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Studies On The Effect Of Natural Antioxdant On The Copper And Zinc Accumulation On The Silkworm, *Bombyx Mori*l.

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Abstract

The best-known type of silk is obtained from the cocoons of the mulberry silkworm (*Bombyx mori* L.). Silkworm totally depends on mulberry leaves for survival.Silkworm fed with good quality mulberry leaves enriched with micronutrients in optimum quantity, results in successful seed cocoon production. Several heavy metals are essential micronutrients for plants but they can damage the plant growth, if they exceed threshold of phytotoxicity. Heavy metals containing industrial effluents and municipal waste water are also being used to irrigate agricultural land. Zn is commonly detected in industrial traceeffluents and municipal waste waters. It has an ability to be accumulated in plants and water. Among micronutrients copper and zinc play a vital role in silkworm growth.Heavy metals may oxidative stress to the mulberry plant. So to reduce oxidative stress tried to prepare high antioxidant rich food used as ingredient in natural diet.Antioxidants offer resistance against oxidative stress by scavenging free radicals, inhibiting lipids peroxidation etc. Studies regarding the catalase activity showed that it was widely distributed in various tissues of *B. mori* larvae. They also detected a relatively high activity in the fat body. Antioxidant enzymes and small molecular weight antioxidant perform an effective response against oxidant in insect. Hence the present investigation was carried out on the oral supplementation of natural antioxidant(rice meal, wheat meal, corn meal and aloe vera gel) on copper and zinc accumulation in haemolymph, muscle and silk gland of *B.mori*larvae.

KEYWORDS: B.mori, antioxidant, natural food,heavy metals andeconomic traits.

INTRODUCTION

Mulberry leaf is the only food for silkworm survival.The quality of mulberry leaves plays an important role in the success of the sericulture industry and direct economics(Choudhury *et al*.,1991).Nutrition of silkworm is the sole factor which almost individually augmented quality and quantity of silkworm cocoon production and productivity(Israr *et al*., 2011). Silkworm fed with good quality mulberry leaves enriched with micronutrients in optimum quantity, results in successful seed cocoon production.It requires about 16 essential nutrient elements for the various metabolic process and for the healthy growth of silkworm (Younus*et al*., 2018). Among micro nutrients copper and zinc play a vital role in silkworm growth. Several heavy metals are essential micronutrients for plants but they can damage the plant growth,if they exceed threshold of phytotoxicity (Bennett,1993).Unlike organic compound metals cannot be degraded or destroyed under biotic condition (Ghosh and Singh,2005). Heavy metals containing industrial effluents and municipal waste water are also being used to irrigate agricultural land Zn is commonly detected in industrial trace effluents and municipal waste waters. It has an ability to be accumulated in plants and animals. In this regard the present study was planned to investigate the heavy metals like copper and zinc accumulation in the haemolymph, muscle and silk gland of *B. mori* larvae and the effort was made to find out the natural antioxidant present in natural ingredients like rice meal,wheat meal, corn starch and aloe vera gel along with mulberry leafagainstinhibits oxidative stress to the*B.mori*.

MATERIALS AND METHODS

In the experimental area soil and water was collected for mineral analysis. Soil and water mineral deposition of experimental mulberry plant growing area were analysed in department of agriculture in Kanyakumari. Atomic absorption spectrophotometer (AAS) Perkin Elmer model A analyst 300 equipped with air acetylene flame, for the analysis of toxic heavy metal i.e., copper and zinc in the samples (Javed *et al*., 2007). These mulberry leaves were used for oral supplementation of experimental *B.mori*larvae.

