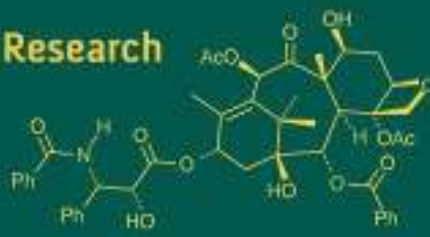
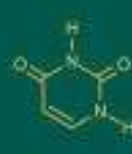
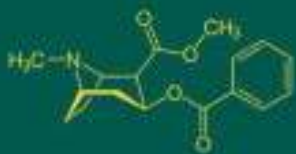


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Chitosan-infused resilience: Uplifting cocoon and post cocoon traits in silkworms infected with bacterial infections

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Abstract

To utilize waste silkworm pupae from the silk-reeling unit by extracting chitosan, an experiment was carried out to evaluate the efficacy of the extracted chitosan against bacterial diseases affecting the silkworm, *Bombyx mori* L. by assessing its impact on cocoon and post cocoon quality parameters. The efficacy of chitosan was evaluated by the bioassay technique using the leaf dip method against silkworms infected with bacterial pathogens, *Bacillus thuringiensis* and *Staphylococcus aureus*. Among the bacterial infected silkworm's, ampicillin applied at 1000 ppm showed the strongest resistance to bacterial stress and recorded the best compared to all the treatments. This was followed by the chitosan concentrations at 5000 ppm, which recorded the highest cocoon weight (1.64 g), pupal weight (1.31 g), shell weight (0.32 g), shell ratio (19.57%), filament length (936.41 m), filament weight (0.27 g) and filament denier (2.60) among the chitosan concentrations. All chitosan concentrations performed comparable to the standard ampicillin treatment and commercial chitosan at 1000 ppm. In contrast, bacterial-infected silkworms without any treatment (absolute control) recorded the lowest cocoon weight (1.44 g), pupal weight (1.16 g), shell weight (0.28 g), shell ratio (19.15%), filament length (733.41 m), filament weight (0.21 g) and filament denier (2.61). Meanwhile, the un-inoculated silkworms recorded the highest cocoon weight (2.04 g), pupal weight (1.62 g), shell weight (0.41 g), shell ratio (20.10%), filament length (1142.63 m), filament weight (0.33 g) and filament denier (2.58) among all the treatments. After the application of chitosan concentrations, *B. thuringiensis* infected silkworms resulted in lowest cocoon parameters with shorter and lighter filaments compared to *S. aureus* indicates the severity of pathogen. A clear dosage and time dependency was observed, where early application (6 HPI) and higher concentrations of chitosan (4500-5000 ppm) significantly enhanced resilience. The results suggest that silkworm-derived chitosan, especially at 5000 ppm, can approximate the effectiveness of antibiotics under controlled rearing conditions.

Keywords: *Bombyx mori*, flacherie, chitosan, cocoon, shell ratio, filament length, filament weight, filament denier, sericulture

Introduction

India stands as a global leader in silk production, with mulberry silk comprising approximately 70 per cent of its total output. The main contributors to this industry in the country are the southern states viz., Karnataka, Tamil Nadu, Telangana and Andhra Pradesh of which Karnataka plays the dominant role. Despite the setbacks due to COVID-19, India's raw silk output increased from 36,582 MT in 2022-23 to 38,913 MT in 2023-24. However, China maintains its lead by consistently producing more than 50,000 MT during the same periods. Further, India's silk export revenue also raised from ₹1,848.96 cores in 2022-23 to ₹2,027.56 cores in 2023-24 (Anon., 2024).

In sericulture, silkworm pupae account for about 60 per cent of the dry weight of cocoons, generating nearly 2 kg of dry pupae for every kilogram of raw silk harvested. India alone yields around 40,000 MT of dry pupae annually, much of which remains untapped. Globally, annual silk production of 1.6 million tonnes results in the generation of approximately 3 million tonnes of pupae, highlighting an immense opportunity for their reuse and valorization (Mahesh *et al.*, 2015) [7]. Nutritionally, silkworm pupae are a rich source of proteins (55 g), lipids (8.5 g) and energy (389.6 kcal per 100 g), along with key minerals such as potassium, phosphorus and magnesium and vitamins including B-complex, A and E.

(Longvah *et al.*, 2011) [6]. Once defatted, the remaining pupal biomass becomes a promising alternative source of chitin traditionally obtained from marine crustaceans. Through deacetylation, this chitin can be converted into chitosan which is known (Suresh *et al.*, 2012) [13].

Chitosan, known for its biocompatible, non-toxic and antibacterial properties, has attracted significant attention for applications in the field of medicine, agriculture, food preservation and environmental protection. Research has demonstrated that incorporating chitosan-enriched feed can enhance silkworm growth and its bactericidal effects which offer a natural substitute for synthetic bactericides.

Diseases of silkworms remain a major adversary in sericulture, with bacterial infections causing 15-47 per cent crop loss in India, higher than in China or Japan. Flacherie disease alone causes 33.88 per cent silkworm mortality, with the autumn season being particularly risky due to temperature shifts and the availability of poor-quality mulberry leaves. Bivoltine silkworm varieties are especially susceptible to diseases compared to multivoltine types (Tayal and Chauhan, 2017) [14].

Among bacterial afflictions, septicemia poses the greatest threat to silkworms (Choudhury *et al.*, 2004) [3], with prevalent pathogens including *Staphylococcus aureus*, *Bacillus thuringiensis*, *Serratia marcescens*, *Pseudomonas fluorescens* and *Escherichia coli*. Notably, *B. thuringiensis* and *S. aureus* are particularly destructive to *Bombyx mori* and the broader sericulture industry. Against this backdrop, recent *in-vivo* studies have focused on evaluating the antimicrobial efficacy of chitosan derived from silkworm pupae against these harmful bacterial pathogens.

Materials and Methods

The chitin and chitosan extraction involved mainly three steps *viz.*, deproteinization, demineralization and deacetylation. The procedure laid out by Suresh *et al.* (2012) [13] was followed for extraction of chitosan. Further, decolorization of chitosan was done by treating with 6 per cent H₂O₂ at 50 °C for 4 hours and resultant product was pure chitosan (Yunjian *et al.*, 2009) [16].

Twenty-five DFL's of third instar FC1×FC2 silkworm hybrid, were procured from the Registered Chawki Rearing Centre, for further rearing and experimentation. Silkworms were reared until the fourth moult by feeding with untreated mulberry leaves by following the standard recommended practices outlined by Dandin and Giridhar (2014) [4]. The pathogens were inoculated orally by feeding mulberry leaves to the silkworms smeared with bacterial suspensions, soon after fourth moult. The mulberry leaf bits of 100 cm² size was prepared and washed in running water and sterilized by using 70 per cent alcohol (by cotton swab). The sterilized leaves were shade dried and such leaves were smeared evenly with bacterial suspension of 10⁸ CFU/mL (*i.e.*, 0.27 mL/ replication of 50 worms) using non-absorbent cotton. The leaves were shade dried and fed to the silkworms and control batches of silkworms were fed only with non-inoculated surface sterilized mulberry leaves (Manjunath Gowda, 2009) [9].

The experiment was carried out in a completely randomized design with nine treatments which were replicated thrice. The treatments are chitosan @ 3500 ppm, 4000 ppm, 4500 ppm and 5000 ppm along with standard ampicillin @ 1000 ppm, commercial chitosan @ 1000 ppm, solvent control, absolute control and bacteria un-inoculated silkworms

The sterilized mulberry leaves were dipped in the above treatments for ten minutes and shade dried (Figure 1). The treated dried leaves were fed to the silkworms after 6 hours post inoculation (hpi), 12 hpi, 18 hpi, 24 hpi and 30 hpi of bacterial suspension. For subsequent feedings, inoculum free leaves suitable for the age was provided for both treated and untreated batches. Further, the ripe worms were hand-picked from each replication and were mounted separately on the mount ages for cocoon spinning. The montages were kept in the mounting hall to provide optimum environmental conditions for spinning. Later on, the cocoons were harvested manually on the sixth day of mounting and economic traits such as cocoon weight (g), pupal weight (g), shell weight (g), shell ratio (%), filament length (m), filament weight (g) and filament denier were recorded. Further, the data was analysed statistically for the test of significance using Fisher's method of analysis of variance as outlined by Sundarraj *et al.* (1972) [12]. The level of significance of F-test was at 5 per cent. The interpretation of data was done using critical difference (CD) values.

