

# Improving silkworm (*Bombyx mori*) growth and survival through mulberry leaves: A study on sustainable sericulture and biodiversity

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## Abstract

The mulberry leaves (*Morus spp.*) are the primary source of food for silkworms, and its quality heavily influences the rearing of silkworms. A randomized block design was used with silkworm larvae (1000 per treatment group) fed on *Morus alba*, *Morus nigra*, and a mixture of both. The proximate analysis of the mulberry leaves showed significant differences in moisture, protein, and fat contents. *Morus alba* exhibited higher protein content, fat, and total ash contents as  $26.16 \pm 0.20\%$ ,  $4.53 \pm 0.12\%$  and  $17.38 \pm 0.06\%$  respectively. Silkworms fed on *Morus alba* demonstrated significantly better growth (final body weight of  $2101.50 \pm 3.50$  mg and body length of  $31.35 \pm 0.03$  mm) as compared to those fed on *Morus nigra* (final weight of  $1993.00 \pm 5.67$  mg and body length of  $28.78 \pm 0.02$  mm). The survival rate was also higher in the *Morus alba* group, particularly during the early molting stages. The absence of extreme temperature-humidity Index (THI) values suggests that no additional environmental stressors were imposed during the trial. The data showed that silkworms fed with *Morus alba* exhibited a high survival probability, followed by mixed leaves and *Morus nigra*, which had the lowest survival. Survival probability was significantly low during specific molting stages, especially in later instars, confirming that molting represents a major biological stressor. The data revealed that both diet and molting stage significantly influence silkworm survival. The research also highlighted the significance of maintaining optimum temperature and humidity for silkworm growth. The semi-controlled environmental conditions proved to be effective in mitigating environmental stress during the experiment. It is recommended that future studies should explore the potential of other mulberry species for sericulture under

diverse environmental conditions. Moreover, investigating how extreme environmental conditions, such as heat waves or excessive rainfall, can influence silkworm productivity would provide valuable insights into optimizing sericulture practices and contribute to the sustainability of the industry.

**Keywords:** Mulberry, *Bombyx mori*, Cocoon yield, Sericulture, *Morus* spp.,

## Introduction

Sericulture is the science of growing mulberry plants and the rearing of silkworms (*Bombyx mori*) to produce silk. Sericulture is an ancient agricultural sector that remains of immense economic value in most regions of the world (Govorushko, 2019). A significant issue in the sericulture sector is the inconsistency of the silk production due to disease outbreaks, malnutrition, and environmental pressure. Mulberry trees (*Morus* spp.) is a species of the Moraceae family and the most important source of food of silk worms (Zhou et al., 2015). The leaves of the trees are a very rich source of nutrients needed to grow and develop the silkworm larvae. They consist of carbohydrate, protein, lipids, vitamins, minerals, and other essential nutrients that silkworms need to form a cocoon. These cocoons are composed of fibrin, a protein of silk which is formed by the food of the silkworm. Nevertheless, the nutritional value of mulberry leaf in various species may differ substantially, and this may influence the quality and amount of silk obtained (Zhang et al., 2019). Quality of mulberry leaves is dependent on various factors, which include, type of mulberry tree, the fertility of the soil, the climate, and the production system. Since the mulberry leaves are the only source of feed for the silkworm, they contain important nutrients required for better growth and cocoon production. As an example, when silkworms are raised on high-protein mulberry leaves, they are likely to develop quickly, give larger cocoons, and produce high-quality silk (Murthy et al., 2013). Conversely, silkworms fed with low-protein or imbalanced mulberry leaves may exhibit slower growth rates and poor cocoon yield. Several species of mulberry are cultivated for sericulture such as *Morus alba*, *Morus indica*, and *Morus nigra*. All of these possessed unique characteristics that influence silkworm development (Katsube et al., 2006).

*Morus alba* (white mulberry) is the predominant species in sericulture. It is known to be a suitable food source with high nutritional value for silkworms. The leaves of *Morus alba* are nitrogen- and protein-rich, which in turn facilitate the fast growth of the silkworms and synthesis of high-quality silk. The white mulberry tree can grow in a range of environmental conditions and can be cultivated on a large scale (Chen et al., 2022). *Morus indica* (Indian mulberry) originates in the Indian subcontinent and is used for silkworm feeding in tropical and subtropical areas. It has

bigger leaves and higher fiber content than *Morus alba*. However, the *Morus indica* trees have low resistance to some pests and diseases, and their less nutrient in which will hinder silkworms' growth and silk production. Nonetheless, it may be more resistant to harsher environmental conditions such as humidity and heat, allowing it to be cultivated in specific areas. Black mulberry (*Morus nigra*) is also sometimes used in sericulture, and it has high antioxidant. It is also more resistant to drought than *Morus alba* and *Morus indica*. But the leaves of *Morus nigra* do not have the high protein content for optimum silk spinning. However, continuous work on its bioactive compounds indicates that it may play a role in silkworm health and environmental stress resistance (Ademola et al., 2017).

Mulberry tree and subsequently silkworm growth and productivity are largely determined by local agro-climatic conditions. Mulberry cultivation depends upon factors like temperature, humidity, soil composition, and availability of water. Mulberry species have different adaptability to these environmental factors. *Morus alba* prefers temperate conditions, but *Morus indica* grows better in hotter, tropical climates. The capacity of specific mulberry species to cope with such environmental stresses (drought or inundation, and large fluctuations in temperature) will determine the extent and dynamism of availability and quality of leaves for silkworm feeding (Zhang et al., 2011).

