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# Biochemical Analysis of Mulberry Leaves (*Morus alba* L.) and Silkworm *Bombyx mori* Enriched with Vermiwash

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## ABSTRACT

A study was carried out to evaluate the effect of vermiwash supplementation on silk worm *Bombyx mori*. The vermiwash was diluted to different concentration as 10, 25 and 50%. Fresh mulberry leaves were sprayed by each concentration of vermi wash. The leaves were then fed to silk worms from 5th instar stage onwards at four times per day. Group 1 larvae received mulberry leaves without vermiwash spray and served as control, group 2 larvae received 10% vermiwash sprayed mulberry leaves, group 3 larvae received 25% vermiwashsprayed mulberry leaves and group 4 larvae received 50% vermiwash sprayed mulberry leaves. Weight of larvae, silk gland, cocoon, pupa and shell weight of silkworm was significantly increased. Minimum larval weight (2.160 g) was in control and maximum (3.416 g) was in 50% enriched larvae. Silk gland weight of control, 10, 25 and 50% enrichment was 0.511, 0.552, 0.661 and 0.738 g, respectively. The cocoon weight of control, 10, 25 and 50% treatment was 1.065, 1.243, 1.296 and 1.374 g respectively. The shell weight of control (0.171 g) was lower than 10% treatment (0.198 g), 25% treatment (0.247 g) and 50% treatment (0.315 g). There was significant increase in the carbohydrate, protein and lipid of fat body and mulberry leaves. Acid phosphatase and alkaline phosphatase are significantly increased in the body of *B. mori*.

**Key words:** Mulberry leaves, *Bombyx mori*, vermi wash, silk gland, cocoon

## INTRODUCTION

Sericulture is one of the oldest industries in India and probably dates back to the beginning of the Christian era. As far as the Indian scenario is concerned sericulture is an especially significant industry, the reasons are varied. India being the second largest producer of silk, next to China at an annual turnover rate of 20,000 metric tonnes and accounting 20% of world production. About 65% of total silk production of India comes from Karnataka alone (Singhvi *et al*., 2002). During the last fifteen years, the production of mulberry silk was increased from 2.258 metric tonnes to 6,900 metric tonnes. The *Bombyx mori* L. (silkworm) is a phytophagous feeder on *Morus alba* L. (mulberry leaves). Scientists have tried alternative hosts for the rearing of silkworm, but they were not cost effective. Based on the nutritional quality, mulberry has a great influence on the silkworm growth, silk yield and disease resistance (Ravikumar, 1988).

Earthworms play a vital role in plant growth. Recently, the commercial vermi culturists have started promoting a product called vermi wash. Vermiwash contains enzyme cocktail of proteases, amylases, urease and phosphatase and secretions of earthworms which would stimulate the growth and yield of crops and even develop resistance in crops receiving this spray. The effect of nitrogen, vitamin and salts supplementation on the growth of silkworm has been investigated by many researchers (Mahmood *et al*., 2002; Kanafi *et al*., 2006; Dhiraj and Venkatesh Kumar, 2012), but the reports on the effect of mulberry leaves enriched with vermiwash were limited. So, the present study was aimed to find out the effective dose of vermiwash application to mulberry leaves to find out its effect on increment of weight of larvae, silk gland, cocoon, pupa and shell weight of silkworm.

## MATERIALS AND METHODS

The eggs of silkworm *B. mori* were collected from Sericulture Training Institute, Nanjikottai, Thanjavur, Tamil Nadu (India). The culture of larvae and mulberry leaves ($MR_2$ variety) were standardized by following the method of Krishnan *et al*. (1995). The larvae were reared in plastic trays (20 larvae/tray) and were fed with mulberry leaves. Fresh mulberry leaves were collected every day morning and stored in wet gunny bags. The larvae were fed four times a day (8:00, 13:00, 18:00 and 27:00 h). Bedding and spacing were adopted carefully at the time of rearing (Krishnaswami *et al*., 1978). Enough care was taken to maintain the humidity (75-80%) and temperature (25-28°C) throughout the study period.

