ZINC TOXICITY EVALUATION ON SILK WORM BOMBYX MORI.L. IN CSR₂XCSR₄

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Abstract

The organisms are exposed to chemicals are considered highly toxic when the LD₅₀ does not reflect any effects from long term exposure. The toxicity can be evaluation by expressing the animals to different doses to successive batches of animals for fixed time and after suitable intervals by accounting the number of animals weight. Keeping in view that the lethal dose of Zinc alone is not sufficient in assessing various responses of the CSR2XCSR4 race of silkworm to the concluded that both lethal and sub lethal doses of zinc has negative impact and sub-sub lethal dose has positive on growth and productivity of silkworm.

Keywords: Silkworm, Zinc toxicity, Lethal, sub lethal dose.

I. INTRODUCTION

Zinc is an essential element both for plants and animals, but present in high concentration in plants and animals it can also be toxic. Hence, Toxicity evaluation of zinc will be useful in final evaluation of designing, safe level (or) tolerable level of zinc to the animals. It helps in making programme for Sericulture rearing. Lethal dose (LD₅₀) is common measure of toxicity that causes death in 50 percent of the test population LD₅₀ is expressed as the dose in milligram (mg) of ten per lillogram (kg) of body weight. Pickering et al. (1962) its development stage (Kamala Deep and Toor, 1977). Sex age nutritional status ion concentration, humidity (Rack et al. 1969 Luckly at al. 1975) have greater role in toxicity studies. When chemical substances sprayed in environment, it reaches to the insect body through the article of insect one has to study the contact toxicity through topical application (Hudhay 1971). The silkworm larvae is separated from chemical environment through the membrane lithe integument, the epithelium of the intestine and epithelium of the respiratory tract.

The II and III instar larvae are sensitive to chemical substances even at lower doses. Hence, the toxicity studies are to be carried out in IV and V instars of silkworm larvae. Many reports and now available on toxicity studies on various insects including B.mori (Kuwana et al. 1967, kashi 1972, Singh and Srivastava, 1984. Srivastava and Rassondi 1985, Christian et.al., 1986, thall and Collin 1986, Venkat Reddy et al. 1989) LD₅₀ values are an important parameter to evaluate the toxicity level and also to determine the sublethal doses, the present toxicity studies begin with 48 hrs. LD₅₀ of zinc CSR₂ X CSR₄ bivoltine silkworm race. The present study is very useful to know the effect of zinc and behavioral changes in silkworm on exposure to a cute and sub acute doses of zinc.

II. MATERIALS AND METHODS

For the evaluation of the zinc the static bioassay is followed, where the biological response of the animal was recorded. So that any charges due to extraneous influences can be easily nullified. For, LD₅₀ determination (Finney 1971). 6 different concentrations of zinc such as 10, 15, 20, 25, 30, 35 mg/kg body wt. were used on trial and error basis. A batch of 50, each of V instar CSR₂XCSR₄ race of
silkworm, Bombyx mori were taken and served as control. Then successive batches CSR₂XCSR₄, race of silkworms were divided into batches 50 each and 5 ml of 6 different concentrations of zinc were given orally controlled conditions during the V instar. The mortality rates were observed in all the doses after 48 hrs. The experiment was repeated those with each concentration LD₅₀ was calculated by graphical plots of with percent mortality as well as probit mortality (Y-axis) versus log dosage of zinc concentration (X-axis) (Finney, 1971). For, subsequent verification, of the zinc LD₅₀’s obtained by graphical methods, of Dragsted-Behrens was employed as given by carpenter (1975). As per this method, silkworm exposed to log. 2 doses of zinc (10, 15, 20, 25, 30, 35, mg/kg body wt) for 48 hrs. the percent mortality at each dose was derived from cumulative mortality value. Using these values LD₅₀’s were calculated by adopting the formula….

