STUDIES ON THE TOXICITY OF COPPER TO THE SILKWORM, BOMBYX MORI L

S. Kalai Mohan¹ and A Vijaya Bhaskara Rao²
¹,² Department of Ecology and Environmental Sciences, Pondicherry University, Puducherry 605014

Abstract
Sericulture is an economic industry in India which contributes substantially to the poor and marginal farmers of drought prone areas. Pesticides usage is common to control fungal, pests and weeds in agriculture as well as sericulture in or around mulberry gardens. This practice results in chemical contamination of mulberry leaves. Feeding of such chemical contaminated leaves to silkworms often leads to poisoning of silkworms and causes lowering of cocoon yield. Therefore it is the need of the hour to establish preventive measure. Copper is known to be the constituent of different fungicides and it effects on non-target organisms and organism were affected by copper in a complex pattern related to growth, reproduction and survival. Hence there exists effect of copper on silkworm and also the studies on silkworm on exposure to copper are needed. For evaluation of copper toxicity the static bio-assay was followed where the biological response of the animal was recorded. LD₅₀ for copper was determined using percent mortality, probit mortality and Dragsted and Beharns method in silkworm, Bombyx mori L(PM X CSR2) of Vth instar. The silkworms were exposed to various doses of copper for a period of 96h acute toxicity. 96h LD₅₀ was established as 17.54 µg/kg b.w. of Cu in Vth instar silkworm larvae.
Keywords: Copper, LD₅₀, Percent mortality, Probit mortality, Bombyx mori, Vth instar larvae

I. INTRODUCTION

Fungicides, pesticides and inorganic fertilizers has long been used to improve agricultural crop yields. However these chemicals exhibit toxic effects not only on target species but also on species that benefit the wider agro ecosystems. It is well known that horticultural and viticultural operation were carried out with copper based fungicide application and resulted in accumulation of copper in soil. Therefore there is a need for a better evaluation of the potential impacts of copper contamination to non-target organisms.

Silkworm belongs to order Lepidoptera and class Bombycidae. Most commonly reared varieties of silkworm are the multivoltine breed in the tropical countries. In India silkworm rearing is one of the important agro based industry. The new silkworm varieties have higher potential of cocoon yield. However one should take care about the management practice particularly in using organic and inorganic fertilizers and pest management. A wide range of pesticides and insecticide is being used in sericulture activity.

Organic copper is known to be a micro nutrient needed for growth and inorganic copper is considered as a nuero toxic. Underwood (1971), reported that copper deficiency leads to defects in myelination in animals. Ryan et. al., (1978), also observed defects in myelination in multiple sclerosis patients. Scheinberg et. al., (1954), reported that copper supplementation should be provided to the mother as nutrient to the fetus. Aspin and Sass (1981), reported that loss of pigmentation of the hair and skin due to copper deficiency. Krishnamachari (1967), observed increased levels of copper absorption
in pellagra. Watts and Heise (1987) observed that copper can affect thyroid function through insulin effect and insulin is known to antagonize thyroid physiological function. Hence the adverse effects of copper toxicity are given more consideration than copper deficiency (Kenneth and Leslie, 1978). Hence it is important to have copper balance in relation to other nutrients in an organism. According to Bat and Akbulut (2001), zinc exhibited the highest toxicity followed by lead and copper.

Copper based fertilizer and fungicides might contaminate and enrich the levels of copper in the soil and a minimal application of fertilizer could lead to metal enrichment in agricultural soil particularly the phosphate fertilizers. Further silkworms are highly susceptible to heavy metals and thus metal contamination could be harmful to sericulture industry. Aerial spraying of insecticides to mulberry fields could result in damage to silkworm rearing. Pesticide used in the mulberry garden either reach the soil water system or it remains in the mulberry plants. Such contamination becomes toxic to the silkworm. In the present study efforts are made mainly to evaluate the LD<sub>50</sub> copper toxicity on the silkworms, Bombyx mori L.