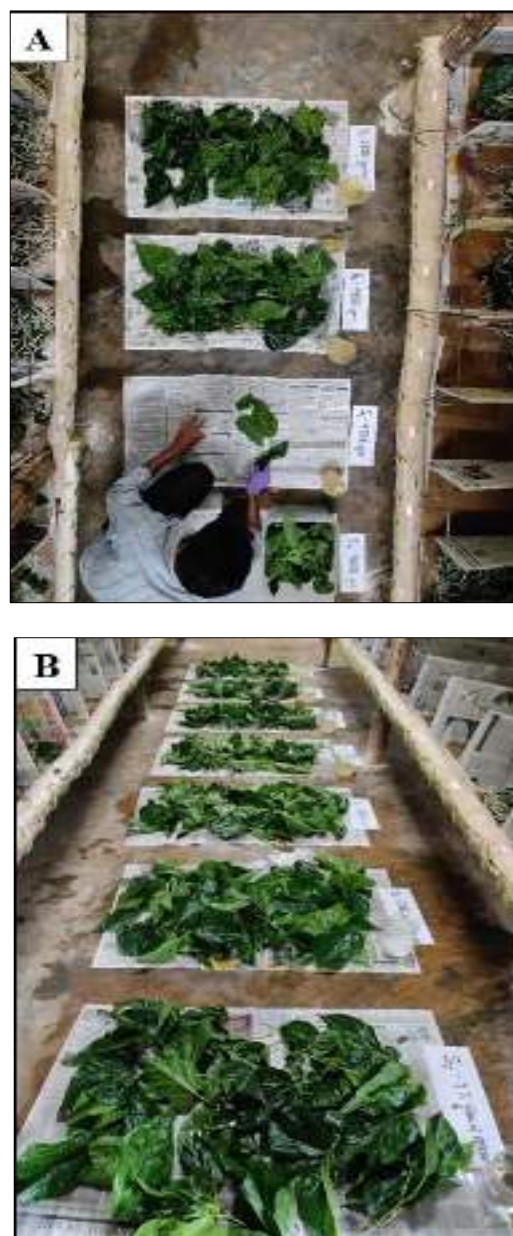


Fig 1: Application of the chitosan to the silkworms by leaf dip method; A-dipping of mulberry leaves in chitosan solution; B- Shade drying of treated mulberry leaves

Results and Discussion

Cocoon weight (g)

The weight of the cocoon was significantly influenced by bacterial strains (A), chitosan concentrations (B) and time of chitosan application after bacterial inoculation (C) for the

silkworms infected with *S. aureus* and *B. thuringiensis* (Table 1). Silkworms infected with *S. aureus* recorded higher overall cocoon weight (1.706 g) compared to those infected with *B. thuringiensis* (1.662 g).

Table 1: Effect of chitosan application on cocoon weight (g) in silkworm infected with *Staphylococcus aureus* and *Bacillus thuringiensis*

Treatments (B)	Time of chitosan application following bacterial inoculation (hpi) (C)										Bacterial strains (A) mean		Treatments (B) mean
	6 hpi		12 hpi		18 hpi		24 hpi		30 hpi				
	<i>Sa</i>	<i>Bt</i>	<i>Sa</i>	<i>Bt</i>	<i>Sa</i>	<i>Bt</i>	<i>Sa</i>	<i>Bt</i>	<i>Sa</i>	<i>Bt</i>	<i>Sa</i>	<i>Bt</i>	
Chitosan @ 3500 ppm	1.666	1.585	1.589	1.551	1.536	1.507	1.600	1.560	1.595	1.443	1.597	1.529	1.563
Chitosan @ 4000 ppm	1.667	1.637	1.624	1.595	1.670	1.468	1.695	1.560	1.566	1.441	1.644	1.540	1.592
Chitosan @ 4500 ppm	1.746	1.659	1.629	1.560	1.638	1.546	1.533	1.502	1.541	1.529	1.617	1.559	1.588
Chitosan @ 5000 ppm	1.796	1.686	1.654	1.608	1.622	1.602	1.669	1.565	1.656	1.545	1.679	1.601	1.640
Ampicillin @ 1000 ppm	1.916	1.808	1.851	1.771	1.794	1.722	1.810	1.713	1.688	1.660	1.812	1.735	1.773
Com. chitosan @ 1000 ppm	1.791	1.701	1.818	1.719	1.772	1.632	1.709	1.569	1.658	1.642	1.750	1.653	1.701
Solvent control	1.591	1.481	1.520	1.455	1.472	1.452	1.485	1.450	1.505	1.386	1.515	1.445	1.480
Absolute control	1.466	1.422	1.466	1.422	1.466	1.422	1.466	1.422	1.466	1.422	1.466	1.422	1.444
Bacteria un-inoculated	2.047	2.034	2.047	2.034	2.047	2.034	2.047	2.034	2.047	2.034	2.047	2.034	2.040
Mean	1.706		1.662		1.633		1.633		1.601		1.681	1.613	
Mean A, B, C	1.647										1.647		1.647

	A	B	AB	C	AC	BC	ABC
F-Test	*	*	*	*	NS	*	*
SEm ±	0.0025	0.0052	0.0074	0.0039	-	0.0117	0.0166
CD @ 5%	0.0049	0.0104	0.0146	0.0077	-	0.0232	0.0328

Note: * Significant; NS-Non-significant; A-Bacterial strains; B-Treatments; C-Hours of post inoculation (hpi); Sa-*Staphylococcus aureus*; Bt-*Bacillus thuringiensis*; Com.-Commercial chitosan

The chitosan concentrations (B) displayed significant differences in increasing the cocoon weight of the silkworms infected with the bacterial pathogens. In the bacteria un-inoculated batches, the cocoon weight remained consistently high (2.040 g), indicating the normal cocoon weight in the absence of bacterial infection. On the contrary, in the infected groups, ampicillin @ 1000 ppm recorded the highest cocoon weight (1.773 g), followed by commercial chitosan @ 1000 ppm (1.701 g) and silkworm pupal chitosan @ 5000 ppm (1.640 g). Lower cocoon weight was observed in the solvent control (1.480 g) and absolute control (1.444 g). This highlights the efficacy of pupal chitosan and ampicillin in minimizing the impact of bacterial infection supporting silkworm growth. Among chitosan concentrations, chitosan @ 5000 ppm showed the best results, suggesting a dose-dependent improvement in cocoon weight. The reduced cocoon weight in absolute and solvent control groups emphasize the severe impact of untreated bacterial infections on silkworm growth and development. Time of chitosan application after bacterial inoculation (C) also had a significant effect on cocoon weight. The highest cocoon weight was observed at 6 hpi (1.706 g), while cocoon weight declined with delayed time of chitosan application after bacterial inoculation such as 18 hpi (1.633 g) and 24 hpi (1.633 g). The decline in cocoon weight with delayed time of chitosan application after bacterial inoculation (18 to 30 hpi) suggests that timely intervention is crucial for minimizing the adverse effects of bacterial infection. The highest cocoon weight recorded at chitosan application at 6 hpi indicates the benefit of early treatment (Figure 2).

The interaction between bacterial strains, chitosan concentrations and time of chitosan application after bacterial inoculation significantly influenced the cocoon weight. The interaction between bacterial strains and chitosan concentrations (A×B) were significant, indicating

that cocoon weight varied depending on both the bacterial strain and the chitosan concentrations applied.

