Research Article

Title:

Laboratory testing of very low-copper-treated water to prolong pupation and emerging time of mosquito larvae: an alternative method to delay mosquito breeding capability

Authors:

Mohamad Reza [a*], Cimi Ilmiawati [b]

[a] *Department of Biology, Faculty of Medicine Andalas University, West Sumatra, Indonesia*

[b] *Department of Pharmacology, Faculty of Medicine Andalas University, West Sumatra, Indonesia*

***Corresponding Author:**

Mohamad Reza, MD, PhD

Department of Biology

Faculty of Medicine Andalas University

Main Campus at Limau Manis, Gedung C Lantai 2

Pauh, Padang, 25166

West Sumatra, Indonesia

e-mail: reza@med.unand.ac.id

Abstract

Introduction: Larvicide application in ovitrap is one of the currently available methods used in mosquito eradication campaign because they eliminate the larval stage. We previously reported that copper in liquid form is a promising candidate due to its potent larvicide properties in a laboratory setting and even in the field. In the field study, several larvae survive in the ovitraps due to the dilution of copper concentration by the rain. The surviving larvae were smaller and less motile. This led our interest to study the effect of sub-lethal dose of copper in ovitrap on larvae development, pupation time and lifespan in adult stage.

Methods: First instar larvae of three species of mosquito (*Aedes albopicus*, *Culex pipiens* and *Anopheles stephensi*) in copper-treated water started from 0.60 ppm, 0.30 ppm, and 0.15 ppm and compared with the control. The surviving larvae were recorded every day in terms of pupa emerging time and adult emerging time. The number of adult mortality was recorded and compare with the control.

Results: Copper showed potent larvicide effect in the 0.60 ppm concentration and demonstrate prolong in pupation and showed significantly lower number of emerging number of mosquitoes up to the lowest 0.15 ppm. Adult lifespan was not different with the control.

Conclusion: This study demonstrated the capability of copper below 1 ppm to prolong pupation time and emerging time of mosquito larvae which provide the possibility in application of copper to cut mosquito breeding capacity in order to reduce their ability to transmit disease.

Keywords: *Aedes albopictus*, *Anopheles stephensi*, *Culex pipiens*, ovitrap, copper, larval control, pupation time

INTRODUCTION

Eradication campaigns for mosquito-borne diseases are of the utmost importance for public health worldwide. Vector-based interventions are considered a more rational and feasible choice than the challenges and expenses associated with discovering cures and vaccines for these diseases. This approach is particularly suitable for under-developed and developing countries with limited financial, technical, and human resources. Over the past century, the dominant methods recommended for vector control have shifted [1] from larval source management (LSM), house screening, and bednets [2] to indoor residual spraying with effective contact pesticides [3, 4].

In urgent efforts to develop new vector control methods, novel approaches have emerged, for example toxic nectar baits [5], repellents [6], oviposition traps [7], and genetic modifications to mosquitoes [8]. These approaches need to be combined in order to optimally function for mosquito eradication, and increasing the potential of combinations of interventions is also required, including LSM [1]

Our recent findings on ability and properties of copper to kill mosquito larvae [9] and laboratory testing of the effect of liquid copper at a concentration of 10ppm on 3 species of mosquito larvae [10] prompted us to perform a field test in a dengue endemic area of West Sumatra, Indonesia. The result is satisfactory (11) however rain dilution became a factor to be considered, since we observed some survived larvae in the outdoor ovitraps. However, during our observation, the survived larvae appear weak and smaller in size in comparison with the control. Another study is required to determine if the survived larvae capable to continue life cycle as mosquito until adults. This urge us to do a laboratory test of sub-lethal

concentration of copper to mosquito larvae to see the effect on mosquito development and breeding capacity.

MATERIALS AND METHODS

Ethical Approval

This research was approved by the ethics committee of Faculty of Medicine Andalas University (Approval No.036/KEP/FK/2019).

