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Research Article

Assessing the rearing performance of *Bombyx mori* (Linnaeus, 1758) on two different mulberry species

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Abstract

The quality and quantity of silk produced are influenced by the types of mulberry leaves fed to the silkworms. The experiment was conducted at the Sericulture Unit, Department of Wildlife and Ecology, University of Veterinary and Animal Sciences (UVAS), Ravi Campus during February-March 2024. A randomized block design was used in three treatment groups. The data was collected on various growth parameters, developmental stages, survival rate, and cocoon weight. The main objective of the current research was to compare the rearing performance of silkworms (*Bombyx mori*) fed with *Morus alba* and *Morus nigra* under semi-controlled environmental conditions. Proximate analysis of mulberry leaves revealed that *M. nigra* have the highest crude protein (19.44 ± 0.24), gross energy (18.12 ± 0.09) and crude fat (7.16 ± 0.09) contents. The results revealed that the growth rate of silkworms was highest in the group fed with black mulberry (*M. nigra)* showing significantly higher weight and length at each instar as compared to those fed on white mulberry (*M. alba*) and mixed mulberry leaves. The survival rate of silkworms (*Bombyx mori*) during the experimental period varied among the different treatment groups. *M. nigra* fed silkworms showed the highest survival rate followed by mixed and *M. alba* fed group. The results showed that the heaviest cocoon was produced from the silkworms that were fed with *M. nigra* leaves **(**2.40 ± 0.14 g, $p < 0.0001$) and the lightest cocoon was produced with *M. alba* leaves (1.74 ± 0.11 g). The present study concluded that *M. nigra* has better nutritional profile, growth performance and cocoon production as compared to *M. alba*. It is recommended that, further studies should also explore the long term effectiveness of using *M. nigra* in sericulture including its economic viability and potential for large scale cultivation. Investigations into other mulberry species and their combinations should also be explored to provide valuable insights into improving silk production.

Keywords: *Morus alba*, *Morus nigra*, Cocoon, Silkworm, Changa Manga forest, Proximate analysis

Introduction

Silkworm is a monophagous insect that mostly feeds on mulberry leaves and plays a leading role in the sericulture industry (Zhang et al., 2022). Sericulture is an agro-based industry, and it includes growing food plants, raising silkworms, and producing silk. A female silkworm can lay between 300 and 500 eggs, which hatch after 12 days. Adult *B. mori* specimens have a creamy white color with faint brownish markings. It rarely flies and has a short lifespan. It goes through four larval stages in its whole metamorphosis, such as egg, larva, pupae, and adult (Indora & Saharan, 2023).

Asia is the world's leading silk-producing region, accounting for 98% of global production (Muzamil et al., 2023). Currently, China and India produce 90 to 92 percent of the world's silk products. South Korea, Japan, Vietnam, Thailand, Uzbekistan, and Brazil account for the remaining 8–10% of industrial output (Bekkamov & Samatova, 2023). There are four types of natural silk. The category of non-mulberry silks includes three varieties, viz., Eri, Tasar, and Muga silks (Saikia & Saikia, 2022). In 1947, sericulture was first established in Taxila, Pakistan, and then spread to forested areas where there were plenty of mulberry plantations. Changa Manga, Chichawatni, Kamlia, Jouhrabad, and Khanewal are the main areas (Shifa et al., 2016).

The various types of mulberry leaves differ in their nutritional profile. The choice of mulberry leaves is crucial to the success of silkworm rearing and sericulture. Water greatly affects the quality of leaves that silkworms eat, and the most crucial elements for a successful silkworm raising process. Mulberry leaves are succulent for silkworms because of their high water content (Bahar et al., 2011). The economics of the mulberry industry are significantly influenced by the leaves quality that is why extensive study and efforts have been made (Chanotra et al., 2019). It is commonly recognized that the quantity and quality of mulberry leaves influences the larvae's growth rate, developmental stage, body weight and survival rate (Ali et al., 2021). The leaves physical and chemical characteristics play a key role in determining their quality (Adeduntan, 2015). The amount and quality of leaves of mulberry eventually influence the growth of silkworms and development (Bahar et al., 2011).

