Research Article

# Assessment of the Effect of Methanolic Herbal Extract on Cocoon Parameters and Tensile Properties of Silk Fiber Spun by *Beauveria bassiana* Infected Muga Silkworm, *Antheraea assamensis* Helfer

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## ABSTRACT

**Aim:** The current study was an attempt to combat white muscardine disease in muga silkworm (*Antheraea assamensis* Helfer) by utilizing herbal extract in an eco-friendly way. **Materials and Methods:** In this study, different cocoon parameters and tensile properties of silk fibres produced by 5$^{th}$ instar muga silkworm larvae infected from white muscardine disease caused by entomopathogenic fungus *Beauveria bassiana* were evaluated by treating with 10% methanolic rhizome extract of *Curcuma longa* (turmeric). The silkworms were topically inoculated with $LC_{50}$ concentration ($1.1\times10^8$ spore/mL) of the fungal spore suspension and then reared on a host plant sprayed with *C. longa* extract. **Results:** Different cocoon parameters such as cocoon weight, pupal weight, shell ratio, filament length, non-breakable filament length, denier, reliability and raw silk percentage were measured and observed to be improved significantly after treating the infected silkworm with the plant extract when compared to the normal control group. Estimation of the weight of sericin and fibroin proteins in cocoons was also accounted for in the study which appeared to be significantly enhanced after treatment of the larvae with *C. longa* Linn. extract. The tensile properties of the muga fibres also followed the same trend of improvement after treatment with the herbal extract. The silk fibre produced by *C. longa*-treated larvae exhibited better tensile strength and breaking strain percentage to a significant extent. **Conclusion:** Thus, the study reflected the curative effect of *C. longa* on the commercial parameters of muga cocoons leading to better silk output probably by restraining the growth of fungal spores in the silkworm body.

**Keywords:** *Beauveria bassiana*, Cocoon, *Curcuma longa,* Muga silkworm.

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## INTRODUCTION

The cultivation of muga silk, the lustrous golden thread has been in existence from time immemorial in Assam which is an agro-based industry and encompasses rural development and economy of the state.[1] This muga silk industry continues to expand due to the high-end demand for raw silk and silk products all over the world. The prospects and sustainability of the silk industry largely depend upon the disease-free rearings of silkworms. However, as muga silkworms (*Antheraea assamensis* Helfer) are semi-domesticated and have to be reared outdoors, they are exposed to several environmental constraints viz., pests and parasites which take a heavy toll on their productivity.[2] White Muscardine Disease (WMD) is one such disease caused by the entomopathogenic fungus *Beauveria bassiana*

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and can be one of the major yield-limiting factors for pre-seed and seed crops during winter.[3,4] The disease accounted for about 60-90% of crop damage under high humid conditions with low temperatures.[5] However, the outbreak of WMD has been increasing over the last few years as muga industry is constantly battling against the changing climatic patterns and becoming more susceptible to this fungal pathogen raising serious concerns among the sericulturists.[6]

So, the management of WMD is becoming increasingly crucial for the muga silk industry. To meet the challenges posed by the fungus, the practice of using fungicides is quite widespread at present. However, the perilous consequences of the widespread use of chemical fungicides have now given rise to the notion of rational use of herbal extracts against it as they are eco-friendly and less expensive. Several studies conducted on WMD in *Bombyx mori* to improve productivity by using traditional medicinal herbs have already been documented well.[7-9] However so far, no such attempts have been made against the WMD in muga silkworm in Assam. The present study is a humble effort to introduce this concept in the muga industry utilizing the common plant *Curcuma longa* Linn. (turmeric) belonging to the family Zingiberaceae (Order: Zingiberals), which possesses known antimicrobial properties.[10] In our study, an effort has been made to evaluate the impact of *C. longa* on different cocoon parameters with the intention of mitigating the disease intensity for better silk output in an eco-friendly environment.

## MATERIALS AND METHODS

### Procurement of *A. assamensis* larvae

Disease-free 5$^{th}$ instar larvae of *A. assamensis* were obtained from the Directorate of Sericulture Office, Khanapara, Guwahati, Assam and reared on an experimental plot on som plants during the winter season (December-January).

### Isolation and identification of *B. bassiana*

The diseased silkworms were collected from a state sericulture farm from Udalguri district, Assam, India and the fungus was isolated in pure form on potato dextrose agar (PDA) media by single spore isolation method.[11,12] The isolate (BB1) was characterized by rDNA ITS sequencing technique at Microbial Type Culture and Gene Bank (MTCC), Chandigarh, India and identified as *B. bassiana*. The genome sequence of the *B. bassiana* isolate is available in NCBI GenBank under the accession number OQ954799.

