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Toxicity of copper treated mulberry (*Morus alba* L.) leaf on commercial rearing characteristics of the silkworm, *Bombyx mori* L.

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Abstract: **The silkworm, *Bombyx mori* L. feeds solely on mulberry (*Morus alba* L.) leaves for all material, micro and macronutrients. An experiment was conducted to examine toxic effects of mulberry leaf (produced treating with various experimental concentrations of copper chloride ($CuCl_2$) employing pot experimental system) on the commercial rearing characteristics of popular bivoltine x bivoltine mulberry silkworm hybrid, CS2 x CSR4. Though 8 experimental Cu concentrations (5, 10, 20, 40, 80, 160, 320 and 640 µg/g soil) apart from control, were planned for production of mulberry leaf, leaf from only 4 experimental Cu concentrations (5, 10, 20 and 40 µg/g soil), apart from the control were used for mulberry (V1 variety) leaf produced as leaf from other experimental conditions was insufficient or leaf not produced at all. Further, experimental mulberry leaf was used only for the fifth instar silkworm larval eating period.**

Only four silkworm commercial rearing parameters; larval weight, cocoon weight, shell weight and shell ratio were considered. Initial two low experimental Cu concentrations (5 and 10 µg/g soil) resulted in increased larval weight, cocoon weight and shell weight, indicating promontory effects of Cu as essential micronutrient. Later higher experimental Cu concentrations (20 and 40 µg/g soil) reduced their count, indicating inhibitory effects as toxicity inducer at these concentrations. Shell ratio on the other hand, had not been influenced by Cu, indicating that shell ratio is race dependent rather than influenced by Cu. Toxicity Index (TI) and Phytotoxicity computed also confirmed the above observations. Results are discussed based on the available literature.

Keywords: **Copper, toxicity, mulberry leaf, *Morus alba*, silkworm, *Bombyx mori*, rearing parameters, Tolerance Index, Phytotoxicity.**

1. INTRODUCTION

The silkworm, *Bombyx mori* L is domesticated, solely for its silk fiber of high quality. The quality and quantity of silk fibre mostly depend on the rearing conditions and nutritive quality of mulberry for optimum growth of various larval stages (Balamani *et al.*, 1995). Silkworm larvae derive all the nutrients required for its growth from the mulberry leaf alone. Supplementing essential nutrients along with its feed to boost up silk production has considerably been worked by researchers in recent times. Variety of sugars, proteins, amino acids, lipids, hormones, vitamins and macronutrients were tried as supplements by a number of investigators to increase the quantity and quality of silk (Ito, 1960; Iwanrat and Ono, 1969; Nagarajan and Radha, 1990; Bajpeyi *et al.*, 1991; Masilamam *et al.*, 1991; Sarker *et al.*, 1995; Bongale and Krishna, 1996; Murugappan *et al.,* 1996). Further, effects of few essential and non-essential metals on the growth of the silkworm have also been worked. The influence of dietary supplementation of cobalt (Arnando, 1954), nickel (Chamundeswari and Radhaknshnamaiah, 1994; Birendronath Saha and Rahman Khan, 1995), copper (Magadum *et al.*, 1992), cadmium, zinc, lead and arsenate (Miyoshi *et al.*, 1978) on the growth and development of the silkworm, *B. mori*, were assessed. Such studies were made through application of known concentrations of zinc (Balamani *et al.*, 1995) and cobalt (Chakrabarthi and Medda, 1978) either by dipping leaves in the solutions or spraying the solution on mulberry leaves and feeding them after air drying. The influence of soil application of essential trace elements on the economic characters of silkworm has also been studied (Viswanath and Krishnamoorthy, 1982; Lokanath *et al.*, 1986; Bose *et al.*, 1995; Yeasmin *et al.*, 1995; Bose and Majumder, 1996; Bongale and Lingaiah, 1997). Such studies focus mainly on fixing optimum application level of essential trace elements in the soil or on the leaf to increase cocoon crop production. Mulberry leaves were produced in pot culture experiments, treating with different experimental Cu concentrations. Mulberry leaf thus produced, was used to feed the silkworm larvae to study toxic effect of these Cu treated mulberry leaf on silkworm. Such studies are not available. Thus, toxicity results of mulberry leaf (produced with different Cu experimental concentrations) on silkworm economic rearing characteristics are reported in the present communication.

2. MATERIALS AND METHODS

For the present study, mulberry leaf was produced from pot culture experiments. Ten Kg of air dried soil with recommended quantity of NPK in polythene bags (to prevent leaching of heavy metal) were placed in well cleaned earthen pots. Ammonium sulphate (6.8 g/10 kg of soil), super phosphate (3.4 g/10 kg of soil) and muriate of potash (0.9 g/10 kg of soil) were the sources of NPK. Soils were then amended with graded concentrations (5, 10, 20, 40, 80, 160 and 320 µg/g) of $CuCl_2$. The soils were given two wetting and drying cycles to ensure better contact between soil and the heavy metal. Three mulberry stem cuttings, pre-treated with 0.005% IBA were planted in each pot. Five replicates were maintained for each experimental Cu concentration. Agronomic operations as described by Krishnaswamy (1978) for mulberry cultivation were followed. Plants were allowed to grow under natural conditions for a period of 95 days. At the end of the experimental period mulberry leaf produced was used to feed the silkworm larvae from the second feeding of fifth larval-to-larval instar till the end of larval period, to assessing the effects of copper treated mulberry leaf on silkworm commercial rearing characters.