Mulberry requires only 6 months to reach maturity to begin raising silkworms. Depending on the degree of care, mulberry can sustain silkworm rearing for 15 to 20 years after it is planted. For women in rural regions, sericulture offers a wealth of opportunities, especially when it comes to earning money from silkworm breeding and reeling. Acres of silkworm farming and mulberry gardens can reduce the need for labourers and save money for the state's sericulture

industry. The sericulture industry is environmentally favorable since mulberry is a persistent crop with good leaf production that creates greenery and helps to preserve soil. In addition, fertilizer for gardens can be made from waste produced by silkworm farming (Chanotra et al., 2019).

Sericulture not only creates job opportunities and boosts farmers' income but also stimulates regional economic growth and enhances environmental sustainability (Fambayun et al., 2022). Despite extensive research, there always remains a necessity for a comprehensive assessment of many species of mulberry leaves to evaluate their suitability under diverse agricultural and weather conditions for sericulture. The goal of this study was to close the gap by thoroughly examining the growth performance and economic characteristics of silkworms fed with two different species of mulberry.

Material and methods

Study area

The research was conducted at the Sericulture Unit, Department of Wildlife and Ecology, UVAS, Ravi Campus in February-March 2024. The research trial took thirty-two days for complete. The rearing room was white-washed, and formaldehyde (2%) solution was used to sanitize all of the instruments, including the rearing trays, stands, and incubator at the start of the experiment (Shah et al., 2007).

Proximate analysis of mulberry leaves

The mulberry species including black mulberry (*M. nigra*), white mulberry (*M. alba) and* mixed mulberry leaves were used to rear silkworms. The proximate composition of mulberry leaves, including dry matter (%), crude fat (%), crude fiber (%), crude protein (%), and gross energy (MJ/kg), was determined (Sree & Vijayalakshmi, 2018).

Timeline of rearing silkworms

The rearing of silkworms was done under semi-controlled environmental conditions, and it includes the incubation of eggs, hatching of eggs, 1st feed, shifting to the grow-out room and feeding, observation of moulting stages, maintaining the required temperature and humidity, cocoon formation, data collection, and observations. *Experimental design*. A randomized block design was applied and experiment was done in duplicates of each of three treatments groups (two species of mulberry including *M. alba, M. nigra* and one control group having mixed mulberry leaves). Each replicate consists of 300 larvae (Andadari, 2021).

Data collection and observations

Data regarding silkworms growth parameters including weight, length, survival, cocoon weight and cocoon production, were noted for each treatment group.

Statistical analysis

Collected data were analyzed through the one-way ANOVA technique (Steel et al., 1997) using PROC GLM in SAS software (version 9.1). For the comparison of significant treatment means, Fisher's least significant difference test was applied. Data were presented in means ± standard errors; the following mathematical model was applied: $Y_{ij} = \mu + \tau_i + \epsilon_{ij}$. Where, Y_{ij} = effect of jth observation on ith treatment group; μ = population mean; τ_i = effect of ith treatment group; ϵ_{ij} = random error. For the survival analysis Kaplan-Meier test was applied.

Results

Silkworm eggs were incubated at 25°C and 75–80% humidity for 10 days to hatch. Newly hatched larvae were shifted from petri plates to trays, and chopped mulberry leaves were provided to the silkworm larvae. The results of proximate analysis revealed distinct variations in dry matter, crude protein, crude fat, crude fiber, and gross energy content. Table 1 summarizes the proximate analysis of *M. alba*, *M. nigra* and mixed mulberry leaves. The statistical analysis revealed that *M. nigra* have the highest crude protein (19.44 ± 0.24), gross energy (18.12 ± 0.09) and crude fat (7.16 ± 0.09) contents. Mixed mulberry leaves exhibited intermediate values for most of the nutritional parameters. These variations are significant as they influence the growth rate and cocoon yield of silkworms.

Table 1. Proximate composition of mulberry species used during experiment (Mean ± S.E, n=5, duration of experiment – 32 days).