### Collection and preparation of *C. longa* rhizomes

The *C. longa* rhizomes were collected from Udalguri district, Assam, India and identified in the Department of Botany, B. Borooah College, Assam. The rhizomes were first washed with tap water and then rewashed with distilled water. The rhizomes were then cut into small slices and dried in shade for 15 days at room temperature. The desiccated rhizomes were then powdered to fine particles by using an electric mixer grinder. For methanolic extract, 10 g of the rhizome powder was mixed with 100 mL of methanol having a concentration of 80%. The sample was then allowed to stand at room temperature for 3 days. The sample was then sieved by using Whatman filter paper no. 1. The solvent was then evaporated in a rotary evaporator (make: Equitron, model: EV11) under reduced pressure at 45°C. The extract was stowed at 4°C in a refrigerator.

### Induction of fungal spore and plant extract

The fresh moult 5$^{th}$ instar silkworm larvae were topically inoculated with $LC_{50}$ concentration ($1.1x10^8$ spore/mL) of *B. bassiana* spore suspension. The first group of larvae was dipped in the spore suspension as per the method of Chavan *et al*.[8] The fungus-inoculated larvae were then reared on a host plant (som) sprayed with 10% methanolic extract of *C. longa* (foliar spray) dissolved in double distilled water. The extract was sprayed for the first three days during the morning. This particular concentration of the extract was selected based on the *in vitro* study conducted earlier.[13] Another group was treated with only *B. bassiana* suspension (inoculated control). The normal control group was fed with som leaves without any pathogenic treatment. For each treatment, three replications with 15 silkworm larvae each were maintained.

### Testing of individual cocoon parameters

12 days after beginning of spinning cocoons, various parameters such as Cocoon weight, Shell weight, Shell ratio (%), Pupal weight, Non-Breakable Filament Length (NBFL), Denier, Reelability, Raw silk percentage, Filament length, were tested as per the methods of Seema *et al*.[12] and Chattopadhyay *et al*.[14] These tests were done as an average of ten cocoons randomly selected from each treatment group.

### Estimation of the weight of sericin and fibroin content in cocoons

It was done by the conventional method of degumming the cocoons with alkali treatment. Individual cocoon shell is taken in a crucible and boiled at 90°C for 30 min by adding 20 mL of 0.3% $Na_2CO_3$.[15] After

39 min, the crucible containing the fibre was washed with boiling distilled water to remove the sericin and dried the fibroin content for 24 hr at 90°C. The weight of sericin and fibroin was calculated by the formulas given below.[16] The experiment was repeated ten times to obtain the mean and presented in the unit of gram.

Sericin content = Initial weight of the shell - Weight of the shell after alkali treatment

Fibroin content = Weight of the shell - Sericin content

### Tensile test

The muga fibres from all the groups were tested for their tensile properties in a computer-equipped Universal Testing Machine (UTM) at the Indian Institute of Technology, Guwahati, Assam (make: Zwick Roell: Z005TN) fitted with 5 KN load cell. A gauge length of 10 cm with a Test speed of 5 mm/min was taken for the test. Before testing, the samples were conditioned at a temperature of 25°±2°C. From each group, 10 specimens were selected to get an average of the test.

### Statistical Analysis

One way Analysis of Variance (ANOVA) was performed to analyse the data obtained by using IBM SPSS 25.0 version. The results of cocoon parameters and silk protein content were presented in mean±SEM. For multiple comparisons within the groups, the data were further subjected to *post hoc* Tukey's HSD (honestly significant difference) to know the differences between means at a significance level $p < 0.05$.

## RESULTS AND DISCUSSION

### Impact on cocoon quality

The impact of the *B. bassiana* infection and subsequent treatment with methanolic plant extract on cocoon quality spun by *A. assamensis* larvae are summarized in Tables 1 and 2. The results clearly indicated that *B. bassiana* infection leads to the production of significantly low-quality cocoons ($p < 0.05$). Reduction in cocoon weight and shell weight eventually influenced the shell ratio which is the quantity of silk that can be produced from each cocoon. However after treatment of *B. bassiana* inoculated group with *C. longa* extract, there is significant elevation ($P < 0.05$) of shell ratio (9.99±0.27) compared to the WMD infected group (7.5±0.45). The shell weight of the plant extract-treated group is also increased (0.41±0.03 g) in comparison to the WMD-infected group (0.21±0.04 g). Similarly, a significant reduction in filament length (119.36±14.9 m), filament weight (0.02±0.02 gm), reelability (15.49±1.31%) and raw silk percentage (2.06±0.39%) was observed in WMD infected group with reference to the healthy ones. When treated with 10% *C. longa* extract the filament length and weight were elevated significantly ($p < 0.05$) than the diseased group with fewer breaks. In the present investigation, the denier of fungus inoculated group was noticed as significantly lower (1.61±0.45) than the control (6.02±0.74) and *C. longa* treated group (4.01±0.19) at $p < 0.05$ level.