LD<sub>50</sub> is a measurement in toxicological studies which is the lethal concentrations that results in the death of 50 percent of test population and the value is expressed as dose/concentration in μg/mg of chemical/toxicant per kilogram(kg) of body weight. The determination of LD<sub>50</sub> is the measurement of toxicity of any toxicant. Further many researchers accepted this method for evaluation of acute toxicity (Doudoroff, 1951; Litchfield and Ferky 1961). LD<sub>50</sub> evaluation is one of the toxicity test on the basis of scientific, economic and ethical ways. According to Finney (1971) the toxicity of a chemical is evaluated by exposing the animals at different concentrations/doses to different groups of animals at fixed period of time and therefore suitable periods by counting the number of animals alive or dead. In general toxicity to an organism depends on season, body size, developmental stage, sex, age, nutrition, and abiotic factors such as temperature, pH, light, relative humidity etc.

As the young age (I-III<sup>rd</sup> instars) silkworms are sensitive to chemical substances the toxicity evaluation can be carried out properly in the 4<sup>th</sup> or 5<sup>th</sup> instar worms. The toxicity evaluation was carried out on the 5<sup>th</sup> instar silkworm in the present study. It is customary to first evaluate LD<sub>50</sub> in toxicological studies. The safe dose is consider as that there are no any adverse physio-chemical changes occur in the biological system. Based on the LD<sub>50</sub> values one can fix the sub lethal and sub-sub lethal dose for further toxicity evaluation such as physiological, biochemical and histological studies.

II. MATERIALS AND METHODS

Disease free laying of silkworm Bombyx mori, L (Race: PM X CSR2) were procured from RSRS, Anantapur, Andhra Pradesh and reared at Pondicherry University according to Krishnaswamy, (1978). The temperature 27±1 °C and humidity 75±5% were maintained throughout the rearing and fresh mulberry leaves were fed ad libitum, Cupric nitrate(BDH make) with molecular formula Cu(NO<sub>3</sub>)<sub>2</sub>.5 H<sub>2</sub>O and molecular weight 293.53 is used in this study. Every 3.802 grams of cupric nitrate contains one gram of copper. This salt form more or less soluble in water. Copper was given to silkworm through oral dosage by spraying on mulberry leaves at different doses. Preliminary tests were conducted to find out the median tolerance limit (LD<sub>50</sub>) of the silkworm for 96h according to Finney, (1971). The dose of copper at which 50% mortality occurred was taken as the median lethal dose LD<sub>50</sub> for 96 hr, which was found to be 17.54 μg/kg b.w. of Cu.

Percent mortality of the silkworm in different doses was determined at 96 hr of exposure. For this purpose silkworms were divided into batches of 50 each and were exposed to different doses of copper ranging from 5-50 μg/kg b.w. This range was given on the trial and error method and the Dragsted and Behernes equation mortality rate was recorded at all the doses of copper at the end of exposure period. A batch of silkworm maintained as control without the exposure of copper. The experiment was repeated thrice. The mean mortality values were converted as percent mortality values from these values probit mortality values were obtained (Finney, 1971). Based on the percent mortality
values, six doses were selected for 96 hr period to plot the dose response curves. The percent mortality and probit mortality values obtained at each exposure period was plotted out as a function of copper dose and the LD\(_{50}\) values were determined from these curves. To verify the LD\(_{50}\) values obtained by graphical methods (Carpenter, 1975) was employed. According to this method the animals were exposed to log\(_2\) concentrations of copper for 96 hr and the percent mortality values were obtained from the cumulative mortality values. By using these values LD\(_{50}\) were calculated by adopting the given equation.

\[ \text{Log LD}_{50} = \text{Log A} + \frac{50-a}{b-a} \times \text{log2} \]

Where,
A = Concentration of copper below 50% mortality
a = Percent kill just below 50% mortality
b = Percent kill just above 50% mortality.

The mean LD\(_{50}\) value was calculated from the values obtained from the above three methods namely percent, probit mortality and Dragstedt and Beheren’s method.

### III. RESULTS AND DISCUSSION

On analysis of data on percent and probit mortality of silkworm at different concentration of copper (\(\mu g/kg\) b.w.) at 96 hr of exposure periods was showed in Fig. 1 and Table 1, it is clear that there was a linear relationship between percent or probit mortality and copper concentration. Thus it can be concluded that percent and probit mortality was increased with the increasing concentration of copper. The percent mortality was plotted against log concentration of copper and yielded sigmoid curve (Fig. 1). Whereas probit mortality plotted against log concentration of copper obtained straight lines (Fig. 2). LD\(_{50}\) values derived from both the percent mortality and probit mortality curves as well as the values calculated from cumulative percent mortality of the silkworm in log\(_2\) concentration of copper using Dragstedt and Behrens method (Carpenter, 1975). All the LD\(_{50}\) values obtained are presented in Table 2 and the mean value of LD\(_{50}\) was taken into consideration to evaluate the level of copper toxicity.