The present investigation was carried on the hybrid CSR CDH.The Disease-FreeLayings (DFLs)of *B.mori* were obtained from the State Government Sericulture Cantre at Thenkasi and incubated at 27°C inant proof racks at 70-80% humidity.The incubation time was 8 days, during which time, the young caterpillars hatched out. The emerging caterpillar were transferred to plastic tray.The fifth instar larvae were selected randomly and grouped in to 5 batches for experiment and control was also set up.Each group consists of 5 replicates with 30 silkworms. Mulberry leaves were collected and the petiole was removed and wash thoroughly to remove the dust particles and left for drying. About 2 gm of rice meal,wheat meal, corn meal and aloevera gel were measured and boiled in distilled water to prepare and fed withsupplement for experimental larvae separately but aloevera gel supplied without boiling. These treatments were carried out in the first day of 5th instar larvae. Silkworm larvae were feed about 3-4 times a day. All the experiment and control larvae were randomly selected and the haemolymph, silk gland and muscles were collected for the analysis of copper and zinc.The experimental data recorded was subjected to statical analysis by Zar (1984).

RESULT AND DISCUSSION

Micronutrients play a significant role in plant growth, photosynthesis, chlorophyll formation, cell wall development,water absorption and resistance to plant disease and enzymatic reactions and improvement for activities of soil microorganism (Yonnus*et al*.,2016).

Table 1showsmineral deposition of soil on mulberry growing area.The experimental soil containsEC(0.06),P^{H}(5.3),Zn(0.86) and Cu(1.06) respectively.Micronutrientssuch as N(0.62),P(0.26) and K(1.01) was observed in the experimental soil.Soil analysis maintain the desired levels of soil nutrients status for cherishing mulberry with enhanced quality mulberry leaf production leading to flourishing with enhanced quality cocoon production. Soil analysis-based prescriptions are necessary to improve crop productivity and to increase nutrient use efficiency.

Table 2 shows the chemical composition of water in treated area are EC(0.06), P^{H}(5.3),Cl(0.90), Ca(0.40), Mg(0.30)& Na(0.70) respectively.Table 3 shows Cu content of haemolymph was decreased (-36.14ppm) when the larvae fed with water of rice meal enriched mulberry leaves when compared to control. Cu content of muscle was significantly decreased (-30.72ppm) when larvae treated with wheat meal water enriched mulberry leaves.Cu content of silk gland was decreased(-3.22ppm) when the larvae treated with corn starch enriched mulberry 3 leaves when compared to control.Cu content of silk gland was decreased(-5.64ppm) when the larvae treated with Aloe vera gel enriched mulberry leaves when compared to control.

Zn content of haemolymph was decreased (-7.79ppm) when the larvae treated with rice was presented in Table 4. Zn content of muscle was decreased (-12ppm) when larvae treated with wheat when compared to control. Zn content of silk gland was decreased (-17.68ppm) when larvae treated with corn when compared to control. Zn content of muscle was decreased (-15.52) when the larvae treated with control. The higher concentration of zinc showed significant decreases in leaf area. This report was in agreement with Rafati *et*

al.(2011), who suggested that Zn and Cu are essential toexcess content is evidently harmful to silkworms. These studies suggest that the influence of copper on the silkworms depend on the concentrations either as supplement or in the feed itself. Tanhan*et al*. (2007) who observed that there was deleterious effect at 50 to 100 ppm levels affecting the survival. This deleterious effect was observed levels affecting the survival.This deleterious effect was observed at higher levels of zinc supplemented with food of silkworms (Blackmore & Wang, 2004).
Table 5 represents the effect of antioxidant on the economic traits of *B.mori*. It is found out the significant improvement was of cocoon weight rice meal (1.680 +1.581) pupa weight of rice meal (1.334 + 1.581) and shell weight of rice meal (350 +1.581) meal treated group. This report was in augmented with Lokanath *et al.* (1986) and who reported that excess of zinc content in mulberry leaves to reduction in cocoon yield. Tsenov and Petkov (1997) assessed that silkworm survival rates were lower when fed with Zn and Cu treated mulberry leaves and the fresh cocoon production per box of silkworm eggs was much lower.It was also observed that rice meal alsoincreases the weight of cocoon and shell.

CONCLUSION

The present study suggests that the natural diet components contain a highly potential antioxidant activity considerably, high content of bioactive phytochemicals (such as phenol, flavanol, soluble sugar, reducing sugarproteinwere estimated in their component. Results also demonstrated that the source of food rather than its intricate quality is very much important for the growth and development of silkworm larvae and for the development of silkworm larvae and for production of silk. Prominent improvement in cocoon shell weight was observed after application rice,wheat, corn,aloe vera gel on mulberry leaf.