Environmental conditions play a critical role in silkworm farming. Silkworms are typically raised in controlled environments to ensure optimal growth and cocoon production. Temperature, humidity, and light exposure are particularly important factors that must be carefully regulated to mimic the natural conditions conducive to silkworm development (Manisankar et al., 2008). The ideal temperature range for silkworm rearing is typically between 23 °C and 28 °C, with a humidity level of 75-85%. The semi-controlled environments for silkworm rearing aim to provide a more sustainable and cost-effective approach compared to fully controlled systems. In these environments, temperature and humidity may fluctuate slightly, but they are still managed to maintain conditions that are within a reasonable range for silkworm development. The semi-controlled conditions allow for a more cost-effective approach to sericulture while still providing adequate control over critical factors (Barcelos et al., 2020).

Despite the significant role of *Bombyx mori* in sericulture, there remains a lack of comprehensive research into the comparative productivity of silkworms when fed different *Morus spp* under semi-controlled environmental conditions. While *Morus alba* is the standard mulberry species used for

silkworm farming, limited studies have explored the potential of other species, such as *Morus nigra* and *Morus indica*, in optimizing silkworm growth and silk yield. The present study was therefore planned to compare the productivity of silkworms reared on different mulberry species under semi-controlled conditions to identify the most suitable species for enhancing sustainable silk production (Oripovich & Ramazonovich, 2020).

### **Materials and methods**

The 32-day experiment was conducted at the Department of Wildlife and Ecology, UVAS, Ravi Campus. The fresh mulberry leaves of two different species were collected from Changa Manga forest (Ajmal et al., 2025; Hussain et al., 2025). The leaves were washed to remove any dirt or debris. After those leaves were dried in an oven at 60 °C until they reached a constant weight. Once dried, leaves were ground to make a fine powder and stored for further analysis. The proximate analysis was done according to standard protocols (AOAC, 2023).

### **Experimental Design**

The first treatment group was provided with *Morus alba*, the second treatment group received *Morus nigra*, and the control group was given a mixture of both *Morus alba* and *Morus nigra* leaves. Each group consisted of 1,000 silkworm larvae (Shah et al., 2007). The experiment was conducted with each treatment group having duplicates. A 17-gram packet of silkworm was obtained from the Punjab Forest Department, Changa Manga Forest, Kasur, Punjab, Pakistan (Hussain et al., 2025). Silkworm's eggs were incubated in petri plates for 7-12 days. After hatching, silkworm larvae were shifted to rearing trays, and 10 to 15 grams of chopped mulberry leaves were provided twice a day. At the end of 1<sup>st</sup> molting, the silkworm caterpillars were shifted to the rearing room, which consists of proper bedding set up to provide enough space to the silkworms and a more efficient cleaning process. The amount of food each larva received was uniform to control the intake of the diet by each treatment. Each treatment group received the same weight of leaves. This design reduced the possible bias of unequal feed access and made the differences in growth and survival due to the type of mulberry leaves, not the amount of feed.

### **Data collection and Statistical analysis**

Silkworms' growth data, including weight, length, survival rate, and cocoon weight, were recorded for each treatment group. The data thus obtained were subjected to analysis through one-way ANOVA technique using PROC GLM in the SAS software (version 9.1). For the comparison of

significant treatment means, Fisher's least significant difference test was applied (Steel et al., 1997) and the following model was applied:

$$Y_{ij} = \mu + \tau_i + \epsilon_{ij}$$

Trend of the temperature-humidity index was calculated using the following formula;

$$THI = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)]$$

Survival analysis was performed using Cox proportional hazards regression to evaluate the effects of treatment and molting stage on silkworm mortality. Kaplan–Meier survival curves were constructed to visualize survival patterns across treatment groups. A p-value of less than 0.05 was considered statistically significant.

## Results

The experiment was conducted under semi-controlled environmental conditions. During the experimental research period, the growth performance, survival rate, and environmental factors were closely monitored to assess the effect of different mulberry species (*Morus alba*, *Morus nigra*, and mixed leaves) on the rearing performance of *Bombyx mori*.

### Proximate composition of mulberry leaves

The proximate composition of different mulberry species is presented in Table 1. The significant differences ( $p \leq 0.05$ ) were observed in moisture, total ash, crude protein and crude fat contents. *Morus nigra* had the highest moisture content ( $8.36 \pm 0.05$ ) followed by *Morus alba* ( $7.46 \pm 0.12$ ) and mixed leaves ( $7.28 \pm 0.17$ ). *Morus alba* had the highest total ash value ( $17.38 \pm 0.06$ ) followed by the mixed leaves ( $13.84 \pm 0.17$ ) and *Morus nigra* ( $12.28 \pm 0.15$ ) with a significant difference ( $p < 0.0001$ ). The crude protein contents were highest in *Morus alba* ( $26.16 \pm 0.20$ ) followed by *Morus nigra* ( $19.50 \pm 0.16$ ), and the mixed leaves were at ( $19.00 \pm 0.07$ ). Similarly, a significant difference ( $p = 0.002$ ) was observed in crude fat content as *Morus alba* showed the highest value ( $4.53 \pm 0.12$ ) followed by the mixed leaves ( $3.91 \pm 0.33$ ) and *Morus nigra* ( $3.20 \pm 0.03$ ). However, there is no statistically significant difference was observed ( $p = 0.951$ ) in crude fiber content with *Morus alba* at  $10.16 \pm 0.07$ , *Morus nigra* at  $10.1 \pm 0.21$ , and the mixed leaves at  $10.12 \pm 0.07$ . The results underline significant differences in moisture, ash, protein, and fat contents, but not in fiber content.