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\log \text{LD}_{50} = \log \frac{A + \frac{50-a}{b-a} \times \log 2}{b-a}
\]

Where A= dose of zinc which kills 50% of the silkworm
a=percent mortality observed immediately below 50%
b= percent mortality observed immediately above 50%

Finally, the mean of the LD₅₀’s obtained from the percent and probit mortality curves and dragsted and behren’s method was taken for further studies.

III. FIXATION OF LETHAL, SUB LATHAL AND SUB-SUB LETHAL DOSES

LD₅₀/ 48 hr of zinc was taken as lethal dose to study the physiological and cocoon commercial characters responses of Zinc. However, knowledge on the concentration of a toxicant that kills 50 percent of test animals in a fixed period of time could become insufficient to assess various responses of the animal to toxicant (Nobbs and Pearu, 1976, Hoppenheit, 1977). Further studies, on acute toxicant have significant limitations such as the occurrence of adaptation of test animal to the imposed toxicity (Stockner and Anita, 1976, Hoppenheit 1977). Hence perkinal (1970) also viewed the need for sub lethal studies because district changes involving a sequence of events in the responses of test animal could occur in sub lethal dose and sub sub lethal doses. So, about one fifth of the 48 hr, LD₅₀ i.e. 4.78 mg/kg body wt. for silkworm Bombyx mori was taken as the sub lethal dose of zinc, and 1/10 of the 48 hrs LD₅₀i.e mg/kg b.w was taken as sub-sub lethal dose of silkworm.

IV. RESULTS AND DISCUSSION

TOXICITY EVALUATION

The silkworm (Bombayx Mori L.) exposed to different doses of zinc showed 13% mortality at 4.29 mg/kg body wt. 25% mortality at 8.58 mg/kg body wt. 50% mortality at 12.88 mg/kg body wt. 75% mortality at 17.17 mg/kg body wt. 88% at mortality 21.46 mg/kg body wt. 100% mortality at 25.75 mg/kg body wt. the computation of percent mortality against different log doses yielded a typical sigmoid curve (Fig.5) the LD₅₀ value obtained from sigmoid curve was 12.16% mg/kg bidy wt. for 46 hrs. When the probit mortality was plotted against log dose of zinc a sigma line was obtained.

The 48 hours of LD₅₀ value obtained from the straight line graph is 12.57 mg/kg b.w. zinc LD₅₀ for v instar silkworm was obtained by talking the mean LD₅₀ derived from percent and probit mortality curves as well as the values calculated from cumulative percent mortalities in log. 2 doses using dragsted and Behrens method (Carpenter 1975). The summary of the present study is given in the table 4 for comparison. The LD₅₀ obtained by these three methods is taken for further studies. Hence, the mean of there was taken in evaluating lebal of zinc toxicity in V instar silkworm (Bombyx Mori L.) the 48 hrs. LD₅₀ obtained for silkworm bombyx mori in V instar.
The purpose of this study was to estimate lethal dose LD\textsubscript{50} (dose estimated that produce 50\% mortality in a test population over a specific period of time on silkworm exposed to zinc. Zinc has been reported as less toxic (Weis et al. 1992). Zinc 48hr-LC\textsubscript{50} for L.Santolla showed to be 25.87 mg/L (Amin et al. 2003). The amount of zinc required, in Lamellidens Marginals for causing 50\% mortality was 12.71, 14.76 and 16.99 ppm in PH5, 7 and 9 respectively and the PH of the medium has a direct influence (Sarala Nair and Tresa radha Krishnan 2005) Free embryo of the sharp tooth catfish C.Garnie princes. Displayed 24 hr LC 50 values of 3.86 and 4.04 mg/L for zinc sulphate and zinc chloride respectively and respective 48 hr LC 50 values of 2.12\% and 3.6\% mg/L (Vilijoen et al. 2003). The information for zinc toxicity to insect is scanty. In the present study the 48 hrs LD\textsubscript{50} estimated for V instar silkworm Bombyx mori 12.26mg/kg body weight and 1/5 of the LD\textsubscript{50} is taken as the sub lethal dose. As seen in the result of the present study it is conclude that zinc has a lesser toxicity because high dose is required to obtain the 50\% mortality LD\textsubscript{50} values are useful in determining the sub lethal doses of zinc.

