B. N. Seal Journal of Science Volume 11, Issue 1, 2023

Research Article

Exploring spinach extract as a remedy for non-spinning syndrome in silkworms linked to pesticide residues

Manojit Chatterjee*

Sericulture Research Laboratory, Post Graduate Department of Zoology, Hooghly Mohsin College, Chinsurah, Hooghly -712101, West Bengal, India

*Corresponding Author's E mail: *manojitchatterjee2007@gmail.com*

Abstract: *Non-spinning syndrome (NSS) in silkworms (Bombyx mori) poses a significant challenge to sericulture, particularly in regions with intensive pesticide use and fluctuating climatic conditions. This study investigates the efficacy of spinach (Spinacia oleracea) extract as a natural intervention to mitigate NSS and enhance overall silk production. F1 hybrid silkworms were reared under controlled conditions, and larvae from NSS-affected areas were treated with varying dilutions of spinach extract (1:100, 1:50, 1:25). The 1:25 dilution emerged as the most effective, significantly improving larval health, cocoon weight (1.72 ± 0.0577 g), and effective rearing rate (68.6 ± 9.05 %). Additionally, treated silkworms exhibited enhanced silk quality, with increased filament length (683 ± 16.2 m) and reelability (78.4 ± 1.36 %). Beyond mitigating NSS, the extract demonstrated potential as a crop-saving agent during adverse weather conditions, promoting synchronized spinning and improved silk parameters even in control silkworms unaffected by NSS. Comparative analysis with existing studies underscores the pivotal role of phytoecdysteroids in the extract, offering a sustainable, eco-friendly approach to address challenges in sericulture. These findings highlight the dual utility of spinach extract as a mitigative agent for NSS and a resilience enhancer in standard rearing practices, paving the way for its broader adoption in sericulture. This research offers a practical, sustainable solution to safeguard silk production against emerging threats, contributing to the resilience and profitability of the sericulture industry.*

Keywords: *Spinach extract; Non-spinning syndrome; Silkworm; Sericulture industry*

1. Introduction

Sericulture, an age-old practice in India, is deeply intertwined with the nation's cultural heritage and rural economy. Believed to have been introduced through the clandestine efforts of Chinese monks smuggling silkworm eggs and mulberry seeds, the industry has evolved over centuries to establish India as the second-largest producer of raw silk globally, with a production of 38,913 metric tons (MT), trailing only China (50,000 MT) as reported by the International Sericultural Commission (2022) . India's unique capability to produce all four types of silk—Mulberry, Eri, Tasar, and Muga—underpins its prominence in the global silk industry. This sector provides livelihoods to over 7.6 million people across 51,000 villages and contributes significantly to the national economy.

Among India's leading silk-producing states, West Bengal holds a prominent position. However, in recent years, the state's sericulture industry has faced unprecedented challenges, including declining raw silk production. Traditional sericulture belts like Murshidabad and non-traditional ones like Nadia have been particularly affected by non-spinning syndrome, a debilitating condition that severely disrupts silk production during critical rearing months from March to October. This syndrome-manifesting as the inability of silkworms to spin cocoons—has emerged as a significant threat to farmers' livelihoods and regional silk production [1].

The emergence of non-spinning syndrome is likely multifactorial, with climatic factors, particularly rising temperatures linked to climate change, playing a major role. Additionally, the indiscriminate use of pesticides in neighboring agricultural fields for non-sericulture crops such as paddy, jute, and potato may contribute to the syndrome by introducing sub-lethal stressors that affect silkworm health [2-7]. Despite its significance, the etiology of non-spinning syndrome in West Bengal remains poorly understood, necessitating targeted research to identify sustainable mitigation strategies.

This study aims to address the problem of non-spinning syndrome by evaluating the efficacy of spinach (*Spinacia oleracea*) extract, which contains phytojuvenoids [8-10] known for their hormonal regulatory properties [11-12]. The research will investigate whether the application of spinach extract can restore the silkworms' ability to spin cocoons and mitigate the adverse effects of the syndrome. Furthermore, the study will explore the potential of spinach extract as a crop synchronizer to improve rearing outcomes even in healthy silkworms unaffected by the syndrome. By focusing on a natural, sustainable intervention, this research seeks to provide practical solutions to the challenges facing the sericulture industry in West Bengal and beyond, thereby safeguarding livelihoods and ensuring the resilience of India's silk economy.