**Table 1.** Proximate composition of various mulberry species fed to silkworms (Mean  $\pm$  S.D, n=5, duration of experiment –32 days).

Parameters	<i>Morus alba</i>	<i>Morus nigra</i>	Mixed Leaves	p-value
Moisture (%)	7.46 <sup>b</sup> ± 0.12	8.36 <sup>a</sup> ± 0.05	7.28 <sup>b</sup> ± 0.17	<0.0001
Total Ash (%)	17.38 <sup>a</sup> ± 0.06	12.28 <sup>c</sup> ± 0.15	13.84 <sup>b</sup> ± 0.17	<0.0001
Crude Protein (%)	26.16 <sup>a</sup> ± 0.20	19.50 <sup>b</sup> ± 0.16	19.00 <sup>c</sup> ± 0.07	<0.0001
Crude Fat (%)	4.53 <sup>a</sup> ± 0.12	3.20 <sup>c</sup> ± 0.03	3.91 <sup>b</sup> ± 0.33	0.002
Crude Fiber (%)	10.16 ± 0.07	10.1 ± 0.21	10.12 ± 0.07	0.951

Superscripts on different means within row differ significantly at  $p \leq 0.05$ .

### Growth Parameters

Growth performance of *Bombyx mori* larvae was assessed by measuring both body weight and body length at four successive molting stages under different dietary treatments, including *Morus alba*, *Morus nigra*, and mixed leaves as a control group. Randomly, 10 silkworm specimens from each treatment group were selected for morphometric measurements during each molting stage. Statistical analysis of growth parameters among different treatment groups is mentioned in Tables 2 and 3. The mean body weight of larvae increased progressively from molting 1 to molting 4 across all treatment groups, reflecting normal larval development. However, significant differences ( $p < 0.0001$ ) were observed among the groups at each stage. Larvae fed with *M. alba* leaves consistently exhibited the highest body weight, reaching an average of  $2101.50 \pm 3.50$  by the fourth molt. This was significantly higher compared to the mixed leaves group ( $2051.00 \pm 2.61$ ) and the *M. nigra* group ( $1993.00 \pm 5.67$ ), indicating superior nutritional efficacy of *M. alba* for larval growth (Fig. 1). The higher weight gain in the *M. alba* group can be attributed to its higher protein and moisture content, which are critical for larval metabolism, tissue synthesis, and silk gland development. In contrast, the *M. nigra* group consistently exhibited the lowest body weights at all stages, suggesting a comparatively lower nutritional suitability or digestibility. Similar to the weight results, body length increased significantly across the molting stages in all treatments, with *M. alba*-fed larvae showing the greatest lengths at each stage. The silkworms fed with *M. alba* reached a mean length of  $31.35 \pm 0.03$  at molting 4, which is significantly longer than those fed on mixed leaves ( $29.39 \pm 0.04$ ) and *M. nigra* ( $28.78 \pm 0.02$ ). The consistent pattern of superior growth in the *M. alba* group further underscores the superior nutrient assimilation and physiological support provided by *M. alba* (Fig. 2). These length differences, although apparently

small it are biologically significant as body length correlates with gut surface area for nutrient uptake capacity.

**Table 2.** Body weight (mg) of silkworms at different molting stages throughout the experiment (Mean  $\pm$  S.D, n=20, duration of experiment –32 days).

Treatment	Molting1	Molting2	Molting3	Molting4
<i>Morus alba</i>	89.50 <sup>a</sup> $\pm$ 2.94	389.00 <sup>a</sup> $\pm$ 3.40	784.00 <sup>a</sup> $\pm$ 2.94	2101.50 <sup>a</sup> $\pm$ 3.50
<i>Morus nigra</i>	72.00 <sup>c</sup> $\pm$ 2.36	365.50 <sup>c</sup> $\pm$ 2.23	727.00 <sup>c</sup> $\pm$ 3.09	1993.00 <sup>c</sup> $\pm$ 5.67
Mixed Leaves	79.00 <sup>b</sup> $\pm$ 1.76	378.50 <sup>b</sup> $\pm$ 2.44	735.50 <sup>b</sup> $\pm$ 2.76	2051.00 <sup>b</sup> $\pm$ 2.61
p-value	<0.0001	<0.0001	<0.0001	<0.0001

Superscripts on different means within columns differ significantly at  $p \leq 0.05$ .