Mosquito Eggs and Mice

An. stephensi (strain SDA 500), were reared in our laboratory under room temperature (26°C), 50-70 % relative humidity, and a 13:11 h light : dark cycle. From the day of emergence, the mosquitos were provided with a 5% fructose solution soaked in a filter paper. Females five days after emergence were allowed to feed on anesthetized mice. Three days later, an ovipositing dish was placed in a cage containing gravid females. The eggs were laid on a filter papers soaking in the dish. The filter paper with eggs was placed on a 12 x 20 cm hatching tray containing 500 ml of water. After hatching, 3-5 mg of carp food per tray was sprinkled on the surface of the water twice daily. Twelve days later, pupae were collected daily and transferred to cages for adult emergence.

Ae. *albopictus* and Cx. *Pipiens'* eggs were purchased and first instar newly hatched larvae were utilized in the experiment. Female BALB/c mice were purchased from SLC (Shizuoka, Japan). The mice were fed *ad libitum* and exposed to a 13:11 h of light : dark cycle. This mosquito-mouse cycle was used to maintain the mosquitoes in the laboratory.

Preparation of Copper Sulfate Solutions

Four $CuSO_4$ solutions were used for the experiments. The copper concentrations were 0.6, 0.3, 0.15, and 0 ppm. Each solution was allocated to a pre washed square plastic plate and cover with a plastic transparent plate. First, we prepared a standard solution of 100 mM of $CuSO_4$ by mixing copper sulfate powder with filtered water (Fine Ceramic Filter NGK insulators, Nagoya, Japan). Then we prepared the four concentration for the experiments by diluting them from the standard solution (100mM $CuSO_4$) and confirming the ppm level using a Copper Measuring Device (Hanna Instruments, Tokyo, Japan) and Z-5010 Polarized Zeeman Flame Atomic Absorption Spectrophotometer (Hitachi Ltd, Tokyo, Japan).

Preparation of Mosquito Larvae

First instar newly hatched larvae were used in this experiment. Fifty larvae were separated and put inside each container. Three containers for each concentration of copper were prepared. Larvae were allowed to hatch and observed every 24 hours under the microscope for mortality. A fine ceramic filter (NGK Insulators, Nagoya, Japan) was used to remove chlorine ions from tap water for the experiments.

RESULT

After 7 days, copper treated larvae of An. *stephensi* and Cx. *pipiens* showed one hundred percent mortality under 0.60 ppm of copper, with some small number of larvae survived in Ae. albopictus group. This followed by about fifty percent mortality under 0.30 ppm copper concentration. The concentration of 0.15

ppm is not showing significant effect on larvae mortality in all three mosquito species (**Figure 1**).

Surviving larvae exposed to 0.15 and 0.30 showed prolonged pupation time in all species observed when compared to the control. We also observed that the surviving larvae of Ae. *albopictus* on 0.60 ppm did not became pupae (**Figure 2**).

Surviving pupae of all observed species exposed to 0.15 ppm of $CuSO_4$ become adult mosquito, albeit at a statistically significantly lower number when compare to the control. Adult mosquito of all observed species emerged at later days compared to control and survived up to 21 days of observation. Almost none of the pupae of all observed species of 0.30 ppm of copper solution emerged as adult mosquito (F**igure 3**).

However, all the adult emerged in copper treated groups and control appear to be viable and showed no difference in term of the lifespan. It seemed that copper effect to the survived larvae is not carried to adult stage and limited to larvae stage and only prolonged emerging in the pupae stage.

DISCUSSION

The result of the present study demonstrated that copper adequately led to a high rate of larval mortality in copper-treated group even below 1 ppm (0.60 ppm). The concentration below that (0.30) still killed half of the larvae and together with the lowest concentration of 0.15 ppm prolonged pupation time and emerging time when compared to the control. The number of mosquitos emerged in afterward is also significantly lower than those in the control group.

However, the copper effect is limited in the larvae and pupae stage. Copper showed no effect to adult stage. The effect on the larvae probably caused by copper effect to commensal bacteria in the larvae midgut, which disrupt their intestinal function. This prevent them to eat properly and collect enough energy for pupation and emerge as adult mosquito. Once the larvae become adult, this copper effect is no longer play a role in their life cycle.

This is an interesting finding, since in the very low concentration of copper prolonged breeding time of mosquito. This might lead to the possibility to utilize copper in the tap water to jeopardize mosquito-breeding capability. Mosquito will need a longer time to breed, which then reduce their capability to transmit mosquito borne disease such as dengue hemorrhagic fever.