Disease Free Layings (DFLs) of popular bivoltine x bivoltine silkworm hybrid, CSR2 x CSR4, procured from Silkworm Seed Production Centre (SSPC), National Silkworm Seed Organization (NSSO), Central Silk Board CSB), Hindupur, were used as experimental animal. The silkworm rearing was conducted as per Krishnaswami (1986), up to first feeding of fifth instar larval period. While the silkworm larvae continued rearing on control mulberry leaf, leaf produced from the pots experimental mulberry with the initial four Cu concentrations, 5, 10, 20 and 40 µg of $CuCl_2$/g soil alone were used for experimental silkworm rearing. Mulberry leaf produced from pot experimental Cu concentration of 80, 160, 320 and 640 µg of $CuCl_2$/g soil was not considered for experimental silkworm rearing as sufficient leaf was not available with these treatments for silkworm rearing. For each treatment, 5 replications of 20 larvae each (limiting factor being the availability of experimental mulberry leaf) were kept. Only four silkworm commercial rearing parameters; larval weight on the fifth day of fifth instar larval period, cocoon weight, shell weight and shell ratio were studied.

Four numbers of larvae from each replication (five replications x 4 = 20 larvae) were randomly picked up on the fifth day of fifth instar larval period at 10 am and weighed on electronic balance. Larval weight was calculated by averaging total 20 larval weight (weight of 20 larval weight/20). At the beginning of spinning in the final instar silkworm larval period, ripened silkworm larvae were collected and mounted on plastic collapsible mountages spread on plastic tray, separately for each treatment and each replication for cocooning. Cocoons were harvested on the 6^{th} day of mounting. From each replication and each treatment, 4 cocoons were randomly selected and weighed. Cocoon weight was determined through averaging 20 cocoon weight (total weight of 20 cocoons/20). The shells of silkworm cocoons that were used for recording single cocoon weight were used for recording single shell weight also. Cocoons were cut opened on one edge carefully, without damaging the live pupa inside the cocoon. The live pupae inside the cut opened cocoons were carefully taken out. All the 20 cut opened cocoon shells were weighed on electronic balance individually and weights recorded. The average shell weight was determined by averaging total weight of 20 empty shells (shell weight = total weight of 20 empty shells/20). Individual cocoon weight and shell weight was separately recorded for each cocoon of each replication and each treatment, including control. Shell ratio for each cocoon was calculated as shell ratio = shell weight/cocoon weight x 100.

Using the recorded data, indices values of tolerance of larval weight, cocoon weight, shell weight and shell ratio to $CuCl_2$ were calculated employing formula of Baker *et al.* (1994). Thus, for TI (Tolerance Index, %) for silkworm larval weight = [(Mean in silkworm larval weight under treatment condition/Mean of silkworm larval weight in control condition)/Mean of silkworm larval weight in treatment] x 100. For calculation of TI for the other commercial silkworm rearing parameters, similar formula was used. Also, phytotoxicity of larval weight, cocoon weight, shell weight and shell ratio was calculated by using formula of Chou and Lin, (1976). Phytotoxicity of silkworm larval weight = [(silkworm larval weight under control condition – silkworm larval weight under treatment condition)/ silkworm larval weight under control condition] x 100. For calculation of phytotoxicity for the other commercial silkworm rearing parameters, similar procedure was followed. Further, the data were analyzed for average and standard deviation (SD). ANOVA and regression was employed to determine the significance or otherwise.

3. RESULTS

Only four silkworm commercial rearing parameters; silkworm larval weight on fifth day of fifth instar, cocoon weight, shell weight and shell ratio were studied and data on these parameters are presented in Table 1.

Table 1: Data on silkworm (CSR2 x CSR4) commercial rearing parameters reared on mulberry treated with different experimental Cu concentrations (µg of $CuCl_2$/g soil) in pot experiments. Values are mean 5 replications (± SD). Note, differences between control and experimental groups are statistically significant ($p < 0.05$) for larval weight, cocoon weight and shell weight only while the same for shell ratio were not significant).

Treatment (µg of $CuCl_2$/g soil)	Larval weight (g)	Cocoon Weight (g)	Shell Weight (g)	Shell Ratio (%)
Control	3.460 ± 0.261	1.700 ± 0.100	0.345 ± 0.017	20.927 ± 0.551
5	3.980 ± 0.164	1.840 ± 0.089	0.373 ± 0.023	20.346 ± 2.255
10	3.940 ± 0.230	1.860 ± 0.152	0.398 ± 0.037	21.424 ± 1.572
20	3.420 ± 0.239	1.620 ± 0.084	0.324 ± 0.012	20.057 ± 1.509
40	2.940 ± 0.230	1.600 ± 0.187	0.302 ± 0.018	19.120 ± 2.583

From the data (Table 1.), it is clear that the larval weight is affected greatly by imposed experimental Cu concentrations. Thus, larval weight of the initial two lower Cu concentrations, 5 and 10 µg/g soil increased over control. However, the other two Cu concentrations, 20 and 40 µg/g soil resulted in reduction of larval weight compared to control. Similarly, cocoon weight and shell weight also affected greatly. Thus, single cocoon weight and shell weight of the first two Cu concentrations, 5 and 10 µg/g soil crossed towards positive side over control. However, the other two Cu concentrations, 20 and 40 µg/g soil resulted in reduction compared to control. The changes in larval weight, cocoon weight and shell weight are significant ($p < 0.05$) while those with shell ratios are not.

a. Effect of copper treated mulberry leaf on silkworm larval weight: Data on the silkworm (CSR2 x CSR4) larval weight taken on the fifth day of fifth instar larval period are furnished in Figure 1. Under control conditions, the larval weight was 3.5 g and it increased to reach 4.0 g with the first two Cu concentrations, 5 and 10 µg/g soil. Later, larval weight showed the decreased trend, gliding down to below 3.5 g (control).