2. Materials and Methods

2.1. Rearing of Silkworms

The study was conducted using *Bombyx mori* L. silkworms of F1 Hybrid, obtained from cross between Nistari Plain and SK6 X SK7 hybrid, reared following the standard procedures outlined by the Central Silk Board (CSB) guidelines. For the control set (C) Silkworm eggs were procured from a certified sericulture center and incubated under controlled environmental conditions. The larvae were reared at optimal humidity (75-85%) and temperature (25-28°C) with a photoperiod of 12:12 hours light-dark cycle. Larvae were reared on fresh mulberry leaves throughout the experiment. For treatment sets T1-4 Vth instar larvae were procured from affected areas. T1 larvae were reared on mulberry leaves procured from affected areas, whereas T2-4 larvae were reared on mulberry leaves procured from affected areas and sprayed with different dilutions (1:100, 1:50 and

1:25 respectively) of spinach extract. For treatment set T5 IVth instar larvae from control set were reared on fresh mulberry leaves sprayed with standardised concentration of spinach extract from 2nd day of Vth instar stage up to spinning.

2.2. Preparation of Spinach Extract and Application

Fresh *Spinacia oleracea* leaves were washed thoroughly with distilled water to remove impurities, shade-dried, and powdered using an electric grinder. The powdered material was extracted with distilled water in a ratio of 1:10 (w/v) at 50°C for 6 hours. The extract was filtered through muslin cloth, and the crude extract was used to prepare three dilutions: 1:100, 1:50, and 1:25 (v/v) in distilled water. These solutions were freshly prepared daily.

The diluted extracts were applied as a foliar spray onto mulberry leaves at a rate of 10 ml per 100 g of leaves per feeding. Treated leaves were air-dried for 10 minutes to ensure uniform adherence of the extract before feeding. The sprays were administered before each meal, three times daily, from the second day onwards of the fifth instar stage. Control groups were fed untreated mulberry leaves.

2.3. Estimation of Rearing Parameters

To evaluate the effects of spinach extract, various biological and economic traits were monitored:

- *Larval Weight:* Individual larval weights were recorded daily using a digital balance.
- *Mortality Rate:* The number of dead larvae in each group was recorded daily.
- *Cocoon Parameters:* Cocoon weight, shell weight, shell ratio [(Shell weight/Cocoon weight) × 100], and filament length were measured post-harvest.
- *Pupal Weight:* Weights of pupae were recorded immediately after cocoon cutting.
- *Spinning Efficiency:* The percentage of larvae successfully spinning cocoons was calculated.

2.3. Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) to determine the significance of differences among the treatment groups. Means were compared using Tukey's HSD test at a 5% significance level. Statistical analyses were performed using statistical software, and results were expressed as mean ± standard deviation (SD).

2.4. Sample Size Justification

Based on prior studies, including Maqbool et al. (2023), a sample size of 50 larvae per replication, with three replications per treatment group, was determined to be sufficient for statistically significant results and reliable interpretation of rearing parameters.

3. Results

3.1. Effect of Spinach Extract Foliar Spray on the Progression of Silkworms to the Spinning Stage During the 5th Instar (Table 1)

The table assesses the progression of silkworms to the spinning stage under different treatment conditions during their 5th instar. The treatments include **Control (C)** larvae with no spinach extract (SE) application, and treatment larvae (T1-4). **T1** with no SE, and **T2, T3, and T4** with increasing concentrations of SE foliar sprays applied at 1:100, 1:50, and 1:25 dilutions, respectively. The numbers in the table indicate the percentage of silkworms progressing to the next stage (spinning).

3.1.1. Control Set (C)

In the control group, where no SE was applied, **15%** of silkworms progressed to spinning by the 6th day, increasing to **89%** by the 7th day. By the 8th day, **97%** reached the spinning stage, and all surviving silkworms completed their progression without mortality or developmental delays.

3.1.2. Treatment Set T1 (No SE Added)

In treatment set T1 with no SE application progression was delayed compared to the control. By the 6th day, only **7%** of silkworms progressed, increasing to **23%** by the 7th day and reaching a maximum of **41%** by the 8th day. A delayed progression was noted as **57%** of silkworms advanced by the 9th day, with no spinning observed beyond this stage.

