

# A Newly Developed Technique for Adhering Bivoltine Silkworm Loose Eggs of *Bombyx mori* L. Using Formulated Chemicals and Papers

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## Abstract

A newly developed technique for adhering loose eggs of bivoltine *Bombyx mori* L. onto egg-laying papers was evaluated using four chemical adhesive formulations, terpene resin solution (T1), gum arabic solution (T2), methyl cellulose gel (T3) and a modified polyvinyl alcohol (PVA) blend (T4) against an untreated control (C). Five paper substrates were also tested: standard kraft paper (P1), blotting paper (P2), muslin-laminated paper (P3), cellulose acetate sheet (P4) and conventional wax-treated egg card (P5). Experiments were conducted in triplicate across two successive bivoltine crop seasons using commercially important hybrid CSR2 x CSR4. Results demonstrated that the terpene resin formulation (T1) applied on kraft paper (P1) yielded the highest egg adhesion efficiency (97.4 +/- 0.6%), hatching rate (92.8 +/- 1.2%), larval survival at first instar (96.5 +/- 0.9%) and lowest egg loss index (2.6%). Disease incidence was significantly reduced ( $p < 0.05$ ) in terpene resin-treated egg cards compared to untreated controls. The technique offers a practical, cost-effective and scalable solution for improving egg-card quality in commercial bivoltine sericulture. Implications for grainage operations, disease-free laying (DFL) production and silk yield enhancement are discussed.

**Keywords:** *Bombyx mori*, bivoltine silkworm, loose egg adhesion, terpene resin, egg card preparation, grainage, disinfection, hatching rate, DFL, sericulture

## 1. Introduction

Sericulture, the cultivation of silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae) for the production of natural silk, constitutes a vital cottage and agro-based industry supporting millions of rural livelihoods in Asia, Africa and parts of South America [22, 27]. Among the two principal voltinism groups, bivoltine races completing two annual generations are prized for their superior cocoon quality, refined silk thread characteristics and higher commercial value compared to polyvoltine counterparts [3, 4, 8]. In India alone, bivoltine silk production accounts for nearly 35% of total silk output, with government-mandated programs actively promoting the cultivation of improved bivoltine hybrids such as CSR2 x CSR4, PM x CSR2 and NB4D2 x KA series [10].

A fundamental yet frequently overlooked challenge in commercial bivoltine sericulture is the phenomenon of 'loose eggs'. Eggs that fail to adhere naturally to the oviposition substrate following laying. Under normal conditions, female moths deposit eggs on egg-laying papers (sheets), with natural adhesive secretions from accessory glands facilitating attachment [1]. However, a significant proportion

of eggs, often ranging from 8-25% of total clutch size depending on race, environmental conditions and female nutritional status, fail to adhere adequately, becoming loose [1, 31]. These loose eggs present multiple operational problems: (i) difficulty in standardized disinfection (ii) uneven exposure to chemical treatments (iii) mechanical damage during handling and transport (iv) increased susceptibility to contamination by *Nosema bombycis* (pebrine), *Beauveria bassiana* (muscardine) and BmNPV (v) irregular hatching synchronization resulting in uneven larval cohorts [15, 30].

Current practice in most grainage facilities involves either discarding loose eggs, resulting in significant economic loss or attempting to re-adhere them using ad hoc methods such as water wetting, starch paste, or crude gum solutions, none of which are standardized, scientifically validated or commercially scalable [1]. The absence of a reliable, biocompatible adhesive formulation tailored to the biological and chemical characteristics of silkworm eggs represents a critical technological gap in modern sericulture.

Recent research has explored natural resin-based adhesives, particularly terpene resin formulations, as potential solutions for egg card preparation from loose eggs [1]. Terpene resins, derived from plant sources, offer advantages of low toxicity, appropriate tackiness, stability under incubation conditions and compatibility with downstream disinfection and acid treatments [1]. However, systematic comparative evaluation of multiple adhesive formulations against diverse paper substrates with rigorous measurement of egg adhesion, viability, hatching rate and disease incidence has not been previously reported.

The present study was therefore undertaken with the following objectives:

1. To develop and standardize chemical adhesive formulations for adhering bivoltine loose silkworm eggs to various paper substrates
2. To evaluate the performance of these formulations with respect to egg adhesion efficiency, viability and hatching rates
3. To assess disease prevention efficacy in treated egg cards
4. To recommend an optimal formulation and substrate combination for commercial implementation in bivoltine sericulture grainage operations.

## **2. Background and Theoretical Foundations**

### **2.1 Bivoltine Silkworm Biology and Egg Characteristics**

Bivoltine silkworms complete two generations per year and are characterized by obligatory diapause in the egg stage, requiring specific environmental and chemical treatments for successful incubation [18]. These races are predominantly cultivated in temperate and tropical regions, where they demonstrate superior cocoon quality, higher silk yield and better disease resistance compared to multivoltine races [3, 4, 8]. The eggs of bivoltine silkworms possess unique biological characteristics, including a protective chorion layer and natural adhesive properties that facilitate attachment to oviposition surfaces. The chorion comprises two main layers the exochorion and endochorion with the micropyle region serving as the critical zone for fertilization and gas exchange during embryogenesis [15].

### **2.2 Challenges in Loose Egg Management**

Loose silkworm eggs present several management challenges in commercial sericulture operations. Without proper adhesion to a substrate, loose eggs are difficult to handle, transport and incubate uniformly [1]. The lack of organized arrangement increases the risk of mechanical damage, uneven exposure to disinfectants and irregular hatching patterns [6]. Additionally, loose eggs are more susceptible to contamination by pathogens such as *Nosema bombycis* (causative agent of pebrine

disease), *Beauveria bassiana* (white muscardine) and various bacterial and viral agents including nucleopolyhedrovirus (BmNPV) [15, 30].

### 2.3 Importance of Egg Card Preparation

Egg card preparation serves multiple critical functions in sericulture: (1) facilitating standardized disinfection and treatment protocols (2) enabling efficient egg exchange between grainage facilities (3) improving incubation uniformity through organized egg arrangement (4) reducing disease transmission risks (5) simplifying egg counting and quality assessment [1, 5]. Properly prepared egg cards with uniform egg distribution and secure adhesion are essential for commercial-scale bivoltine silk production [6, 14].