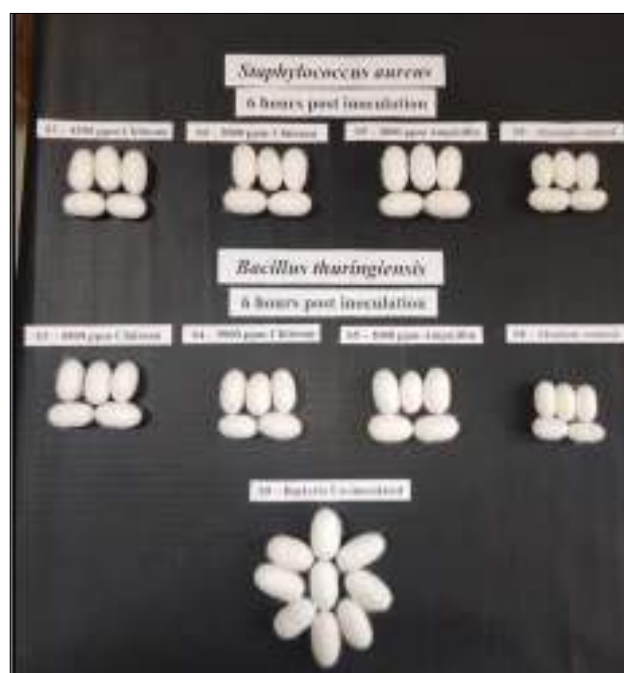


Fig 2: Cocoons of the *Staphylococcus aureus* and *Bacillus thuringiensis* infected silkworms treated (6 hpi) with the chitosan at different concentrations

For example, ampicillin maintained higher cocoon weight for both strains compared to other chitosan concentrations, while lower chitosan concentrations were less effective in it. In contrast, the interaction between bacterial strains and time of chitosan application after bacterial inoculation (A×C) was non-significant, indicating similar trends in weight reduction over time for both bacterial strains. However, the interaction between chitosan concentrations and time of chitosan application after bacterial inoculation

(B×C) was significant, revealing variation in the effectiveness of treatments at different time of application. Ampicillin and higher concentrations of chitosan consistently maintained higher weight across all time points, while lower doses of chitosan showed more pronounced reductions at later stages. Furthermore, the three-way interaction (A×B×C) was significant, demonstrating the influence of combination of bacterial strain, chitosan concentrations and its application time. For instance, ampicillin and high chitosan concentrations exhibit sustained weight maintenance for both bacterial strains across all time points, while solvent control and absolute controls consistently showed the least weights.

Among the interactions, highest cocoon weight (1.916 g) was observed with ampicillin @ 1000 ppm application 6 hours after the *S. aureus* inoculation. Among the chitosan concentrations, highest cocoon weight (1.796 g) was observed in the treatment of chitosan @ 5000 ppm application 6 hours after the *S. aureus* inoculation followed by 1.746 g of cocoon weight was observed with chitosan @ 4500 ppm application 6 hours after the *S. aureus* inoculation and 1.686 g of cocoon weight was observed with chitosan @ 5000 ppm application 6 hours after the *B. thuringiensis* inoculation. Similarly, bacteria un-inoculated silkworms

were found with highest cocoon weight of 2.047 g, on contrary the least cocoon weight (1.422 g) was observed in the silkworms of absolute control with the *B. thuringiensis* inoculation.

Overall, the study highlights the superior efficacy of ampicillin and chitosan-based concentrations in mitigating the negative effects of bacterial infection. Chitosan, particularly at higher concentrations, can serve as an effective and environmentally friendly alternative to antibiotics. Early chitosan application plays a critical role in ensuring optimal cocoon weight.

Pupal weight (g)

The effect of different dosages and time of application of chitosan concentrations on the pupal weight (g) of silkworms infected with *S. aureus* and *B. thuringiensis* were analysed and the results are presented in the table 2 which reveals that the bacterial strains (A) have significant impact on the pupal weight of silkworms. The pupal weight of silkworms infected with *B. thuringiensis* (1.288 g) were lower compared to those infected with *S. aureus* (1.341 g), highlights the relatively higher pathogenicity of *B. thuringiensis* and induces more severe stress and weight loss in silkworms.

Table 2: Effect of chitosan application on pupal weight (g) in silkworm infected with *Staphylococcus aureus* and *Bacillus thuringiensis*

Treatments (B)	Time of chitosan application following bacterial inoculation (hpi) (C)										Bacterial strains		Treatments (B) mean
	6 hpi		12 hpi		18 hpi		24 hpi		30 hpi		(A) mean		
	Sa	Bt	Sa	Bt	Sa	Bt	Sa	Bt	Sa	Bt	Sa	Bt	
Chitosan @ 3500 ppm	1.330	1.275	1.264	1.243	1.219	1.201	1.282	1.244	1.280	1.150	1.275	1.223	1.249
Chitosan @ 4000 ppm	1.327	1.325	1.297	1.270	1.332	1.175	1.341	1.247	1.255	1.141	1.310	1.232	1.271
Chitosan @ 4500 ppm	1.389	1.341	1.301	1.248	1.300	1.236	1.227	1.194	1.234	1.222	1.290	1.248	1.269
Chitosan @ 5000 ppm	1.433	1.361	1.309	1.281	1.290	1.277	1.337	1.237	1.325	1.234	1.339	1.278	1.308
Ampicillin @ 1000 ppm	1.532	1.444	1.473	1.413	1.432	1.373	1.444	1.365	1.352	1.327	1.446	1.384	1.415
Com. chitosan @ 1000 ppm	1.428	1.359	1.449	1.374	1.418	1.303	1.365	1.253	1.327	1.310	1.397	1.320	1.359
Solvent control	1.265	1.180	1.218	1.159	1.172	1.165	1.185	1.160	1.205	1.106	1.209	1.154	1.181
Absolute control	1.178	1.140	1.178	1.140	1.178	1.140	1.178	1.140	1.178	1.140	1.178	1.140	1.159
Bacteria un-inoculated	1.624	1.614	1.624	1.614	1.624	1.614	1.624	1.614	1.624	1.614	1.624	1.614	1.619
Mean	1.364		1.325		1.303		1.302		1.279		1.341	1.288	
Mean A, B, C	1.315										1.315		1.315

	A	B	AB	C	AC	BC	ABC
F-Test	*	*	*	*	NS	*	*
SEm ±	0.0026	0.0055	0.0078	0.0041	-	0.0124	0.0175
CD @ 5%	0.0051	0.0109	0.0154	0.0081	-	0.0244	0.0345

Note: * Significant; NS-Non-significant; A-Bacterial strains; B-Treatments; C-Hours of post inoculation (hpi); Sa-*Staphylococcus aureus*; Bt-*Bacillus thuringiensis*; Com.-Commercial chitosan

Pupal weight of the silkworm was also significantly influenced by the chitosan concentrations (B). Among the chitosan concentrations, those with higher concentrations of chitosan significantly reduced bacterial infection, resulting in higher pupal weight in the infected silkworms. This indicates a dosage-dependent effect of chitosan. Among the other concentrations, ampicillin at 1000 ppm showed the maximum pupal weight (1.415 g), followed by commercial chitosan at 1000 ppm (1.359 g) and chitosan at 5000 ppm (1.308 g). Lower pupal weight of 1.181 g and 1.159 g was observed with the solvent control and absolute control treatments respectively, confirming the adverse effect of infection without any treatment. The highest pupal weight was observed in bacteria un-inoculated silkworms, with a mean pupal weight of 1.619 g, indicating no bacterial infection and serving as a control.