A significant fall in the synthesis of sericin (0.013±0.01 g) and fibroin (0.072±0.02 g) was recorded in the diseased cocoons relative to the control group in terms of

**Table 1: Effect of *C. longa* rhizome extract on different post-cocoon characters of silkworm *A. assamensis* infected with *B. bassiana*. Different small letters indicate significant differences within each group of cocoon characters ($p < 0.05$).**

| Groups | Concentrations | Cocoon weight (g) | Shell weight (g) | Pupal weight (g) | Shell ratio (%) |
|---|---|---|---|---|---|
| *C. longa* treated | 10% | 4.07 ± 0.24$^{a}$ | 0.41 ± 0.03$^{ab}$ | 3.65 ± 0.22$^{a}$ | 9.99 ± 0.27$^{a}$ |
| *B. bassiana* Inoculated | 1.1x10$^{8}$ spore/mL | 1.97 ± 0.21$^{b}$ | 0.21 ± 0.04$^{a}$ | 1.76 ± 0.24$^{b}$ | 7.5 ± 0.45$^{b}$ |
| Control | - | 6.36 ± 0.17$^{c}$ | 0.69 ± 0.03$^{b}$ | 5.67 ± 0.17$^{c}$ | 11.85 ± 0.48$^{c}$ |

**Table 2: Effect of *C. longa* rhizome extract on different filament characters of silkworm *A. assamensis* infected with *B. bassiana*. Different small letters indicate significant differences within each group of cocoon characters ($p < 0.05$).**

| Groups | Concentrations | Filament length (m) | Filament weight (g) | Non-Broken Filament Length (NBFL) (g) | Denier | Reliability (%) | Raw silk percentage (%) |
|---|---|---|---|---|---|---|---|
| *C. longa* treated | 10% | 292.52±19.21$^{a}$ | 0.16±0.02$^{a}$ | 87.34±7.9$^{a}$ | 4.01±0.19$^{a}$ | 35.73±4.48$^{a}$ | 4.25±0.17$^{a}$ |
| Fungus Inoculated | 1.1x10$^{8}$ spore/mL | 119.36±14.93$^{b}$ | 0.02±0.02$^{b}$ | 18.95±0.94$^{a}$ | 1.61±0.45$^{b}$ | 15.49±1.31$^{ab}$ | 2.06±0.39$^{b}$ |
| Control | - | 436.98±24.35$^{c}$ | 0.36±0.04$^{c}$ | 267.26±14.7$^{b}$ | 6.02±0.74$^{c}$ | 63.28±15.3$^{ac}$ | 7.01±0.32$^{c}$ |

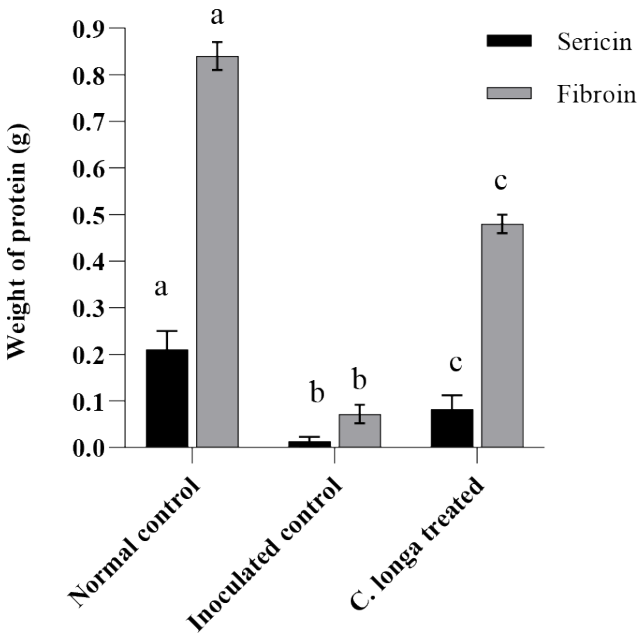


Figure 1: Effect of ***C. longa*** rhizome extract on sericin (g) and fibroin (g) content of silkworm ***A. assamensis*** infected with ***B. bassiana.*** Different small letters indicate significant differences within groups of each testing parameter ($p < 0.05$).