The literature available on the effects of zinc in insects on short term studies, carried out at lethal doses of toxicity, which exhibit the sequence of sequence of events involving in the response of the test animal to the sub-lethal doses (Sprogue, 1971’ Hoppewhite, 1977). Hence, it is important to obtain such lethal doses and to compare the responses of the animal at the sub lethal doses lethal doses with those of the lethal dose. Behavior responses have been used previously as a tool in studies of metal toxicity, involving the determination of LD\textsubscript{50}.

The controlled silkworm exhibited on their usual manner i.e the silkworm were very actively feeding and movement were well co-ordinate, silkworm were about and at any subject disturbance money very sensitively. The silkworm exposed to lethal dose of fluoride becom irritable and hyper excited movements abnormal crawling movements were exhibited, other symptoms that have been observed are slowly becoming restlessness slowly becoming sluggishness with sharp jerky movements on exposure to lethal dose finally the silkworm settled down at same place with the loss of equilibrium and caused to death. A sub lethal dose did not kill the silkworm in a short period of time but could affect its ability to respond to the environment and therefore shortening its life during sub lethal dose the symptoms appeared were include same hyper excitable movement and slight vomiting. At the initial hours and decreased food consumption.

Zinc is a complex mode of action and the knowledge of toxicity studies behavioral observations are certainly useful to establish limited and levels of suspect ability of the silkworm Bombyx Mori to zinc is very active. Hence, on the 6th day i.e. at 144 hours after IV moult for V instar were fed with zinc treated leaves at different intervals of 6.00; 10.00 16.00 and 22.00 hours. After words the instar physiological and cocoon commercial were studied.

V. CONCLUSION

As LD50 is very useful in establishing the tolerance limits and safety levels of toxic agents for the insects and also for the evaluation of lethal and sublethal and sub-sublethal doses, the present investigation has been carried with determination of Zinc. LD50 to CSR2XCSR4 race of silkworm by dose response curves. Keeping in view that the lethal dose of zinc alone is not sufficient in assessing various responses of the CSR2XCSR4 race of silkworm to the trace element Zinc.Investigation has carried out in silkworms exposed to lethal ,sub lethal ,sub –sub lethal dose. CSR2XCSR4 race of silkworm to the concluded that both lethal and sub lethal doses of zinc has negative impact and sub-sub lethal dose has positive on growth and productivity of silkworm.
Table 1: Mortality of silkworm (Bombyx Mori) at different does of Zn after 48 hrs of exposure. Mortality expressed in both percent kill and probit kill (Each value is represents an average of five replication)

| S.No. | Dose of Zinc mg/kg body weight | Log dose | No. of silkworm exposed | No. of silkworm dead | % kill | Probit Kill |
|-------|-------------------------------|----------|-------------------------|----------------------|--------|-------------|
| 1     | 4.25                          | 0.6324   | 8                       | 1                    | 13     | 3.87        |
| 2     | 8.58                          | 0.9334   | 8                       | 2                    | 25     | 4.33        |
| 3     | 12.68                         | 1.1095   | 8                       | 4                    | 50     | 5.00        |
| 4     | 17.17                         | 1.2347   | 8                       | 5                    | 75     | 5.67        |
| 5     | 21.17                         | 1.3316   | 8                       | 7                    | 88     | 6.18        |
| 6     | 25.75                         | 1.4108   | 8                       | 8                    | 100    | 8.09        |

Fig.1. 48 hours percent and probit mortality values of CSR2 X CSR4 race of silkworm Bombyx mori in different doses of zinc with morus alba at 24°C and 70±5% RH. Each value is a mean of five replicates.

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