The pupal weight significantly decreased as the time of chitosan application after bacterial inoculation (C) increased, particularly at 24 hpi and 30 hpi, reflecting the progression of infection. At 6 hpi, higher pupal weight (1.364 g) was observed compared to later time of application. The gradual decrease in pupal weight with increasing hours post-inoculation signifies the progressive impact of bacterial infection. The effect was more pronounced at 30 hpi (1.279 g), where the lowest weight was recorded, and indicating cumulative damage over time. The interactions between bacterial strains, chitosan concentrations and time significantly influenced pupal weight. The interaction between bacterial strains and chitosan concentrations (A×B) was significant, indicating variation of pupal weight with bacterial strain and the chitosan concentrations. For example, ampicillin and high

chitosan doses showed greater effectiveness in maintaining higher pupal weight for both strains, while untreated controls recorded the lowest weight. In contrast, the interaction between bacterial strains and time of chitosan application after bacterial inoculation (A×C) was found to be non-significant, indicating similar trend in pupal weight reduction over time for both the bacterial strains. However, the interaction between chitosan concentrations and time of chitosan application after bacterial inoculation (B×C) was significant, illustrating variation in the effectiveness of chitosan concentrations over time. Ampicillin and higher concentrations of chitosan consistently maintained better pupal weight across all time points, whereas untreated controls showed continuous weight decline. Furthermore, the three-way interaction (A×B×C) was significant, showing that pupal weight was influenced by the combined effect of bacterial strain, chitosan concentrations and time. For instance, ampicillin and higher doses of chitosan maintained higher weight consistently, while solvent and absolute controls showed the lowest weight across all time points and both strains.

Among the interactions of the infected silkworms, highest pupal weight (1.532 g) was noticed with ampicillin @ 1000 ppm application 6 hours after the *S. aureus* inoculation. Among the chitosan concentrations, highest pupal weight (1.433 g) was observed with chitosan @ 5000 ppm application 6 hours after the *S. aureus* inoculation followed by 1.389 g of pupal weight was observed with chitosan @ 4500 ppm application 6 hours after the *S. aureus* inoculation and 1.361 g of pupal weight was observed with chitosan @ 5000 ppm application 6 hours after the *B. thuringiensis* inoculation. Similarly, bacteria un-inoculated silkworms had the highest pupal weight of 1.624 g. Whereas, the least (1.140 g) was observed with chitosan @ 4000 ppm application 30 hours after the *B. thuringiensis* inoculation.

Shell weight (g)

Significant differences were observed in all factors, including bacterial strains (A), chitosan concentrations (B) and time of chitosan application after bacterial inoculation (C) (Table 3). For the effect of bacterial strains (A), across all the treatments and time points, silkworms infected with *B. thuringiensis* (0.315 g) showed consistently lower shell weight compared to those infected with *S. aureus* (0.330 g), indicating a higher pathogenicity of *B. thuringiensis*.

Significant differences in the shell weight were also observed by the chitosan concentrations (B). The highest shell weight was registered with bacteria un-inoculated treatment across all time points, with a mean shell weight of 0.410 g, indicating no bacterial stress and serving as the benchmark for healthy silkworms. Among infected silkworms, ampicillin @ 1000 ppm recorded the maximum shell weight (0.348 g), followed by commercial chitosan @ 1000 ppm (0.333 g) and chitosan @ 5000 ppm (0.321 g). The solvent control and absolute control showed significantly lower shell weight with means of 0.290 g and 0.277 g, respectively, confirming the adverse impact of bacterial infection without treatment. Shell weight decreased

progressively with delayed time of chitosan application after bacterial inoculation (C). The shell weight was highest at 6 hpi (0.332 g) and declined gradually with the lowest values observed at 30 hpi (0.313 g). This trend reflects the cumulative effect of bacterial infection over time. For example, in chitosan @ 5000 ppm, shell weight decreased from 6 hpi (0.353 g) to 30 hpi (0.321 g). The interactions between bacterial strains, chitosan concentrations and time of application significantly influenced shell weight. The interaction between bacterial strains and chitosan concentrations (A×B) was significant, with ampicillin and higher chitosan concentrations consistently maintaining higher shell weight for both strains. Furthermore, the interaction between bacterial strains and time of chitosan application after bacterial inoculation (A×C) was significant, indicating that shell weight declined more sharply over time for *B. thuringiensis*. The interaction between chitosan concentrations and time of chitosan application after bacterial inoculation (B×C) was also significant, revealing that ampicillin @ 1000 ppm and chitosan @ 5000 ppm concentrations showed better shell weight retention across time points. Notably, the three-way interaction (A×B×C) was significant, demonstrating that the combination of ampicillin or higher doses of chitosan with early application (6 to 12 hpi) was most effective in mitigating weight loss.

Among the infected silkworms, highest shell weight (0.374 g) was observed with ampicillin @ 1000 ppm application 6 hours after the *S. aureus* inoculation. Among the chitosan concentrations,

highest shell weight (0.353 g) was observed with chitosan @ 5000 ppm application 6 hours after the *S. aureus* inoculation followed by 0.347 g of shell weight with chitosan @ 4500 ppm application 6 hours after the *S. aureus* inoculation and 0.334 g of shell weight was observed with chitosan @ 5000 ppm application 6 hours after the *B. thuringiensis* inoculation. Similarly, it was observed that the highest shell weight of 0.413 g with bacteria un-inoculated silkworms. On the contrary, the least shell weight (0.274 g) was observed in absolute control with *B. thuringiensis* infection.

The study reveals that ampicillin is the most effective treatment in minimizing shell weight losses caused by bacterial infection, followed by high-dosage of chitosan concentrations. The higher dose of silkworm pupal chitosan (5000 ppm) performed better than lower doses, suggesting a dosage-dependent effect of chitosan in combating bacterial infection. Commercial chitosan also demonstrated substantial efficacy. *B. thuringiensis* has a greater detrimental effect on shell weight compared to *S. aureus* and the impact of infection worsens with time. Shell weight decreased significantly with increasing time of application. This progressive decline, particularly at 24 hpi and 30 hpi, indicates that bacterial infections intensify over time, causing cumulative stress and weight loss. Timely intervention with effective chitosan concentrations, especially at higher doses, is critical to improve shell weight in infected silkworms.

Table 3: Effect of chitosan application on shell weight (g) in silkworm infected with *Staphylococcus aureus* and *Bacillus thuringiensis*

Treatments (B)	Time of chitosan application following bacterial inoculation (hpi) (C)										Bacterial strains (A) mean		Treatments (B) mean
	6 hpi		12 hpi		18 hpi		24 hpi		30 hpi				
	<i>Sa</i>	<i>Bt</i>	<i>Sa</i>	<i>Bt</i>	<i>Sa</i>	<i>Bt</i>	<i>Sa</i>	<i>Bt</i>	<i>Sa</i>	<i>Bt</i>	<i>Sa</i>	<i>Bt</i>	
Chitosan @ 3500 ppm	0.326	0.301	0.315	0.298	0.307	0.297	0.309	0.307	0.306	0.285	0.313	0.298	0.305
Chitosan @ 4000 ppm	0.330	0.302	0.319	0.315	0.329	0.284	0.333	0.304	0.303	0.291	0.323	0.299	0.311
Chitosan @ 4500 ppm	0.347	0.311	0.319	0.303	0.328	0.300	0.297	0.299	0.298	0.298	0.318	0.302	0.310
Chitosan @ 5000 ppm	0.353	0.316	0.334	0.317	0.321	0.316	0.322	0.307	0.321	0.301	0.330	0.311	0.321
Ampicillin @ 1000 ppm	0.374	0.354	0.369	0.347	0.353	0.341	0.356	0.338	0.327	0.321	0.356	0.340	0.348
Com. chitosan @ 1000 ppm	0.353	0.334	0.356	0.336	0.346	0.320	0.335	0.307	0.322	0.323	0.342	0.324	0.333
Solvent control	0.316	0.293	0.292	0.288	0.292	0.277	0.291	0.283	0.291	0.272	0.296	0.283	0.290
Absolute control	0.279	0.274	0.279	0.274	0.279	0.274	0.279	0.274	0.279	0.274	0.279	0.274	0.277
Bacteria un-inoculated	0.413	0.407	0.413	0.407	0.413	0.407	0.413	0.407	0.413	0.407	0.413	0.407	0.410
Mean	0.332		0.327		0.321		0.320		0.313		0.330	0.315	
Mean A, B, C	0.323										0.323		0.323

	A	B	AB	C	AC	BC	ABC
F-Test	*	*	*	*	*	*	*
SEm ±	0.0011	0.0022	0.0032	0.0017	0.0024	0.0050	0.0071
CD @ 5%	0.0021	0.0044	0.0062	0.0033	0.0046	0.0099	0.0139

Note: * Significant; NS-Non-significant; A-Bacterial strains; B-Treatments; C-Hours of post inoculation (hpi); Sa-*Staphylococcus aureus*; Bt-*Bacillus thuringiensis*; Com.-Commercial chitosan

Shell ratio (%)

There was no significant difference in shell ratio between silkworms infected with two bacterial strains (A), the time of chitosan application after bacterial inoculation (C) and also their interactions (Table 4).