3.1.3. Treatment Set T2 (SE Dilution 1:100)

T2 demonstrated a marked increase in the percentage of silkworms progressing to spinning compared to T1, showing the positive effect of SE application at a low concentration. By the 6th day, **31%** of silkworms advanced, increasing to **73%** by the 8th day. The maximum progression occurred on the 9th day, with **78%** of silkworms reaching the spinning stage, though some developmental delays and mortality were observed.

3.1.4. Treatment Set T3 (SE Dilution 1:50)

The higher concentration of SE in T3 led to a greater percentage of silkworms reaching the spinning stage earlier than in T2. On the 6th day, **49%** progressed, rising to **71%** by the 7th day and **93%** by the 8th day. By the 9th day, **96%** of silkworms had reached the spinning stage, indicating enhanced progression with increased SE concentration.

3.1.5. Treatment Set T4 (SE Dilution 1:25)

The highest concentration of SE (1:25 dilution) resulted in the greatest percentage of silkworms progressing to the spinning stage. Progression began earlier, with **10%** advancing by the 5th day, increasing to **49%** on the 6th day and **71%** on the 7th day. By the 8th day, **93%** reached the spinning stage, and **96%** progressed by the 9th day, similar to T3 but with slightly earlier progression.

- In the treatment set T1 (no SE), silkworm progression to the spinning stage was slower and lower in percentage compared to the control set and other treatment sets T2, T3, and T4.
- The application of spinach extract (T2–T4) increased the percentage of silkworms reaching the spinning stage, with the highest concentration (T4) showing the most rapid and widespread progression.
- The results suggest that increasing concentrations of spinach extract positively influence the maturation and spinning behavior of silkworms, with T4 yielding the best outcomes in terms of timing and progression percentage.

Table 1: Effect of Spinach Extract Foliar Spray on the Progression of Silkworms to the Spinning Stage During the 5th Instar

Sl. No.	Duration in 5th Instar (Days)	Control sets		Treatment sets		
		C	T1	T2	T3	T4
		Maturation Percentage (%)				
1.	2nd	-	-	Application of SE started	Application of SE started	Application of SE started
2.	3rd	-	-	-	-	-
3.	4th	-	-	-	-	-
4.	5th	-	-	-	-	10
5.	6th	15	-	-	7	49
6.	7th	89	-	23	31	71
7.	8th	97	-	41	73	93
8.	9th	-	-	57	78	96
9.	10th	-	-	60	-	-
10.	11th	-	No maturation	-	-	-
11.	12th	-	Maximum mortality	-	-	-

* *values in cells represent maturation % of silkworm larvae in respective days of 5th instar stage.*

3.2. Assessment of Different Treatment Effects on Rearing Parameters (Table 2)

This table presents the effects of different treatments (C, T1, T4, T5) on larval and cocoon parameters. T5 represents Vth instar control larvae fed with fresh mulberry leaves using selected dose (1:25) of SE as foliar spray before each feeding.

- *Larval Duration (LD):* The longest larval duration (29.2 ± 0.602) was observed in the T1 group, significantly higher than other groups. T4 (25.4 ± 0.528) showed an intermediate effect, while T5 (23 ± 0.537) and the control (23.2 ± 0.534) had the shortest larval durations, statistically similar to each other.
- *Mean Larval Weight (MLW):* The T1 group also recorded the highest mean larval weight (39.4 ± 1.14), followed closely by T4 (38.3 ± 1.11). Both T5 (36.2 ± 1.15) and the control (36.4 ± 1.15) exhibited similar and lower weights.
- *Cocoon Weight (CW):* T4 (1.72 ± 0.0577) had the highest cocoon weight, followed by T5 (1.49 ± 0.0655). The control group recorded intermediate cocoon weights (1.6 ± 0.0612), while T1 showed no cocoon formation (0 ± 0) due to non-spinning syndrome.
- *Shell Weight (SW):* No significant difference in shell weight was observed among the control (0.246 ± 0.0261), T4 (0.236 ± 0.0261), and T5 (0.254 ± 0.0252) groups, all of which were statistically similar. T1 recorded no shell weight (0 ± 0), consistent with the lack of cocoon formation in this group.
- *Shell Ratio (SR):* The highest shell ratio (16.9 ± 0.982) was observed in T5, followed by the control (15.1 ± 1.05) and T4 (13.6 ± 1.06), all statistically similar. T1 showed no shell ratio (0 ± 0).
- *Effective Rate of Rearing (ERR):* The highest ERR was recorded in T5 (73.4 ± 9.13), followed closely by the control (72.2 ± 9.13) and T4 (68.6 ± 9.05), indicating better survival rates in these treatments. T1 recorded zero ERR (0 ± 0), suggesting a complete loss during rearing.