It is also known that *Aedes* sp. had been resistant to insecticide in indoor or outdoor spraying. A study conducted in Sri Lanka showed *Aedes aegypti* and *Aedes albopictus* highly resistant to DDT and they now have the ability to oviposit indoors and outdoors [12]. Another study in Bangladesh reported abundant potential larval habitats for *Aedes* sp. in containers or jars spread around the city [13]. These situations need another approach of vector control, such as laying ovitraps or another effort to cut or delayed mosquito breeding capability, or even provide insecticide in the tap water if possible.

The US EPA is suggesting 1 ppm as safe limit of copper concentration in the drinking water (14), thus make the application of very low 0.15 ppm of copper might be logically acceptable in the future.

We believed that, together with the application of copper as ovitrap, the concentration of 0.15 ppm of copper in the tap water in houses and large scale water reservoir might be a potent strategy in cutting mosquito breeding capability

and their ability to transmit mosquito borne disease, especially dengue hemorrhagic fever, which mainly breed in the city water reservoir and container. This result might be a chance to reevaluate the allowance of copper in drinking water as one of possible strategy in eradicating mosquito borne disease in the future.

Authors` contributions: MR conceived and designed the study protocol. MR and CI formulated the study proposal and obtained funding. MR conducted research in the laboratory. MR carried out data analysis and interpretation and wrote the manuscript while CI conducted the statistical analysis. All authors read and approved the final manuscript.

Acknowledgement: The authors thank Prof. Hirotomo Kato, PhD for kind permit to use his laboratory in Japan for this research.

Funding: This study was supported by a grant from the Ministry of Research, Technology, and Higher Education of Indonesia (KEMENRISTEKDIKTI, Skim Penelitian Dasar, No: 163/SP2H/LT/DRPM/2019) to MR and CI. The funder had no role in the study design, data collection and analysis, preparation or publication of the manuscript.

Conflict of interest: The authors report no conflicts of interest.

References

1. Brady OJ, Charles HJ, Tatem AJ, et al. Adult vector control, mosquito ecology and malaria transmission. Int Health 2015;7:121-29

2. Ross R. The Prevention of Malaria. New York: E.P. Dutton & Company;1910.

3. World Health Organization. Terminology of Malaria and of Malaria Eradication. Report of a Drafting Committee. WHO, Geneva, 1963.

4. Davey & Wilson. Control of Disease in the Tropic. London : H.K. Lewis & Co . Ltd. 3rd Edition, 1965 ; p. 168–185.

5. Muller GC, Beier JC, Traore SF, Toure MB, et al. Successful fiel trial of attractive toxic sugar bait (ATSB) plant-spraying methods against malaria vectors in the *Anopheles gambiae* complex in Mali, West Africa. Malar J 2010; 9:210.

6. Achee NL, Bangs MJ, Farlow R, et al. Spatial repellents: from discover and development to evidence-based validation. Malar J 2012; 11:164.

7. Rapley, LP, Johnson PH, Williams CR, et al. A lethal ovitrap-based mass trapping scheme for dengue control in Australia: II. Impact on populations of the mosquito *Aedes aegypti*." Medical and veterinary entomology 2009; 23(4) : 303-316.

8. Galizi R, Doyle LA, Menichelli M et al. A synthetic sex ratio distortion system for the control of the human malaria mosquito. Nat Commun 2014; 5:3977.

9. Reza M, Yamamoto DS, Matsuoka H. Low concentration copper solution jeopardizes larval movement and ability to survive predation: New insight into malaria eradication via vector control. Med Entomol Zool 2012;**63**:217-22.

10. Reza M, Yamamoto DS, Matsuoka H. Larvicidal and ovipositional preference test of copper solution for mosquitos. Med Entomol Zool 2014; 65(3):147-150.

11. Reza M, Ilmiawati C, Matsuoka H. Application of copper-based ovitraps in local houses in West Sumatera, Indonesia, A field test of a simple and affordable larvicide for mosquito control. Trop Med Health 2016 44:11

12.Dharsini S, Vinobaba M, Jude PJ et al. Prevalence and insecticide susceptibility of dengue vectors in the district of Batticaloa in eastern Sri Lanka. Trop Med Health 2011; 39:47-52.