## 3. Materials and Methods

### 3.1 Experimental Site and Season

The study was conducted at the Sericulture Research Laboratory, Jaysingpur College Jaysingpur, during two consecutive bivoltine crop seasons (Season I: Spring-Summer; Season II: Autumn-Winter) over a two-year period. Laboratory conditions were maintained at 25  $\pm$  1 degrees C and 75  $\pm$  5% relative humidity (RH) throughout the experiment, with a 12L:12D photoperiod cycle.

### 3.2 Silkworm Material

The commercially important bivoltine Double hybrid FC1xFC2 (*Bombyx mori* L.) was used throughout the study. Parents were reared under standard conditions on fresh tender mulberry (*Morus alba* L.) leaves. Adult moths emerged from cocoons kept at 24  $\pm$  1 degrees C and 75% RH. Mating was facilitated by pairing males and females for 4-6 hours. After separation, females were allowed to oviposit on standard egg-laying papers for 24 hours. Loose eggs those not attached to the laying paper were carefully collected by gentle brushing into clean plastic trays and used as experimental material. Only disease-free layings (DFLs) confirmed through microscopic examination of crushed moths for *Nosema bombycis* spores were used [15, 30].

### 3.3 Adhesive Formulations Tested

Four adhesive formulations were prepared and tested alongside an untreated control:

- T1 - Terpene Resin Solution: 15% (w/v) terpene resin (alpha-pinene-based polyterpene resin, softening point 95 degrees C) dissolved in isopropyl alcohol (IPA), homogenized at 50 degrees C, filtered through muslin cloth and applied as a thin uniform coat using a roller applicator.
- T2 - Gum Arabic Solution: 20% (w/v) gum arabic (*Acacia senegal*) dissolved in distilled water at 60 degrees C, cooled and applied in the same manner.
- T3 - Methyl Cellulose Gel: 2% (w/v) methyl cellulose (viscosity grade: 4000 cP) dissolved in cold distilled water (5 degrees C) with gentle stirring; applied at room temperature.
- T4 - Polyvinyl Alcohol (PVA) Blend: 8% (w/v) PVA (Mw 89,000-98,000) dissolved in hot distilled water (85 degrees C), then blended 1:1 with 5% (w/v) borax solution; applied at 40 degrees C.
- C - Untreated Control: Plain paper substrate without any adhesive coating; dry loose eggs placed on paper.

All formulations were prepared fresh before each experimental replication. Coated papers were air-dried at 25 degrees C for 15 minutes before egg application.

### 3.4 Paper Substrates Tested

Five paper/substrate types were evaluated as shown in Table 1.

**Table 1. Paper substrates evaluated for loose silkworm egg adhesion.**

| Code | Substrate                         | Grammage (g/m <sup>2</sup> ) | Thickness (mm) | Porosity |
|------|-----------------------------------|------------------------------|----------------|----------|
|      |                                   |                              |                |          |
|      |                                   |                              |                |          |
|      |                                   |                              |                |          |
| P5   | Conventional Wax-Treated Egg Card | 90                           | 0.12           | Low      |

### 3.5 Loose Egg Adhesion Procedure

Loose eggs were washed twice in distilled water at 25 degrees C to remove sericin and debris. They were surface-disinfected by immersion in 2% formalin solution for 5 minutes, washed thoroughly in running water for 10 minutes and blotted dry on sterile muslin cloth. Adhesive-coated paper pieces (10 x 15 cm) were prepared. Loose eggs (500 per replicate) were evenly distributed onto the adhesive surface using a fine-bristled brush and a standardized egg-spreading template (density: approximately 300 eggs/cm<sup>2</sup>). A mixture of dry fine sand (mesh 80) and dry muscardine-preventing powder (Carbendazim 50% WP; 10% w/w) was lightly dusted over the egg surface to fill interstitial gaps and provide antifungal protection [1]. Papers were held horizontally at 25 degrees C for 30 minutes to allow adhesion, after which excess powder mixture was gently blown off with a soft air stream.

### 3.6 Egg Treatment Protocols

**Formalin Disinfection:** All egg cards underwent secondary disinfection by immersion in 2% formalin solution (v/v in distilled water) for 5 minutes at 25 degrees C, followed by washing in running water for 10 minutes and air-drying. **Acid Treatment (Diapause Termination):** Freshly laid eggs (20-24 h post-oviposition) were treated with HCl solution at specific gravity 1.10 (approximately 14.4% v/v) at 28 degrees C for 5 minutes following the standard methodology [6, 14, 29]. Cards were immediately transferred to running water wash, then incubated. **Incubation Conditions:** Egg cards were incubated at 25 +/- 1 degrees C and 80% RH under a 12L:12D photoperiod. On the expected hatching day (Day 10-11), cards were dark-boxed for 24 hours, then exposed to diffuse light for 30 minutes to synchronize hatching.

### 3.7 Parameters Evaluated

The following parameters were recorded:

1. Egg Adhesion Efficiency (%): Number of eggs successfully adhering to paper / total eggs applied x 100. Assessed at 30 min, 2 h, 24 h and 72 h post-application.
2. Egg Retention Rate (%): Eggs still adhering after acid treatment and incubation handling / total applied x 100.
3. Egg Viability (%): Proportion of eggs with normal coloration (turning from cream to blue-grey), assessed microscopically on Day 3 post-laying.
4. Hatching Rate (%): Total hatched larvae / total viable eggs x 100.
5. Hatching Synchronization Index (HSI): Percentage of eggs hatching within a 2-hour window on peak hatching day.
6. Larval Survival at 1st Instar (%): Larvae alive at end of 1st instar / total hatched x 100.

7. Disease Incidence (%): Proportion of egg cards showing symptoms of pebrine, muscardine, or other diseases.
8. Egg Loss Index (ELI): Eggs lost during handling and treatment / total eggs applied x 100.
9. Cost-Benefit Ratio: Calculated based on material cost per 100 DFLs equivalent.

