Environment and Ecology 39 (1A) : 180—187, January—March, 2021
ISSN 0970-0420

# Toxicity Evaluation of Cupric Sulfate on Rearing Performanc of CSR2X4 Silkworm

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Received 3 September 2020, Accepted 24 November 2020, Published on 9 January 2021

## ABSTRACT

An experimental study was carried out to test the cupric sulfate toxic effect on rearing performanceof CSR2X4 silkworm. Cupric sulfate fortified mulberry leaves after feeding to silkworms decreases the cocoon quality characters including shell cocoon ratio, raw silk percentage, Denier and Renditta, Floss shell ratio by decreasing floss protein synthesis and further it was also made alterations on the fecundity of silk moths.

**Keywords:** Silkworm, Cocoon quality characters, Cupricsulfate, Denier.

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## INTRODUCTION

Sericulture is the science of rearing of silkworm for the commercial production of raw silk and includes all the operation which is required for the production of silk fibers (Ganie *et al.* 2012). Sericulture is an agro based cottage industry which plays an important role in improving the rural economy because it possesses high employment and income generation capacity (Basavarajappa *et al.* 2002).

Fungicides, pesticides and inorganic fertilizers have 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 (Shivakumar *et al.* 2016).The pesticide poisoning of silkworm causes various types of damage to silkworm, depending on kinds and quality of pesticides. It shows effects like delayed and uneven growth, weak body, light and small or uneven thickness of the filament, shortened cocoon filament, poor reel ability of cocoons, reduced number of eggs (Bizhannia *et al.*2008, Bora *et al.* 2012). All of them results in economic loss.

The most frequent case of the silkworm damage by pesticide occurs by feeding silkworm with pesticide contaminated mulberry leaves,i.e.increase pesticidal effectiveness without causing damage to silkworms, an appropriate pesticide for the purpose of pesticidal control should be used by a proper method at the time when no risk of causing residual toxicity to silkworm is anticipated (Munhoz *et al*. 2013).

Copper based fertilizer and fungicides might con-

taminated 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(Kalai Mohan and Vijaya Bhaskara Rao 2017). Copper is a chemical element with symbol Cu and atomic number 29. It is a malleable and ductile metal with very high thermal and electrical conductivity. A freshly exposed surface of pure copper has a reddish-orange color. Organic copper is known to a micro nutrient needed for growth and inorganic copper is considered as a neuron toxic. Further silkworms are highly susceptible to heavy metals and thus metal contamination could be harmful to sericulture industry.Hence, the present investigation was made to evaluate the effects of cupric sulfate both on the rearing performance and toxicity by using silkworm *Bombyx mori* CSR2 × 4 as a model organism.

## MATERIALS AND METHODS

The eggs of bivoltine hybrid silkworm (CSR2 × CSR4) were collected from silkworm seed production center. Silkworms were reared in good laboratory conditions in duplicate batches. V1 variety of mulberry (*Moras* spp.) leaves were used from the well-maintained field. Appropriate environmental conditions were provided to the silkworms as suggested by Krishnaswamy *et al*.(1973).

### Preparation of standard stock solution

For the preparation of standard stock solution 3.802g of cupric sulfate was dissolved in 1000 ml of distilled water, it contains 1000 µg of copper. From this solution, 20ml was taken and added 80 ml of distilled water and sprayed the chemical every 4 times per day up to 96 hours.

### Cocoon parameters

Ten silkworms from each batch were taken randomly and the each weight in grams, length in cm and width in cm was recorded and average of the same was calculated.

### Effective rate of rearing (%)

Effective rate of rearing is calculated by using this formula.

$$\text{ERR} = \frac{\text{Number of cocons harvested}}{\text{Number of worms brushed}} \times 100$$

### Shell with floss and without floss (g)

For shell with floss cocoon, ten shells from both batches were taken and the weights were recorded and the single weight was calculated in grams. For shell with without floss twenty cocoons were taken and floss layer was removed for their cocoons. Then the weights of these shells were calculated in grams. The floss layer of twenty shells was removed and weighed accurately. The weight of the floss of single shell was calculated in grams.