13. Ferdousi F, Yoshimatsu S, Ma E et al. Identification of essential containers for Aedes larval breeding to control dengue in Dhaka, Bangladesh. Trop Med Health 2015, *inpress*

14. New Jersey Department of Health and Senior Service. Hazardous Substance Fact Sheet, Copper Acetoarsenite. nj.gov/health/eoh/rtkweb/documents/fs/0529.pdf.

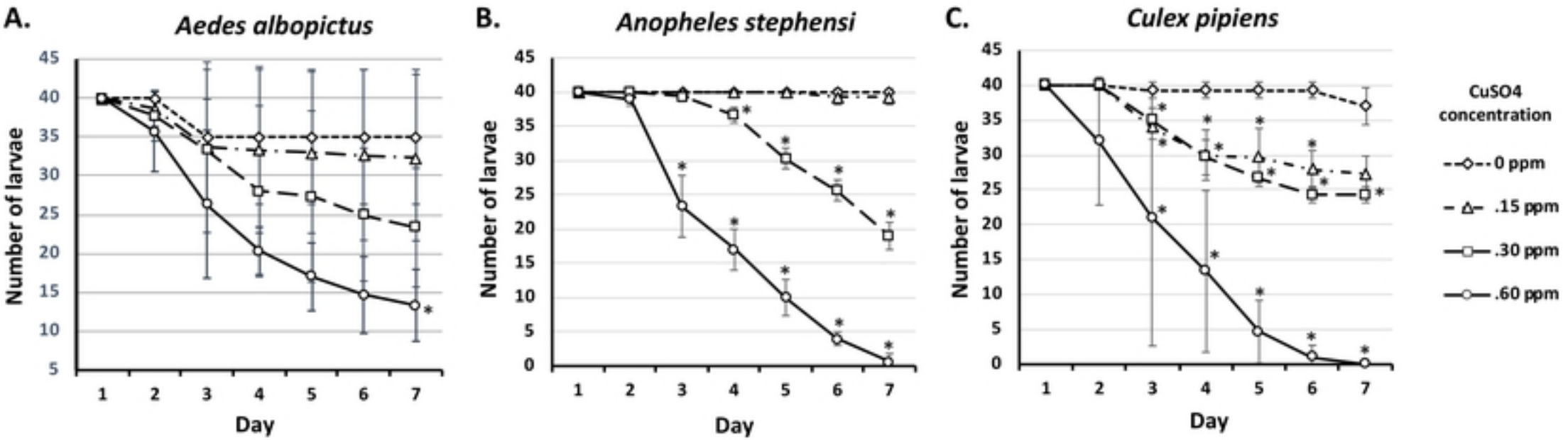

Figure 1. The observed mortality of *A. albopictus*, *A. stephensi*, and *C. pipiens* larvae on exposure to 0.15, 0.30, and 0.60 ppm of $CuSO_4$. *significantly different from control, $p<0.05$ (t-test or Mann-Whitney U test);

