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**ORIGINAL ARTICLE** **OPEN ACCESS**

# Characters of cocoons of *Bombyx mori* immunized with fungal pathogen *Beauveria bassiana* (Bals.) Vuill.

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**ABSTRACT**
*Sericulture is a sustainable agro-based enterprise providing employment opportunities for millions of the rural and semi-urban folk, preventing migration from villages to cities in search of livelihood and alleviation of poverty. The major quantity of the silk is produced from mulberry silkworm Bombyx mori. The silkworm is tiny insect with a lot of economic significance; it is very sensitive and prone to different diseases such as pebrine, grasserie, flacherie and muscardine. The main constraint of the sericulture industry in India is frequent outbreak of silkworm diseases that causes cocoon crop loss. The fungal pathogen Beauveria bassiana (Bals.) Vuill.is one of the most harmful pathogens and highly contagious causing white muscardine disease in silkworm Bombyx mori. The incidence of the disease is high in rainy and winter seasons. As per Chandrasekhar and Nataraju (2008), the average cocoon crop loss due to diseases in India is to the tune of 15–47%. Out of the total cocoon crop loss, 10-40% loss is accounted for muscardine disease caused by fungal pathogen Beauveria bassiana. With this background the authors carried out an experiment, to understand the commercial characteristics of silk cocoons inoculated with fungal pathogen Beauveria bassiana compared to healthy silkworms.*
***Keywords:*** *Bombyx mori, Beauveria bassiana, inoculation, Economic characters.*

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## INTRODUCTION

India is an agriculture based country, agriculture and its allied activities are the major source of livelihood for more than 50 percent of the country's population. Sericulture can provide employment opportunities in various sectors of the industry and to heterogeneous groups of the people of the society. The entire gamut of sericulture industry comprises of many stages that begins with culturing of silkworm for the production of cocoons, the basic raw material for the production of raw silk. Around 70 percent of silk production is from mulberry silkworm and remaining 30 per cent silk production is from non-mulberry silkworms. Silkworm *Bombyx mori* is completely domesticated insect, due to continuous domestication, it has lost its wild characters; subsequently it has become susceptible to different types of diseases caused by different groups of micro-organisms. Major constraint of the sericulture industry is frequent outbreak of diseases such as Grasserie, Flacherie, Muscardine and Pebrine caused by viral, bacterial, fungal and protozoan microorganisms that affect the cocoon crops and cause huge loss to the sericulture farmers. Among these diseases, white muscardine caused by *Beauveria bassiana* is the most common one occurring during rainy and winter seasons in India, low humidity and low temperature favour the multiplication and spread of the fungal disease. Fast multiplication and fast spread are the main characteristic features of the *Beauveria bassiana* and white muscardine develops into an epizootic within no time if the conditions are congenial. Bassi [3] revealed the infectious nature of the disease and identified the fungal causative agent and recommended the appropriate measures for prevention of the fungal disease i.e., muscardine Ainsworth [1]. The recurrent occurrence of diseases is one of the major problem for the development of the sericulture industry. Janakiraman [6], Ayuzawa [2], Chandrasekharan and Nataraju [3] reported that 10-40 percent silkworm cocoon crop loss is due to the fungal disease caused by the pathological agent *Beauveria bassiana.* Therefore, the study was carried out to realize the

effect of the fungal disease i.e., white muscardine on various economical parameters of the cocoons that influence the quality of silk.

## MATERIAL AND METHODS

The popularbivoltine double hybrid (CSR2 X CSR27) X (CSR6 X CSR26) was chosen for the current investigation. The silkworms stock has been maintained with required conditions for the healthy growth and development of silkworms to carry out the experiment in the laboratory as recommended by Dandin *et al* [5]. Simultaneously pure *Beauveria bassiana* culture was maintained by using Potato dextrose agar medium. Conidia were collected from the 3-week-old *Beauveria bassiana* pure culture and prepared sterile inoculum in a beaker that contained 50 ml sterile double distilled water and a drop of tween-20. LD50 value was calculated for the 5th instar silkworms by following the probit analysis (Leora software, 1987). Then the silkworms were inoculated immediately after passing the 4th moult, with sub-lethal concentration (2.15 X $10^4$ conidia/ml) and healthy silkworms were treated with double distilled water and used as control. For the experiment 4 replications were maintained with 100 silkworms in each replication. On the seventh day of fifth instar matured larvae were noticed, the mature worms were transferred to mountages. On sixth day after mounting the cocoons were harvested and assessed for all the economic parameters of cocoons such as weight of cocoon, shell weight, shell percentage, length of the silk filament, non-breakable filament length, number of breaks and denier by using the formulas mentioned below.