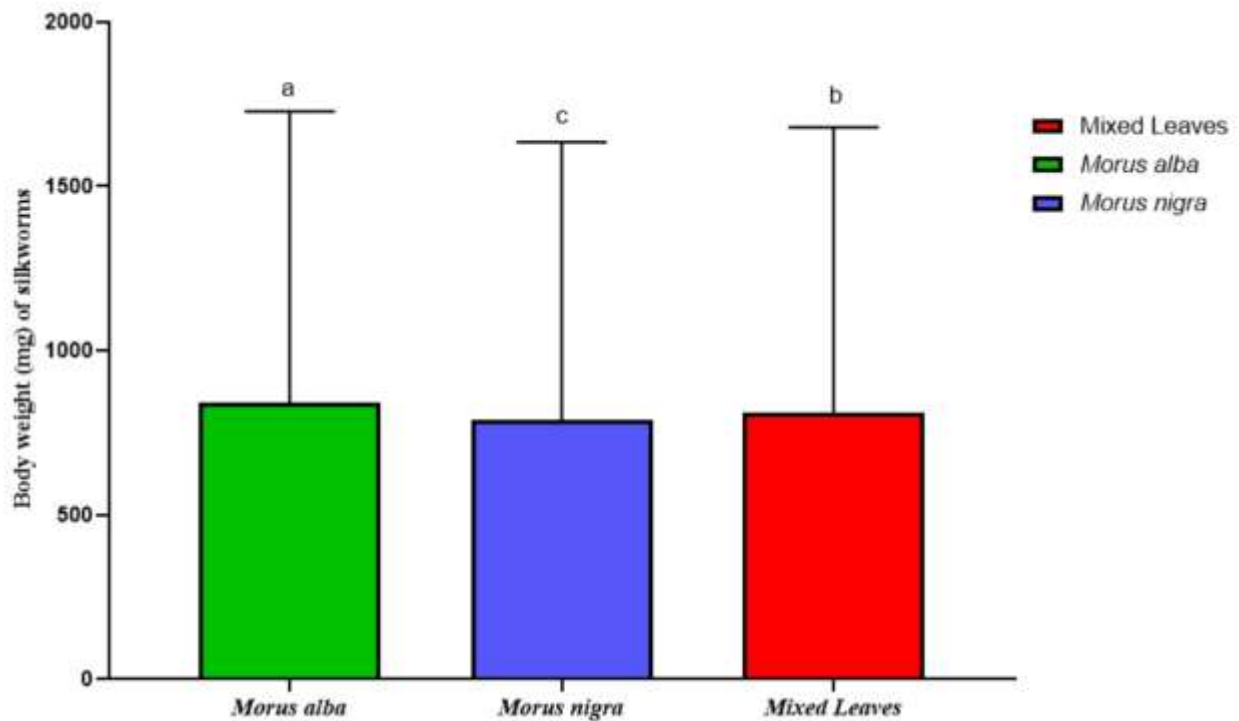
**Table 3.** Body length (mm) of silkworms at different molting stages throughout the experiment (Mean  $\pm$  S.E, n=20, duration of experiment –32 days).

Treatment	Molting1	Molting2	Molting3	Molting4
<i>Morus alba</i>	3.26 <sup>a</sup> $\pm$ 0.03	10.76 <sup>a</sup> $\pm$ 0.02	14.80 <sup>a</sup> $\pm$ 0.02	31.35 <sup>a</sup> $\pm$ 0.03
<i>Morus nigra</i>	2.77 <sup>c</sup> $\pm$ 0.02	9.70 <sup>c</sup> $\pm$ 0.02	12.77 <sup>c</sup> $\pm$ 0.02	28.78 <sup>c</sup> $\pm$ 0.02
Mixed Leaves	2.91 <sup>b</sup> $\pm$ 0.04	10.13 <sup>b</sup> $\pm$ 0.05	13.44 <sup>b</sup> $\pm$ 0.03	29.39 <sup>b</sup> $\pm$ 0.04
p-value	<0.0001	<0.0001	<0.0001	<0.0001

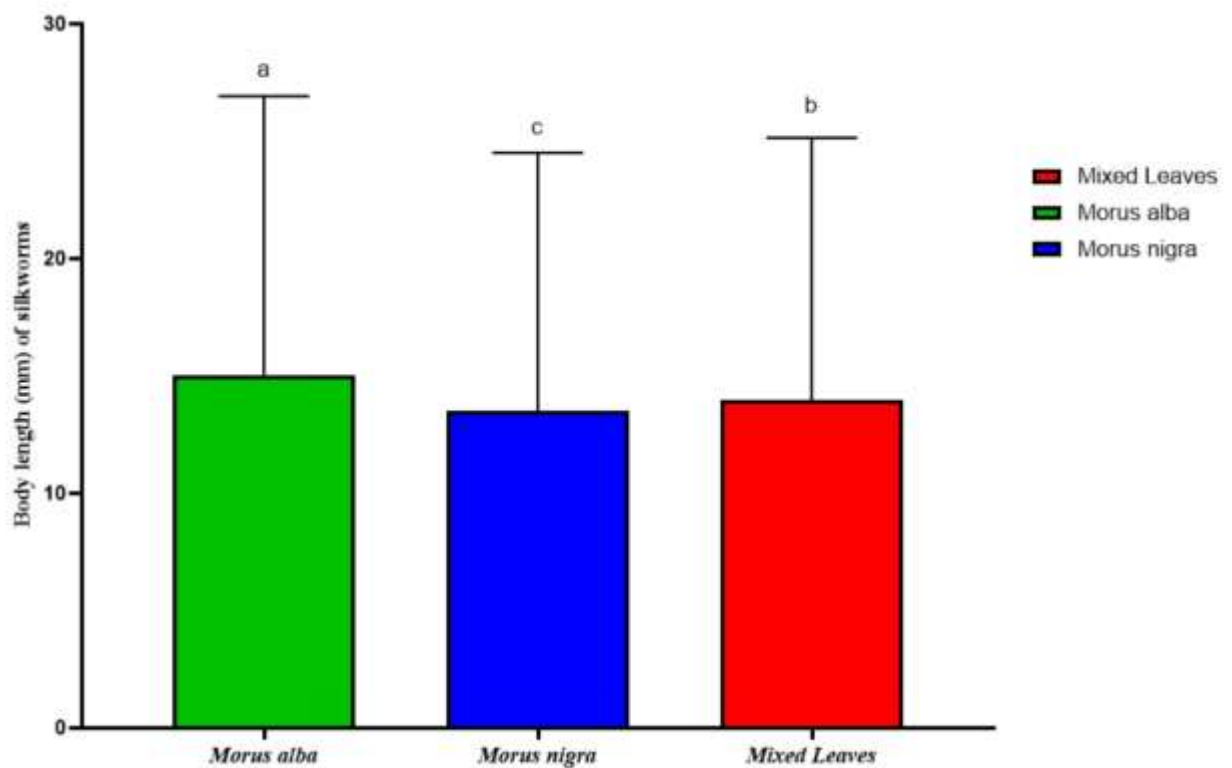
Superscripts on different means within column differ significantly at  $p \leq 0.05$ .