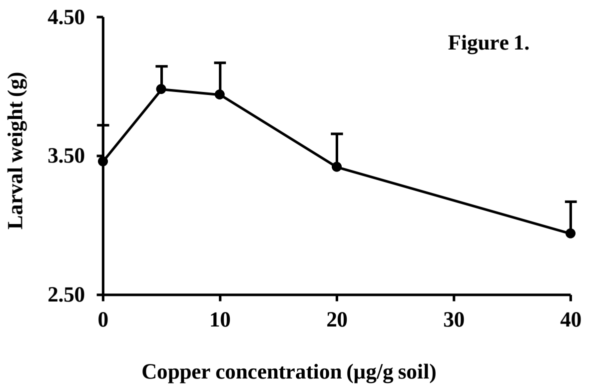

Figure 1: Silkworm, *Bombyx mori* (CSR2 x CSR4) larval weight on the fifth day of fifth instar, reared on mulberry leaf produced in pot experiments with different Cu concentrations (µg/g soil). Values are mean of 5 replications (± SD). Note that differences between control and experimental values are significant ($p < 0.05$).

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b. Effect of copper treated mulberry leaf on cocoon weight of silkworm: Single cocoon weight recorded was statistically processed and presented in Figure 2. The trend in results on cocoon weight resembled those of larval weight. Thus, average cocoon weight of control batch silkworm larvae was 1.7 g. Clear-cut increase in average cocoon weight was observed for the first two Cu concentrations only (5 and 10 µg/g soil), increasing from 1.7 g (control) to 1.84 g (for 5 µg/g soil) and 1.86 g (for 10 µg/g soil). From Cu concentration of 20 µg/g soil, the cocoon weights reduced and it reached 1.6 g of weight for cocoon weight at 20 and 40 µg/g soil treatments. Beyond this treatment, no survival of larvae was observed and therefore, no data on cocoon weight was collected.

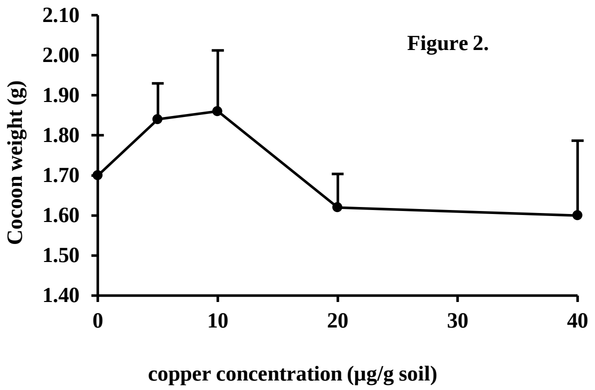

Figure 2: Silkworm, *Bombyx mori* (CSR2 x CSR4) cocoon weight reared on mulberry leaf produced in pot experiments with different Cu concentrations (µg/g soil). Values are mean of 5 replications (± SD). Note that, differences between control and experimental values are significant ($p < 0.05$).

c. Effect of copper treated mulberry leaf on silkworm shell weight: Observed data on the cocoon shell weight after feeding mulberry leaf from the plants treated with different concentrations of Cu are presented in Figure 3. Remarkably, the trends in data on shell weight followed that of both larval weight and cocoon weight. The shell weight of silkworm cocoons under controlled conditions was 0.345 g while it increased for the first two initial Cu treatments (5 1nd 10 µg/g soil). From treatment of 20 µg/g soil onwards, the cocoon shell decreased progressively.

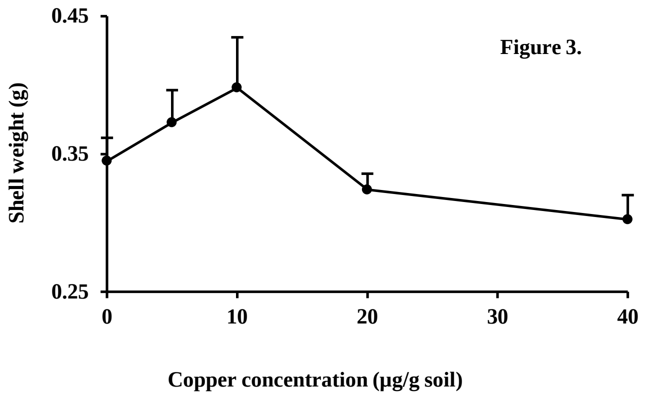

Figure 3: Silkworm, *Bombyx mori* (CSR2 x CSR4) cocoon shell weight reared with mulberry leaf produced in pot experiments with different Cu concentrations (µg/g soil). Values are mean of 5 replications (± SD). Note that the differences between control and experimental values are significant ($p < 0.05$).

d. Effect of copper treated mulberry leaf on silkworm cocoon shell ratio: Similar results, as those of larval weight, cocoon weight and shell weight were observed for cocoon shell ratio also for silkworm reared on mulberry leaf produced with different concentrations of Cu (Figure 4.). Thus, the shell ration of cocoons under controlled conditions was 20.3% under controlled conditions and the same increased for the first two initial low Cu concentrations; 20.35% for Cu concentration of 5 µg/g soil and 21.42% for Cu concentration of 10 µg/g soil. However, the observed differences were not statistically significant. Further, SR% decreased from Cu concentration of 20 µg/g soil, which was also not significant (Figure 4.).