Shell ratio only showed a significant difference for the effect of chitosan concentrations (B), with the highest shell ratio observed in the bacteria un-inoculated group, which had a mean value of 20.10 per cent, showing the natural shell ratio in the absence of bacterial infection. Among the infected groups of silkworms, all the chitosan concentrations (3500 to 5000 ppm) were found to be on par with ampicillin at 1000 ppm, which recorded a shell ratio of 19.63 per cent. Increasing chitosan concentration did not lead to a clear improvement, suggesting a plateau effect. Ampicillin at 1000 ppm recorded the highest shell ratio of 19.63 per cent in bacteria infected silkworms, indicating its efficacy in mitigating the effect of bacterial infections. Commercial chitosan recorded a shell ratio of 19.59 per cent, similar to other chitosan concentrations. The absolute control group consistently recorded the lowest shell ratio of 19.15 per cent, emphasizing the negative impact of bacterial infection. The interactions between bacterial strains (A), chitosan concentrations (B) and time of chitosan application after bacterial inoculation (C) had varying effect on shell ratio. The interaction between bacterial strains and chitosan concentrations (A×B) was non-significant, suggesting that the chitosan concentrations had similar effects on both strains. On contrast, the interaction between bacterial strain and time of chitosan application after bacterial inoculation (A×C) was significant, with shell ratio declining more sharply over time for *B. thuringiensis*. However, the interaction between chitosan concentrations and time of chitosan application after bacterial inoculation (B×C) was non-significant, indicating that variation among chitosan concentrations were consistent across time points. The three-way interaction (A×B×C) was non-significant, indicating no interactive influence of all factors on shell ratio.

The improvement in the economic parameters (cocoon weight, pupal weight, shell weight and shell ratio) were found among the infected silkworms fed with the chitosan concentrations because the positively charged amino groups of chitosan form a strong bond with the teichoic acid present

in the bacterial cell membrane, inducing cell wall distortion and disruption. Consequently, this exposes the cell membrane to osmotic shock, triggering the leakage of cytoplasmic content (Vishu Kumar *et al.*, 2005) [15]. Furthermore, the binding of chitosan to teichoic acid, accompanied by the potential extraction of bacterial membrane lipid, ultimately culminates in bacterial death. This phenomenon was observed in the chitosan treated silkworm batches, leading to a significant enhancement of economic parameters compared to the absolute control and the solvent control among the batches treated with *B. thuringiensis* and *S. aureus*. Consequently, the highest concentration of chitosan (5000 ppm) yielded the most favourable economic parameters, including mean cocoon weight (16.79 g), pupal weight (13.39 g), shell weight (3.30 g) and shell ratio (19.67%), for silkworm infected with pathogens.

The present findings closely align with those of Priyadharshini *et al.* (2018) [11], who reported the highest economic parameters for silkworm infected with both pathogens after treatment with 2.5 per cent chitosan (25000 ppm). This treatment yielded the highest single cocoon weight (1.82 g for *S. aureus* and 1.62 g for *B. thuringiensis*), pupal weight (1.50 g for *S. aureus* and 1.34 g for *B. thuringiensis*) and shell weight (0.32 g for *S. aureus* and 0.27 g for *B. thuringiensis*). Manimegalai and Chandramohan (2008) [8] obtained similar results, with single cocoon weight of 1.79 g, shell weight of 0.33 g and shell ratio of 18.46 per cent, using a 1000 ppm treatment of doxycycline hydrogen chloride (an antibiotic) for silkworms infected with *B. thuringiensis*. Priyadharshini *et al.* (2009) [10] also found that treatment with botanicals, such as amla, yielded the best economic parameters for silkworms infected with *Bacillus* sp., with single cocoon weight of 1.76 g, shell weight of 0.30 g and shell ratio of 17.04 per cent. The results were also in accordance with Anusha and Bhaskar (2017) [2], who reported that lowest cocoon weight (1.000 g), single shell weight (0.100 g) and shell ratio percentage (10.00) were noticed in silkworm infected with bacterial combination of *Alcaligenes faecalis* + *Bacillus subtilis* + NPV followed by *A. faecalis* + *B. subtilis* + *Lysinibacillus sphaericus* combination with cocoon weight (1.12 g), single shell weight (0.12 g) and shell ratio percentage (10.71).

Table 4: Effect of chitosan application on shell ratio (%) in silkworm infected with *Staphylococcus aureus* and *Bacillus thuringiensis*

Treatments (B)	Time of chitosan application following bacterial inoculation (hpi) (C)										Bacterial strains (A) mean		Treatments (B) mean
	6 hpi		12 hpi		18 hpi		24 hpi		30 hpi				
	<i>Sa</i>	<i>Bt</i>	<i>Sa</i>	<i>Bt</i>	<i>Sa</i>	<i>Bt</i>	<i>Sa</i>	<i>Bt</i>	<i>Sa</i>	<i>Bt</i>	<i>Sa</i>	<i>Bt</i>	
Chitosan @ 3500 ppm	19.59	19.02	19.82	19.21	20.01	19.69	19.28	19.68	19.14	19.72	19.57	19.47	19.52
Chitosan @ 4000 ppm	19.80	18.41	19.64	19.74	19.70	19.36	19.64	19.45	19.33	20.17	19.62	19.43	19.53
Chitosan @ 4500 ppm	19.86	18.74	19.58	19.46	20.04	19.43	19.36	19.92	19.37	19.50	19.64	19.41	19.52
Chitosan @ 5000 ppm	19.65	18.71	20.23	19.75	19.78	19.73	19.29	19.66	19.40	19.47	19.67	19.46	19.57
Ampicillin @ 1000 ppm	19.52	19.58	19.91	19.62	19.68	19.79	19.67	19.74	19.40	19.35	19.64	19.62	19.63
Com. chitosan @ 1000 ppm	19.73	19.64	19.60	19.51	19.51	19.61	19.59	19.60	19.44	19.63	19.57	19.60	19.59
Solvent control	19.89	19.79	19.24	19.79	19.86	19.05	19.59	19.50	19.30	19.63	19.58	19.55	19.56
Absolute control	19.02	19.27	19.02	19.27	19.02	19.27	19.02	19.27	19.02	19.27	19.02	19.27	19.15
Bacteria un-inoculated	20.19	20.01	20.19	20.01	20.19	20.01	20.19	20.01	20.19	20.01	20.19	20.01	20.10
Mean	19.47		19.64		19.65		19.58		19.52		19.61	19.54	
Mean A. B. C	19.57										19.57		19.57

	A	B	AB	C	AC	BC	ABC
F-Test	NS	*	NS	NS	*	NS	NS
SEm ±	-	0.145	-	-	0.153	-	-
CD @ 5%	-	0.286	-	-	0.301	-	-

Note: * Significant; NS-Non-significant; A-Bacterial strains; B-Treatments; C-Hours of post inoculation (hpi); Sa-*Staphylococcus aureus*; Bt-*Bacillus thuringiensis*; Com.-Commercial chitosan

Filament length (m)

Significant differences were observed for bacterial strains (A), chitosan concentrations (B) and time of chitosan application after bacterial inoculation (C) showing that the effects of bacterial strains, chitosan concentrations and time of application acted independently. But there were no significant differences among the interactions between factors (A×B, A×C, B×C and A×B×C), (Table 5).