### Shell ratio (%)

Shell ratio is measured by using the following formal.

$$\text{Shell ratio} = \frac{\text{Single shell weight}}{\text{Single cocoon weight}} \times 100$$

### Silk quality parameters

*Floss shell ratio (%)*

The floss shell ratio was calculated by using the following formula.

$$\text{Floss shell ratio} = \frac{\text{Weight of the floss}}{\text{Weight of the shell}} \times 100$$

*Filament length (m)*

Ten cocoons were randomly selected from each batch and was reeled to find out the single filament length of the cocoon using mono cocoon reeling unitL=RX1.125mt

Here, R= number of revolutions recorded by reeling unit

1.125= circumference of reeling unit in meter

*Filament weight (g), Denier and Renditta*

The weight of the single filament was calculated in grams.This denotes the thickness of the filament, 9000

meters of the silk filament weighing 1g is considered as 1 Denier.

$$\text{Denier} = \frac{\text{Filament weight}}{\text{Filament Length}} \times 9000$$

This is a measure actual silk available from the cocoons. The Renditta was expressed as the quantity of cocoons required to get a kg of raw silk.

$$\text{Renditta} = \frac{\text{Cocoon weight}}{\text{weight of the silk}}$$

*Silk percentage (%)*

It was calculated as the quantity of silk produced from a unit weight of cocoons.

$$\text{Silk} = \frac{\text{Silk weight}}{\text{Cocoon Weight}} \times 100$$

*Reeling breaks*

The reeling breaks were calculated at the time of reeling, which are significant in accessing filament quality.

The non-broken length of silk filament was calculated by using the formula

$$\text{Non – broken filament length} = \frac{\text{Silk Length}}{\text{No.of Cocoons Taken for Realing+No.of Breaks}}$$

*Fibroin and Sericin (%)*

The cocoon shell was treated with 2% KDH at 70-80◦C for few minutes and constantly string till the cocoon become pluffy. The pluffy material was then washed thoroughly in tap water and further treated with dilute acetic (1g/lt) to neutralize the alkalinity. After through wash in water, the pluff was dried at 90-100◦C cocoon shell were calculated using the following formula.

$$\text{Fibroin} = \frac{\text{weight of fibroin}}{\text{Weight of Cocoon Shell}} \times 100$$

Sericin % is calculated by using the formulae.

Sericin= 100 – fibroin %

**Grainage parameters**

*Papal weight and duration*

After obtaining the cocoon weight they were cut open to record the pupal weight (g).The pupal duration was calculated from the time the worms stopped spinning time to the emergence of moths.

*Rate of moth emergence (%)*

Moth emergence percentage was calculated based on the number of pupae transformed into adults and was computed using the formula.

$$\text{Rate of moth emergence} = \frac{\text{No. moth emergence}}{\text{No.of Pupae Kept for moth emergence}} \times 100$$

**RESULTS**

Two batch of CSR2×4 silkworms were nourished with V1 variety of mulberry leaves by treated and control. The larval quality parameters are measured in 5$^{th}$ instar larvae. The silkworm weight got decreased when fed with mulberry leaves treated with the cupric sulfate. The control weight was 3.12g (± 0.191) and the treated weight was 2.89 g (± 0.043).The CSR2×4 silkworm fed with V1 variety mulberry leaves noted maximum larval length 7.2 cm (± 0.166), but the treated ones length was 6.7 cm (± 0.130).The silkworm feed with mulberry leaves width was about 3.5 cm (± 0.138) but in case of treated silkworm measured 3.1

**Table 1.** Larvae quality parameters. ± Standard error,% Average.

| Parameters | Control | Treated |
|---|---|---|
| Silk Worm Weight (g) | 3.12 g | 2.89 g |
| | ±0.191 | ±0.043 |
| | 24.24 % | 21.03 % |
| Silk Worm Length (cm) | 7.2 cm | 6.7 cm |
| | ±0.166 | ±0.130 |
| | 21.2 % | 19.7 % |
| Silk Worm Width (cm) | 3.5 cm | 3.1 cm |
| | ±0.138 | ±0.070 |
| | 27.2 % | 22.42 % |
| Effective Rate of Rearing | 96.00 % | 85.55 % |