Table 2: Assessment of different treatment effects on rearing parameters

Rearing Parameters	**Treatment sets**			
	C	**T1**	**T4**	**T5**
LD	23.2 ± 0.534^{c}	29.2 ± 0.602^{a}	25.4 ± 0.528^{b}	23 ± 0.537^{c}
MLW	36.4 ± 1.15	39.4 ± 1.14	38.3 ± 1.11	36.2 ± 1.15
CW	1.6 ± 0.0612^{ab}	0 ± 0^{c}	1.72 ± 0.0577^{a}	1.49 ± 0.0655^{b}
SW	0.246 ± 0.0261^{a}	0 ± 0^{b}	0.236 ± 0.0261^{a}	0.254 ± 0.0252^{a}
SR	15.1 ± 1.05^{a}	0 ± 0^{b}	13.6 ± 1.06^{a}	16.9 ± 0.982^{a}
ERR	72.2 ± 9.13^{a}	0 ± 0^{b}	68.6 ± 9.05^{a}	73.4 ± 9.13^{a}

Values are means ± SEM. Means in a row without a common superscript letter differ (P<0.05) as analyzed by one-way ANOVA and the TUKEY test.

3.3. Assessment of Different Treatment Effects on Silk Quality Parameters (Table 3)

Similar to table 2, this table evaluates the silk quality parameters for different treatments C, T4, and T5 as no cocoon formation was observed in T1 set due to non-spinning syndrome.

- *Filament Length (FL):* Filament length was highest for T5 (691 ± 15.8), followed by T4 (683 ± 16.2) and the control (675 ± 16.1), though the differences were not statistically significant.
- *Denier:* The highest filament size (denier) was observed in T5 (3.39 ± 0.0735), followed by T4 (3.09 ± 0.0653). The control group exhibited the lowest denier value (2.83 ± 0.032), indicating finer filament production. All differences were statistically significant.
- *Reliability (%):* The T5 group achieved the highest reliability (82.1 ± 1.1), followed by T4 (78.4 ± 1.36) and the control (75 ± 1.67). While the control and T4 were not significantly different, T5 stood out as significantly better.
- *Non-Broken Filament Length (NBFL):* The highest non-broken filament length was observed in T5 (646 ± 35.1), followed by T4 (603 ± 34.2). The control recorded the shortest length (566 ± 35.1), indicating reduced resilience in untreated conditions.
- *Renditta (Kg):* Renditta values gradually increased from the T5 (9.02 ± 0.172) to control (9.29 ± 0.221) and T4 (9.56 ± 0.212), although the differences were not statistically significant.

Table 3: Assessment of different treatment effects on silk quality parameters

Silk quality Parameters	Treatment sets		
	C	**T4**	**T5**
FL	675 ± 16.1	683 ± 16.2	691 ± 15.8
DENIER	2.83 ± 0.032^{c}	3.09 ± 0.0653^{b}	3.39 ± 0.0735^{a}
REELABILITY	75 ± 1.67^{b}	78.4 ± 1.36^{ab}	82.1 ± 1.1^{a}
NBFL	566 ± 35.1	603 ± 34.2	646 ± 35.1
RENDITTA	9.29 ± 0.221	9.56 ± 0.212	9.02 ± 0.172

Values are means ± SEM.

Means in a row without a common superscript letter differ ($P<0.05$) as analyzed by one-way ANOVA and the TUKEY test.

In Table 1, T5 emerges as the most favorable treatment for larval and cocoon parameters, demonstrating high ERR and shell ratio. Similarly, Table 2 highlights the superior silk quality

parameters in the T5 group, showcasing the benefits of this treatment over others. T1 showed severe adverse effects, while the control and T4 groups offered intermediate outcomes.