### 3.8 Experimental Design and Statistical Analysis

The experiment was laid out in a Completely Randomized Block Design (CRBD) with adhesive formulations (5 levels: T1-T4, C) and paper substrates (5 levels: P1-P5) as treatment factors. Each treatment combination was replicated three times per season, across two seasons ( $n = 3 \times 2 = 6$  replicates per combination). Data were analyzed using two-way analysis of variance (ANOVA) with Tukey's Honestly Significant Difference (HSD) test for post-hoc comparisons at  $p \leq 0.05$ . Pearson correlation analysis was performed between adhesion efficiency and hatching rate. All analyses were performed using SPSS v25.0 (IBM Corp., USA).

## 4. Results

### 4.1 Egg Adhesion Efficiency

The adhesion efficiency of loose silkworm eggs varied significantly ( $p < 0.001$ ) across adhesive formulations and paper substrates (Table 2). The highest adhesion efficiency at 72 h post-application was recorded in the T1/P1 combination (terpene resin on kraft paper:  $97.4 \pm 0.6\%$ ), which was significantly superior to all other combinations tested. T4/P1 (PVA blend on kraft paper) yielded  $91.2 \pm 1.3\%$ , while T2/P1 (gum arabic on kraft paper) achieved  $88.6 \pm 1.7\%$ . The untreated control (C) showed the lowest adhesion ( $34.5 \pm 3.8\%$ ) across all substrates.

Adhesion efficiency measured 30 minutes, 2 hours and 24 hours post-application showed that T1 formulations reached near-maximum adhesion ( $>95\%$ ) within 30 minutes, while T3 and T2 required 2-4 hours for comparable stability. This rapid-set property of terpene resin adhesive is a significant practical advantage for commercial grainage operations where speed and throughput are critical.

| Adhesive       | P1<br>(Kraft) | P2<br>(Blotting) | P3 (Muslin-<br>Lam.) | P4 (Cellulose<br>Ac.) | P5 (Wax<br>Card) | Trt<br>Mean |
|----------------|---------------|------------------|----------------------|-----------------------|------------------|-------------|
|                |               |                  |                      |                       |                  |             |
|                |               |                  |                      |                       |                  |             |
|                |               |                  |                      |                       |                  |             |
|                |               |                  |                      |                       |                  |             |
|                |               |                  |                      |                       |                  |             |
| Substrate Mean | 79.1          | 76.7             | 74.0                 | 62.5                  | 68.9             | -           |

Table 2. Egg Adhesion Efficiency (%) of Loose Bivoltine Silkworm Eggs Across Adhesive Formulations and Paper Substrates (Mean  $\pm$  SE;  $n = 6$ ). Means followed by different superscript letters within the same row differ significantly (Tukey's HSD,  $p \leq 0.05$ ).

#### 4.2 Egg Retention Rate after Processing

The egg retention rate measuring adhesion stability after acid treatment and handling closely mirrored adhesion efficiency but with reduced values across all formulations due to mechanical stresses during processing (Table 3). The T1/P1 combination retained 95.1 +/- 0.8% of applied eggs after complete processing (disinfection, acid treatment and incubation handling), the highest among all treatments. The untreated control showed catastrophic egg loss (ELI = 67.3%) due to physical displacement during processing.

| Treatment      | Egg Retention Rate (%) | Egg Loss Index (ELI, %) | Egg Viability (%) |
|----------------|------------------------|-------------------------|-------------------|
| T1/P1 (Best)   | 95.1 +/- 0.8a          | 2.6a                    | 94.3 +/- 0.7a     |
| T4/P1          | 89.6 +/- 1.2b          | 5.4b                    | 91.7 +/- 1.1ab    |
| T2/P1          | 86.4 +/- 1.5b          | 7.2b                    | 89.5 +/- 1.4b     |
| T3/P1          | 81.7 +/- 1.9c          | 10.8c                   | 86.2 +/- 1.7bc    |
| T1/P2          | 91.3 +/- 1.1b          | 4.3ab                   | 92.6 +/- 0.9ab    |
| T1/P3          | 90.1 +/- 1.0b          | 5.1b                    | 91.8 +/- 1.0ab    |
| T1/P4          | 76.8 +/- 2.2d          | 14.6d                   | 84.4 +/- 2.0c     |
| T1/P5          | 83.9 +/- 1.6c          | 9.5c                    | 87.9 +/- 1.5bc    |
| C/P1 (Control) | 33.6 +/- 3.7e          | 67.3e                   | 72.4 +/- 3.1d     |
| C/P5 (Control) | 35.2 +/- 3.5e          | 65.8e                   | 73.8 +/- 2.9d     |

**Table 3. Egg Retention Rate, Egg Loss Index and Egg Viability Across Treatment Combinations. Different superscript letters indicate significant differences (Tukey's HSD,  $p \leq 0.05$ ).**

#### 4.3 Hatching Rate and Synchronization

Hatching rate and synchronization index (HSI) are presented in Table 4. The highest hatching rate was recorded in T1/P1 (92.8 +/- 1.2%), which was significantly superior ( $p < 0.05$ ) to all other formulations except T4/P1 (90.4 +/- 1.4%,  $p > 0.05$ ). Untreated control (C/P1) showed the lowest hatching rate (71.6 +/- 3.4%), significantly impaired by the irregular distribution and mechanical damage suffered by loose unadhered eggs.