Species	Dry Matter (%)	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)	Gross Energy (MJ/kg)
M. alba	90.88 ± 0.16^{b}	18.34 ± 0.25^{b}	4.28 ± 0.15^{c}	9.89 ± 0.09^{c}	16.95 ± 0.06^{c}
M. nigra	92.78 ± 0.27^{a}	19.44 ± 0.24^{a}	7.16 ± 0.09^{a}	12.14 ± 0.10^{a}	18.12 ± 0.09^{a}
Mixed mulberry leaves	91.42 ± 0.37^{b}	19.01 ± 0.06^{a}	6.01 ± 0.06^{b}	11.04 ± 0.07^{b}	17.66 ± 0.03^{b}
p-value	0.001	0.008	<0.0001	<0.0001	<0.0001

Note: superscripts on different means within columns differ significantly at $p \leq 0.05$.

Growth and developmental stages of B. mori. Overall, a total of 300 silkworm larvae were kept in each treatment group. At each instar randomly 5 specimens were randomly selected for morphometric measurements. The weight and length of the silkworms fed on different

mulberry species were recorded at various instars. The results indicated significant differences in growth patterns among the treatment groups. The statistical analysis revealed that the growth rate of silkworms was highest in the group fed on black mulberry (*M. nigra)* showing significantly higher weight and length at each instar as compared to those fed on white mulberry (*M. alba*) and mixed mulberry leaves. Statistical analysis of body length (mm) and body weight (g) of silkworms fed with *M. alba, M. nigra* and mixed mulberry leaves (*M. alba* and *M. nigra*) are mentioned in Table 2 and 3.

Table 2. Statistical analysis of body length (mm) of silkworms fed with *M. alba*, *M. nigra* and mixed mulberry leaves (x ± S.E, n=5, duration of experiment – 32 days).

Treatment	Molting 0	Molting 1	Molting 2	Molting 3	Molting 4
M. alba	1.78 ± 0.02^{c}	3.12 ± 0.07^{c}	11.80 ± 0.06^{c}	13.82 ± 0.08^{c}	43.15 ± 0.32^{c}
M. nigra	2.28 ± 0.04^{a}	4.06 ± 0.06^{a}	14.50 ± 0.08^{a}	25.11 ± 0.14^{a}	52.80 ± 0.27^{a}
Mixed leaves	2.00 ± 0.05^{b}	3.50 ± 0.04^{b}	13.20 ± 0.07^{b}	19.72 ± 0.06^{b}	47.27 ± 0.08^{b}
p-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

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

Table 3. Statistical analysis of body weight (g) of silkworms fed with *M. alba, M. nigra* and mixed mulberry leaves (x ± S.E, n=5, duration of experiment – 32 days).

Treatment	Molting 0	Molting 1	Molting 2	Molting 3	Molting 4
Morus alba	0.0186c ± 0.0005	0.0826c ± 0.0007	0.414c ± 0.0062	0.740c ± 0.0306	1.110c ± 0.0407
Morus nigra	0.0324a ± 0.0003	0.169a ± 0.0053	0.580a ± 0.0052	0.997a ± 0.0175	2.100a ± 0.0816
Mixed leaves	0.0242b ± 0.0003	0.098b ± 0.0004	0.518b ±0.0071	0.842b ± 0.007	1.600b ± 0.0471
p-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

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

The temperature and humidity during the rearing periods played a crucial role in the growth and development of the silkworms. The mean temperature and humidity were 23.62°C and 54.78%, respectively.

Correlation between temperature and humidity on the growth performance of silkworms fed with *M. alba.*

The correlation coefficient between humidity and gain in weight was calculated as 0.928. The result revealed that there is a strong positive correlation. Similarly, the correlation coefficient between humidity and the increase in length was 0.642. This suggests a moderate positive correlation. The correlation coefficient between temperature and gain in weight was calculated as 0.911. The result showed a strong positive correlation, meaning that higher temperatures significantly enhance the weight gain of silkworms with *M. alba* leaves. Similarly, the correlation coefficient between temperature and the increase in length was 0.843. These coefficients suggest that temperature is a key factor in both weight gain and length increase for silkworms reared on *M. alba,* with weight gain being slightly more sensitive to changes in temperature.