The mulberry trees were enriched with the vermiwash in three different concentrations such as 10, 25 and 50% once in a week by using foliar spray for 4 weeks. For each concentration of vermiwash application, separate plots were used and untreated control plot was maintained separately in the neighboring field. The worms were fed with these enriched leaves every day and were supplied for the entire period of 5th instar larvae. Finally, the larval weight, cocoon weight, pupa weight and shell weight were measured and estimation of biochemical parameters of mulberry leaves and fat body of silkworm larvae were carried out. The carbohydrate was estimated by the Anthrone method of Seifter *et al*. (1950), protein was estimated by the method of Lowry *et al*. (1951) and lipid were estimated by chloroform methanol method (Folch *et al*., 1957). Comparison was made between all the treatment groups by drawing bar charts using Microsoft excel.

## RESULTS

The changes in the larval and cocoon characteristics of silkworm *B. mori* were studied when the larvae fed on mulberry leaf enriched with vermiwash (Fig. 1, 2). There was increased weight in enriched larvae when compared to control larvae. Minimum larval weight (2.160 g) was in control and maximum (3.416 g) was in 50% enriched larvae. The silk gland weight is significantly increased in the vermiwash enriched larvae. Silk gland weight of control, 10, 25 and 50% enrichment was 0.511, 0.552, 0.661 and 0.738 g, respectively. The mean weight of cocoon was increased in the vermiwash enriched larvae. The cocoon weight of control, 10, 25 and 50% treatment was 1.065, 1.243, 1.296 and 1.374 g, respectively. The data obtained on the pupal weight of the control and enriched group were recorded. The increase in the pupal weight was clearly noticed in the vermi wash enriched groups. Weight of pupa in the control, 10, 25 and 50% was

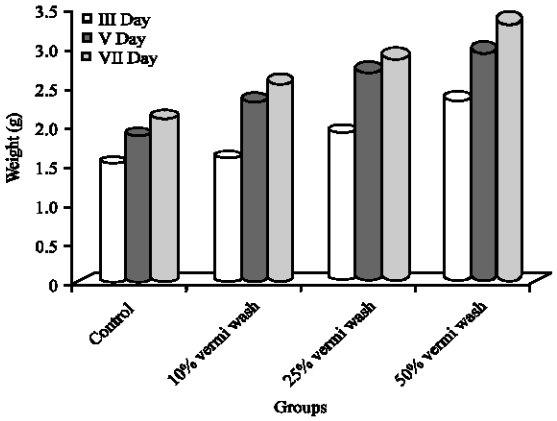


Fig. 1: Weight of 5th instar

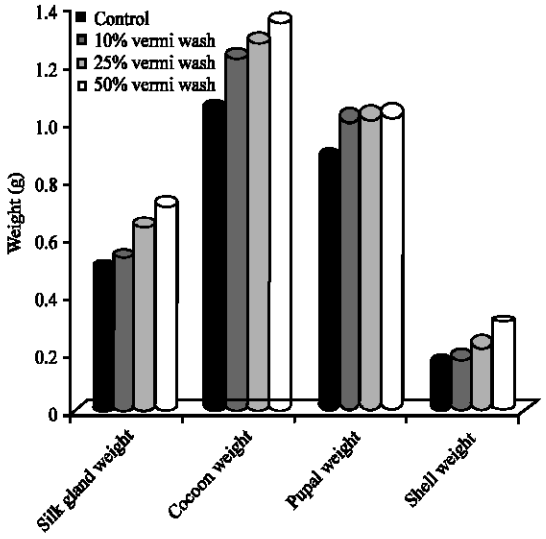


Fig. 2: Weight of silk gland, cocoon, pupal and shell weight of enriched and control

0.894, 1.045, 1.049 and 1.059 g. The shell weight was significantly increased in the enriched groups when compared to the control. The shell weight of control (0.171 g) was lower than 10% treatment (0.198 g), 25% treatment (0.247 g) and 50% treatment (0.315 g).