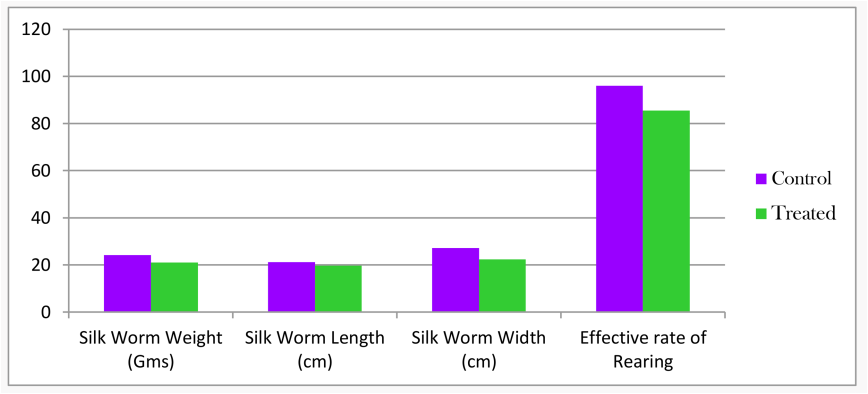


**Fig. 1.** Larvae quality parameters.

cm (± 0.070) it shows enlargement in larval width in control silkworm compare to exposed to silkworm (Table 1 and Fig. 1).

Silkworm fed on mulberry leaves showed the maximum weight of 1.354g (± 0.0027) but the cupric sulfate effects on the cocoon weight where it compress and decreases the body weight to 0.992g (± 0.0021).CSR2×4 silkworms nourished on mulberry plant leaves noted maximum weight of shell with floss of 0.331g (±0.003). Where as in case of treated shows decreased weight to 0.248 (± 0.026).The normal CSR2×4 silkworm feds with mulberry plants recorded maximum weight of shell without floss was 0.312g (± 0.029). The shell weight without floss was higher in normal than treated 0.236g (± 0.0022). Hybrid silkworm fed on variety mulberry leaves shown the maximum weight of floss 0.0019 g (± 0.023) but those fed with cupric sulfate caused effect on the weight of floss 0.0012g (±0.015) (Table 1and Fig.1).

The cocoon quality parameters are presented in Table 2 and Fig. 2. Hybrid silkworm fed on mulberry plant leaves noticed maximum shell ratio 24.4g (± 0.27). The effective shell ratio shows those treated with cupric sulfate 25.2g (±0.3516) it clearly indicates that shell ratio was higher in treated than in control. The CSR2×4 silkworm nourished on mulberry leaves

**Table 2.** Cocoon parameters.

| Parameters | Control | Treated |
|---|---|---|
| Cocoon Weight (g) | 1.354g | 0.992g |
| | ±0.0027 | ±0.0021 |
| | 22% | 20.08% |
| Weight of Shell with Floss (g) | 0.331g | 0.248g |
| | ±0.0033 | ±0.0026 |
| | 19.92% | 19.85% |
| Weight of Shell without Floss (g) | 0.312g | 0.236g |
| | ±0.0029 | ±0.0022 |
| | 24.7% | 18.3% |
| Weight of Floss (g) | 0.0019g | 0.0012g |
| | ±0.00023 | ±0.00015 |
| | 23.24% | 19.67% |
| Shell Ratio | 24.4 | 25.2 |
| | ±0.2732 | ±0.3516 |
| | 24.34 % | 19.78 % |
| Floss- Shell Ratio | 0.543 | 0.483 |
| | 0.0057 | ±0.0042 |
| | 27.2% | 21.72% |

**Table 3.** Silk quality parameters.

| Parameters | Control | Treated |
|---|---|---|
| Filament Length (m) | 985.5 m | 819 m |
| | 38.6 % | 34.2 % |
| Filament Weight (g) | 0.217 g | 0.137 g |
| | 22.5 % | 19.7 % |
| Denier | 1.991 | 1.506 |
| | 32.7 % | 30.5 % |
| Renditta | 7.403 | 5.216 |
| | 29.4 % | 27.2 % |
| Raw Silk % | 19.2 % | 13.50 % |
| Non-broken Filament | 164.5 m | 102.37 m |
| Length (m) | 27.5 % | 22 % |
| Reeling Breaks (in No.) | 1 | 3 |
| | 20 % | 60 % |
| Fibroin % | 64.50 % | 62.25 % |
| Sericin % | 37.75 % | 35.50 % |