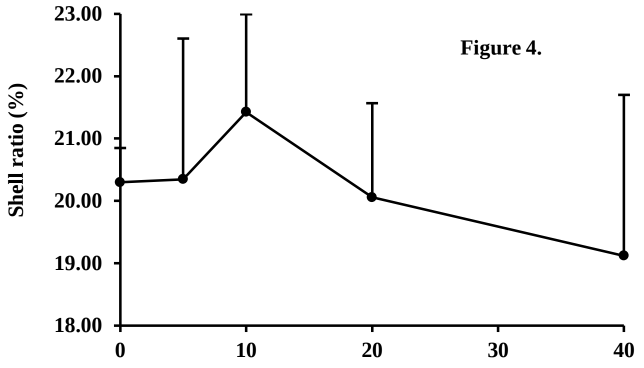

Figure 4: Silkworm, *Bombyx mori* (CSR2 x CSR4) cocoon shell ratio reared with mulberry leaf produced in pot experiments with different Cu concentrations (µg/g soil). Values are mean of 5 replications (± SD). Note that the differences in shell ratio are not significant.

II. Tolerance Index (TI) for the silkworm commercial rearing characters reared on copper treated mulberry leaf: Computation of Tolerance Index (TI) is one of the important measurements, indicating intensity of toxicity to plant or an organism. In the present study, Cu was taken as essential micronutrient and heavy metal as well to the mulberry silkworm sole food plant, mulberry. Cu treated mulberry leaves were fed to the silkworm, the commercial characters were recorded and the TI (Tolerance Index) was calculated. The analyzed data on TI derived from concerned formula for commercial cocoon characters are presented in Table 2.

Table 2: Tolerance Index values for the commercial cocoon characters of the silkworm (*Bombyx mori* L., CSR2 x CSR4) reared on mulberry (*Morus alba* L. V1 variety), treated with different Cu concentrations (µg/g soil) in pot experiments. Values are mean 5 replications (± SD). Note that the difference in TI for larval weight, cocoon weight and shell weight are alone statistically significant ($p < 0.05$) while those for shell ratio are not.

Treatment (µg of $CuCl_2$/g soil)	Tolerance Index (TI) values			
	Larval weight (%)	Cocoon weight (%)	Shell weight (%)	Shell Ratio (%)
0	100.000 ± 0.000	100.000 ± 0.000	100.000 ± 0.000	100.000 ± 0.000
5	115.622 ± 10.934	108.603 ± 9.191	108.295 ± 8.002	100.195 ± 10326
10	114.442 ±11.749	109.926 ± 13.569	115.740 ± 12.718	105.564 ± 7.443
20	99.402 ± 11.561	95.490 ± 6.430	94.148 ± 5.650	98.954 ± 8.959
40	85.704 ± 12.443	94.869 ± 16.514	87.772 ± 4.587	94.400 ± 14.165

In the case of Toxicity Index (TI), data indicated that TI for control is 100%, implying that no Cu is present in control mulberry leaf. However, TI for the first two Cu concentrations increased further over 100%. TI for experimental Cu concentration of 5 µg/g soil ranged from 100% (shell ratio) to 115% (larval weight. Similar TI values were observed for Cu concentration of 10 µg/g soil, ranging from105% (shell ratio) to 115% (shell weight). From Cu concentration of 20 µg/g soil onwards, the trend in TI reduced. Least TI values were observed for larval weight at 40 µg/g soil concentrations for larval weight. Notably, the TI values did not vary for shell ratio for all Cu concentrations indicating that the shell ratio is race dependent rather than the Cu treatment related.

a. Tolerance Index (TI) for silkworm larval weight: Data on the Tolerance Index (TI) for fifth instar fifth day silkworm larval weight reared on copper treated mulberry leaf are graphically represented in Figure 5. Data on TI (Toxicity Index) clearly indicated that the first two Cu concentrations, 5 and 10 µg/g soil increased the tolerance capacity of silkworm larvae reared. The rest of Cu concentrations (20 and 40 µg/g soil) resulted in decrease in TI. Differences among TI values are statistically significant at ($p < 0.05$).

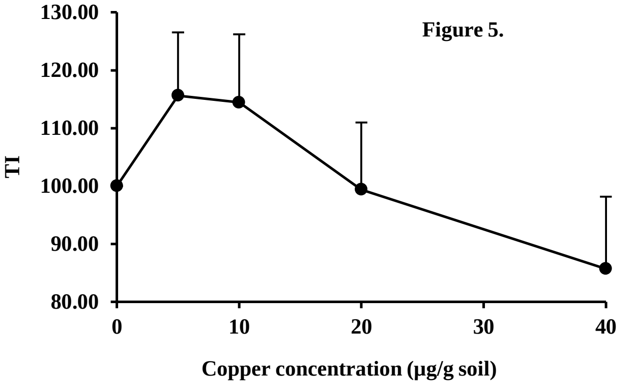

Figure 5: Graphical representation of Toxicity Index (TI) for fifth instar fifth day silkworm larval weight of the commercial Silkworm, *Bombyx mori* L. (CSR2 x CSR4) reared on the mulberry, *Morus alba* L. (V1) leaf produced in pot experiments treated with different Cu concentrations (µg/g soil). Values are mean of 5 replications (± SD).

b. Tolerance Index (TI) for cocoon weight: Data on TI for silkworm cocoon weight reared on copper treated mulberry leaf are graphically represented in Figure 6. In the case of silkworm cocoon weight also, the initial 2 Cu concentrations, 5 and 10 µg/g soil inflicted increase in TI values and the later Cu concentrations reduced Tolerance Index values. Differences in data are statistically highly significant at 5% level ($p < 0.05$).