4. Discussion

The findings of this study highlight the significant impact of spinach extract in mitigating non-spinning syndrome in silkworms and improving overall sericulture productivity. Consistent with prior research, including our survey-based studies [1], the emergence of non-spinning syndrome can be attributed to adverse weather conditions and the indiscriminate use of pesticides in non-sericulture agriculture, such as paddy, potato, and jute cultivation in nearby fields [2,3,4,5,6,7]. These stressors likely compromise silkworm physiology, as evidenced by reduced spinning efficiency and other rearing challenges observed in affected areas.

This study demonstrates that spinach extract, particularly at a 1:25 dilution dose (T4 & T5), is a promising natural intervention for addressing non-spinning syndrome and synchronising spinning in normal silkworm. Data from the rearing parameters (Table 2) and silk quality parameters (Table 3) substantiate its efficacy. Notably, T4 silkworms exhibited almost similar or statistically non-significant deviation from control data in most of the cases, whereas T5-treated silkworms exhibited significant improvements in larval duration (23.0 ± 0.537 days), cocoon weight (1.49 ± 0.0655 g), and effective rearing rate (73.4 ± 9.13%) compared to untreated controls. Additionally, T5 achieved superior silk quality, with the highest filament length (691 ± 15.8 m), denier value (3.39 ± 0.0735), and reelability (82.1 ± 1.1%). Similar findings on the effect spinach extract was reported by other authors as well (Selvi, 2014), they also demonstrated a dose-dependent relationship, where higher concentrations (3%) led to increased cocoon and silk quality. These findings were suggestive of the facts that crude spinach extract in 1:25 dilution is effective in mitigating non-spinning syndrome during the Vth instar stage and the ability of spinach extract to enhance silk quality even in control silkworms (T5 group) without non-spinning syndrome further underscores its broader utility. Improvements in spinning synchronization and resilience during adverse weather conditions position spinach extract as a crop saver in silkworm rearing as well, other researchers [13] also reported similar impact of spinach extract as crop saver. Importantly, the 1:25 dilution dose achieved the best balance of larval growth and cocoon development, highlighting its optimal concentration for field application.

The physiological basis for these outcomes likely resides in the phytojuvenoids and other bioactive compounds present in spinach [8,9,10] as evident from numerous studies, which may act as hormonal regulators to alleviate stress-induced disruptions in silkworms [11,12]. Similar observation was made in Eri silkworm as well [14]. While further biochemical studies are

warranted, these findings align with earlier observations that natural plant extracts can support insect development under stress conditions.

5. Conclusion

This study confirms the effectiveness of spinach extract as a viable remedy for non-spinning syndrome in silkworms, a condition linked to both climatic fluctuations and pesticide exposure from neighboring agriculture. The 1:25 dilution dose emerged as the most effective treatment, enhancing spinning efficiency, rearing parameters, and silk quality. These results have significant implications for the sericulture industry, particularly in regions like West Bengal, where non-spinning syndrome has disrupted livelihoods and silk production. While most of the previous studies [13,15,16] focussed on high ethanol or methanol concentrations and offered insights into laboratory-level efficacy, present study emphasised on a field-viable aqueous extract reflecting a sustainable approach to sericulture. Moreover, unlike these studies, which primarily address controlled or extreme stress conditions, the present study uniquely highlights spinach extract's dual role: a remedial measure for non-spinning syndrome and a quality enhancer in standard rearing practices. This broader applicability underscores its potential in optimizing silk production across varying environmental and operational scenarios.

6. Future Scope of Study

Further research is essential to deepen our understanding of the biochemical pathways through which spinach extract mitigates stress in silkworms. Additionally, long-term field trials should evaluate its efficacy across diverse environmental conditions and silkworm strains. Exploring the integration of other natural plant-based remedies with spinach extract could provide a comprehensive, sustainable strategy for enhancing sericulture resilience in the face of climatic and agricultural challenges.