### Temperature-Humidity Index (THI)

Environmental factors play a vital role in the physiological performance of silkworms. To monitor ambient rearing conditions, the Temperature-Humidity Index (THI) was calculated on the environmental data collected on daily basis during the experimental period. The THI values, as shown in Figure 3, demonstrated moderate fluctuations but remained within the optimal physiological range for *Bombyx mori* (75–80) throughout the rearing period. This indicates that the semi-controlled environmental conditions were effective in minimizing thermal and moisture stress, ensuring a stable microclimate conducive for silkworm development. The absence of extreme THI values suggests that no additional environmental stressors were imposed during the trial.

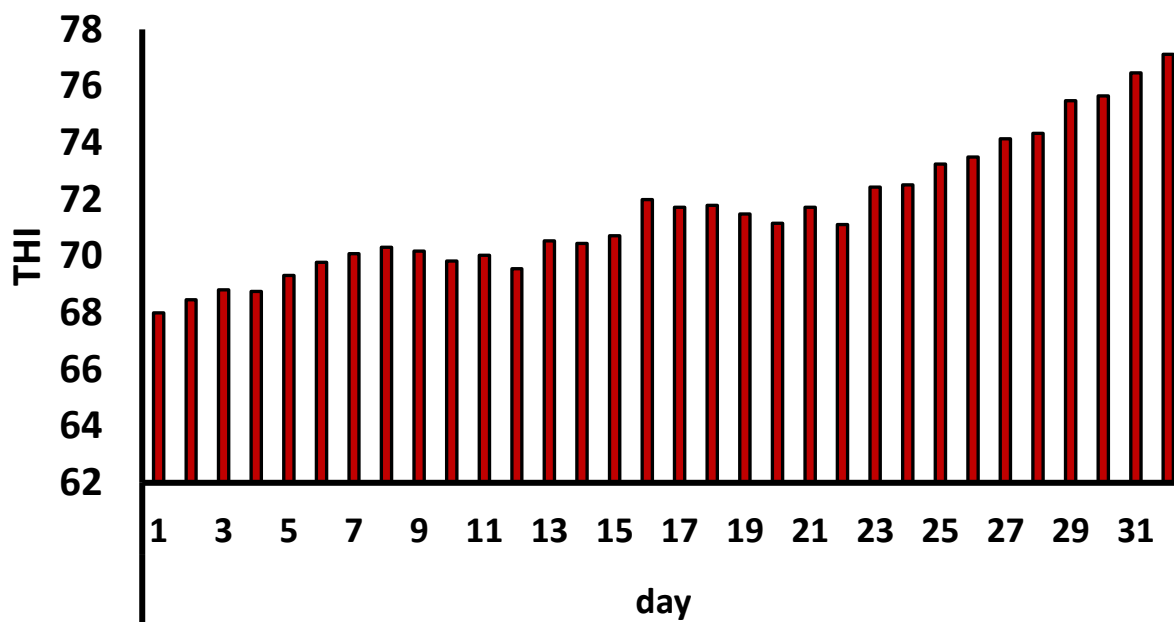


**Figure 1.** Body weight (mg) of silkworms at different molting stages.



**Figure 2.** Body length (mm) of silkworms at different molting stages





**Figure 3.** Trend of Temperature-Humidity Index (THI) during experimentation period.

### Survival Analysis

Survival patterns of *Bombyx mori* larvae were analyzed to determine the effects of different dietary treatments (*Morus alba*, *Morus nigra*, and mixed leaves) and developmental stages on larval mortality. A Cox proportional hazards regression model was used to evaluate the individual and interactive contributions of these variables. The Omnibus tests of model coefficients confirmed the overall significance of the Cox regression model. The likelihood ratio chi-square value was 343.151 with 3 degrees of freedom ( $p < 0.001$ ), indicating that the predictors collectively explained a significant proportion of variation in silkworm survival (Table 3). Further analysis of individual predictors is presented in Table 4. Treatment (Diet type) showed a trend towards significance ( $p = 0.073$ ), with a hazard ratio of 0.782. This indicated that a 21.8% reduction in mortality risk was observed when larvae were fed with *Morus alba* as compared to *Morus nigra*, though the difference was not statistically significant at the 0.05 level. Molting Stage was a highly significant predictor ( $p < 0.001$ ) with a very low hazard ratio of 0.002, indicating that mortality risk varied greatly between molting stages, which is likely due to the physiological stress of ecdysis. The interaction term (Treatment  $\times$  Molting Stage) was statistically significant ( $p = 0.001$ ), with a hazard ratio of 1.248. This finding demonstrates that the effect of dietary treatment on survival varies

across molting stages, implying that the benefits or risks of each diet are not uniform across the silkworm's rearing life cycle.