Correlation between temperature and humidity on growth performance of silkworms fed with *M. nigra*

The correlation coefficient between humidity and gain in weight was calculated as 0.911. It indicated that higher humidity seems to be positively impact the weight gain of silkworms when fed on *M. nigra* leaves. Similarly, the correlation coefficient between humidity and the increase in length was 0.673. This suggests a moderate positive correlation. The correlation coefficient between temperature and gain in weight was calculated as 0.973. The result indicated a very strong positive correlation meaning higher temperatures significantly enhance the weight gain of silkworms fed with *M. nigra* leaves. Similarly, the correlation coefficient between temperature and the increase in length was 0.861. The value showed a strong positive correlation.

Correlation between temperature and humidity on growth performance of silkworms fed with mixed mulberry leaves (*M. alba* and *M. nigra*)

The correlation coefficient between humidity and gain in weight was calculated as 0.923. The result indicated a very strong positive correlation. Increased humidity levels are likely to facilitate greater weight gain in silkworms. Similarly, the correlation coefficient between humidity and the increase in length was 0.673. The result indicated a moderate positive correlation. The correlation coefficient between temperature and gain in weight was calculated as 0.947. The result indicated a very strong positive correlation. Higher temperatures contribute to a considerable weight increase in silkworms. Similarly, the correlation coefficient between temperature and the increase in length was 0.864. The value revealed a strong positive

correlation, though the relationship is slightly weaker than for weight gain when reared on mixed mulberry leaves.

Survival rate*:* The survival rate of silkworms (*B. mori*) during the experimental period varied among the different treatment groups (Fig. 1). *M. nigra* fed silkworms showed the highest survival rate followed by mixed and *M. alba* fed groups (Fig. 2).

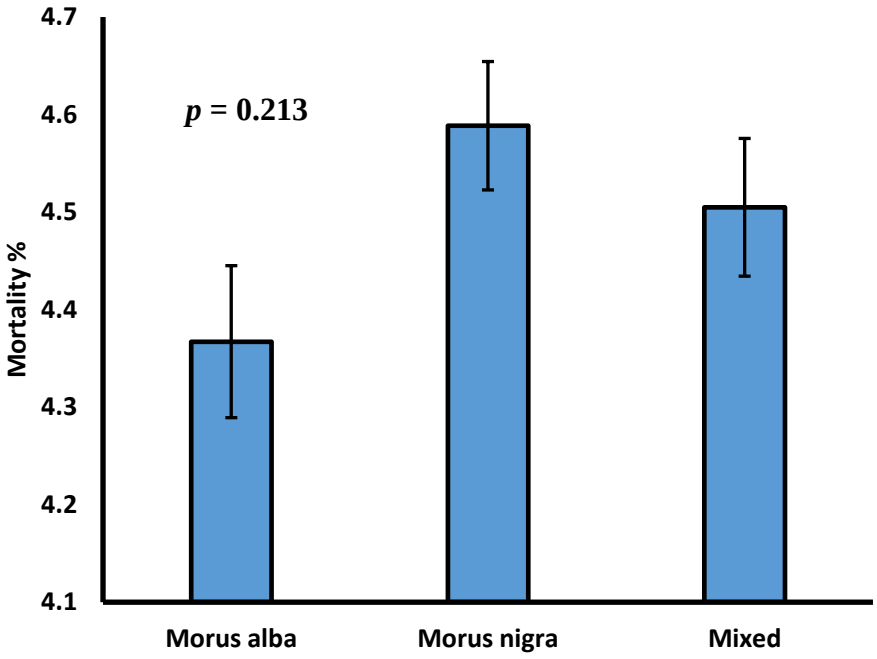

Figure 1. Mortality percentage of silkworms at different developmental stages during the experiment (n=300, duration of experiment – 32 days)