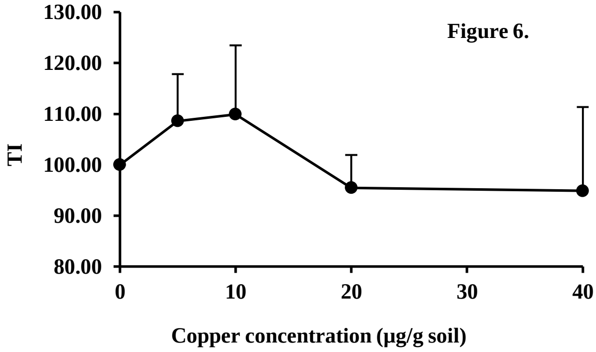

Figure 6: Graphical representation of Toxicity Index (TI) for silkworm cocoon weight of the commercial Silkworm, *Bombyx mori* L. (CSR2 x CSR4) reared on the mulberry, *Morus alba* L. (V1) leaf produced in pot experiments treated with different Cu concentrations (µg/g soil). Values are mean of 5 replications (± SD).

c. Tolerance Index (TI) for shell weight: Data on the Tolerance Index (TI) for shell weight (Figure 7.) reared on copper treated mulberry leaf clearly demonstrated the same trend observed for the above parameters (larval weight and cocoon weight). Thus, the TI increased for the first two Cu concentrations (5 and 10 µg/g soil). For the rest of Cu concentrations (20 and 40 µg/g soil), the TI decreased. Maximum TI decrease was observed with Cu concentration of 40 µg/g soil, indicating high toxicity to the silkworm larvae at this particular concentration (and beyond?), thereby recording least TI value of 88%.

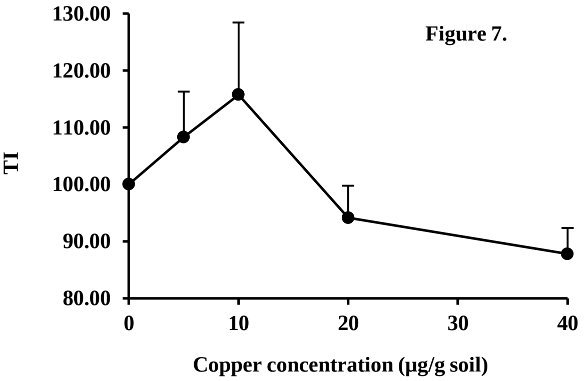

Figure 7: Graphical representation of Toxicity Index (TI) for shell weight of the commercial Silkworm, *Bombyx mori* L. (CSR2 x CSR4) reared on the mulberry, *Morus alba* L. (V1) leaf produced in pot experiments treated with different Cu concentrations (µg of CuCl2/g soil). Values are mean of 5 replications (± SD).

d. Tolerance Index (TI) for shell ratio: Data on the Tolerance Index (TI) for shell ratio are graphically represented in Figure 8. As against to the TI values for larval weight, cocoon weight and shell weight, the TI values for shell ratio exhibited a different trend. Though the initial two Cu concentrations (5 and 10 µg/g soil) increased beyond 100% and that for the other two Cu concentrations (20 and 40 µg/g soil) decreased slightly below 100%, the increase or the decrease in TI values from that of control (100%) was not much, as the differences between TI value are not significant statistically.

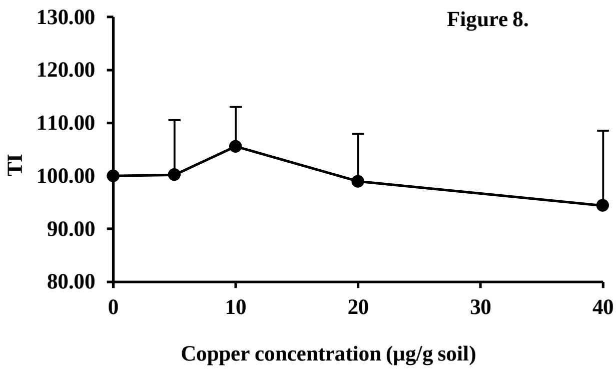

Figure 8: Graphical representation of Toxicity Index (TI) for shell ratio of the commercial Silkworm, *Bombyx mori* L. (CSR2 x CSR4) reared on the mulberry, *Morus alba* L. (V1) leaf produced in pot experiments treated with different Cu concentrations (µg/g soil). Values are mean of 5 replications (± SD). Note little differences in TI values compared to control. The differences in TI are not significant statistically.

III. Phytotoxicity for the silkworm commercial rearing characters reared on copper treated mulberry leaf: Phytotoxicity of silkworm commercial cocoon characters is another indicator towards understanding the level of toxicity that the silkworm, *Bombyx mori* L. (CSR2 x CSR4) can withstand after feeding mulberry (*Morus alba* L, V1 variety) leaves produced from the pot experiments treated with different concentrations of Cu (5, 10, 20, 40 and 80 µg/g soil). Data on Phytotoxicity are presented in Table 3. Perused Table 3, it is clear that phytotoxicity varied among treatments as well as parameters studied. The phytotoxicity for control silkworm commercial cocoon characters was zero, while it decreased to negative levels for all the commercial cocoon characters with treatment of Cu at 5 and 10 µg/g soil. Thereafter, the phytotoxicity increased. Maximum variations are observed for larval weight, cocoon weight and shell weight while those were very minimal for shell ratio.