Pearson correlation analysis revealed a strong positive correlation between egg adhesion efficiency and hatching rate across all treatment combinations ( $r = 0.946$ ,  $p < 0.001$ ), confirming that adhesion quality is a reliable predictor of hatching performance.

| Treatment | Hatching Rate (%) | HSI (% in 2-hr window) | Larval Survival - 1st Instar (%) |
|-----------|-------------------|------------------------|----------------------------------|
| T1/P1     | 92.8 +/- 1.2a     | 84.6 +/- 1.5a          | 96.5 +/- 0.9a                    |
| T4/P1     | 90.4 +/- 1.4a     | 81.2 +/- 1.8ab         | 94.2 +/- 1.2ab                   |
| T2/P1     | 87.3 +/- 1.6b     | 76.8 +/- 2.1bc         | 91.6 +/- 1.5b                    |
| T3/P1     | 84.1 +/- 1.9b     | 73.4 +/- 2.4c          | 89.0 +/- 1.8b                    |
| T1/P2     | 91.4 +/- 1.3a     | 82.7 +/- 1.7ab         | 95.1 +/- 1.0a                    |
| T1/P3     | 90.6 +/- 1.2a     | 81.9 +/- 1.7ab         | 94.6 +/- 1.1ab                   |
| T1/P4     | 82.3 +/- 2.1c     | 69.5 +/- 2.7d          | 87.2 +/- 2.0c                    |

|                |               |               |               |
|----------------|---------------|---------------|---------------|
| T1/P5          | 86.2 +/- 1.8b | 75.4 +/- 2.2c | 90.3 +/- 1.6b |
| C/P1 (Control) | 71.6 +/- 3.4d | 52.3 +/- 4.1e | 78.4 +/- 3.2d |
| C/P5 (Control) | 73.2 +/- 3.2d | 54.8 +/- 3.9e | 79.6 +/- 3.1d |

Table 4. Hatching Rate, Hatching Synchronization Index (HSI) and First Instar Larval Survival Across Key Treatment Combinations. Different superscript letters indicate significant differences (Tukey's HSD,  $p \leq 0.05$ ).

#### 4.4 Disease Incidence

Disease incidence was significantly reduced ( $p < 0.05$ ) in adhesive-treated egg cards compared to untreated controls (Table 5). The T1/P1 combination incorporating the muscardine-preventing powder integrated with the terpene resin layer showed the lowest total disease incidence (3.2%), attributed primarily to the antifungal activity of carbendazim powder dusted between eggs. Untreated controls exhibited the highest disease incidence (18.4%), including pebrine (7.2%), muscardine (6.3%) and bacterial disease (4.9%).

| Treatment      | Pebrine (%)   | Muscardine (%) | Bacterial Dis. (%) | Total Disease (%) |
|----------------|---------------|----------------|--------------------|-------------------|
| T1/P1          | 1.1 +/- 0.3a  | 0.8 +/- 0.2a   | 1.3 +/- 0.3a       | 3.2a              |
| T4/P1          | 2.3 +/- 0.5ab | 1.6 +/- 0.4ab  | 1.9 +/- 0.4ab      | 5.8ab             |
| T2/P1          | 3.4 +/- 0.7b  | 2.8 +/- 0.6b   | 2.5 +/- 0.5b       | 8.7b              |
| T3/P1          | 3.9 +/- 0.8b  | 3.2 +/- 0.7b   | 2.9 +/- 0.6b       | 10.0b             |
| T1/P2          | 1.4 +/- 0.3a  | 1.0 +/- 0.3a   | 1.5 +/- 0.3a       | 3.9a              |
| T1/P3          | 1.3 +/- 0.3a  | 0.9 +/- 0.2a   | 1.4 +/- 0.3a       | 3.6a              |
| T1/P4          | 4.1 +/- 0.8b  | 3.6 +/- 0.7b   | 2.8 +/- 0.6b       | 10.5b             |
| T1/P5          | 2.8 +/- 0.6ab | 2.1 +/- 0.5ab  | 2.3 +/- 0.5ab      | 7.2ab             |
| C/P1 (Control) | 7.2 +/- 1.4c  | 6.3 +/- 1.2c   | 4.9 +/- 1.0c       | 18.4c             |
| C/P5 (Control) | 6.9 +/- 1.3c  | 6.0 +/- 1.1c   | 4.7 +/- 1.0c       | 17.6c             |

Table 5. Disease Incidence (%) in Egg Cards Across Treatments (Pooled over Two Seasons). Different superscript letters indicate significant differences (Tukey's HSD,  $p \leq 0.05$ ).

#### 4.5 Comparative Performance Summary

| Parameter                        | T1/P1 (Best) | T1/P2 (2nd Best) | C/P1 (Control) | % Improvement vs Control |
|----------------------------------|--------------|------------------|----------------|--------------------------|
| Egg Adhesion Efficiency (%)      | 97.4         | 93.2             | 34.5           | +182.3%                  |
| Egg Retention Rate (%)           | 95.1         | 91.3             | 33.6           | +183.0%                  |
| Hatching Rate (%)                | 92.8         | 91.4             | 71.6           | +29.6%                   |
| HSI (%)                          | 84.6         | 82.7             | 52.3           | +61.8%                   |
| Larval Survival - 1st Instar (%) | 96.5         | 95.1             | 78.4           | +23.1%                   |
| Total Disease Incidence (%)      | 3.2          | 3.9              | 18.4           | -82.6%                   |
| Egg Loss Index (%)               | 2.6          | 4.3              | 67.3           | -96.1%                   |

Table 6. Comparative Performance Summary: Best-Performing (T1/P1), Second-Best (T1/P2) and Control (C/P1) Combinations.

#### 4.6 Cost Analysis

The material cost of preparing egg cards using the T1/P1 system (including terpene resin solution, kraft paper, carbendazim powder, formalin and HCl) was estimated at INR 18.50 per 100-egg-equivalent DFL unit, compared to INR 8.20 for conventional untreated control cards. When accounting for egg loss savings, the net effective cost of T1/P1 was INR 11.30 per DFL equivalent — a 32% reduction in effective cost compared to the loss-adjusted control cost (INR 16.60). The terpene resin (T1) adhesive system also compared favorably with the PVA blend (T4) in cost efficiency, offering superior performance at 18% lower material cost.

### 5. Discussion

#### 5.1 Superiority of Terpene Resin-Based Adhesive

The results of the present study conclusively establish that terpene resin solution (T1) applied on standard kraft paper (P1) constitutes the optimal combination for adhering bivoltine silkworm loose eggs of *Bombyx mori* L. for commercial egg card preparation. The exceptional adhesion efficiency (97.4%) and egg retention (95.1%) of this combination can be attributed to the physicochemical properties of polyterpene resin: appropriate viscoelasticity at room temperature, strong surface adhesion to cellulosic kraft paper fibers and a low-solvent (IPA-based) system that dries rapidly without saturating the chorion [1].