### Cocoon Weight

Randomly 10 cocoons were selected from each replication of experimental and control batches and weighed the cocoons. Average of single cocoon weight was calculated in gram units.

### Shell weight

After taken cocoon weight, the cocoons were cut open and taken the average weight of 10 cocoon shells in grams.

### Shell Percentage

Shell percentage is one of the important parameter to assess the quality of cocoon that indicates the quantity of silk available in each cocoon. Shell percentage was computed by using the formula as given below.

$$\text{Shell Percentage} = \frac{\text{Weight of the cocoon shell}}{\text{Weight of the cocoon}} \times 100$$

### Length of the silk filament

By using eprouvettei.e., single cocoon reeling unit the silk filament length (circumference of 1.125m), was reeled and the length of the silk filament was calculated, by using the formula given below measured in meters recommended by Sonwalkar [12]

Filament length (m) = Number of rotations × Circumference of the eprouvette wheel

(Circumference of the eprouvette wheel = 1.125m)

### Non-breakable filament length and Number of Breaks

Non-breakable filament length indicates the length of silk filament reeled from a cocoon without any break and calculated the non-breakable filament length by using the formula and recorded the number of breaks.

$$\text{Average Non-breakable filament Length} = \frac{\text{Total filament length}}{1 + \text{No.of breaks}}$$

### Denier

Thickness of silk yarn is known as denier, uniformity of denier indicates the evenness and quality of silk filament. The denier was calculated by using the formula [12]:

$$\text{Denier(D)} = \frac{\text{Weight of filament (g)}}{\text{Length of filament (m)}} \times 9000$$

### Statistical analysis

The data collected from the four replications of experiment and control batches was statistically analysed by following one way ANOVA.

## RESULTS AND DISCUSSION

It is obvious from the results that growth and development and the commercial characteristics of treated silkworms with *Beauveria bassiana* a fungal pathogen have been effected drastically. The data collected on economic characters during experimentation are shown in Table-1and Graphs- 1-7.

Significant reduction of silk cocoon parameters were recorded in experimental silkworms viz., cocoon weight (1.060g), shell weight (0.120 g), shell percentage (11.83%), length of silk filament (728.570m) and non-breakable filament length (614.900m) compared to healthy control. But the number of breaks during the process of reeling of silk was more and the thickness of the silk yarn (3.870 d) is high in treated batch than untreated control (3.120 d).

Silk cocoons are the basic raw material for the silk production. The commercial characters of cocoons i.e., cocoon weight, shell weight, shell percentage, length of silk filament, number breaks and denier indicate the yield of raw silk from the cocoon. Compactness, resilient firmness of cocoon, weight of cocoon are the important commercial characters in price fixation. The shell weight of a cocoon is important to understand the qualitative and quantitative yield parameters of silk and to determine the amount of raw silk that can be obtained. Shell percentage accurately denote the amount of raw silk that can be reeled from the cocoons and aids in assessing renditta i.e., units of cocoons required for the production of one unit of silk. Silk is a continuous filament within each cocoon, having a usable length of about 900 to 1200 metres; the length of silk filament in a cocoon indicates the reliability of silk filament from a cocoon. Cocoons with a longer filament length have greater silk reeling productivity with fewer feeding ends. Silk reeling efficiency also depends on the length of the silk filament with less number or no breaks during reeling process. The size of the cocoon silk filament is expressed in denier [12, 8].