Table 3: Phytotoxicity for the commercial cocoon characters of the silkworm (*Bombyx mori* L. CSR2 x CSR4) reared on mulberry (*Morus alba* L. V1 variety), treated with different Cu concentrations (µg/g soil) in pot experiments. Values are mean 5 replications (± SD). Note that the difference in Phytotoxicity for larval weight, cocoon weight and shell weight are alone statistically significant ($p < 0.05$) while those for shell ratio are not.

Treatment (µg of $CuCl_2$/g soil)	Phytotoxicity values			
	Larval weight (%)	Cocoon weight (%)	Shell weight (%)	Shell Ratio (%)
0	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
5	-15.029 ± 10.934	-8.235 ± 9.191	-8.121 ± 8.002	-0.241 ± 10.326
10	-13.873 ± 11.749	-9.412 ± 0.560	-15.429 ± 12.718	-5.553 ± 7.443
20	1.156 ± 11.561	4.706 ± 6.430	6.032 ± 5.650	1.179 ± 8.959
40	14.035 ± 9.805	1.235 ± 0.260	6.667 ± 3.830	4.675 ± 14.872

a. Phytotoxicity for silkworm larval weight: Data on the Phytotoxicity for fifth instar fifth day silkworm larval weight reared on copper treated mulberry leaf are graphically represented in Figure 9. From the graph, it is clear that the initial two Cu concentration (5 and 10 µg/g soil) recorded negative phytotoxicity while the same for the control silkworm larvae was 0%. The other two treatment Cu concentrations, 20 and 40 µg/g soil recorded higher (positive) phytotoxicity, indicating that the above Cu concentrations, 20 and 40 µg/g soil are highly toxic to silkworm larvae in its expression of larval weight.

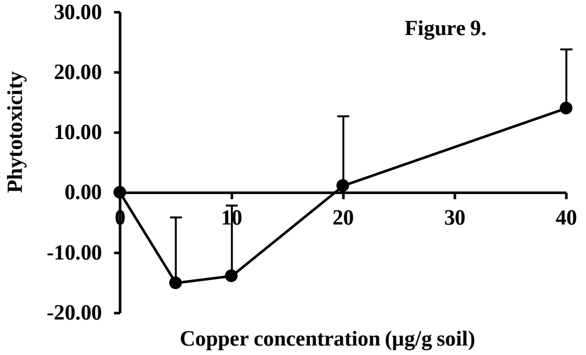

Figure 9: Graphical representation of Phytotoxicity for fifth instar fifth day silkworm larval weight of the commercial Silkworm, *Bombyx mori* L. (CSR2 x CSR4) reared on the mulberry, *Morus alba* L. (V1) leaf produced in pot experiments treated with different Cu concentrations (µg/g soil). Values are mean of 5 replications (± SD).

b. Phytotoxicity for cocoon weight: Data on the Phytotoxicity for cocoon weight reared on copper treated mulberry leaf are graphically represented in Figure 10. In the case of cocoon weight also, the initial two Cu concentrations (5 and 10 µg/g soil) recorded negative phytotoxicity, while the other two treatments (20 and 40 µg/g soil) imposed positive phytotoxicity.

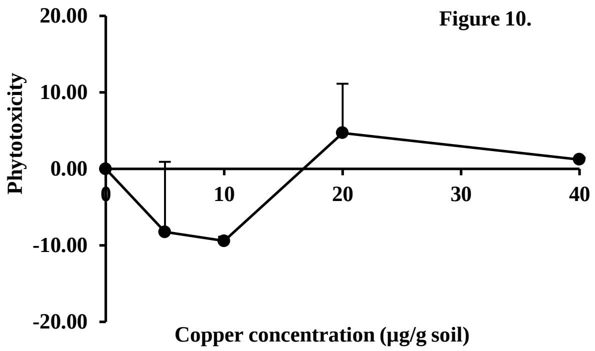

Figure 10: Graphical representation of Phytotoxicity for cocoon weight of the commercial Silkworm, *Bombyx mori* L. (CSR2 x CSR4) reared on the mulberry, *Morus alba* L. (V1) leaf produced in pot experiments treated with different Cu concentrations (µg of CuCl2/g soil). Values are mean of 5 replications (± SD).

c. Phytotoxicity for shell weight: Data on the Phytotoxicity for cocoon shell weight reared on copper treated mulberry leaf are graphically represented in Figure 11. Similar trends in phytotoxicity for cocoon shell weight were observed as observed for cocoon weight. Thus, the initial two Cu concentrations (5 and 10 µg of CuCl2/g soil) recorded negative phytotoxicity, while the other three treatments (20, 40 and 80 µg of CuCl2/g soil) imposed positive phytotoxicity. In the case of shell weight also phytotoxicity was not much different from control batches at Cu concentrations of 20 and 40 µg of CuCl2/g soil, the phytotoxicity was not much differed from that of control, recording very close values of phytotoxicity of control (Figure 8.15), which may also be an experimental error.