The filament length was significantly affected by the bacterial strain (A) where, *B. thuringiensis* infected silkworms generally exhibited lower filament lengths than *S. aureus* infected silkworms across all chitosan concentrations (B) and time of chitosan application after bacterial inoculation (C). The mean filament length for *S. aureus* infected silkworms was 923.96 m, compared to 895.38 m for *B. thuringiensis* infected silkworms, highlighting the

more severe impact of *B. thuringiensis* on silk production. Among the chitosan concentrations (B), the highest filament length was recorded in the bacteria un-inoculated group, with a constant mean of 1142.63 m, indicating the optimal filament length in the absence of bacterial infection. Among the infected groups, chitosan concentrations displayed a dose-dependent trend, with higher concentrations producing better results. The mean filament length ranged from 849.39 m to 936.41 m. Ampicillin @ 1000 ppm produced the longest mean filament length (988.84 m), suggesting its effectiveness in mitigating the adverse effects of bacterial infection. Commercial chitosan had a filament length of 956.03 m, comparable to the higher doses of chitosan. The absolute control exhibited the shortest filament length (733.41 m), reflecting the detrimental effects of bacterial infection without any chitosan concentrations (Figure 3).

Table 5: Effect of chitosan application on filament length (m) in silkworm infected with *Staphylococcus aureus* and *Bacillus thuringiensis*

Treatments (B)	Time of chitosan application following bacterial inoculation (hpi) (C)										Bacterial strains (A) mean		Treatments (B) mean
	6 hpi		12 hpi		18 hpi		24 hpi		30 hpi				
	Sa	Bt	Sa	Bt	Sa	Bt	Sa	Bt	Sa	Bt	Sa	Bt	
S ₁ : Chitosan @ 3500 ppm	890.25	880.50	901.13	848.06	894.38	830.63	843.75	819.94	792.75	792.56	864.45	834.34	849.39
S ₂ : Chitosan @ 4000 ppm	957.56	944.44	900.00	866.44	892.50	825.19	880.69	852.75	852.38	772.13	896.63	852.19	874.41
S ₃ : Chitosan @ 4500 ppm	952.88	938.81	957.00	885.56	903.00	856.69	900.75	803.25	832.88	787.13	909.30	854.29	881.79
S ₄ : Chitosan @ 5000 ppm	1024.88	998.81	958.69	966.94	951.00	922.13	936.94	904.13	845.25	855.38	943.35	929.48	936.41
S ₅ : Ampicillin @ 1000 ppm	1074.38	1042.50	1047.75	993.75	1007.25	996.00	954.94	937.88	912.00	921.94	999.26	978.41	988.84
S ₆ : Com. chitosan @ 1000 ppm	1029.38	1017.56	994.69	993.19	975.19	905.25	985.88	899.63	935.81	823.69	984.19	927.86	956.03
S ₇ : Solvent control	845.81	862.88	829.13	849.56	830.06	809.63	827.63	814.69	780.56	791.25	822.64	825.60	824.12
S ₈ : Absolute control	753.19	713.63	753.19	713.63	753.19	713.63	753.19	713.63	753.19	713.63	753.19	713.63	733.41
S ₉ : Bacteria un-inoculated	1142.63	1142.63	1142.63	1142.63	1142.63	1142.63	1142.63	1142.63	1142.63	1142.63	1142.63	1142.63	1142.63
Mean	956.26		930.22		908.39		895.27		858.21		923.96	895.38	
Mean A, B, C	909.67										909.67		909.67

	A	B	AB	C	AC	BC	ABC
F-Test	*	*	NS	*	NS	NS	NS
SEm ±	8.133	17.253	-	12.859	-	-	-
CD @ 5%	16.049	34.046	-	25.376	-	-	-

Note: * Significant; NS-Non-significant; A-Bacterial strains; B-Treatments; C-Hours of post inoculation (hpi); Sa-*Staphylococcus aureus*; Bt-*Bacillus thuringiensis*; Com.-Commercial chitosan

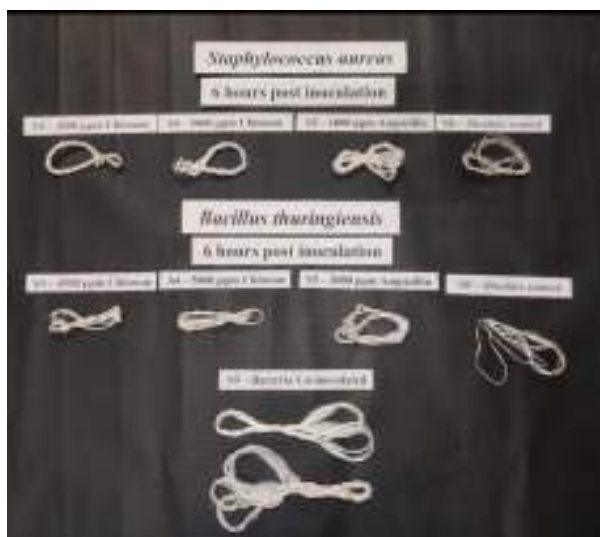


Fig 3: Filaments of the *Staphylococcus aureus* and *Bacillus thuringiensis* infected silkworms treated (6 hpi) with the chitosan at different concentrations

Time of chitosan application after bacterial inoculation (C) significantly affected filament length, with the highest filament lengths were observed at 6 hpi (956.26 m), followed by a gradual decline across delayed time of chitosan application after bacterial inoculation, with the lowest values recorded at 30 hpi (858.21 m). This trend indicates that earlier chitosan application after bacterial inoculation is critical for maintaining filament length, as the infection's impact becomes more pronounced over time. Filament length was highest at 6 hpi and progressively declined with time, emphasizing the importance of early intervention. Chitosan concentrations mitigated the adverse effects of bacterial infections but could not restore filament length to the level of the un-inoculated group. *B. thuringiensis* infected silkworms produced shorter filaments compared to *S. aureus* infected silkworms, highlighting the greater virulence of *B. thuringiensis*.

Filament weight (g)

The effect of dosage and time of chitosan application on filament weight in silkworms infected with *S. aureus* and *B. thuringiensis* was studied from the table 6. The analysis revealed significant effects of bacterial strains (A), chitosan concentrations (B) and time of chitosan application after bacterial inoculation (C) on filament weight, while interactions (A×B, A×C, B×C and A×B×C) were mostly non-significant, suggesting that the main factors independently influenced filament weight.

The filament weight was significantly affected by the bacterial strain (A), where the filament weight was slightly higher for *S. aureus* compared to *B. thuringiensis*, with means of 0.267 g and 0.253 g, respectively. This highlights the more severe impact of *B. thuringiensis* on filament weight, as well as the lesser impact of chitosan concentrations on *B. thuringiensis*.

The filament weight was also significantly affected by the chitosan concentrations (B). Among the groups of infected silkworms, chitosan at 5000 ppm maintained a significant filament weight (0.270 g) compared to lower doses of chitosan, with chitosan at 3500 ppm recording the least filament weight (0.240 g) among chitosan concentrations. Ampicillin at 1000 ppm recorded the highest filament weight (0.285 g), followed by commercial chitosan at 1000

ppm with 0.273 g. The bacteria un-inoculated group had the highest filament weight (0.328 g) across all time points, establishing the baseline for optimal filament production. The lowest filament weights were observed in the untreated groups, specifically the solvent control with 0.231 g and the absolute control with 0.213 g.

Time of chitosan application after bacterial inoculation (C) have the significant impact on filament weight where, it declined with delayed chitosan treatment application of bacteria post-inoculation, from 0.276 g at 6 hpi to 0.240 g at 30 hpi. Early intervention was more effective in mitigating the adverse effects of bacterial infection.