**Table 3.** Omnibus tests of model Coefficients for the Cox Proportional Hazards Model.

Omnibus Tests of Model Coefficients <sup>a</sup>									
-2 Log Likelihood	Overall (score)			Change From Previous Step			Change From Previous Block		
	Chi-square	df	Sig.	Chi-square	Df	Sig.	Chi-square	df	Sig.
2668.457	343.151	3	.000	607.485	3	.000	607.485	3	.000
a. Beginning Block Number 1. Method = Enter									

**Table 4.** Cox regression coefficients for treatment, molting stage and their interaction.

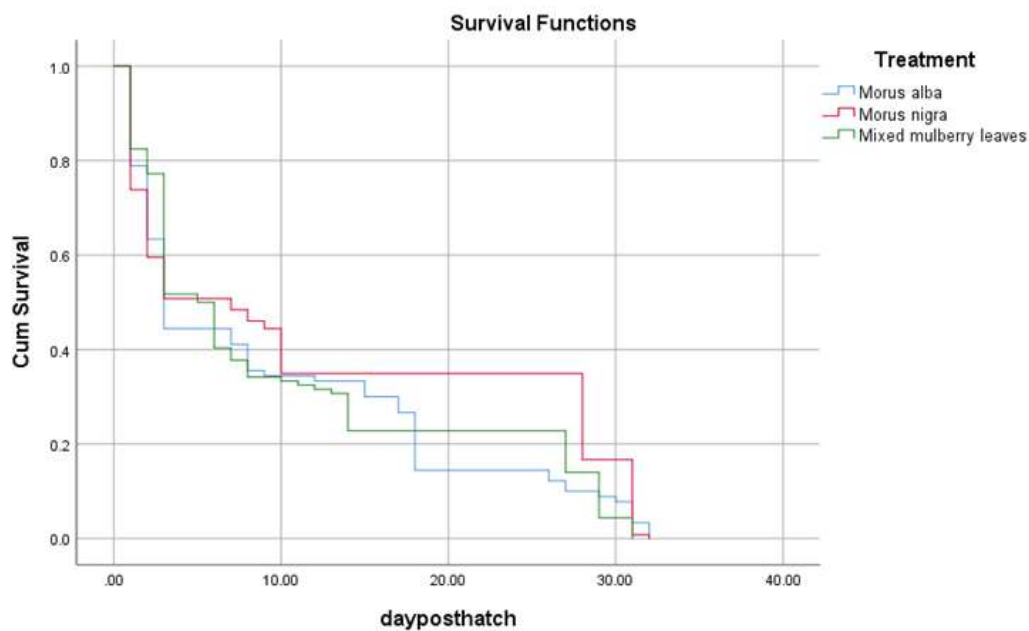
	Variables in the Equation					
	B	SE	Wald	df	Sig.	Exp (B)
Treatment	-.246	.137	3.223	1	.073	.782
Molting stage	-6.488	1.030	39.668	1	.000	.002
Molting stage*Treatment	.221	.065	11.648	1	.001	1.248

### Survival Patterns

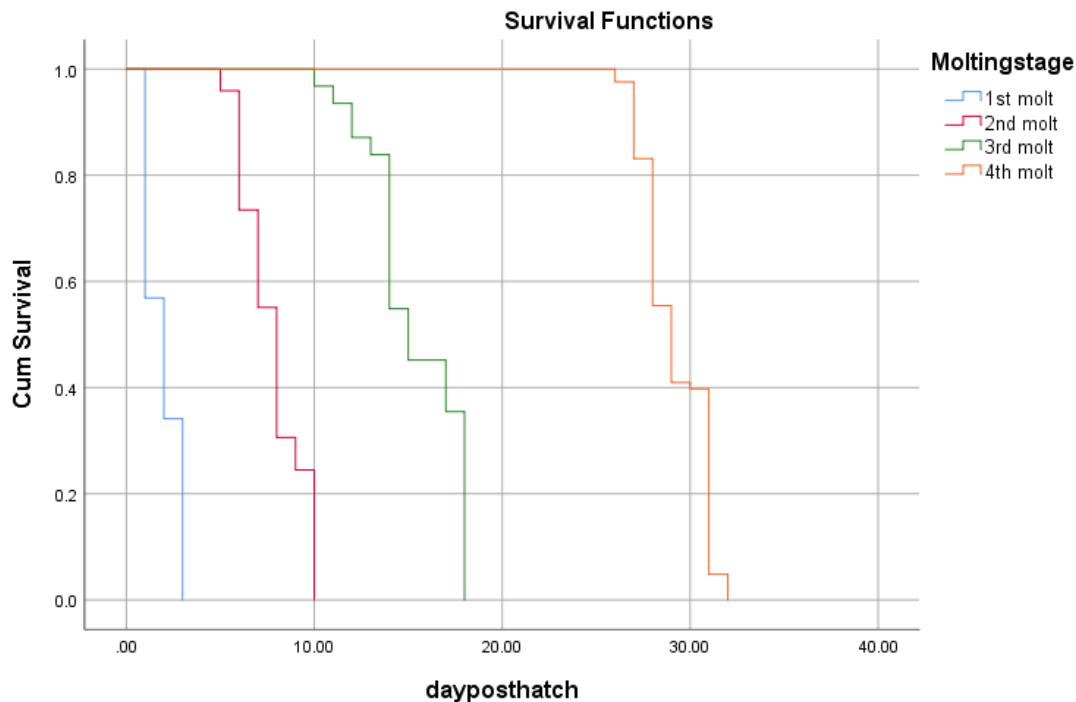
The results indicated that the *Morus alba* diet is generally associated with improved survival rate, particularly during early molting phases. The interaction effect revealed the importance of timing in diet efficacy. For example, the type of mulberry species may be more beneficial during certain stages of the life cycle and less effective or even detrimental at other stages. While treatment alone did not reach statistical significance, its strong interaction with molting stage highlights the complex relationship between nutritional quality and physiological stress points in silkworm development.