**Biochemical studies from mulberry leaf:** In all treatments, the level of carbohydrate is higher than the control. Carbohydrate levels in control leaf was 14 mg, in 10% vermiwash enriched leaf it was 14.40 mg, in 25% enriched leaf it was 14.80 mg and in 50% enriched leaf it was 15.0 mg. The protein content was significantly higher in vermiwash enriched leaf than control. The protein level of the control leaf was 2.693 mg. The protein content of 10% enriched leaf was 2.704 mg, 25% enriched leaf was 2.714 mg and 50% enriched leaf was 2.721 mg. The lipid level of control leaf was 0.052 mg. In 10, 25 and 50% enrichment, it was 0.063, 0.076 and 0.094 mg, respectively (Fig. 3).

**Biochemical studies from silkworm:** The level of carbohydrate in the total body tissue of the control, 10, 25 and 50% vermiwash enriched larvae was 16, 16.40, 16.80 and 17.40 mg, respectively. The protein level in the control, 10, 25 and 50% enriched larvae was 1.975, 1.989, 1.996 and 2.007 mg, respectively. The lipid level in the control, 10, 25 and 50% enriched larvae was 0.056, 0.062, 0.080 and 0.089 mg, respectively (Fig. 4).

**Enzyme studies:** The acid phosphatase level in the control larvae was 2.1 IU $L^{-1}$ and significant increase was observed in 10% (3.5 IU $L^{-1}$), 25% (7.2 IU $L^{-1}$) and 50% enriched larvae (9.4 IU $L^{-1}$). The alkaline phosphatase level in the control was (33 IU $L^{-1}$) lower than 10% (76 IU $L^{-1}$), 25% (124 IU $L^{-1}$) and 50% enriched (163 IU $L^{-1}$) body tissue of *B. mori* (Fig. 5).