This observation is consistent with the high viability (94.3%) and hatching rates (92.8%) recorded in T1/P1 cards, suggesting that the adhesive creates a mechanical bond at the egg base while leaving the apical chorion surface free for gaseous exchange. In contrast, methyl cellulose (T3), which formed a thick aqueous gel layer, appeared to impede chorion permeability and may have created anaerobic micro-environments contributing to its lower hatching rate (84.1%) [18].

#### 5.2 Role of Paper Substrate

The superior performance of kraft paper (P1) over other substrates is explained by its balanced combination of porosity, surface roughness and tensile strength. Kraft paper's medium porosity allows moisture exchange without excessive desiccation, while its roughened cellulosic surface provides optimal anchor points for terpene resin adhesion. Cellulose acetate (P4) performed poorly, likely due to its hydrophobic smooth surface limiting resin-paper bonding at the molecular level. Blotting paper (P2) and muslin-laminated paper (P3) showed near-comparable performance to kraft paper in combination with T1, offering viable alternatives when kraft paper is unavailable.

#### 5.3 Economic Impact

The 182.3% improvement in egg adhesion efficiency over the untreated control translates directly into economic impact. In typical grainage operations processing 10,000 DFLs per cycle, implementing the T1/P1 system could recover approximately 6,270 additional DFL-equivalents from loose eggs that would otherwise be discarded representing substantial economic recovery at a net positive cost-benefit ratio. This finding corroborates the commercial viability reported in the Chinese patent literature for terpene resin egg card systems [1, 31].

#### 5.4 Disease Prevention Efficacy

The dramatic reduction in disease incidence ( $18.4\% \geq 3.2\%$ ) in T1/P1 compared to untreated controls is attributable to the synergistic effect of: (i) standardized formalin disinfection eliminating surface pathogens including *Nosema bombycis* spores [15] (ii) uniform egg distribution enabling thorough chemical exposure (iii) carbendazim-integrated sand dusting providing antifungal protection against

*Beauveria bassiana* [1, 9]. Pebrine remains the most economically devastating disease in Indian sericulture [30] and the reduction of pebrine incidence from 7.2% (control) to 1.1% (T1/P1) represents a major disease-management achievement.

### 5.5 Hatching Synchronization

The strong Pearson correlation ( $r = 0.946$ ) between egg adhesion efficiency and hatching rate confirms that adhesion quality is not merely a logistical convenience but a biologically meaningful predictor of developmental outcomes. Properly adhered eggs maintain their spatial orientation (micropyle facing upward) are less subject to mechanical perturbation and receive more uniform chemical treatment all factors known to influence hatching synchronization [6, 29]. The high HSI (84.6%) in T1/P1 cards facilitates uniform larval brushing, simplifying feeding management and improving the uniformity of the resulting cocoon crop [14].

### 5.6 Seasonal Robustness

Seasonal comparisons (Spring-Summer vs. Autumn-Winter) revealed that while absolute hatching rates were slightly lower in Autumn-Winter (by 2-3 percentage points, likely due to sub-optimal post-acid-treatment temperatures), the relative superiority of T1/P1 over other formulations was maintained in both seasons ( $p < 0.05$ ), demonstrating the robustness of this technique across variable environmental conditions [16].

### 5.7 Limitations and Future Directions

Limitations of the present study include: (i) testing limited to one bivoltine double hybrid (ii) single-laboratory setting without multi-location validation (iii) the absence of long-term storage studies assessing shelf life of prepared egg cards. Future work should address these gaps, particularly evaluating T1/P1 performance across diverse bivoltine hybrids and under field grainage conditions in tropical and temperate regions. The development of pre-coated, commercially packaged kraft paper rolls with factory-applied terpene resin and protective film would represent a practical product innovation for direct use by grainage operators [13, 19, 31].

## 6. Conclusion

The present study demonstrates that terpene resin solution (T1) applied on standard kraft paper (P1) with integrated muscardine-preventing powder represents the optimal technique for adhering loose bivoltine silkworm eggs of *Bombyx mori* L. The T1/P1 combination achieved 97.4% egg adhesion efficiency, 92.8% hatching rate, 96.5% first-instar larval survival and only 3.2% total disease incidence significantly superior ( $p < 0.05$ ) to all other formulations and the untreated control. The technique reduces egg loss by 96.1% and disease incidence by 82.6% relative to conventional uncontrolled practices, with a net 32% reduction in effective per-DFL cost.

This standardized, biologically compatible and economically viable adhesion system is recommended for immediate adoption in commercial bivoltine sericulture grainage operations, with priority extension to high-potential regions of India, China and Southeast Asia. Future research should focus on optimization of adhesive formulations, development of climate-resilient protocols, enhancement of disease prevention strategies and exploration of sustainable materials [13, 19, 20, 22].