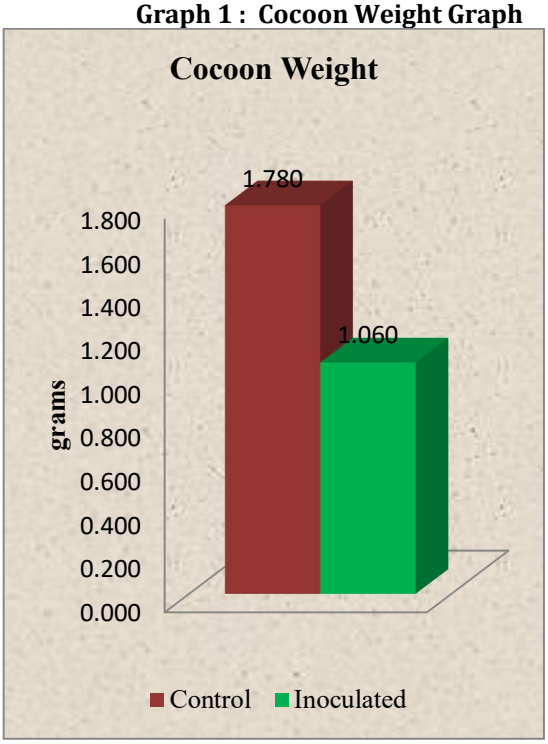
Economical cocoon parameters directly reveal the health status of the silkworm *Bombyx mori* and its growth and development. Lakshmi Velide *et al*. [11] noticed decline in commercial characteristics of cocoons in tasar silkworm *Antheraea mylitta* infected with protozoan pathogen *Nosema bombysis.* Rajitha and Savithri [9] *stated that Beauveria bassiana* infection influenced the qualitative and quantitative commercial characters of cocoon like spinning silkworm weight, weight of cocoon and shell, shell ratio, silk filament length, number of breaks and length of non-breakable silk filament and size of the filament i.e., denier and suggested that it may be due to the cessation of feed and metabolic trauma caused by the *Beauveria bassiana.* Rath *et al* [10] have noticed the reduction of shell weight in tasar silkworm *Antheraea mylitta* infected with protozoan pathogen Nosema sp. that causes pebrine disease. Jhansi Lakshmi [7] reported that the reduction of commercial cocoon characters in silkworm *Bombyx mori* may be due to stress induced by the infestation of white muscardine disease and reduction of silk synthesis due to damage caused during the progress of the fungal pathogen and cessation of ingestion of feed. Mulberry silkworm is a completely domesticated insect with commercial significance and reared indoors and *Beauveria bassiana* is a destructive fungal pathogen that causes white muscardine. The disease occurrence in silkworm results in reduction in the commercial characteristics of cocoons; this has a direct effect on the quality of cocoon crops and disheartens the sericulture farmers.

**Table-1: Changes in Commercial Characteristics of Cocoons of silkworms immunized with *Beauveria bassiana* (Bals.) Vuill compared to Unimmunized**

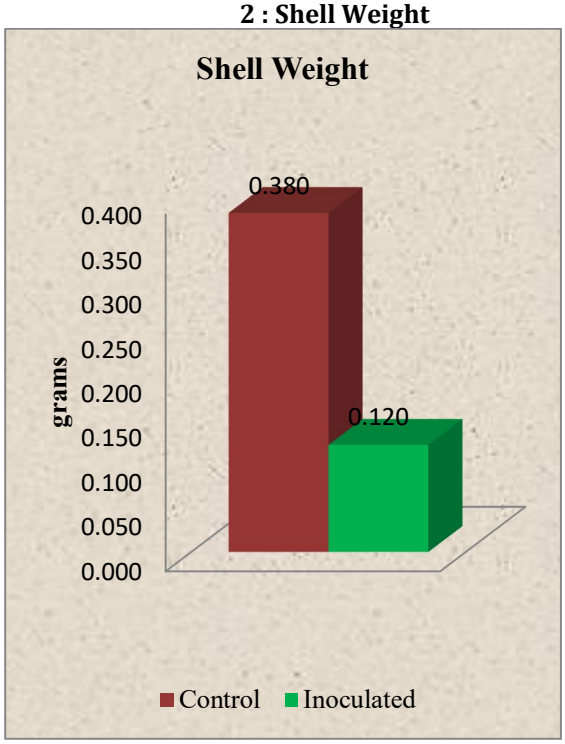
| S.NO | Name of the parameter | Treatments | Mean ±sd | t-value | p value | sig |
|---|---|---|---|---|---|---|
| 1 | Cocoon weight | Control | 1.780±0.077 | 12.619 | 0.000 | *** |
| | | Inoculated | 1.060±0.116 | | | |
| 2 | Shell weight | Control | 0.380 ±0.018 | 16.732 | 0.000 | *** |
| | | Inoculated | 0.120 ±0.032 | | | |
| 3 | Shell Ratio | Control | 21.110±1.827 | 6.169 | 0.000 | *** |
| | | Inoculated | 11.830±3.199 | | | |
| 4 | Filament length | Control | 999.180±0.502 | 874.453 | 0.000 | *** |
| | | Inoculated | 728.570±0.568 | | | |
| 5 | Non- breakable filament length | Control | 847.500±0.463 | 1019.000 | 0.000 | *** |
| | | Inoculated | 614.900±0.314 | | | |
| 6 | Number of breaks in the silk filament | Control | 0.200±0.009 | 77.213 | 0.000 | *** |
| | | Inoculated | 5.100±0.155 | | | |
| 7 | Denier of the silk filament | Control | 3.120± 0.172 | 5.858 | 0.000 | *** |
| | | Inoculated | 3.870± 0.262 | | | |

