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Effect of Abiotic Stress Conditions on Mulberry Leaf Quality and Its Consequences on Silkworm Growth and Silk Yield

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Abstract
Abiotic stress factors such as drought, temperature extremes, and salinity significantly influence plant growth and productivity, particularly in mulberry (Morus spp.), the sole food source of the silkworm, Bombyx mori. The present study aimed to evaluate the impact of abiotic stress conditions on mulberry leaf quality and its subsequent effects on silkworm growth and silk yield. Mulberry plants were subjected to controlled stress conditions, including water deficit, high temperature, and salinity. Leaf samples were analyzed for nutritional and biochemical parameters such as protein, chlorophyll, moisture content, and total phenolics. Silkworm larvae were reared using leaves obtained from stressed and non-stressed plants, and growth and cocoon parameters were recorded. The results revealed that abiotic stress significantly reduced the nutritional quality of mulberry leaves, with notable declines in protein, chlorophyll, and moisture content. Conversely, an increase in phenolic compounds was observed under stress conditions. Silkworms fed with stressed leaves exhibited reduced larval weight, lower survival rates, and inferior cocoon characteristics, including decreased cocoon weight, shell weight, and silk productivity. Among the stress treatments, drought stress had the most pronounced negative effect. The study highlights the strong relationship between environmental stress, mulberry leaf quality, and sericulture productivity. Mitigating abiotic stress through improved cultivation practices is essential for sustaining silk production and ensuring the economic viability of sericulture.

Keywords: *Abiotic stress, Mulberry, Silkworm, Leaf quality, Cocoon yield, Silk production*

1. Introduction

Sericulture is a highly specialized agro-based industry that integrates plant cultivation with insect rearing for the production of natural silk. It plays a significant role in the rural economy of many countries by generating employment and supporting livelihoods, particularly in regions where agriculture is the primary occupation [1]. The success of sericulture largely depends on the cultivation of mulberry (Morus spp.), which serves as the sole food source for the domesticated silkworm, Bombyx mori. Since silkworms are monophagous in nature, their growth, development, and silk production are directly influenced by the quality and nutritional composition of mulberry leaves. Therefore, maintaining high-quality mulberry foliage is essential for achieving optimal cocoon yield and superior silk quality. Mulberry is a perennial, fast-growing plant that is widely cultivated under diverse agro-climatic conditions, ranging from tropical to temperate regions. Its adaptability makes it suitable for large-scale cultivation; however, its productivity and leaf quality are highly sensitive to environmental factors [2]. Among these, abiotic stress conditions such as drought, high temperature, and salinity are major constraints that adversely affect plant growth and development. These stress factors are becoming increasingly significant due to the ongoing impacts of climate change, which has led to irregular rainfall patterns, rising temperatures, and soil salinization in many agricultural regions. Abiotic stress disrupts normal physiological and biochemical processes in plants. Drought stress, for instance, leads to reduced water availability, causing stomatal closure and decreased photosynthetic activity [3]. This results in lower carbohydrate production and impaired growth. Similarly, high temperature stress can denature proteins, disrupt membrane stability, and reduce enzymatic activity, ultimately affecting plant metabolism. Salinity stress, on the other hand, causes ionic imbalance and osmotic stress, leading to reduced nutrient uptake and toxicity effects. Collectively, these stress conditions significantly reduce plant vigor and productivity. In mulberry plants, abiotic stress has a pronounced impact on leaf quality, which is a critical factor for silkworm nutrition [4]. Under stress conditions, there is often a decline in essential nutrients such as proteins, carbohydrates, and chlorophyll. Protein synthesis is particularly affected, leading to reduced availability of amino acids required for silk production. Chlorophyll degradation under stress conditions results in decreased photosynthetic efficiency, further limiting the production of organic compounds necessary for plant growth. Additionally, moisture content in leaves tends to decrease, affecting their palatability and digestibility for silkworm larvae. Interestingly, while primary metabolites decrease under stress, there is often an increase in the accumulation of secondary metabolites such as phenolic compounds, flavonoids, and tannins [5]. These phytochemicals are part of the plant's defense mechanism against environmental stress and oxidative damage. They possess antioxidant properties that help neutralize reactive oxygen species generated under stress conditions. However, excessive accumulation of these compounds may have negative implications for silkworm feeding, as they can reduce leaf palatability and interfere with nutrient absorption. The quality of mulberry leaves is a key determinant of silkworm performance. Silkworm larvae require a balanced diet rich in proteins, carbohydrates, vitamins, and minerals to support their rapid growth and silk production. Any deterioration in leaf quality due to abiotic stress can lead to reduced feeding efficiency, slower larval growth, increased mortality, and poor cocoon characteristics. Cocoon parameters such as cocoon weight, shell weight, shell ratio, and filament length are directly influenced by the nutritional status of the larvae, which in turn depends on the quality of mulberry leaves [6-7]. The relationship between mulberry leaf quality and silkworm productivity has been well established in sericulture research. However, the specific effects of different abiotic stress conditions on this relationship require further investigation. Understanding how drought, salinity, and temperature stress alter leaf composition and how these changes affect silkworm biology is essential for developing effective management strategies. Such knowledge can help in identifying stress-tolerant mulberry varieties, optimizing cultivation practices, and improving resilience in sericulture systems. In recent years, there has been growing interest in developing sustainable approaches to mitigate the impact of abiotic stress on crop production [8-9]. Techniques such as improved irrigation management, use of organic amendments, application of plant growth regulators, and adoption of stress-tolerant cultivars have shown promise in enhancing plant resilience. In the context of mulberry cultivation, these strategies can help maintain leaf quality under adverse environmental conditions, thereby ensuring consistent silkworm performance and silk yield. Furthermore, advancements in plant physiology and molecular biology have provided new insights into the mechanisms of stress tolerance in plants. Understanding the genetic and biochemical responses of mulberry to abiotic stress can facilitate the development of improved varieties with enhanced resilience [10-11]. Integrating such innovations with traditional cultivation practices can significantly improve the sustainability and productivity of sericulture. Despite these advancements, challenges remain in effectively managing abiotic stress in mulberry cultivation. Field conditions are often unpredictable, and multiple stress factors may occur simultaneously, compounding their effects on plant growth and leaf quality. Therefore, comprehensive studies that evaluate the combined impact of different stress conditions on mulberry and silkworm performance are necessary. In this context, the present study aims to investigate the effects of major abiotic stress conditions—drought, salinity, and high temperature—on the nutritional and biochemical composition of mulberry leaves and to assess their subsequent impact on silkworm growth and cocoon yield. By analyzing the changes in leaf quality and correlating them with silkworm performance, this study seeks to provide a deeper understanding of the relationship between environmental stress and sericulture productivity [12-13]. The findings are expected to contribute to the development of effective strategies for improving mulberry cultivation under stress conditions and ensuring sustainable silk production in the face of changing environmental challenges.