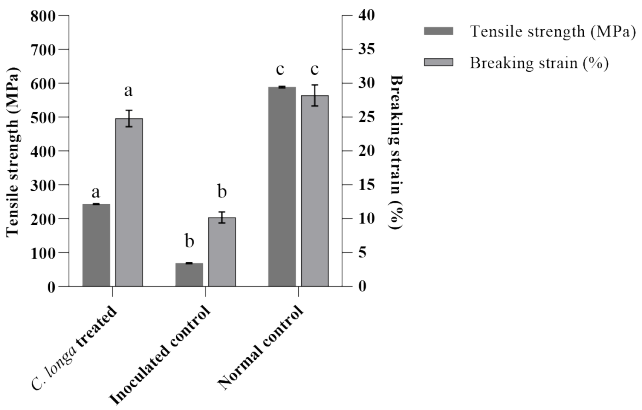


Figure 2: The tensile strength vs. breaking strain curve to show the effect of 10% methanolic extract of ***C. longa*** rhizome on the tensile properties of ***Antheraea assamensis*** silk fiber spun by ***B. bassiana*** infected silkworm. Different small letters indicate significant differences within each testing parameters ($p < 0.05$).

weight as depicted in Figure 1. Again there was a significant improvement of the weight of both the proteins after treatment with 10% *C. longa* extract at $p < 0.05$.

### Impact on tensile properties

The tensile strengths of the degummed muga fibres from all the test groups are presented in Figure 2. A tensile strength versus strain graph was plotted (Figure 2) where it is evident that the fibres spun by the normal control group showed maximum tensile strength (588.7±2 Mpa) with great extensibility (28.1±1.5%) as indicated by the breaking strain percentage. In comparison with the normal control group, the fungus-inoculated group exhibited significantly less tensile strength (68.8±1.3 MPa). Taking into account the breaking strain percentage, the inoculated control group exhibited significantly less extensibility (10.2±0.8%) contrary to the normal control group. On the other hand, when treated with 10% methanolic *C. longa* extract, there is a significant improvement in both tensile strength (~243.6±1.2 MPa) and breaking strain (24.8±1.2 MPa) as compared to the inoculated control group.

## DISCUSSION

The present study resulted in low-quality cocoon production in terms of its weight, shell weight, pupal weight and shell ratio spun by the *B. bassiana* infected silkworms. These findings of the present study are in accordance with previous experiments conducted on *Bombyx mori* infected with *B. bassiana* by Seema *et al*.[12] A study conducted by Rath and Sinha[17] on Uzi fly infestation also reported a similar decrease in cocoon and shell weight of infected *Antheraea mylitta* larvae. The production of inferior-quality cocoons was explained by the authors to be the result of the physiological stress induced by the fungal pathogen *B. bassiana* which may be true in the case of the muga silkworm larvae too.[18] These parameters are improved after treating the WMD-infected group with the methanolic *C. longa* extract which is again in conformity with the study of Devi and Bai[7] where ethanolic *Ocimum sanctum* leaf extract was used to treat WMD in *Bombyx mori*. Similarly, the significant reduction in filament length, filament weight, and reelability observed in WMD infected group is also in agreement with the results of Rajitha and Savithri.[18] Treatment with 10% *C. longa* extract again elevated the filament length and weight which in turn improved the reelability of the cocoons giving rise to better quality of raw silk percentage. Similar results were earlier reported by Saad *et al*.[9] who tried to control bacterial and fungal diseases in *Bombyx mori* by using mulberry and basil leaves. Denier is another important unit which is used to indicate the thickness of the silk fibre which was observed to be significantly lower in *B. bassiana* infected group compared to the *C. longa* treated group which is again in correspondence with the results of Soumya,[19] who applied five botanical leaf extracts as bed disinfectant to manage rearing performances of WMD infected silkworm, *B. mori*. The common reason offered from the results is explained as the physiological

tension induced by the fungal infection that might cause the oozing out of the silk proteins erratically in lumps which can be the reason for deterioration of the filament quality.[18] The results of the current study also reflected a decrease in the weight of the silk proteins sericin and fibroin in the infected silkworm group in comparison with the *C. longa* treated group and the normal control group. This result is synonymous with the study of Devi and Bai[7] who reported a significant reduction of weight of sericin and fibroin secreted by 5$^{th}$ instar *B. mori* larvae inoculated with *B. bassiana* compared to normal control. The reason for the decline of silk proteins probably is the fungal pathogen which could directly or indirectly affect the growth and development of silk glands.[18]