### Kaplan–Meier Survival Curves

Kaplan–Meier survival curves were constructed to visualize survival trends. The results revealed that silkworms fed with *Morus alba* exhibited the highest survival probabilities over time, followed by mixed leaves and *Morus nigra*, which had the lowest survival (Figure 2). Survival probability was significantly low during specific molting stages, especially in later instars, confirming that molting represents a major biological stressor (Figure 3). The data revealed that both diet and molting stage significantly influence silkworm survival, and their interaction is a critical factor for optimizing rearing practices in sericulture.



**Figure 1.** Kaplan Meier analysis for survival test among treatment groups



**Figure 2.** Kaplan Meier analysis for survival test among different molting stages

### Cocoon weight

The average weight of the cocoons was varied significantly in each treatment group. The data showed that the treatment group fed with *Morus alba* produced the heaviest cocoons ( $2.45 \pm 0.16$ ) followed by mixed leaves ( $2.11 \pm 0.16$ ) and *Morus nigra* ( $1.78 \pm 0.10$ ). These findings demonstrated that the type of mulberry feed greatly influences the cocoon's weight. The higher cocoon weight of *Morus alba* can be attributed to higher protein contents that help in the increased production of silk proteins in silkworms.

**Table 5:** Average cocoon weight, g produced by silkworms during experiment (Mean  $\pm$  S.D, n=10, duration of experiment –32 days).

Treatment group	Cocoon weight
<i>Morus alba</i>	$2.45 \pm 0.16$
<i>Morus nigra</i>	$1.78 \pm 0.10$
Mixed mulberry	$2.11 \pm 0.16$

### Discussion

The present study was planned to check the impact of different mulberry species available in the study area on the growth and survival of *Bombyx mori*. The results clearly demonstrated that the silkworms fed with *Morus alba* exhibited a higher growth rate and development as compared to those fed with *Morus nigra* and the mixed leaves (*Morus alba* and *Morus nigra*).

The proximate analysis done during the present study revealed significant differences in terms of protein, fat, and ash contents. The analysis showed that significant differences ( $p \leq 0.05$ ) were observed in moisture, total ash, crude protein, and crude fat contents. The results are in line with the research conducted by Moulick et al. (2023), who found that silkworms that consumed *Morus alba* had superior growth, especially in body weight and silk production. As it is reported by Rattanapans et al. (2024), protein is an essential nutrient in silkworm development since it helps in the production of silk fibers. Silk fibers contain protein fibroin as the major constituent, which is made by the salivary glands of the silkworms.

Likewise, Urbanek et al. (2022) found that the quality of the mulberry leaves directly influences the development and production of silk by the silkworms. The increased protein levels in *Morus alba* are essential to silkworms' metabolic functioning, which allows them to grow faster and form a better cocoon. Conversely, reduced protein content in *Morus nigra* reduced growth. *Morus nigra* might contain certain antioxidants unique to it, which might in turn benefit the health of silkworms, but it does not appear to directly affect the growth or cocoon quality.

The results of the present study were further reinforced by the findings of Ram et al. (2016). However, there is no statistically significant difference was observed ( $p = 0.951$  in crude fiber content with *Morus alba* at  $10.16 \pm 0.07$ , *Morus nigra* at  $10.1 \pm 0.21$ , and the mixed leaves at  $10.12 \pm 0.07$ ). The results underline significant differences in moisture, ash, protein, and fat contents, but not in fiber content.

Sujatha et al. (2024) reported that the choice of mulberry species plays a critical role in determining the success of sericulture operations. The results of growth rate during the present study are in line with Yang et al. (2023), who documented that silkworms fed with *Morus alba* showed better cocoon yield and feed conversion ratios. The better growth performance of *Morus alba* is linked to its higher protein and moisture contents. It has been regarded that *Morus alba* is the gold standard for silkworm nutrition. The higher levels of nitrogen and protein play a critical role in silkworm growth and the synthesis of silk fibers. This is consistent with the study of Khan

et al. (2010), who reported that *Morus alba* is nutritionally superior as compared to other species, particularly in terms of protein content.

In contrast, black mulberry (*Morus nigra*) showed slower growth and lower body weight in silkworms. This aligns with the findings of Yang et al. (2023), who documented that while *Morus nigra* has notable benefits, including drought resistance, its lower protein content makes it less optimal for silkworm rearing. *Morus nigra* is known for its antioxidant properties, but this study and others, such as those by Memete et al. (2022), suggested that these benefits do not directly impact the growth rate and cocoon production. These findings reported the ongoing challenge in sericulture in selecting the right mulberry species for optimal silkworm development, particularly in varying environmental conditions.

The intermediate results were found in the mixed leaves diet group (*Morus alba* and *Morus nigra*). Nevertheless, the degree of growth in the silkworms was enhanced compared to silkworms fed on *Morus nigra* only. The result indicated that the interbreeding of mulberry species lack the same efficiency as that of *Morus alba* in facilitating the optimum growth. The findings align with Memete et al. (2022), who have also found that mixed mulberry leaves do not produce the same performance as the use of more nutritious and higher-quality mulberry species, such as *Morus alba*.

