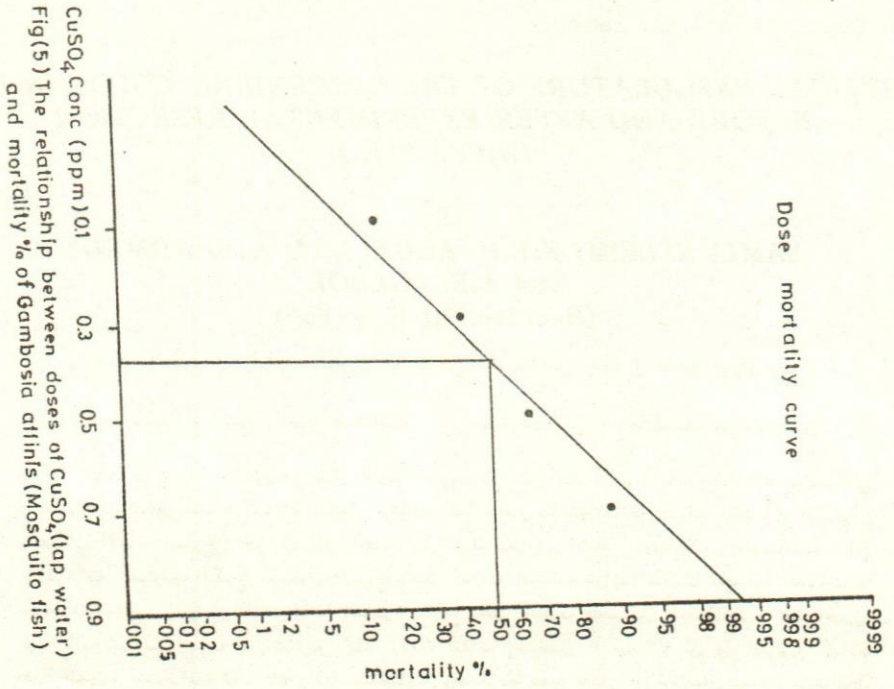
LC₅₀ with 19/20 confidence limits: 0.084 (0.066 to 1.006) ppm



Fig(1) the relationship between doses of CuSO₄ (tap water) and mortality % of Tilapia Nilotica Juveniles.

Fig(2) The relationship between doses of CuSO₄ (distilled water) and mortality % of Tilapia Nilotica Juveniles.

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Fig(5) The relationship between doses of CuSO₄(tap water) and mortality % of Gambusia affinis (Mosquito fish)

Fig(6) The relationship between doses of CuSO₄(distilled water) and mortality % of Gambusia affinis (Mosquito fish)