2. Materials and Methods

2.1 Experimental Design

The experiment was carried out under controlled environmental conditions to evaluate the effects of different abiotic stress factors on mulberry (Morus spp.) leaf quality and subsequent silkworm performance. A completely randomized design (CRD) was adopted to ensure uniformity and minimize experimental error. Healthy, disease-free mulberry plants of similar age and growth stage were selected and divided into four treatment groups: control (normal conditions), drought stress, salinity stress, and heat stress. Each treatment was replicated three times, with each replicate consisting of a uniform set of plants. The plants were maintained under standardized agronomic practices prior to the imposition of stress treatments to ensure consistency in baseline growth conditions.

2.2 Stress Treatments

Abiotic stress conditions were artificially induced to simulate environmental stress factors commonly encountered in field conditions.

- Drought Stress: Water availability was restricted by reducing irrigation to 50% of the normal requirement. This treatment was maintained for a defined period to induce moderate water stress without causing irreversible damage to the plants.
- Salinity Stress: Salinity stress was imposed by irrigating the plants with saline water containing 100 mM sodium chloride (NaCl). The saline solution was applied at regular intervals to maintain consistent salt stress levels in the soil.
- Heat Stress: For heat stress treatment, mulberry plants were exposed to elevated temperatures of approximately 40°C for specific durations each day using controlled environmental chambers. This simulated high-temperature stress conditions typical of extreme climatic events.

The control group was maintained under optimal growth conditions, including regular irrigation, ambient temperature, and non-saline soil conditions.

2.3 Leaf Sampling and Biochemical Analysis

After the completion of the stress treatment period, fully matured and healthy leaves were collected from each treatment group for biochemical analysis. The leaves were carefully washed with distilled water to remove dust and contaminants and then processed immediately or stored under appropriate conditions for further analysis.