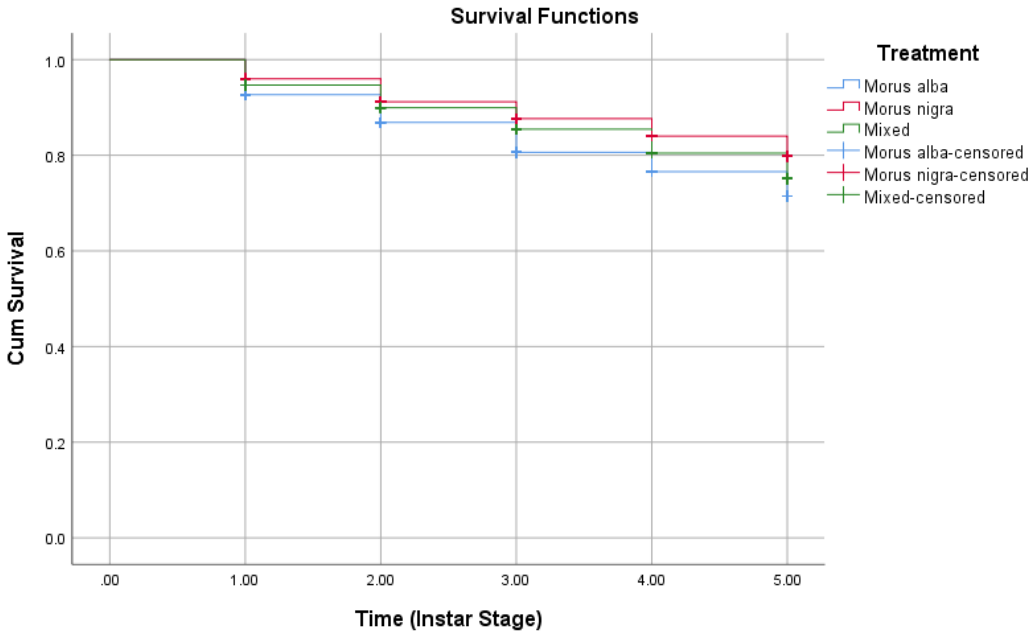

Figure 2. Survival analysis of silkworms at different developmental stages during the experiment (n=300, duration of experiment – 32 days)

Cocoon weight

The average weight of the cocoons produced by the silkworms varied significantly with the type of mulberry leaves they were fed. The statistical analysis showed that the heaviest cocoon was produced from the silkworms that were fed with *M. nigra* leaves weighting 2.40 ± 0.14 ($p < 0.0001$). In contrast, the lightest cocoon was produced by silkworms fed with *M. alba* leaves as 1.74 ± 0.11 g weight was recorded. The statistical analysis showed that the p-value is less than 0.0001, indicating a statistically significant difference in cocoon weight between the groups. This suggests that the type of mulberry leaves has a significant impact on cocoon weight with *M. nigra* being the most favorable for higher cocoon weights (Table 4).

Table 4. Statistical analysis of cocoon weight (g) in different treatment groups (x ± S.E, n=300, duration of experiment – 32 days).

Treatment group	Cocoon weight
M. alba	1.74 ± 0.11^{c}
M. nigra	2.40 ± 0.14^{a}
Mixed mulberry	2.02 ± 0.14^{b}
p-value	< 0.0001

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

Discussion

Present study investigated the nutritional content of *M. alba*, *M. nigra*, and mixed mulberry leaves and their subsequent effects on silkworms growth performance, survival rates and cocoon quality. The proximate analysis indicated that *M. nigra* have the highest crude protein (19.44 ± 0.24), gross energy (18.12 ± 0.09) and crude fat (7.16 ± 0.09) contents as to *M. alba* and mixed mulberry leaves. Mixed mulberry leaves showed varying proximate analysis values that are in between *M. alba* and *M. nigra*. These variations are important because they influence how fast silkworms grow and how many cocoons they will produce. The findings of our study are align with Iqbal et al., (2012), who found that *M. nigra* leaves have more content of protein as compared to *M. alba* leaves, leading to better growth and higher silk production in silkworms. Similarly, Yu et al. (2018) reported that higher levels of protein in mulberry leaves result in higher weight of larvae and increased cocoon output.