Filament denier

The study evaluated the impact of dosage and time of treatment application on filament denier in silkworms infected with *S. aureus* and *B. thuringiensis* (Table 7). The bacterial strains (A), chitosan concentrations (B), time of chitosan application after bacterial inoculation (C) and their interactions have the significant impact on the filament denier of the silkworm infected with the bacterial pathogens except the interaction of chitosan concentrations and the time of chitosan application after bacterial inoculation (B×C) which was non-significant.

The significant impact of filament denier by the bacterial strains (A) demonstrates that, *B. thuringiensis* slightly outperformed *S. aureus* in maintaining higher denier values, with an overall mean of 2.601 for *B. thuringiensis* and 2.541 for *S. aureus*.

The filament denier was also varied significantly by the effect of chitosan concentrations (B), where the highest filament denier was recorded in the absolute control at 2.613. All the chitosan concentrations from 3500-5000 ppm were found to be on par with ampicillin at 1000 ppm, with a filament denier of 2.596. Among the chitosan concentrations, the highest filament denier was observed in chitosan at 5000 ppm, with a denier of 2.596, followed by chitosan concentrations of 4500 ppm, 4000 ppm and 3500 ppm, which recorded filament deniers of 2.570, 2.549 and 2.548, respectively. Commercial chitosan showed competitive results (2.568), slightly lower than those of higher chitosan doses. Bacteria un-inoculated silkworms recorded a filament denier of 2.581, serving as the baseline without bacterial infection. Filament denier declined with delayed time of chitosan application after bacterial inoculation (C), from 2.594 at 6 hpi to 2.524 at 30 hpi.

Early chitosan application showed better results in maintaining denier. The highest denier was recorded for the batch with 6 hpi (2.594) followed by, 12 hpi (2.592), 18 hpi (2.582) and 24 hpi (2.561) were found to be on par with the 6 hpi.

The interactions between bacterial strains, chitosan concentrations and time significantly influenced filament denier. The interaction between bacterial strains and chitosan concentrations (A×B) was significant, indicating that differences in denier values across chitosan concentrations were strain-dependent. Furthermore, the interaction between bacterial strains and time of chitosan application after bacterial inoculation (A×C) was significant, with denier values decreasing more sharply over time for *B. thuringiensis*. In contrast, the interaction between chitosan concentrations and time of chitosan application after bacterial inoculation (B×C) was non-significant, indicating consistent chitosan concentrations effects across time points. Notably, the three-way

interaction (A×B×C) was significant, reflecting complex interactions among all factors and highlighting the intricate relationships between bacterial strains, chitosan concentrations and time in influencing filament denier.

Among the interactions, highest filament denier (2.779) was observed in the concentrations chitosan @ 5000 ppm applied 30 hours after the *S. aureus* inoculation followed by 2.737 of filament denier was observed in the concentrations of chitosan @ 4500 ppm applied 24 hours after the *S. aureus* inoculation and 2.700 of filament denier was observed in the concentrations of chitosan @ 5000 ppm applied 6 hours after the *S. aureus* inoculation among all the treatments. Contrary, the least filament denier (2.295) was observed in the concentrations of chitosan @ 4000 ppm applied 30 hours after the *S. aureus* inoculation.

The enhancement in the reeling parameters (filament length, filament weight and filament denier) were found among the infected silkworms fed with the chitosan concentrations. The reduction of bacterial cell numbers in the chitosan treated infected silkworm batches is caused by cell-surface alterations and loss of barrier functions. Chitosan, with its

positive charge, easily reacts with negatively charged bacteria, further inhibiting bacterial growth. Surface interference is a possible mechanism of the bactericidal properties (Helander *et al.*, 2001) [5]. The bacterial death in silkworms caused by chitosan and antibiotic treatments helps maintain the immune system of infected silkworms, resulting in the best outcome for reeling parameters. The results of the present study are also in accordance with the findings of Manimegalai and Chandramohan (2008) [8], who reported the highest silk filament length of 787 m with streptomycin, followed by doxycycline hydrogen chloride (782 m), lincomycin (768 m) and erythromycin (727 m) for silkworms infected with *B. thuringiensis*.

These findings are consistent with those of Anusha and Bhaskar (2017) [2], who reported that the shortest cocoon filament length (568 m) and lowest filament denier (1.10) were recorded in silkworms infected with a combination of pathogens (*Alcaligenes faecalis* + *Bacillus subtilis* + NPV). In contrast, control silkworms without bacterial infection showed significantly better results, with a filament length of 778.0 m and a filament denier of 2.31.

Table 6: Effect of chitosan application on filament weight (g) in silkworm infected with *Staphylococcus aureus* and *Bacillus thuringiensis*

Treatments (B)	Time of chitosan application following bacterial inoculation (hpi) (C)										Bacterial strains		Treatments (B) mean
	6 hpi		12 hpi		18 hpi		24 hpi		30 hpi		(A) mean		
	Sa	Bt	Sa	Bt	Sa	Bt	Sa	Bt	Sa	Bt	Sa	Bt	
S ₁ : Chitosan @ 3500 ppm	0.257	0.244	0.267	0.238	0.255	0.228	0.244	0.221	0.230	0.220	0.251	0.230	0.240
S ₂ : Chitosan @ 4000 ppm	0.279	0.265	0.268	0.242	0.264	0.229	0.255	0.239	0.217	0.220	0.257	0.239	0.248
S ₃ : Chitosan @ 4500 ppm	0.284	0.260	0.277	0.251	0.263	0.249	0.273	0.225	0.215	0.224	0.262	0.242	0.252
S ₄ : Chitosan @ 5000 ppm	0.306	0.280	0.278	0.274	0.279	0.263	0.271	0.252	0.257	0.238	0.278	0.261	0.270
S ₅ : Ampicillin @ 1000 ppm	0.315	0.314	0.302	0.281	0.297	0.286	0.273	0.263	0.262	0.261	0.290	0.281	0.285
S ₆ : Com. chitosan @ 1000 ppm	0.299	0.297	0.288	0.302	0.286	0.253	0.269	0.258	0.253	0.226	0.279	0.267	0.273
S ₇ : Solvent control	0.244	0.240	0.242	0.231	0.235	0.227	0.236	0.227	0.199	0.224	0.231	0.230	0.231
S ₈ : Absolute control	0.225	0.201	0.225	0.201	0.225	0.201	0.225	0.201	0.225	0.201	0.225	0.201	0.213
S ₉ : Bacteria un-inoculated	0.328	0.328	0.328	0.328	0.328	0.328	0.328	0.328	0.328	0.328	0.328	0.328	0.328
Mean	0.276		0.268		0.261		0.255		0.240		0.267	0.253	
Mean A, B, C	0.260										0.260		0.260

	A	B	AB	C	AC	BC	ABC
F-Test	*	*	NS	*	NS	NS	NS
SEm ±	0.003	0.005	-	0.004	-	-	-
CD @ 5%	0.005	0.011	-	0.008	-	-	-

Note: * Significant; NS-Non-significant; A-Bacterial strains; B-Treatments; C-Hours of post inoculation (hpi); Sa-*Staphylococcus aureus*; Bt-*Bacillus thuringiensis*; Com.-Commercial chitosan

Table 7: Effect of chitosan application on filament denier in silkworm infected with *Staphylococcus aureus* and *Bacillus thuringiensis*