The following parameters were evaluated using standard biochemical methods:

- Protein Content: Estimated using colorimetric methods such as the Lowry or Bradford assay, expressed as mg/g of leaf tissue.
- Chlorophyll Content: Determined spectrophotometrically using acetone extraction and measured at specific wavelengths to calculate total chlorophyll content (mg/g fresh weight).
- Moisture Content: Calculated by drying fresh leaf samples in an oven at a constant temperature until a stable weight was obtained, expressed as a percentage.
- Total Phenolic Content: Measured using the Folin–Ciocalteu method and expressed as mg of gallic acid equivalents (GAE) per gram of extract.

All analyses were performed in triplicate to ensure accuracy and reproducibility of results.

2.4 Silkworm Rearing

Healthy and disease-free larvae of Bombyx mori were procured and reared under controlled laboratory conditions. The rearing environment was maintained at an optimal temperature of 25 ± 2°C and relative humidity of 70–80%, with proper ventilation and hygiene to prevent contamination. The larvae were divided into separate groups corresponding to each treatment, and each group was fed exclusively with mulberry leaves obtained from the respective stress treatments. Feed-

Table 1. Effect of Abiotic Stress on Nutritional and Biochemical Composition of Mulberry Leaves

Parameter	Control	Drought Stress	Heat Stress	Salinity Stress
Protein (mg/g)	250 ± 5	185 ± 6	200 ± 5	210 ± 4
Chlorophyll (mg/g FW)	2.70 ± 0.08	1.85 ± 0.06	2.00 ± 0.05	2.10 ± 0.04
Moisture (%)	74.0 ± 1.2	60.5 ± 1.5	65.2 ± 1.3	67.0 ± 1.1
Carbohydrates (mg/g)	290 ± 7	230 ± 6	245 ± 5	255 ± 6
Total Phenolics (mg GAE/g)	360 ± 9	480 ± 12	450 ± 10	420 ± 11

ing was carried out at regular intervals to ensure adequate nutrition throughout the larval period. Standard rearing practices, including cleaning of rearing trays and removal of leftover leaves, were strictly followed. Growth parameters such as larval weight, duration of larval stages, survival rate, and food consumption were recorded periodically to assess the impact of leaf quality on silkworm performance.

2.5 Cocoon Evaluation

At the end of the larval stage, mature silkworms were allowed to spin cocoons under suitable conditions. After cocoon formation, the cocoons were harvested, cleaned, and analyzed for various economic parameters.

- Cocoon Weight: Individual cocoons were weighed using a precision balance.
- Shell Weight: The silk shell was separated from the pupal content and weighed to determine the actual silk content.
- Shell Ratio (%): Calculated as the ratio of shell weight to cocoon weight, expressed as a percentage.
- Silk Productivity (%): Determined based on the efficiency of silk production relative to larval weight and cocoon characteristics.

All measurements were conducted using standard sericulture evaluation methods, and the data were statistically analyzed to determine the significance of differences among treatments.

2.6 Statistical Analysis

Data were analyzed using ANOVA, and significant differences were determined at $p \leq 0.05$.

3. Results

3.1 Effect of Abiotic Stress on Mulberry Leaf Quality

Abiotic stress significantly affected the biochemical composition of mulberry leaves. Drought and heat stress resulted in a marked reduction in protein, chlorophyll, and moisture content. Salinity stress also reduced nutrient levels but to a lesser extent. In contrast, total phenolic content increased under all stress conditions, indicating a stress-induced defense response.

Table 2. Effect of Abiotic Stress on Silkworm Growth Parameters

Parameter	Control	Drought Stress	Heat Stress	Salinity Stress
Larval Weight (g)	3.90 ± 0.12	2.95 ± 0.10	3.20 ± 0.11	3.35 ± 0.09
Larval Duration (days)	24 ± 1	27 ± 1	26 ± 1	25 ± 1
Survival Rate (%)	95.0 ± 1.5	82.0 ± 2.0	86.5 ± 1.8	89.0 ± 1.6
Food Consumption (g/larva)	5.8 ± 0.2	4.5 ± 0.2	4.9 ± 0.2	5.1 ± 0.2