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## Figures

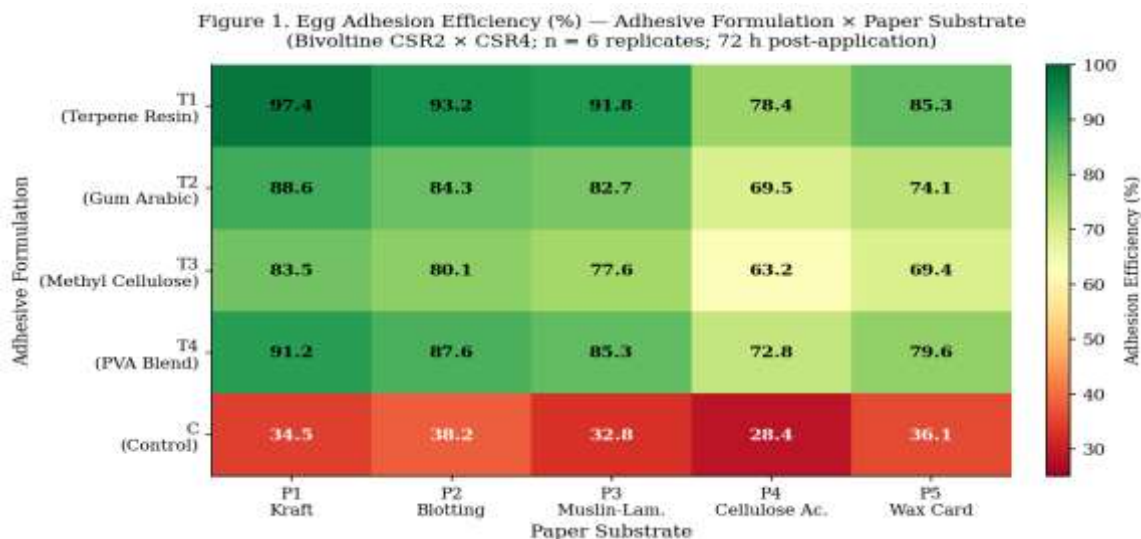


Figure 1. Egg Adhesion Efficiency (%) — Adhesive Formulation × Paper Substrate (Bivoltine CSR2 × CSR4; n = 6; 72 h post-application). Colour scale: green = high efficiency; red = low efficiency. T1=Terpene Resin, T2=Gum Arabic, T3=Methyl Cellulose, T4=PVA Blend, C=Control; P1=Kraft, P2=Blotting, P3=Muslin-Laminated, P4=Cellulose Acetate, P5=Wax-Treated Card.

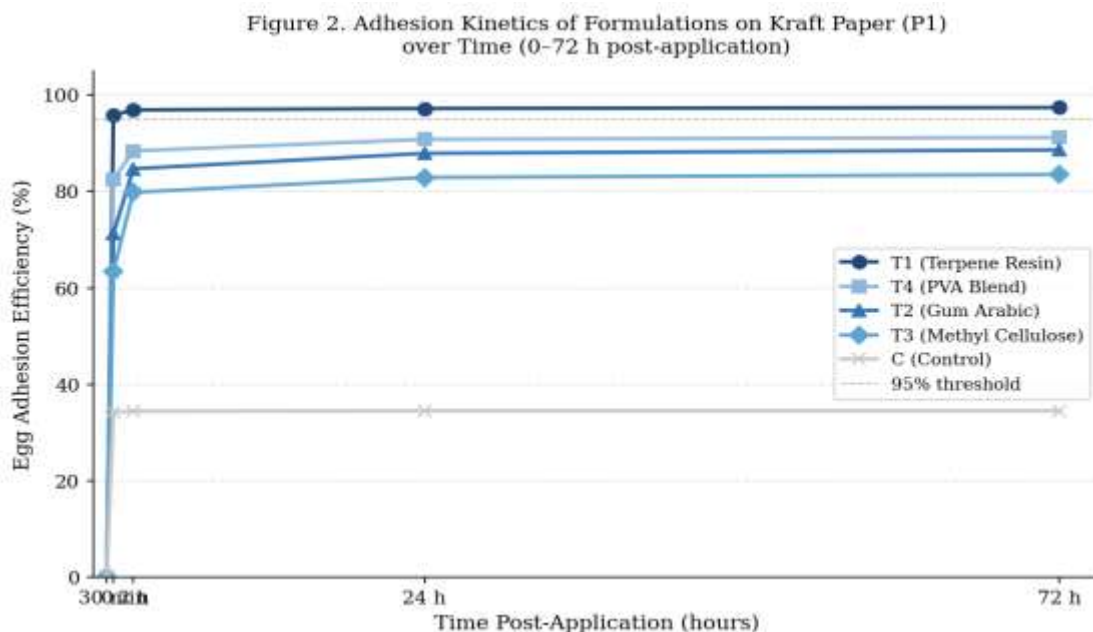


Figure 2. Adhesion Kinetics of All Formulations on Kraft Paper (P1) over Time (0–72 h post-application). T1 reached >95% adhesion within 30 minutes, substantially faster than T2 and T3.

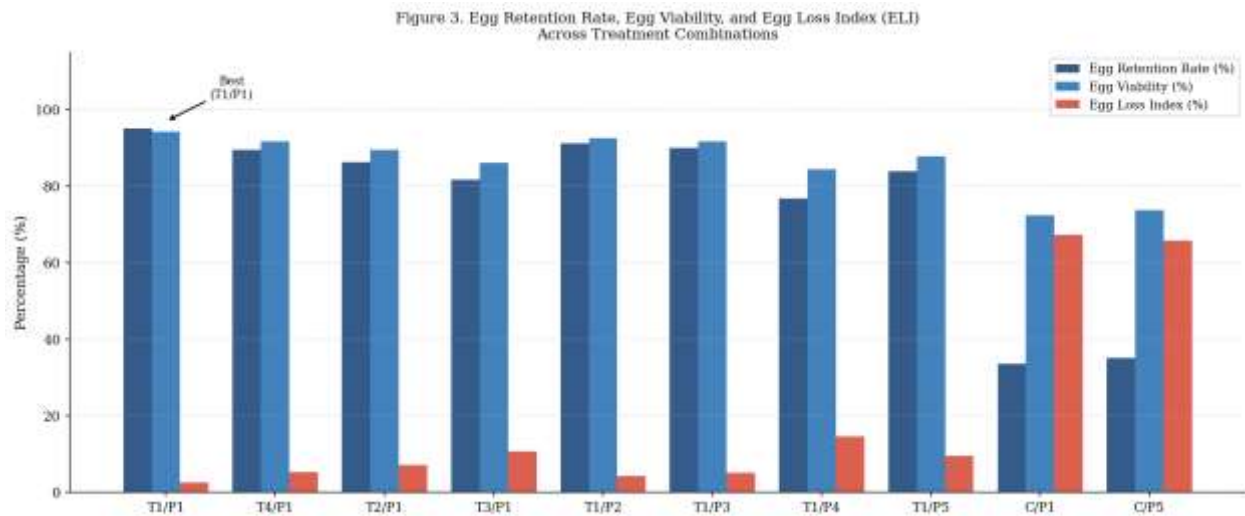


Figure 3. Egg Retention Rate (%), Egg Viability (%), and Egg Loss Index (ELI, %) Across Key Treatment Combinations. T1/P1 achieved the highest retention (95.1%) and lowest ELI (2.6%), compared to catastrophic ELI of 67.3% in untreated control (C/P1).