Acute toxicity of copper (Cu) on silkworm *Bombyx mori* was evaluated by exposing the Vth instar larvae to a graded concentration of copper. The LD\(_{50}\) of copper at 96 hr was determined as 17.58 \(\mu g/kg\) b.w. The toxicity studies of copper were carried out by some researchers on different organisms. Lethal concentrations were estimated in different aquatic insects. Copper toxicity can vary among different larvae of different species of insects. The 96 hr LC\(_{50}\) for the larvae *Chromonus Tentans* was 14.46 mg/L (Nebeker et. al., 1994) and 17.2 mg/L for copper chloride, 18.55 mg/L for copper sulphate and 18.21 mg/L for copper nitrate (Warrin et. al., 2009). Kuribayashi et. al., (1988) reported that LC\(_{50}\) was 20.91 ppm for third instar larvae of silkworm. Many research reports are available on LC\(_{50}\) in fresh water fishes on exposure to copper (Thompson et. al., 1980; Bangri et. al., 1986). However their reports for 96 hr LC\(_{50}\) values ranges from 0.1 - 10 mg/L. This wide range may account for interspecies variability and test conditions such as pH, water hardness, size, sex, and temperature modify the copper toxicity (Howarth and Sprague, 1978). LD\(_{50}\) values are useful in determining the sub-lethal doses and there is need for sub-lethal toxicity studies which proved to be of most practical value (Perkins, 1979). In fact the knowledge on LD\(_{50}\) alone is insufficient to assess various responses of an animal to the toxicant (Hoppenheit, 1977). As it is known that the responses to lethal doses are quite different from the response to sub-lethal doses (Hoppenheit, 1977 and Perkins, 1979). Hence it is equally important to study sub-lethal toxicity for assessing the overall toxicity of a xenobiotic in the test organism. Hence to derive sub-lethal concentrations and to evaluate the responses to lethal doses are needed. Further various symptoms of poisonings can also be obtained from studies involving the determination of LD\(_{50}\). In our
study control silkworm exhibited normally in the sense that they were very active in feeding and were well coordinated in their movements. However in lethal dose of copper the silkworm developed irritability and hyper excitability. Similar observation were concurrent with the earlier reports on exposures to lethal dose copper (Dashmuk and Maratae, 1980; Vidyunamala et. al., 2010)

Table 1: 96 hr percent and probit mortality values *Bombyx mori* in different doses of copper fed with *Morus alba* at 24 °C and 70±5% RH.

| S. No | Dose μg/kg b. wt. | Log dose | No. of larvae exposed | No. of larvae dead | % mortality | Probit mortality |
|-------|-------------------|----------|-----------------------|--------------------|-------------|-----------------|
| 1     | 5                 | 0.6789   | 50                    | 5                  | 10          | 3.71            |
| 2     | 10                | 1        | 50                    | 10                 | 20          | 4.1             |
| 3     | 15                | 1.1761   | 50                    | 20                 | 40          | 4.75            |
| 4     | 20                | 1.3016   | 50                    | 30                 | 60          | 5.25            |
| 5     | 25                | 1.3979   | 50                    | 35                 | 70          | 5.52            |
| 6     | 30                | 1.4771   | 50                    | 40                 | 80          | 5.84            |
| 7     | 35                | 1.5441   | 50                    | 45                 | 90          | 6.25            |

Table 2: LD$_{50}$ values of silkworm larvae at 96 hr

| S. No | Name of the Method                      | LD$_{50}$ values (mg/kg b.w.) |
|-------|-----------------------------------------|-------------------------------|
| 1     | Percent mortality(sigmoid curve)        | 17.50                         |
| 2     | Probit mortality (linear curve)         | 17.59                         |
| 3     | Dragstedt and Beheren’s method           | 17.54                         |
| 4     | Mean                                    | 17.54                         |
| 5     | ± SD                                    | ±2.49                         |

Figure 1. A sigmoid curve between percent mortality (% kill) of V$^{th}$ instar silkworm larvae against log dose of copper.
Figure 2. A linear curve between probit mortality of V\textsuperscript{th} instar silkworm larvae against log dose of copper.

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