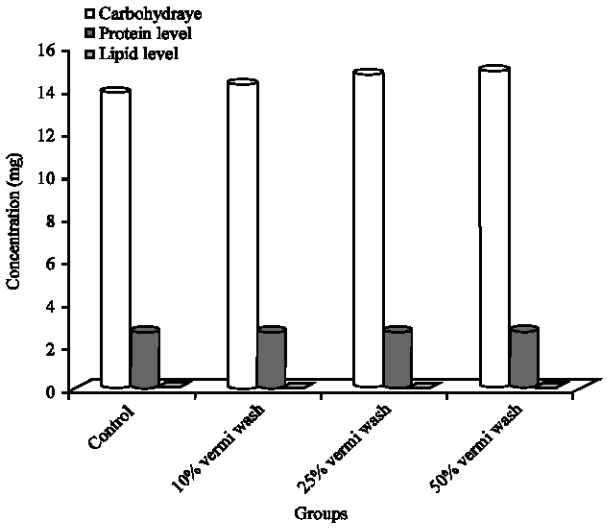


Fig. 3: Proximate composition of enriched and control mulberry leaf

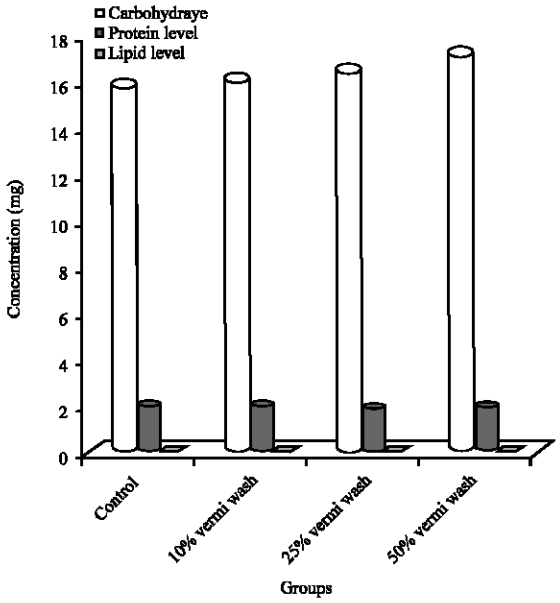


Fig. 4: Proximate composition of enriched and control fifth instar larvae

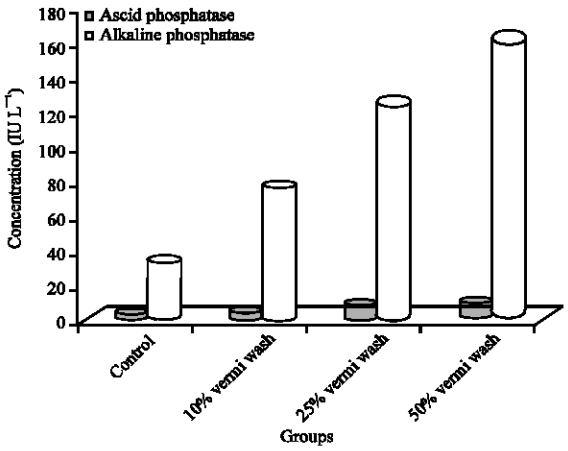


Fig. 5: Acid and alkaline phosphatase of enriched and control

## DISCUSSION

In the present study, the fifth instar larvae of *B. mori* were fed on mulberry leaves which was enriched with vermiwash in different concentrations. The increases in the larval, pupal and shell weight of fifth instar larvae of *B. mori* were observed. The consumption of mulberry leaf by *B. mori* is significantly increased when they enriched with vermiwash and correspondingly increased the assimilation and production efficiency of the larvae. It was due to the volume of enzyme secreted in the digestive system resulted in the improvement of digestion. In the present investigation; larval, silk gland, cocoon, pupal and shell weight of *B. mori* were increased when larvae fed with vermiwash enriched mulberry leaves than the untreated control. Chikkaswamy *et al.* (2001) and Mahmood *et al.* (2002) reported that application foliar sprays of green leaf significantly improve the larval, silk gland, cocoon, pupal and shell weight of *B. mori*. larvae fed with mulberry leaves enriched with Amway protein showed significant enhancement larval, cocoon, shell weight (Rani *et al.*, 2011). Singhvi *et al.* (2002) reported foliar application of 'seriboost' significant increase larval weight.

In the present study, biochemical composition of mulberry leaves enriched with vermiwash and control leaf were analysed. The level of the carbohydrate, protein and lipid is greater in vermiwash enriched leaves than the control. Foliar sprays of mulberry leaves increased the biochemical parameters (Chikkaswamy *et al.*, 2001). The growth rate of plants is also increased in the vermiwash enriched than control. Dhiraj and Venkatesh Kumar (2012) reported that foliar sprays influence the performance of mulberry varieties in terms of physiological parameters of plants viz., carbohydrates, protein etc. Ashfaq *et al.* (2000) studied the various minerals viz., N, P, K, Ca, Mg and Cu, respectively in various combination were enhanced silk production. Venkataramana *et al.* (2009) studied the effect of enrichment of different concentration of vermiwash and cow dung wash to improve the biochemical and yield attributing parameters and yield of mulberry. The biochemical analysis of *B. mori* fed with vermiwash enriched leaves showed the increase in carbohydrate, protein and lipid content in the total body tissue of fifth instar larvae than control. These results are in accordance with the finding of Dhiraj and Venkatesh Kumar (2012). Singhvi *et al.* (2002) reported foliar application of seriboost significant increase in the biochemical parameters of silk worm in his study. Santhi *et al.* (2003) reported that protein content increased with the application of humic acid in rice, maize and sugarcane. Nandakumar *et al.* (2004) also reported that foliar

application of Humic acid (HU) in combination with NPK increased soil nutrients (N, P, K, Fe, Mn, Zn and Cu) which is essential growth factors of rice. Carbohydrate, protein and lipid are the main sources of energy at the time of larval-larval, larval-pupal, pupal-adult transformation Venkataramana *et al.* (2009). The level of enzymes, such as alkaline phosphatase and acid phosphatase, in the body of fifth instar larvae showed significant increase when they fed with vermiwash enriched leaves.

In this study, it is understood that the vermiwash enriched larvae showed an increased larval weight which leads to higher gain of cocoon weight and increased silk production. By knowing this, it is suggested that the farmers are advised for vermiwash enriched larval culture techniques.

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