References

1. Chatterjee, M. (2022). Emergence of Non-Spinning Syndrome in Silkworms (Bombyx mori L.) in Nadia District: First report from West Bengal. *B. N. Seal Journal of Science*, *10*(1), 15–28.
2. Jyothi, N. B., Bavachikar, P. N., Maribashetty, V. G., & Radhakrishna, P. G. (2019). *Effect of pesticide residue in soil on silkworm, Bombyx mori L - survey analysis. 38*(2), 31–37. https://doi.org/10.7852/ijie.2019.38.2.31
3. Kuribayashi, S. (1988). Damage of Silkworms Caused by Pesticides and Preventive Measures. *Jarq-Japan Agricultural Research Quarterly*, *21*, 274–283.

4. Park, K.-H., Kim, B., Park, Y.-K., Lee, H.-D., Jeong, M.-H., You, A.-S., Sohn, B.-H., Kang, P.-D., & Kyung, K. (2007). Relationship between the non-spinning syndrome of silkworm, Bombyx mori and pesticides. *The Korean Journal of Pesticide Science*, *11*.
5. Sahan, U., Gul, Z., & Buyukuysal, R. (2020). Biochemical changes in hemolymph of spinning and non-spinning silkworm larvae, Bombyx mori (L., 1758) (Lepidoptera: Bombycidae), reared on fresh mulberry leaves: Possible reasons for non-spinning syndrome. *Turkish Journal of Entomology*, *44*, 571–579. https://doi.org/10.16970/entoted.737773
6. Ümran, Ş., & Levent, B. (2019). *A Survey Possible Reasons of Non-Spinning Syndrome in Sericulture Industry and Its Incidence in Turkey*. 9th Bacsa International Conference "Sericulture preservation and revival – problems and prospects""SERIVIVAL" 2019, Batumi, Georgia.
7. Vassarmidaki, M. E., Harizanis, P. C., & Katsikis, S. (2000). Effects of Applaud on the Growth of Silkworm (Lepidoptera: Bombycidae). *Journal of Economic Entomology*, *93*(2), 290–292. https://doi.org/10.1603/0022-0493-93.2.290
8. Bakrim, A., Maria, A., Sayah, F., Lafont, R., & Takvorian, N. (2008). Ecdysteroids in spinach (Spinacia oleracea L.): Biosynthesis, transport and regulation of levels. *Plant Physiology and Biochemistry*, *46*(10), 844–854. https://doi.org/10.1016/j.plaphy.2008.06.002
9. Gorelick, J., Iraqi, R. H., & Bernstein, N. (2020). Ecdysteroid Content and Therapeutic Activity in Elicited Spinach Accessions. *Plants*, *9*(6). https://doi.org/10.3390/plants9060727
10. Hunyadi, A., Herke, I., Lengyel, K., Báthori, M., Kele, Z., Simon, A., Tóth, G., & Szendrei, K. (2016). Ecdysteroid-containing food supplements from Cyanotis arachnoidea on the European market: Evidence for spinach product counterfeiting. *Scientific Reports*, *6*(1), 37322. https://doi.org/10.1038/srep37322
11. Cheng, H., Wang, Y., Chang, M., Zhang, K., Cai, Z., Qian, Y., & Li, D. (2022). Sperm maturation screening and the effect of ecdysone on sperm development of silkworm Bombyx mori. *Journal of Asia-Pacific Entomology*, *25*(2), 101916. https://doi.org/10.1016/j.aspen.2022.101916
12. Khyade, V. B., Shukla, K. K., & Sarawade, J. P. (2012). *Juvenoid activity of some non mulberry plant extractives through inhibition of chitin deposition in the integument of fifth instar larvae of silkworm, Bombyx mori (L)(Race: PM x CSR2)* https://api.semanticscholar.org/CorpusID:55895332.
13. Maqbool, S., Rufai, Z., Mf, B., & Parvez, S. (2023). *Use of spinach (Spinacia oleracea) containing phytoecdysteroid (β-ecdysone) as a crop saver in sericulture industry*.
14. Lalmuankimi, C., Gogoi, I., & Singha, T. A. (2020). Effect of Plant Extracts on Larval Growth Parameters of Eri Silkworm, Samia ricini Boisd. *International Journal of Current Microbiology and Applied Sciences*. https://api.semanticscholar.org/CorpusID:234525417.
15. Selvi, S. (2014). *Effect of Spinacia oleracea (spinach) on morphometric parameters of silk worm Bombyx mori*. https://api.semanticscholar.org/CorpusID:215797313.