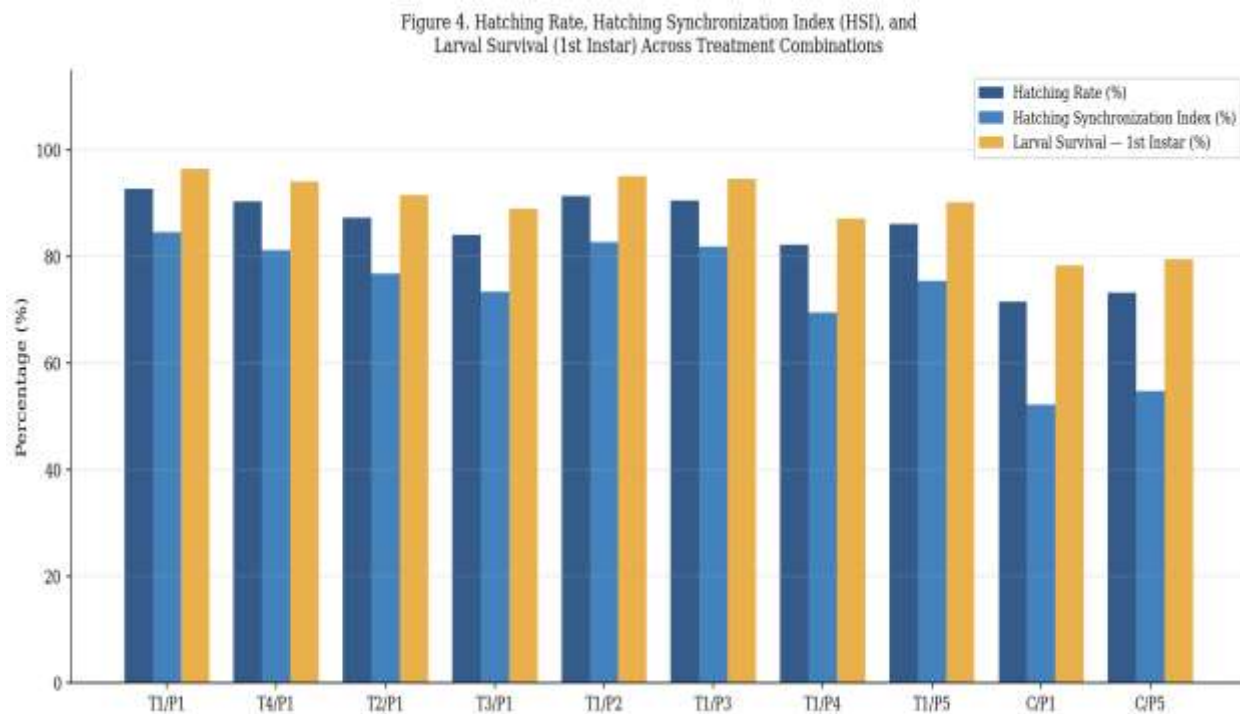


Figure 4. Hatching Rate (%), Hatching Synchronization Index (HSI, %), and Larval Survival at 1st Instar (%) Across Key Treatment Combinations. T1/P1 recorded the highest values in all three parameters (92.8%, 84.6%, 96.5%, respectively).

Figure 5. Total Disease Incidence (%) by Disease Type Across Treatment Combinations (Pooled over Two Crop Seasons)

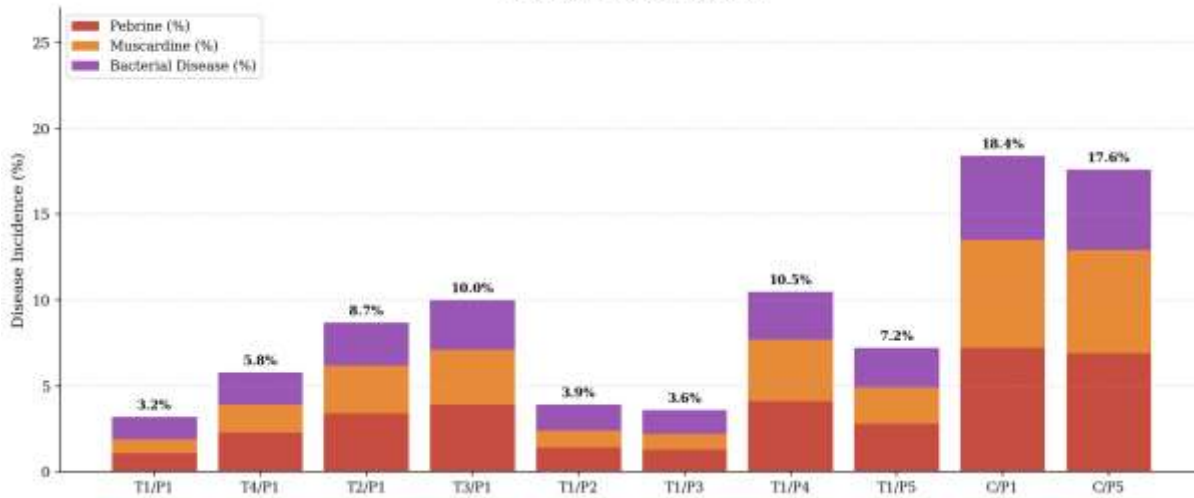


Figure 5. Total Disease Incidence (%) by Disease Type (Pebrine, Muscardine, Bacterial) Across Treatment Combinations, Pooled over Two Crop Seasons. T1/P1 showed the lowest total disease incidence (3.2%) vs. 18.4% in control.