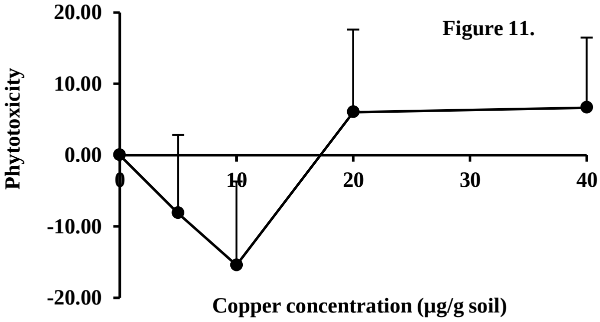

Figure 11: Graphical representation of Phytotoxicity for cocoon shell weight of the commercial Silkworm, *Bombyx mori* L. (CSR2 x CSR4) reared on the mulberry, *Morus alba* L. (V1) leaf produced in pot experiments treated with different Cu concentrations (µg of CuCl2/g soil). Values are mean of 5 replications (± SD).

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d. Phytotoxicity for shell ratio: Data on the Phytotoxicity for cocoon shell ratio reared on copper treated mulberry leaf are graphically represented in Figure 12. Though the initial two Cu concentrations (5 and 10 µg/g soil) slightly recoded negative values, those for the other Cu concentrations (20 and 40 µg/g soil) increased positively slightly from 0.0%, the increase or the decrease in phytotoxicity values from that of control (0.0%) was not much, as the differences between phytotoxicity value are not significant statistically. Thus, the trends in shell ratio for phytotoxicity are identical.

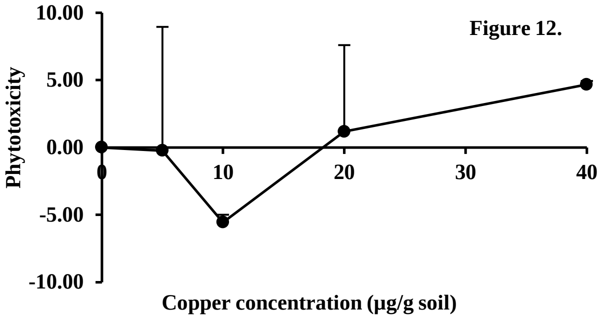

Figure 12: Graphical representation of Phytotoxicity for cocoon shell ratio of the commercial Silkworm, *Bombyx mori* L. (CSR2 x CSR4) reared on the mulberry, *Morus alba* L. (V1) leaf produced in pot experiments treated with different Cu concentrations (µg/g soil). Values are mean of 5 replications (± SD). Note, differences in phytotoxicity values are not significant.

4. DISCUSSIONS

Since the mulberry silkworm, *Bombyx mori* is a monophagous insect, more emphasis is given on the improvement of its only host plant, mulberry (*Morus alba* L.) on one side and enhancing the production and productivity of the insect on the other, to increase the yield of silk fibres of high quality (Balamani *et al.*, 1995). Since mulberry leaf is the only feed for silkworm, growth of silkworm larvae in turn depends principally on the nutrient content of mulberry leaves (Krishnaswami *et al*., 1971) as it derives almost all the nutrients required for its growth from the mulberry leaf itself. Different varieties of macro and micronutrients have been supplements by various researchers in order to increase the quantity and quality of silk (Ito, 1960; Iwanrat and Ono, 1969; Nagarajan and Radha, 1990; Bajpeyi *et al*., 1991; Masilamam *et al*., 1991; Sarker *et al*., 1995; Bongale and Krishna, 1996; Murugappan *et al*., 1996). Further, some essential and nonessential metals have also been tried in this direction. Effectiveness of dietary supplementation of cobalt (Arnando, 1954), nickel (Chamundeswari and Radhaknshnamaiah, 1994; Birendronath Saha and Rahman Khan, 1995), copper (Magadum *et al*., 1992; Prince, 1999), cadmium, zinc, lead and arsenate (Miyoshi *et al*., 1978) were assessed. Such effects have also been assessed by applying known concentrations of zinc (Balamani *et al.*, 1995) and cobalt (Chakrabarthi and Medda, 1978) either by dipping or spraying on the leaves and feeding them after air drying. On the other hand, influence of soil application of essential trace elements on economic characters of silkworm has also been studied (Viswanath and Krishnamoorthy, 1982; Lokanath *et al.*, 1986; Bose *et al.*, 1995; Yeasmin *et al.*, 1995; Bose and Majumder, 1996; Bongale and Lingaiah, 1997). However, these studies are mainly aimed at determining the optimal dosage of metals.

In commercial silkworm rearing, the economic cocoon characters are more important, deciding the profitability or otherwise of silkworm cocoon crop. In the present study, the impact of mulberry leaf grown in plot experiments treated with different concentrations of copper (5, 10, 20 and 40 µg/g soil) was studied. On average, the average weight of the silkworm larva in four Cu treated groups was less than that of the control. When the individual treatments are considered, the silkworm larval weight increased over that of control for the initial two Cu concentrations (5 and 10 µg/g soil) only, the increase being statistically significant. Significant decrease in silkworm larval weight was recorded for the remaining two Cu treatment concentrations (20 and 40 µg/g soil). The other two commercial cocoon characters (cocoon weight and shell weight) also followed the trend observed for silkworm larval weight. An interesting observation was made for the shell ratio. In the case of shell ration also, there occurred increase in shell ratio for the initial two Cu concentrations, 5 and 10 µg/g soil and the rest two Cu concentrations, 20 and 40 µg/g soil infused decrease in shell ratio over that of control. While the differences for three cocoon economic rearing characters, silkworm larval weight, cocoon weight and shell weight are statistically significant ($p < 0.05$) level, non-significant differences were encountered for shell ratio. Similar results were reported for the cocoon commercial characters (Prince, 1999). Mulberry leaf alone should not be considered as responsible for such increase or decrease in silkworm economic rearing characters. It should be copper present in leaf due to treatment of mulberry plants with different experimental Cu concentrations in pot culture experiments. Mulberry leaf did not receive Cu directly, but through transport or translocation from soil through root system. The amount of Cu that is transported to stem or leaf by root system is always less than that acquired by root system (Yruela, 2005, 2009) from experimental soils in pot. Copper is reported to influence almost all biochemical and macronutrient contents in mulberry leaf. Thus, implications of Cu on nitrogen (Prince, 1999), chlorophyll (De *et al.,* 1985; Keshan and Mukherji, 1992), carbohydrate and crude fibre contents (Narwal *et al.*,1990; Prince, 1999) should be some of the supporting facts.