During the rearing of the silkworms, environmental conditions are very important as the ideal temperature and humidity conditions are needed to enhance the growth and survival of silkworms. Our results are also consistent with those of Suresh et al. (2012), which proved that optimal environmental conditions are necessary to reduce stress and maximize the growth potential of silkworms. The measurements of Temperature-Humidity Index (THI) showed that the semi-controlled environmental conditions were kept in the optimal range as the experiment progressed. It is a substantial contribution because in most studies, attention has been given to fully controlled systems, which may not be realistic in large-scale sericulture operations because of high cost. To identify the influence of various dietary treatments (*Morus alba*, *Morus nigra*, and combined leaves) and developmental stages on larval mortality, the survival patterns of *Bombyx mori* larvae were studied.

The finding revealed that silkworms fed with *Morus alba* had the highest survival rate, particularly at the early stages of development. Si et al. (2021) reported similar findings as silkworms fed on *Morus alba* leaves had a higher survival rate during the critical molting phases. The higher survival

rate in the *Morus alba* group can also be attributed to the improved nutritional quality, which enhances the silkworms' ability to handle stress. The high protein contents in *Morus alba* are likely to contribute to better health and resilience, enabling the silkworms to better cope with the physiological stress during molting stages. In contrast, the survival rate in the *Morus nigra* group was low, which is due to the lower nutritional composition and the less digestible nature of the leaves, which in turn affects the overall health of the larvae. These findings are consistent with Si et al. (2021), who reported that the importance of providing a high-quality diet to ensure a better survival rate, particularly during stressful developmental stages.

Moreover, the mixed leaves group showed a survival rate better *Morus nigra* group but lower than the *Morus alba* group. This indicates that while a combination of mulberry species may offer a more balanced nutrient profile, it does not provide the optimal support for silkworm survival when compared to *Morus alba*. These findings further supported the study of Andadari et al. (2005), who reported that while mixed mulberry feed could be used in some cases, they tend to fall short in supporting the full growth potential of silkworms, as compared to single-species diets like *Morus alba*.

In addition to biological performance, there is the direct economic aspect of the decision made in the selection of mulberry species in regard to sericulture. *Morus alba* is commonly planted and tends to be more affordable in terms of production expenses because it is not so specific and has a high yield of leaves. It needs fewer resources from farmers because it can withstand various climatic conditions, thus lowering the costs of inputs. Comparatively, *Morus nigra* is not as extensively cultivated, despite being more resistant to drought, which makes it more expensive to procure and most of them are unavailable in most geographical areas. The high protein levels and a stable increase in growth rates of *M. alba* also lower the cost per unit of the silk made since better-quality cocoons are reaped with minimal feed expenditure. Thus, both biologically and economically, *M. alba* is the most economical choice of sustainable silk production, whereas other species can only be more appropriate in small niche markets where being robust to stressors is more important than maximum productivity. In areas where *Morus alba* cultivation is feasible, sericulturists can use this species as the primary feed for silkworms to maximize silk yield and improve growth performance. However, *Morus nigra* and mixed diets can also be used, particularly in areas where drought resistance or environmental resilience is more prominent.

Even though the current research was carried out in semi-controlled environmental conditions, the design has certain limitations as far as generalizing the findings to the full field sericulture conditions is concerned. Semi-controlled systems also aid in the minimization of environmental stressors, which include extreme changes in temperature and humidity, hence making a better evaluation of the dietary effects. Nonetheless, when released in the open field, silkworms tend to face other issues such as unpredictable weather patterns, infestations by pests, as well as fluctuation in the quality of soil and leaves that might affect the growth of mulberry and the productivity of the silkworm. Accordingly, the superiority of *Morus alba* as here observed is probably applicable in the general cases, but the extent of its advantages might vary when the species is exposed in a full field. Future studies should then confirm such findings on a large scale and field-based sericulture in order to have a better understanding of interactions among the environmental variability, mulberry species, and silkworm performance.

One limitation of the present study is that it did not include an analysis of secondary metabolites or bioactive compounds present in mulberry leaves. Previous studies have shown that mulberry species, particularly *Morus nigra*, contain diverse phytochemicals such as flavonoids, phenolics, and antioxidants that can contribute to improved stress tolerance, immunity, and overall health of silkworms. While our results clearly demonstrate the superior growth and cocoon yield with *Morus alba*, the potential health-promoting effects of these bioactive compounds remain unexplored. Incorporating phytochemical profiling in future research would therefore provide a more comprehensive understanding of the nutritional and functional role of different mulberry species in sericulture.

Furthermore, because the present experiment was conducted under semi-controlled conditions, environmental variation was reduced compared to what typically occurs in open-field rearing systems. While this approach improves internal validity, it also limits external generalizability. Validation of these findings under full field conditions, where silkworms encounter greater fluctuations in climate, pest pressures, and mulberry leaf variability, would further strengthen the applicability of our conclusions for large-scale sericulture operations.

## **Conclusion**

The findings of the present study showed that *Morus alba* has better nutritional values as compared to *Morus nigra*. The group fed with *Morus alba* had higher body weight, body length, and heavy cocoon weight. The mixed mulberry leaves showed intermediate results, revealing that mixing



various mulberry species does not offer better results than using *Morus alba* exclusively. The research also highlighted the significance of maintaining optimum temperature and humidity for silkworm growth. The semi-controlled environmental conditions proved to be effective in mitigating environmental stress during the experiment. It is recommended that future studies should explore the potential of other mulberry species for sericulture under diverse environmental conditions. Moreover, investigating how extreme environmental conditions, such as heat waves or excessive rainfall, can influence silkworm productivity would provide valuable insights into optimizing sericulture practices and contributing to the sustainability of the industry.

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