The results indicate that ricemeal has maximum impact on improving cocoon shell weight when compared with control. Our result indicates that the mulberry leaves when applied with specific diet may improve the quantity of silk as revealed from enhancement of cocoon shell.Weight with additional diet components. The knowledge may have serious implication in sericulture industry applied for silkworm rearing.According to the overall results of this of this study the rice meal is highly recommended for rearing silkworms because of its beneficial effects on silkworm biological and economic productivity.

Table 1 Mineral deposition of soil on mulberry growing area

Parameters	Observed Value
EC	0.06
P^H	5.3
Zn	0.86
Cu	1.06
N	0.62
P	0.26
K	1.01

Table 2 Chemical composition of water in mulberry growing area

Parameters	Observed Value
EC	0.06
P^H	5.3
Cl	0.90
Ca	0.40
Mg	0.30
Na	0.70

Table 3 Effect of natural antioxidant on copper content of haemolymph, muscle and silk gland of *B.mori* larvae.

Treatment	Haemolymph (ppm)	Muscle (ppm)	Silk gland (ppm)
Control	0.166+ 0.023	0.192+4.728	0.124+4.562
Rice	0.106+ 0.010 (-36.14)*	0.148+ 0.021 (-22.9)*	0.122+0.016 (-16.12)*
Wheat	0.139+0.010 (-16.26)*	0.133+ 8.249 (-30.72)*	0.118+0.010 (-4.83)*

Corn	0.138 +9.37 (-16.86)*	0.120 + 4.958 (-37.49)*	0.120+ 9.278 (-3.22)*
Aloe vera gel	0.089 + 4.484 (-46.38)*	0.131 + 5.411 (-31.77)*	0.117+ 0.027 (-5.64)*

Percent deviation over control values in parentheses N=30 * not significant P≤ 0.05 (t-test)

Table 4Effect of natural antioxidant on Zinc content of haemolymph, muscle and silk gland of *B.*mori larvae.

Treatment	Haemolymph	Muscle	Silkgland
Control	0.372 + 0.189	0.625 + 0.033	0.277 + 0.017
Rice	0.343 + 0.040 (-7.79)*	0.545 + 0.002 (-12.8)*	0.249 + 0.027 (-10.10)*
Wheat	0.268 + 0.020 (-26.88)*	0.550 + 0.021 (-12)*	0.262 + 0.030 (-5.41)*
Corn	0.256 + 0.028 (-31.18)*	0.539 +0.052 (-13.76)*	0.228 + 0.038 (-17.68)*
Aloevera gel	0.163 + 0.024 (-56.18)*	0.528 + 0.030 (-15.52)*	0.296 + 0.021 (-2.88)*

Percent deviation over control values in parenthesesN=30
* not significant at P≤ 0.05 (t-test) 6

Table 5 Effect of antioxidant on the economic traits of *B.mori*

Treatment	Cocoon weight (mg)	Pupa weight (mg)	Shell weight (mg)	Shell ratio (%)
Control	1650 + 158.10	1400+ 1.788	250+ 1.643	15.15+ 0.01
Rice	1680 + 137.21 (1.77)*	1370+118.19 (-2.13)*	320 +108.51 (28)	19.04+1.61 (25.67)
Wheat	1601 + 145.08 (-2.89)*	1321 + 130.33 (-5.60)*	280+ 140.38 (12)	17.48+1.36 (15.37)
Corn	1621 + 158.11 (-1.71)*	1321 + 120.38 (-5.60)*	300+158.21 (20)	18.50+1.61 (22.11)
Aloevera gel	1580 + 114.08 (-4.13)*	1309+ 128.41 (-6.46)*	271 + 128.13 (8.4)*	17.15+1.36 (13.2)

Percent deviation over control values in parentheses N=30
* not significant All other deviations significant at P≤ 0.05 (t-test)

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