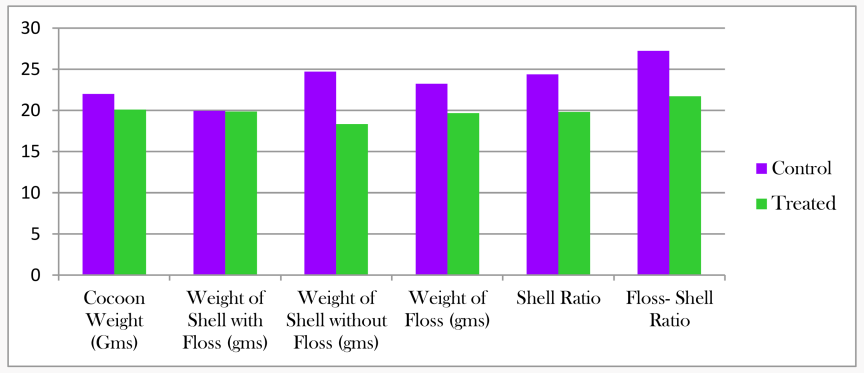


**Fig. 2**. Cocoon parameters.

recorded maximum floss shell ratio 0.543 (± 0.057) but those treated with cupric sulfate shown lesser ratio of 0.483 (±0.042). Silk quality parameters are given in Table 3 and Fig. 3. Filament length is one of the major contributing quantitative traits of silkworm. The hybrid silkworm fed on V1 variety mulberry leaves noticed maximum filament length 985.5cm, but exposed with cupric sulfate; it shows decreased in the filament length 819cm.The hybrid silkworm fed with mulberry leaves, noticed maximum filament weight 0.217, contrast to that these was a decreased filament weight 0.137 in case of exposed one.

In case of control silkworm the denier was about 1.991d but in case of exposed silkworm it was about 1.506d. The Renditta was about 5.216 but in case of exposed silkworm it was about 7.403.The control cocoon gives good raw silk percentage. The CSR2×4 when fed with mulberry leaves that gives maximum percentage of raw silk i.e. about 19.2% but the cupric sulfate consumed raw silk there was decreased percentage 13.50%.The breakages in filament length in normal feed CSR2×4 was 164.25m and the effective treating non broken filament length was 102.37m. Reeling breaks determines the quality of silk. The hybrid fed with mulberry plant leaves it shows minimum reeling break only one time but in case of treated shows maximum reeling breaks 3 times. The fibroin content was higher 64.50% in control silkworm compared to 62.25% exposed silkworm. As in case of sericin higher in control 37.75% rather than treated silkworm 35.50%.

The grainage parameters are presented in Table 4 and Fig. 4. The maximum pupal weight 1.023g was

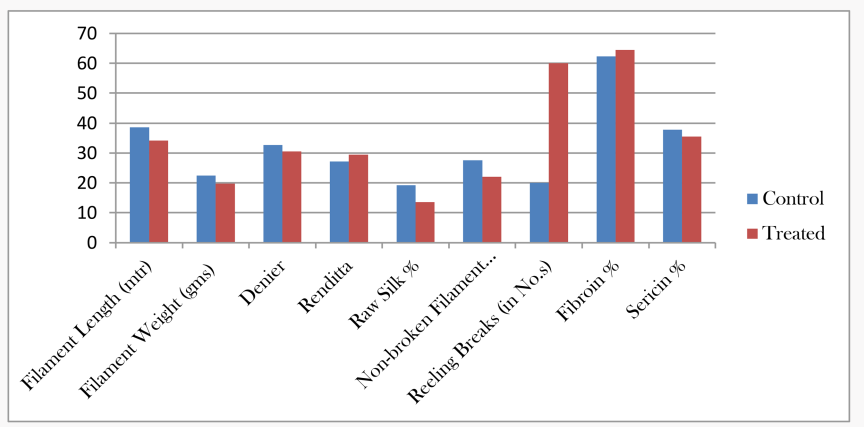


**Fig. 3.** Silk quality parameters.

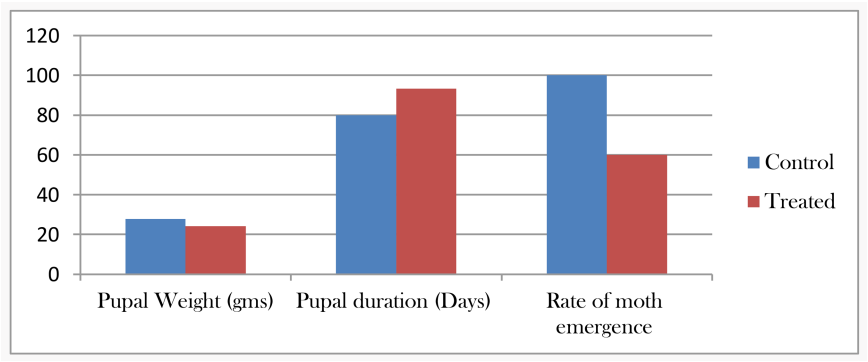


**Fig. 4.** Grainage parameters.

recorded in normal than the pupal weight exposed 0.744g. The pupal duration is very important in showing cocoon quality. The normal silkworm taken pupal duration for 12 days and the treated pupal duration isabout 14 days. The moth emergence is the major step in rearing of silkworm. The normal hybrid fed on mulberry leaves shown 90% of moth emerges but the treated worms showed only 60% of moth emergence.