*** Highly significant

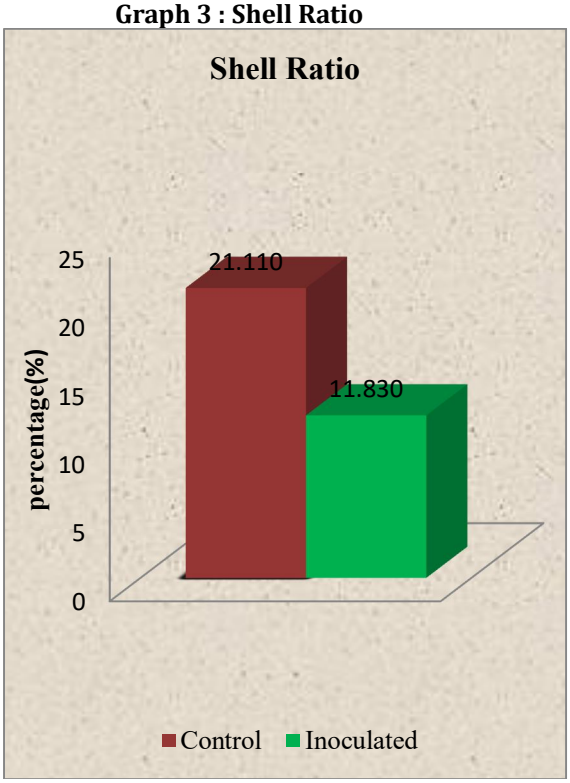
Graph 1 : Cocoon Weight Graph



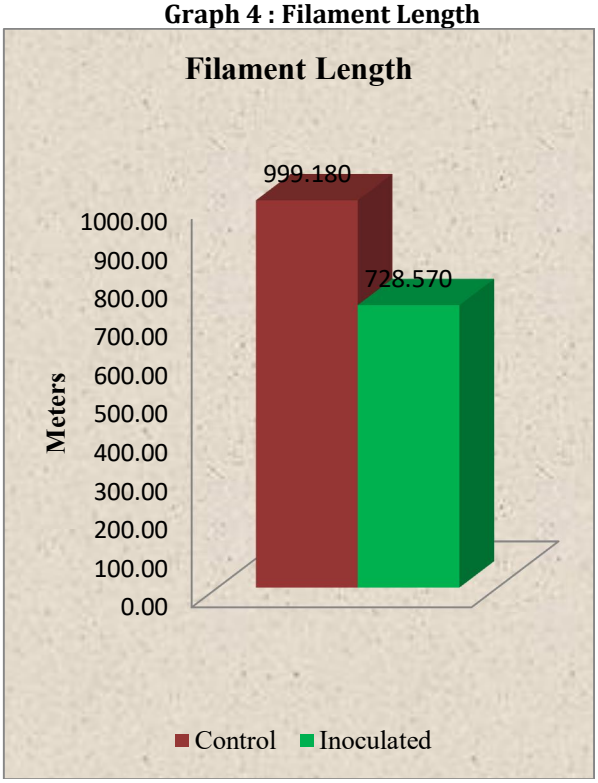

2 : Shell Weight



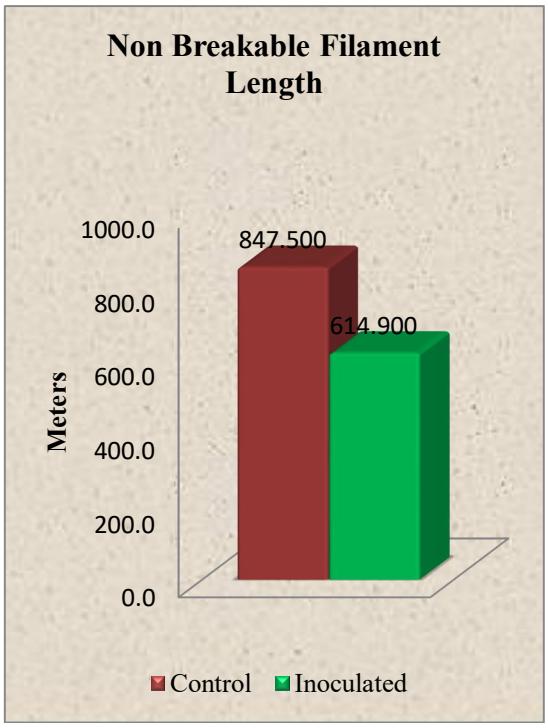

Graph 3 : Shell Ratio



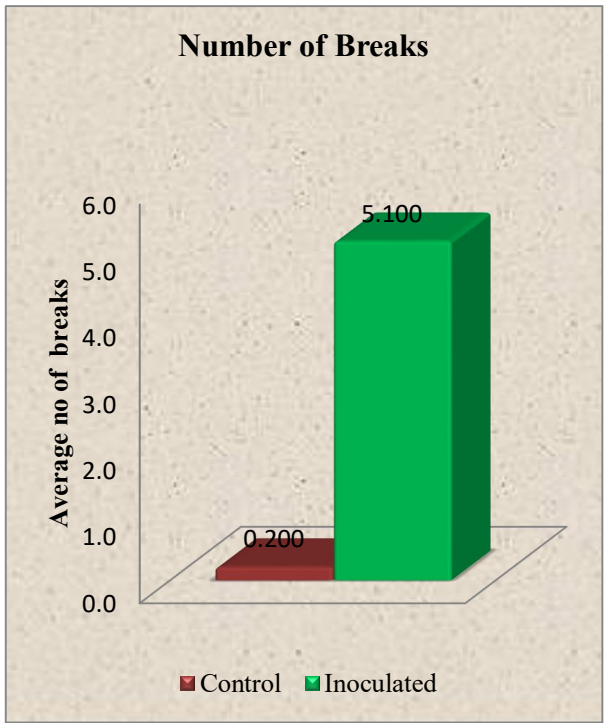

Graph 4 : Filament Length



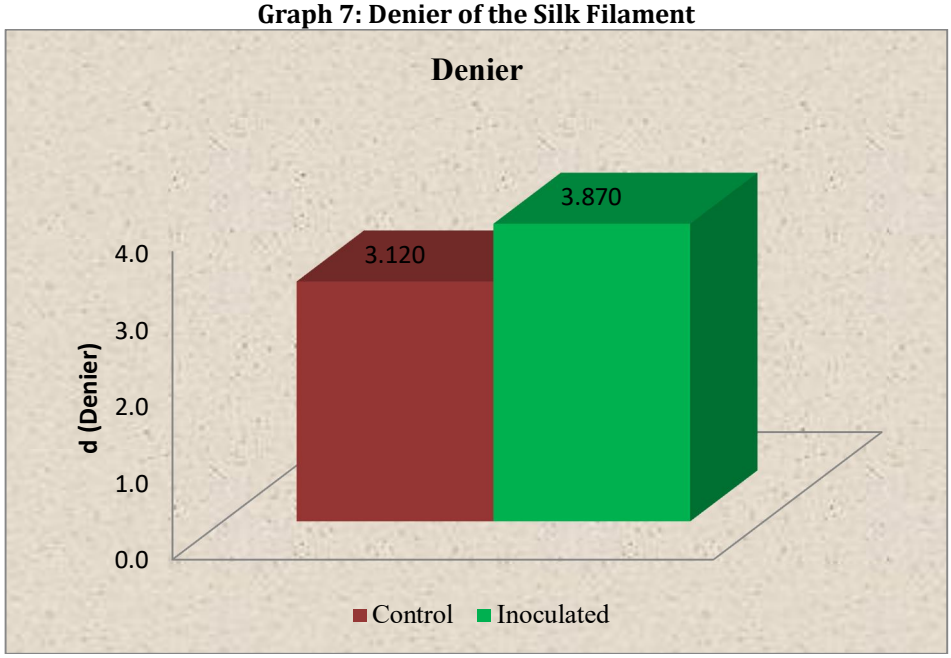
**Graph 5 : Non Breakable Filament Length**




**Graph 6 : Number of Breaks in the Silk Filament**




**Graph 7: Denier of the Silk Filament**




## CONCLUSION

The study on cocoon economical parameters treated with fungal pathogen *Beauveria bassiana* (Bals.) Vuill in silkworm *Bombyx mori* will be useful to understand and to assess the level of cocoon crop loss. If the diseases could be controlled below the economic threshold level, there will be an elevation of qualitative and quantitative characters of cocoon without expansion of the mulberry acreage. Hence, it is the responsibility of each and every personnel involved in the industry and practicing sericulture to explore preventive and control measures of the incidence of the disease to improve the cocoon yield parameters. Further, it is necessary to carry out research on these lines to understand the accurate cocoon crop loss due to the disease and to develop and design appropriate strategies to avoid the outbreak of diseases and to elevate the important economical traits of cocoon crops.

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