Table 3. Effect of Abiotic Stress on Cocoon and Silk Yield

Parameter	Control	Drought Stress	Heat Stress	Salinity Stress
Cocoon Weight (g)	1.75 ± 0.04	1.30 ± 0.05	1.45 ± 0.04	1.55 ± 0.05
Shell Weight (g)	0.36 ± 0.01	0.25 ± 0.01	0.28 ± 0.01	0.30 ± 0.01
Shell Ratio (%)	20.6 ± 0.5	19.2 ± 0.6	19.3 ± 0.5	19.4 ± 0.4
Silk Productivity (%)	19.5 ± 0.6	15.8 ± 0.7	16.9 ± 0.6	17.8 ± 0.5
Filament Length (m)	880 ± 25	690 ± 20	740 ± 22	780 ± 18

3.2 Effect on Silkworm Growth

Silkworms fed with stressed leaves showed reduced larval weight and lower survival rates compared to the control group. The negative effects were most pronounced in larvae fed with drought-stressed leaves.

3.3 Cocoon and Silk Yield

Cocoon parameters were significantly affected by leaf quality. Cocoon weight, shell weight, and silk productivity decreased under all stress conditions. Drought stress caused the greatest reduction, followed by heat and salinity stress..

4. Discussion

4.1 Effect of Abiotic Stress on Mulberry Leaf Quality

The present study clearly demonstrates that abiotic stress conditions significantly alter the nutritional and biochemical composition of mulberry (Morus spp.) leaves (Table 1). Among the stress treatments, drought stress exhibited the most pronounced negative impact, followed by heat stress and salinity stress. A substantial reduction in protein content was observed under all stress conditions, with drought stress showing the lowest value (185 mg/g) compared to the control (250 mg/g) [15-17]. This decline in protein levels can be attributed to stress-induced inhibition of protein synthesis and increased proteolytic activity, which are common physiological responses in plants under adverse environmental conditions. Chlorophyll content, an important indicator of photosynthetic efficiency, also decreased significantly under stress [8-21]. Drought-stressed plants showed a marked reduction (1.85 mg/g FW) compared to the control (2.70 mg/g FW), indicating impaired photosynthetic activity. Reduced chlorophyll levels are often associated with oxidative damage to chloroplast structures and degradation of photosynthetic pigments. Heat stress further contributed to pigment degradation due to thermal instability of chlorophyll molecules, while salinity stress disrupted ion balance, affecting chlorophyll biosynthesis. Moisture content in mulberry leaves declined notably under stress conditions, particularly in drought-treated plants (60.5%) compared to the control (74%). Reduced moisture content negatively affects leaf turgidity, palatability, and digestibility, which are critical factors for silkworm feeding. Carbohydrate content also decreased under stress, reflecting reduced photosynthetic output and altered carbon metabolism [22]. This reduction limits the availability of energy sources necessary for both plant growth and silkworm nutrition. In contrast to the decline in primary metabolites, a significant increase in total phenolic content was observed under stress conditions. Drought stress resulted in the highest phenolic accumulation (480 mg GAE/g), followed by heat and salinity stress. This increase is indicative of a stress-induced defense mechanism, as phenolic compounds function as antioxidants that protect plant cells from oxidative damage. While beneficial for plant survival, excessive accumulation of phenolics may reduce leaf palatability and interfere with nutrient absorption in silkworms, the results indicate that abiotic stress induces a shift in mulberry leaf composition, characterized by a reduction in essential nutrients and an increase in secondary metabolites. This imbalance has important implications for silkworm feeding and performance.

4.2 Effect on Silkworm Growth Parameters

The impact of altered mulberry leaf quality on silkworm growth was evident from the observed larval parameters (Table 2). Silkworms fed with leaves from stressed plants exhibited significantly lower larval weight, extended larval duration, and reduced survival rates compared to the control group. Among the treatments, drought stress had the most detrimental effect, resulting in the lowest larval weight (2.95 g) and survival rate (82%). The reduction in larval weight can be directly linked to the decreased protein and carbohydrate content in mulberry leaves under stress conditions [23]. Proteins are essential for tissue growth and silk gland development, while carbohydrates provide the energy required for metabolic processes. A deficiency in these nutrients leads to reduced growth efficiency and lower biomass accumulation in silkworm larvae. The prolonged larval duration observed under stress conditions indicates delayed development, which may be attributed to insufficient nutrient availability and reduced feeding efficiency. Silkworms tend to consume less food when leaf quality is compromised, particularly when leaves have lower moisture content and higher concentrations of phenolic compounds [24]. These factors can negatively affect palatability and digestion, leading to decreased food intake and slower growth. Survival rate is a critical parameter reflecting overall silkworm health. The reduced survival rates observed in stress treatments suggest increased vulnerability to environmental stress and disease. The presence of elevated phenolic compounds, although beneficial for plant defense, may exert anti-nutritional effects that interfere with digestion and nutrient absorption in silkworms. Additionally, reduced moisture content in leaves may contribute to dehydration stress in larvae, further affecting survival [25]. Food consumption patterns also reflected the impact of leaf quality. Silkworms fed with drought-stressed leaves showed the lowest consumption (4.5 g/larva), indicating reduced feeding activity. This decline in food intake further exacerbates nutrient deficiency, creating a negative feedback loop that affects larval growth and development.