Treatments (B)	Time of chitosan application following bacterial inoculation (hpi) (C)										Bacterial strains (A) mean		Treatments (B) mean
	6 hpi		12 hpi		18 hpi		24 hpi		30 hpi		Sa	Bt	
	Sa	Bt	Sa	Bt	Sa	Bt	Sa	Bt	Sa	Bt			
S ₁ : Chitosan @ 3500 ppm	2.613	2.494	2.674	2.522	2.570	2.464	2.602	2.428	2.615	2.496	2.615	2.481	2.548
S ₂ : Chitosan @ 4000 ppm	2.627	2.525	2.688	2.504	2.662	2.508	2.598	2.517	2.295	2.568	2.574	2.524	2.549
S ₃ : Chitosan @ 4500 ppm	2.683	2.496	2.598	2.553	2.611	2.618	2.737	2.515	2.326	2.560	2.591	2.548	2.570
S ₄ : Chitosan @ 5000 ppm	2.700	2.522	2.615	2.543	2.631	2.568	2.598	2.505	2.779	2.503	2.665	2.528	2.596
S ₅ : Ampicillin @ 1000 ppm	2.631	2.705	2.601	2.550	2.649	2.580	2.571	2.520	2.592	2.557	2.609	2.583	2.596
S ₆ : Com. chitosan @ 1000 ppm	2.610	2.617	2.606	2.735	2.641	2.502	2.471	2.586	2.438	2.469	2.553	2.582	2.568
S ₇ : Solvent control	2.596	2.487	2.626	2.452	2.553	2.530	2.563	2.505	2.306	2.547	2.529	2.504	2.517
S ₈ : Absolute control	2.691	2.535	2.691	2.535	2.691	2.535	2.691	2.535	2.691	2.535	2.691	2.535	2.613
S ₉ : Bacteria un-inoculated	2.581	2.581	2.581	2.581	2.581	2.581	2.581	2.581	2.581	2.581	2.581	2.581	2.581
Mean	2.594		2.592		2.582		2.561		2.524		2.601	2.541	
Mean A, B, C	2.571										2.571		2.571

	A	B	AB	C	AC	BC	ABC
F-Test	*	*	*	*	*	NS	*
SEm ±	0.013	0.027	0.038	0.020	0.028	-	0.084
CD @ 5%	0.025	0.053	0.074	0.039	0.055	-	0.166

Note: * Significant; NS-Non-significant; A-Bacterial strains; B-Treatments; C-Hours of post inoculation (hpi); Sa-*Staphylococcus aureus*; Bt-*Bacillus thuringiensis*; Com.-Commercial chitosan

Conclusion

The cocoon parameters such as cocoon weight, pupal weight, shell weight and shell ratio were significantly higher in chitosan treated *S. aureus* infected silkworms (1.68 g, 1.34 g, 0.33 g and 19.61%, respectively) than in chitosan treated *B. thuringiensis* infected ones (1.61 g, 1.28 g, 0.31 g and 19.54%, respectively). Chitosan @ 5000 ppm recorded the best cocoon traits among chitosan concentrations. Ampicillin (1000 ppm) and uninoculated controls had the highest values overall, while absolute control had the lowest. Early chitosan application after 6 hours of bacterial inoculation resulted in the higher values of cocoon parameters (1.70 g, 1.36 g, 0.33 g and 19.47%, respectively), compared to delayed application.

The post cocoon traits of silkworm were significantly influenced by the bacterial strain, chitosan concentrations and timing of chitosan application, each acting independently. The reeling parameters such as filament weight, filament length and filament denier were notably higher in chitosan treated *S. aureus* infected silkworms (923.96 m, 0.267 g and 2.601, respectively) than in chitosan treated with *B. thuringiensis* infected silkworms (895.38 m, 0.253 g and 2.541, respectively). Among the silkworm pupal chitosan concentrations, the highest record of reeling parameter values was observed with chitosan @ 5000 ppm (S4: 936.41 m, 0.270 g and 2.596, respectively), while the lowest were recorded at 3500 ppm (S1). The uninoculated control group yielded the longest (1142.63 m) and heaviest (0.328 g) filaments, highlighting the detrimental effect of infection on filament quality. Early chitosan intervention, ideally at 6 hours post-inoculation, proved most effective in preserving filament length, weight and denier, with delayed application progressively reducing benefits. The interactions have no significant effect on the reeling parameters.

Early chitosan concentrations applications (6 hpi) yielded the highest economic values, with significant declines seen at later interventions. Interactions among bacterial strain, chitosan concentrations and application timing significantly influenced results, although shell ratio was only affected by chitosan concentrations, not strain or timing. The study demonstrates that high-dose, early-applied chitosan especially at 5000 ppm is nearly as effective as antibiotics in reducing bacterial damage and optimizing silk production metrics. Chitosan thus represents a promising, eco-friendly alternative to conventional antibiotics in sericulture.

References

1. Anonymous. Performance of Indian silk industry. Ann Rep, CSB, Bangalore; 2024, p. 19.
2. Anusha HG, BHASKAR RN. Identification of new pathogenic bacteria (*Alcaligenes faecali*) and assessment of pathogenicity with mixed infection on rearing and cocoon parameters of silkworm (*Bombyx mori* L.). Res Crops. 2017;19(1):120-126.
3. Choudhury A, Guha A, Yadav A, Kumari J, Unni Bg, Roy Mk. Induced immunity in *Antheraea assamensis* Ww larvae against flacherie causing *Pseudomonas aeruginosa*. Exp Parasitol. 2004;106:75-84.
4. Dandin SB, Giridhar k. Handbook of Sericulture Technologies. CSB, Bangalore. 2014. pp. 335-385.
5. Helander IM, Lassila NEL, Ahvenainen R, Rhoades J, Roller S. Chitosan disrupts the barrier properties of the

- outer membrane of gram-negative bacteria. Int J Food Microbiol. 2001;71:235-244.
6. Longvah T, Mangthya K, Ramulun P. Nutrient composition and protein quality evaluation of ERI silkworm (*Samia cynthia ricini*) prepupae and pupae. Food Chem. 2011;128:400-403.
7. Mahesh DS, Vidhathi BS, Narayanaswamy TK, Subbarayappa Ct, Muthuraju R, Shruthi P. Bio-nutritional science of silkworm pupal residue to many new ways for utilization. Int. J Adv Res Biol Sci. 2015;2(9):35-40.
8. Manimegalai S, Chandramohan N. Antibiotics for the management of bacterial flacherie of silkworm, *Bombyx mori* L. Madras Agril J. 2008;95(1-6):218-222.
9. Gowda M. Inheritance of tolerance to BmNPV among popular breeds of silkworm, *Bombyx mori* L. (Ph.D. Thesis). Bangalore: UAS; 2009, p. 182.
10. Priyadharshini P, Mahalingam CA, Shashidhar KR. Evaluation of antibacterial efficacy of certain botanicals against bacterial pathogen *Bacillus* sp. of silkworm, *Bombyx mori* L. Int J Indus Ento. 2009;18(1):49-52.
11. Priyadharshini P, Mahalingam CA, Prabhu S, Thangamar A, Umopathy G. *In vivo* antibacterial effect of chitosan against *Staphylococcus aureus* and *Bacillus thuringiensis* and its impact on economic parameters of silkworm, *Bombyx mori* L. J Pharmacognosy Phytochem. 2018;7(2):2448-2451.
12. Sundarraj N, Nagaraju S, Ventataramu MN, Jagannath MK. Design and analysis of field experiments. Directorate of Research, UAS, Bangalore; 1972, p. 139.
13. Suresh HN, Mahalingam CA, Pallavi. Amount of chitin, chitosan and chitosan based on chitin weight in pure races of multivoltine and bivoltine silkworm pupae *Bombyx mori* L. Int J Sci Nature. 2012;3:214.
14. Tayal MK, Chauhan TP. Silkworm Diseases and Pests. In: Ind. Entomol. Springer. 2017;10:265-289.
15. Kumar VAB, Varadaraj MC, Gowda LR, Tharanathan RN. Characterization of chito-oligosaccharides prepared by chito-sanolysis with the aid of papain and pronase and their bacterial action against *Bacillus cereus* and *Escherichia coli*. Biochem J. 2005;391:167-175.
16. Yunjian D, Yuqiao Z, Shuchao D, Bao Y. Preparation of water-soluble chitosan from shrimp shell and its antibacterial activity. Innov Food Sci Emerg Tech. 2009;10:103-107.