Copper is reported to influence almost all biochemical and macronutrient contents in mulberry leaf. Thus, nitrogen levels increased according to increase in treatment Cu concentration (Prince, 1999). The results of this study are well supported by the studies reported by Shaikh *et al*. (2013) and Yerranna *et al*. (2018), the negative effects of copper on shoot have a direct relationship to their toxicity on shoots and roots. Further, there was no appearance of sprouting at very high Cu concentrations (Yerranna et al., 2018), indicating the relative importance of Cu for shoot generation and leaf functions. Nitrogen content of the leaves is reported to increase according to increasing treatment concentration of Cu (Prince, 1999). Accordingly, protein content of leaves also increased in Cu treatments (Prince, 1999). Free amino acid content of treated mulberry leaves also increased for the initial low concentrations of experimental Cu concentrations as observed for total soluble proteins. Amino acids being the building blocks for the synthesis of polypeptides, may serve as compensating agent in nitrogen requirement of silkworm larvae. From the above observations, it is viewed that both amino acids and soluble proteins in experimental mulberry leaves recorded increase in their quantum for the initial experimental Cu concentrations compared to control mulberry leaf For the Cu concentrations above the initial experimental low concentrations, these contents declined as the Cu concentration increased. Degradation and thereby reduction in chlorophyll content may the result of enhanced activity of chlorophyllase (De *et al.,* 1985; Keshan and Mukherji, 1992), resulting in decrease in the chlorophyll content of the leaves and their interference in photosynthetic process. Heavy metals have been shown to affect the synthesis of sugars (Narwal *et al.*,1990). With regards to fibre contents, Prince (1999) reported that crude fibre content of the leaves showed a steady decrease in Cu treatment.

While Cu is an essential micronutrient, exposure to excess Cu has a detrimental effect on plant growth. The affect of Cu toxicity is largely on root growth and morphology. Copper tends to accumulate in the root tissue with little translocated to the shoots (Marschner, 1995). Based on the Cu concentrations, two different Cu contents are defined; deficiency and excess in Cu. Copper deficiency is expressed in many symptoms of chlorosis, which is initially observed in young leaves, and may evolve necrotic lesions (Abdel-Ghany and Pilon, 2008), reduced expression of genes associated with the cell wall (Printz *et al*., 2016), depletion in electron transport chain of photosynthesis, leading to a reduction in non-photochemical electron extinction, mainly due to impairment of plastocyanin (Abdel-Ghany and Pilon, 2008). At high concentrations Cu can become phytotoxic affecting plant development due to direct or indirect interference with numerous physiological processes (Maksymiec, 1997; Vangronsveld and Clijsters, 1994). Symptoms of copper phytotoxicity include stunted growth, leaf chlorosis (Baron *et al.,* 1995; Fernandes and Henriques, 1991). Excess copper triggers phenotypic changes, such as reduced root and shoot development, reduced cell viability at root ends and premature induction of root lignification (Lequeux *et al*., 2010).

The above reports are well explained through computation of Tolerance Index (TI) and phytotoxicity studies, in the present study. With the control batches, the TI was 100%. If this TI level is taken as base line for comparison of TI values for different treatments, the TI increased for cocoon economic characters with the initial two Cu concentrations. Thus this increase in TI should be referred to as '*over-tolerant* or *supra-tolerant*' at *infra-Cu concentrations*, crossing positively the base TI value, 100% of control. On the other hand, TI of silkworm cocoon economic characters decreased from the third Cu experimental concentration, 20 µg/g soil onwards, as the concentration of copper increased and inverse relation established. Thus, with supra-concentrations of Cu, TI may be called as '*less tolerant* or *infra-tolerant*' to *supra-Cu concentrations*, gliding below the base TI value, 100% of control. Phytotoxicity for the cocoon economic parameters, on the other hand showed a direct relation towards the Cu concentration, showing negative phytotoxicity for the initial two Cu concentrations and positively increased phytotoxicity for the later Cu concentrations. Thus, at *infra-Cu concentrations*, resulted in '*negative-phytotoxicity*'. *Supra-Cu concentrations*, on the other hand resulted in '*supra-phytotoxicity*'. Most importantly, the TI (Tolerance Index) and Phytotoxicity of silkworm larval weight, cocoon weight and shell weight are profoundly influenced by imposed Cu concentrations. However, TI and phytotoxicity did not show much influence on shell ratio. Clearly the issue of shell ratio can be presumed that it is a derived value from directly recorded cocoon weight and shell weight, thus implying that the shell ratio is solely dependent on silkworm race rather than imposed experimental Cu concentrations. There are supporting reports that shell ratio is silkworm race specific or dependent (Datta, 1992; Suresh Kumar *et al*., 2001; Rahmathulla, 2012). Therefore, it can safely be inferred that different Cu concentrations applied in plot experiments did affect drastically on silkworm cocoon economic characters like silkworm larval weight, cocoon weight and shell weight while these Cu concentrations impose a little impact on shell ratio.

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