The results of the analysis of the tensile parameters obtained from the present study are comparable to the previously reported data. As per the reports from earlier research studies, the muga silk fibres possess the highest tensile strength among all the commercially exploited silk.[20,21] In the present investigation, the maximum tensile strength was shown by the normal control group followed by the plant extract-treated group and fungus-inoculated group. These results appeared to be comparable to the handful of data reported earlier on the tensile properties of muga silk.[15,22] The minor variations can be due to the differences in degumming process, testing protocol, rearing season and environmental changes. The inferior strength of the silk from fungus inoculated control group indicates the poor structure of the fiber that can be co-related with the down secretion of silk proteins (Figure 1) which is needed to be investigated further. It is evident from from the study that the breaking strain of the plant extract treated group has enhanced to a great extent even though the tensile strength is still significantly lower than control group. However, due to the non-availability of data on the tensile parameters of silk fibres produced by diseased larvae, it is difficult to compare the current data. The production of low-strength fibre can be the consequence of the disturbances pertaining to the formation of cocoons due to the diseased state of the silkworm.

The current research reflected an improvement of all the parameters after the use of 10% *C. longa* methanolic extract. The restorative influence of the botanicals can be accredited to the presence of certain secondary metabolites such as flavonoids, steroids, alkaloids, terpenoids, phenols etc. that exhibit toxicity against microbes in several ways.[23,24] The varied responses of curcumin derived from *C. longa* rhizomes against a number of plant pathogenic fungi have already been documented well by Kim *et al*.[25] Curcumin exerts its fungicidal effect in as many ways as by a decrease in ergosterol present in the fungal cell wall which results in apoptosis through ROS generation, lessening in the secretion of proteinase and changes in properties of ATPase activity associated with membrane thereby resulting in the disruption of the fungal plasma membrane.[26,27] Besides curcumin there are several other major phytoconstituents present in *C. longa* such as turmerone, curdione, elemene, germacrone and curcumol are proven to have antimycotic activity.[28,29] Thus as depicted by the present research, the corrective effect of *C. longa* on different commercial traits of muga cocoons may be due to the antagonistic or synergistic effect of these various bioactive compounds which subsequently reduced or inhibited the germination of *B. bassiana* spores in the muga silkworm body.

## CONCLUSION

From the Results obtained in the current study, the rhizome extract of *C. longa* is found to be promising as it helped to impart overall better performances of the silkworm group than the *B. bassiana* inoculated group in terms of some of the important economic traits. Further study on the screening of the active substances present in such growth-promoting extract may prove to be helpful in enhancing commercial muga silk production. Thus, the present study provides insights into the utilization of botanical extracts to combat silkworm diseases in the cheapest ways for better silk output.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**WMD:** White Muscardine Disease; **NBFL:** Non-Breakable Filament Length; **ANOVA:** Analysis of Variance; **MTCC:** Microbial Type Culture and Gene Bank; **ITS:** Internal Transcribed Spacer; **rDNA:** Ribosomal DNA.

## SUMMARY

The present study investigated different economic parameters of muga silk cocoons formed by white muscardine disease-infected muga larvae caused by the entomopathogenic fungus *Beauveria bassiana*. The larvae were subsequently treated with 10% methanolic *Curcuma longa* rhizome extract and observed its effect on the commercial parameters of cocoons. The results were compared with the cocoons spun by the group of larvae reared under normal conditions. The data were subjected to ANOVA for comparison of the mean. Different parameters of the cocoon from the infected group such as cocoon weight, shell weight, pupal weight, shell ratio, filament length, NBFL, denier, reelability and raw silk percentage exhibited significant deterioration as compared to the normal control group. The weight of the silk proteins sericin and fibroin from the cocoons was also measured which showed a similar trend of production of inferior quality cocoons as reflected from the results. However, after treating the *B. bassiana* infected larvae with *C. longa* extract, the larvae spun cocoons with significant improvement in all the parameters at $p < 0.05$ level. The tensile strength of the muga fibres obtained from the three groups of larvae was also tested. According to the result of the tensile test, the tensile strength of the fibres from the WMD-infected group was significantly lower than that of the plant extract-treated group and normal control group with significantly lower extensibility. Thus the results obtained from the current study displayed a positive impact of the plant extract probably because the presence of antimicrobial phytoconstituents might inhibit the germination of fungal spores on the silkworm body. However further investigations are required so as to dig out the mechanism by which the plant constituents exerted its positive effect against WMD.

### Author Contribution

All the experiments and data analysis were done by Sanghamitra Saharia, Shibani Kalita while Dimpimoni Kalita, Anjumani Ojah helped in literature search and manuscript review was done by Dr. Sunayan Bardoloi. All the authors read and approved the final manuscript.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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