## DISCUSSION

Silkworm hybrid fed with V1 variety of mulberry leaves showed increased in weight 3.12g, length 7.2 cm, width 3.5 cm, when compared to treated with chemical cupric sulfate showed decrease in weight 2.89 g, length 6.7 cm,width 3.1cm respectively. The increase in larval weight, width, length in control may due to rich nutrient content of mulberry leaves. These results are in conformity with those of Vanishree *et al*.(1996). Who reported that increases in larval weight, length, width attributed to the increased protein synthesis and subsequent accumulation of storage protein in the body an account of additional oral proteins.

**Table 4.** Grainage parameters.

| Parameters | Control | Treated |
|---|---|---|
| Pupal Weight (g) | 1.023 g | 0.744 g |
| | 27.7 % | 24.2 % |
| Pupal duration (Days) | 12 | 14 |
| | 80 % | 93.33 % |
| Rate of moth emergence % | 100 % | 60 % |

Silkworm fed with mulberry shows maximum cocoon weight 1.354g (± 0.0027) but treated shows when fed with cupric sulfate its minimum weight shows 0.992g (± 0.0021). Significant increase in cocoon weight may be due to feeding of mulberry leaves with rich nutrient constant enhanced weight this results are line with the earlier observation of Shashidhar *et al.* (2009). The result showed that inappropriate doses of cupric sulfate significantly lower the cocoon weight.

The CSR2×4 fed with mulberry leaves shows maximum weight shell 0.331g, without floss 0.312 g, weigh of floss 0.0019g. The changes occurs when CSR2×4 silkworm treated with cupric sulfate it effects on weight of shell 0.248g, without floss 0.236g and weight of floss 0.0012g.

This results clearly indicates that cupric sulfate adversely influences the growth and development of silkworm. The increase in shell weight, floss, and shell weight floss is maximum in control.The CSR2×4 fed on mulberry leaves show maximum floss shell ratio 0.543 but it got effected by treated with cupric sulfate 0.483, the maximum shell ratio showed when treated with cupric sulfate 25.2, but in control worms shell ratio shows 24.4. Increase in the shell ratio might be due to enhance silk productivity by supplementation of nutrient in mulberry leaves. These results are also supported by the observation of Shashidhar *et al.* (2009).

The CSR2 × 4 silkworm nourished with mul-

berry leaves of V1 shows maximum length 985.5 m, weight 0.217 m but in case of treated, the trend was decrease in the length 819 m, weight 0.137g, these results are in conformal in the finding of Gaviria *et al*.(2006) who reported that supplementation of V1 variety leaf enhance the filament .The present result suggested that the filament length and its weight can be decreased after supplemented with the chemical cupric sulfate, possible due to the effect of $CuSO_4$ on the silkgland activity. The Denier was also higher in control ones rather than the treated.

The treated larvae silk filament length was significantly decreased than with normal fed with mulberry leaves. This is due to the chemical activity of cupricsulfate.This results was supported with (Sharma *et al.* 2017).The CSR2×4 fed with mulberry leaves showed the maximum renditta while in treated it was less. Similar result was also observed by Subhan *et al.* (2013).

Silk is the most important in silkworm rearing. In case of control CSR2×4 is fed with mulberry leaves achieved good raw silk percentage 19.2% but in treated it was decreased i.e. about 13.50%. The reeling breaks depends on quality of silk cocoons. The normal CSR2×4 nourishes with mulberry leaves shows minimum breaks of one time but on treated with cupric sulfate breaks three times.The Fibro in and Sericin protein content were found to be higher in control rather than treated with cupric sulfate. The findings are supported by the work of Gulrajani (1988). From the results, it revealed that the pupal weight of control is higher than the treated. The shrinkage of pupal is due to cupric sulfate consumption results was similar with Lakshmi Devi and Yellamma (2013) work.

The chemical also showed effect on moth emergence. The rate of moth emergence in the treated was 60% which was much lesser compared to control (90%) indicating high fecundity.

## CONCLUSION

Based on the above result it may be concluded that, the treatment of copper sulfate had shown negative effect on the growth of silkworm larva and cocoon quality parameters. Hence it is suggested that high dosage of copper sulfate usage in terms of pesticide in sericulture must be meticulous.

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