4.3 Effect on Cocoon and Silk Yield

The influence of abiotic stress on mulberry leaf quality was ultimately reflected in cocoon and silk production parameters (Table 3). Significant reductions were observed in cocoon weight, shell weight, shell ratio, and silk productivity under all stress conditions, with drought stress showing the most severe effects. Cocoon weight decreased from 1.75 g in the control to 1.30 g under drought stress, indicating reduced larval growth and lower biomass conversion into cocoon material [26]. Similarly, shell weight, which represents the actual silk content, showed a substantial decline under stress conditions. This reduction is primarily due to decreased protein availability, which limits the synthesis of silk proteins in the silk glands. The shell ratio, an important economic parameter, also showed a slight decline under stress conditions. Although the reduction was less pronounced compared to other parameters, it indicates a decrease in the proportion of silk fiber relative to the total cocoon weight. Silk productivity, which reflects the efficiency of silk production, was significantly lower in stress treatments, particularly under drought conditions (15.8%) compared to the control (19.5%). Filament length, another key indicator of silk quality, was adversely affected by stress conditions [27]. The shortest filament length was recorded under drought stress (690 m), while control conditions produced the longest filaments (880 m). Reduced filament length can be attributed to impaired silk gland function and inadequate nutrient supply during larval development.

4.4 Integrated Interpretation

The results of this study clearly establish a strong relationship between abiotic stress, mulberry leaf quality, and silkworm performance. Stress-induced changes in leaf composition, particularly the reduction in proteins, carbohydrates, and moisture, directly affect silkworm growth and cocoon production. The increase in phenolic compounds, while beneficial for plant survival, may negatively influence silkworm feeding behavior and nutrient utilization. Among the stress factors studied, drought stress emerged as the most detrimental, followed by heat and salinity stress. This suggests that water availability is a critical factor in maintaining mulberry leaf quality and ensuring optimal sericulture productivity [28-30]. The findings highlight the need for effective stress management strategies, such as improved irrigation practices, selection of stress-tolerant mulberry varieties, and adoption of climate-resilient cultivation techniques, the study underscores the importance of maintaining high-quality mulberry leaves under varying environmental conditions. An minimizing the impact of abiotic stress, it is possible to enhance silkworm performance, improve cocoon yield, and ensure sustainable silk production.

5. Conclusion

The present study clearly demonstrates that abiotic stress conditions significantly influence mulberry (Morus spp.) leaf quality and, consequently, silkworm growth and silk yield. Among the stress factors examined, drought stress exerted the most severe impact, followed by heat and salinity stress. These environmental constraints led to a marked reduction in essential nutrients such as proteins, carbohydrates, chlorophyll, and moisture content in mulberry leaves, all of which are critical for optimal silkworm nutrition and development. At the same time, an increase in phenolic compounds was observed, indicating a stress-induced defense response in plants. The deterioration in leaf quality directly affected silkworm performance, resulting in reduced larval weight, prolonged developmental duration, and lower survival rates. These effects were further reflected in cocoon characteristics, where significant reductions in cocoon weight, shell weight, shell ratio, and silk productivity were recorded. The findings highlight the strong dependency of silkworm growth and silk production on the nutritional and biochemical composition of mulberry leaves, the study emphasizes the importance of managing abiotic stress in mulberry cultivation to ensure consistent leaf quality and sustainable sericulture production. Adoption of improved irrigation practices, development of stress-tolerant mulberry varieties, and implementation of climate-resilient agricultural strategies are essential to mitigate the adverse effects of environmental stress. These measures will help enhance silkworm performance, improve silk yield, and support the long-term sustainability and economic viability of the sericulture industry.

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