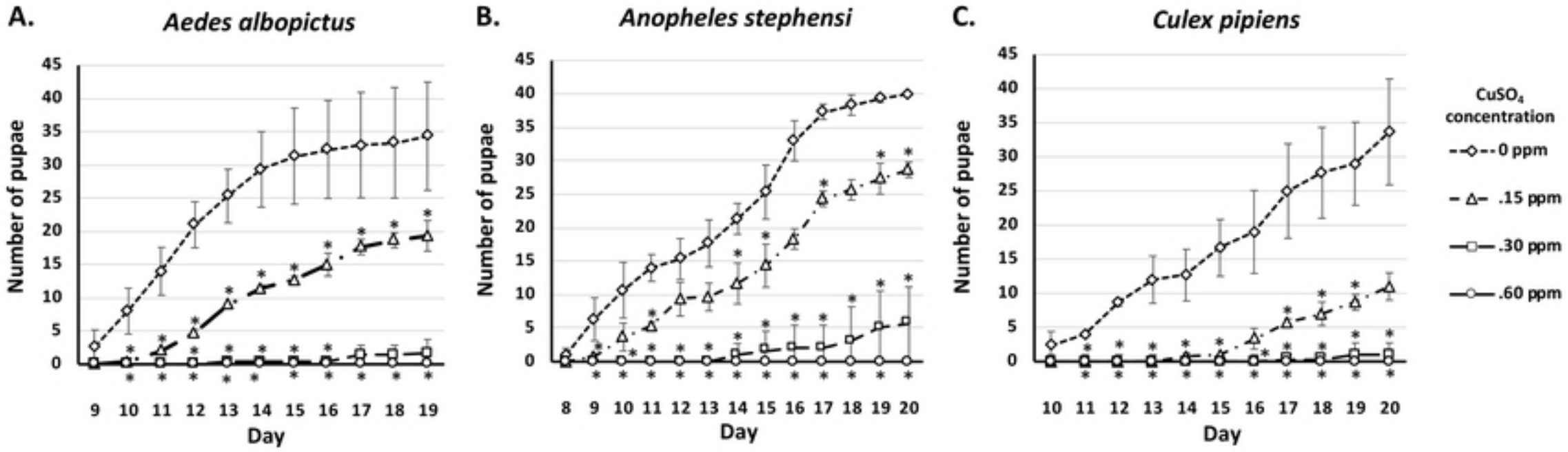

Figure 2. The number of emerging pupae of *A. albopictus*, *A. stephensi*, and *C. pipiens* on exposure to 0.15, 0.30, and 0.60 ppm of $CuSO_4$. *significantly different from control, $p<0.05$ (Mann-Whitney U test)

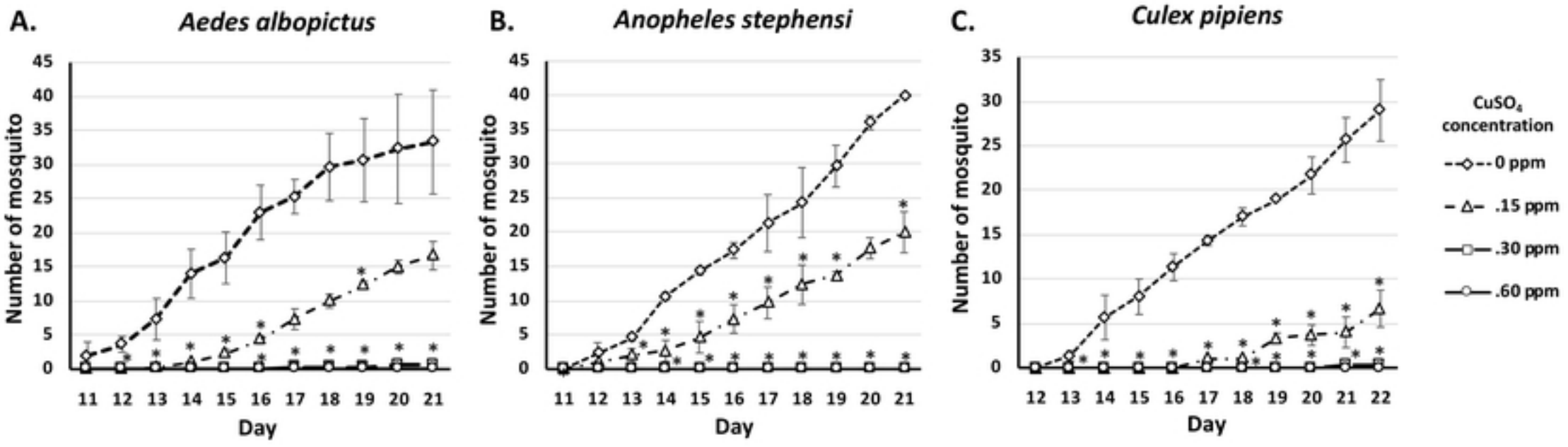

Figure 3. The number of emerging *A. albopictus*, *A. stephensi*, and *C. pipiens* mosquito on exposure to 0.15, 0.30, and 0.60 ppm of $CuSO_4$. *significantly different from control, $p<0.05$ (Mann-Whitney U test)