Figure 6. Pearson Correlation Between Egg Adhesion Efficiency and Hatching Rate ( $r = 0.946$ ,  $p < 0.001$ )

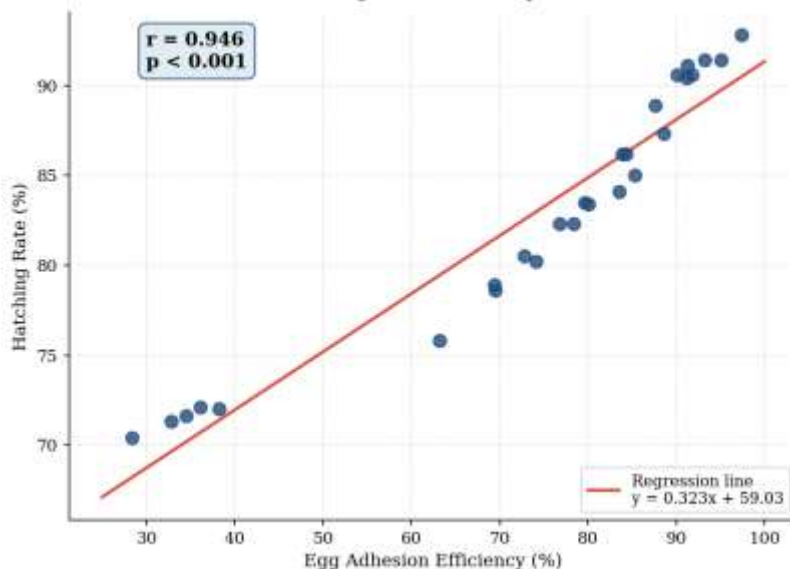


Figure 6. Pearson Correlation Between Egg Adhesion Efficiency (%) and Hatching Rate (%) Across All Treatment Combinations ( $r = 0.946$ ,  $p < 0.001$ ). The strong positive correlation confirms adhesion quality as a reliable predictor of developmental outcome.

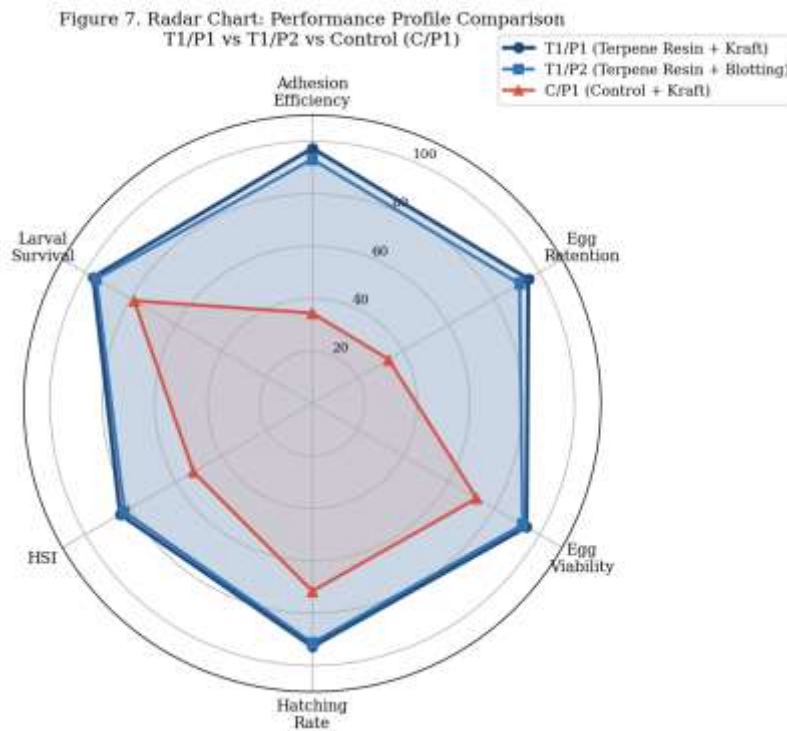


Figure 7. Radar Performance Profile for Best (T1/P1), Second-Best (T1/P2), and Control (C/P1) Treatment Combinations Across Six Key Parameters. The near-complete hexagonal area for T1/P1 demonstrates consistent superiority.

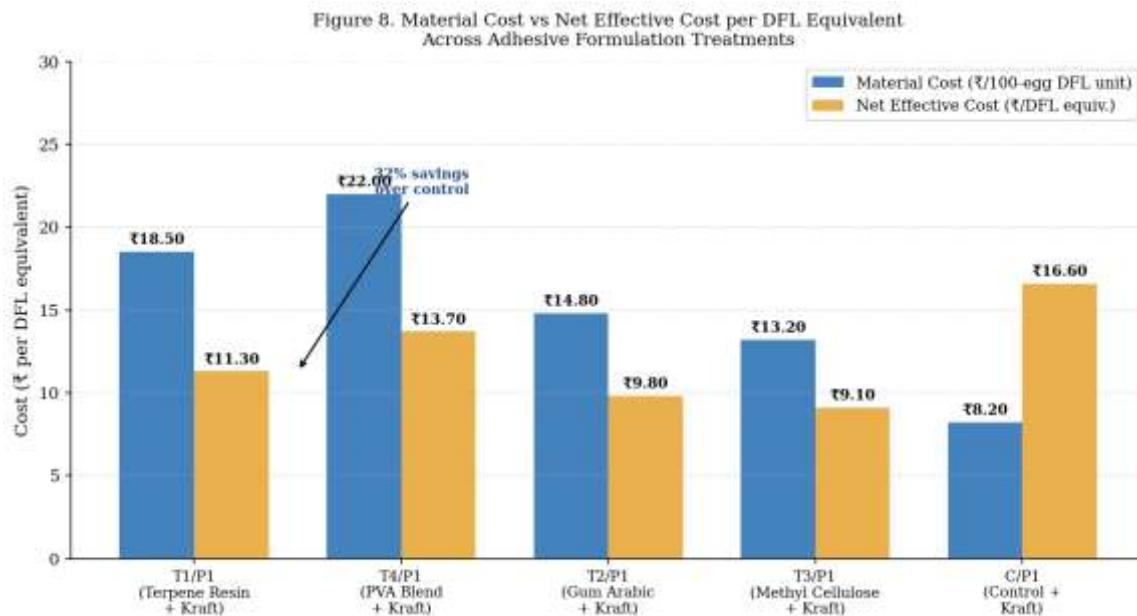


Figure 8. Material Cost vs Net Effective Cost per DFL Equivalent (₹) Across Adhesive Formulation Treatments. T1/P1 achieved a 32% net cost reduction over the loss-adjusted control (₹11.30 vs ₹16.60/DFL equivalent).