The morphometric analysis of this study indicated that the silkworms showed significantly higher growth in length and body weight during all the instars when fed with *M. nigra* leaves as compared to mixed leaves and *M. alba* leaves. The study conducted by Hosamani et al. (2019) indicated a significant relationship between the varieties of mulberry and the species of silkworms, particularly concerning larval length, larval weight, and shell ratio. These results are supported by the research of Shifa et al. (2016), which indicated that silkworms reared on higher protein mulberry types gained more weight and had enhanced cocoon features. Another study conducted by Zannoon et al., (2008), highlighted that *M. nigra* is the most effective mulberry species for silkworms, contributing to enhanced growth rates, larger cocoon weights and greater survival rates. Similarly, Adeduntan (2013) showed that the protein found in mulberry leaves plays a key role in the healthy growth and development of silkworms.

The results of our study revealed that higher temperature and humidity levels are closely related to the weight gain and length increase of silkworms. Maintaining a temperature of 23.62°C and a humidity of 54.78% provided a suitable environment for the development of silkworms. The findings align with the study by Hussain et al. (2011), which highlighted the importance of maintaining the right temperature and humidity for better growth of silkworms and production of cocoon. When humidity rises, it speeds up the metabolism of larvae, likely due to improved hydration that aids in nutrient absorption, as noted by Saha et al. (2022). Our findings are also in line with Rahmathulla (2012), who noted that temperature plays a key role in the growth of silkworms. Gupta & Dubey (2021) also highlighted the importance of managing environmental factors to improve larval development and the production of silk.

The highest survival was noted in *M. nigra* group (97.25%.), mixed leaves group (96.57%) and the *M. alba* group (95.65%). This trend continued with cocoon weights, where the *M. nigra* group formed the heaviest cocoons. These results are consistent with the research conducted by Tuigong et al. *(*2015), which showed that diets rich in protein support the growth of silkworms and increase their survival rates. The superior production of cocoon in *M. nigra* is consistent with the results of Ayandokun & Alamu (2020) which revealed that silkworms fed with high-quality leaves of mulberry showed improved cocoon quality. The results of our study align with the findings of Gaviria et al. (2006), as they reported that the weight of the cocoon and the weight of the shell are crucial traits linked to productivity. Furthermore, a similar study conducted by Nguyen et al. (2024) discovered that higher protein contents resulted in better quality of cocoons and silk.

Kabita et al. (2023) reported that sericulture has the potential to significantly improve the agro-based economy of the rural population in Sujapur, Malda district of West Bengal, India. The research aimed to examine key commercial traits such as the duration and weight of larvae, weight of cocoon, shell weight, and larval size, as well as the environmental factors of temperature (24℃) and humidity (49%) for three popular silkworm breeds. The F1 hybrid breed displayed much more variation in all the measured factors, including larval duration (24.67 ± 0.88), larval weight (33 ± 3.21), weight of cocoon (1.63 ± 0.07), shell weight (0.31 ± 0.0058), and larval size (6.10 ± 0.06). This research highlighted that the F1 variety is well-suited for cultivation in Sujapur, Malda, during spring and shows promise for a hybrid program. Our research highlights the importance of mulberry species in sericulture. The research indicated that using *M. nigra* in silkworm diets could lead to major gains in productivity, particularly through higher survival rates, better growth rates and improved quality of cocoon. This conclusion matches the suggestions from Thrilekha et al. (2024), who proposed that a high level of protein in mulberry varieties should be used to increase the production of silk.

Conclusion

The results revealed that silkworms fed with *M. nigra* had the highest weight gain, maximum body length and produced the heaviest cocoons as compare to *M. alba* and mixed mulberry leaves. Although the growth performance of silkworm fed with mixed mulberry leaves was satisfactory but it results highlighted that *M. nigra* is more suitable feed source for silkworms. It is recommended that, further studies should explore the long term effectiveness of using *M. nigra* in sericulture including its economic viability and potential for large scale cultivation.

Investigations into other mulberry species and their combinations should also be explored to provide valuable insights into improving silk production in sericulture.

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