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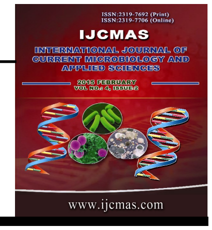

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Original Research Article

Studies on symptomological and economic parameters of silk cocoons of *Bombyx mori* inoculated with *Beauveria Bassiana* (Bals.) Vuill.

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A B S T R A C T

Beauveria bassiana infection influenced the growth and development of silkworm larvae and ultimately the economical cocoon characters like matured larval weight, cocoon weight, shell weight, shell percentage, filament length, non-breakable filament length, number of breaks and denier. Significant reduction of matured larval weight (2.08 g), cocoon weight (0.72 g), shell weight (0.09 g), shell ratio (12.80%), filament length (478.9 m), non-breakable filament length (120.8 m) and more number of breaks (4.1) and higher denier (2.62 d) was recorded in the experimental silkworm compared to control.

Keywords

Beauveria bassiana, Bombyx mori, Symptomological changes cocoon yield parameters

Introduction

Obviously, the success of sericulture industry primarily depends on the successful harvest of cocoon crops. The major problem of sericulture in a tropical country like India is the high incidence of diseases. The major diseases affecting mulberry silkworm are muscardine (fungal disease), flacherie (bacterial diseases) grasserie (viral diseases) and pebrine (protozoan disease). Silkworm crop loss occurs in all the silkworm growing areas of the world, but the type of severity varies. They differ from region to region, crop to crop and even from farmer to farmer. Leaving aside minor variations, it has been found that crop loss is generally more in the tropics than in the temperate regions.

The magnitude of the disease damage is on the higher side in India. It is a general observation that out of 5-6 crops per year, two are usually lost due to diseases and other reasons and even the successful once are partially lost. Thus the frequent outbreak of diseases is one of the main handicaps for the progress of sericulture industry. The reasons attributed, are poor hygienic conditions and continuous silkworm rearing all round the year, the climatic conditions that favour faster multiplication of disease causing germs. The accumulated germs under favourable conditions become active and cause the outbreak of diseases.

Beauveria bassiana (Bals) Vuill is one of the most destructive fungal pathogen of silkworm *Bombyx mori* L causing white muscardine disease, which is common in all sericulture zones of the world. The fungal disease is common during winter and rainy seasons. White muscardine was the first disease described in insects caused by a microorganism. Bassi (1835) demonstrated the contagious nature of the disease and identified the cause as a fungus and suggested measures to control the disease (Ainsworth, 1956). The fungus was named after Agostino Bassi Vuillemin in his honour as *Betrytis bassiana* by Balsamo and created a genus *Beauveria bassiana* (Balsamo) Vuillemin. It was a first microorganism to be recognized as a disease causing agent (Bassi, 1835). This disease wiped out the entire sericulture industry in Italy and France during 1920–1925. In India 10–40 percent of loss has been accounted for white muscardine in total loss due to diseases (Janakiraman, 1961; Ayuzawa, 1972; Chandrasekharan and Nataraju, 2008). In the year 2005, heavy rain in major silk producing states of Karnataka, Tamil Nadu and Andhra Pradesh has led to a widespread outbreak of muscardine among the silkworm cocoon crops, threatening the silk output in the country. In this backdrop, the study was carried out to examine the symptological changes in *Bombyx mori* during the progress of the disease and its influence on economical characters of the cocoons.

Materials and Methods

PM x CSR_2 was selected for the study and the silkworms were reared under optimum conditions with meticulous coordination of many activities such as maintenance of mulberry garden, preparation and disinfection of the rearing room and appliances, procurement and handling of silkworm eggs and incubation, young and late age silkworm rearing, general hygiene, moulting care, mounting, spinning, harvesting of cocoons *etc.* (Dandin *et al.*, 2003). On the 1st day of the fifth instar, the larvae were inoculated by dipping in sublethal concentration of fungal conidia spore suspension (2.15 x 10^6 conidia spores/ ml @ 50 ml/100 worms for 45 Sec) and larvae treated with double distilled water were used as control. From the day of inoculation to the end of the 5th instar the silkworms were kept under continuous surveillance to examine the symptomplogical changes during the development of fungal pathogen *Beauveria bassiana* and taken photographs.

By the end of the fifth instar the silkworm showed the symptoms of spinning i.e., cessation of feed, body shrinkage, translucent light yellow colour skin etc. These larvae are known as matured worms, the weight of the worms was recorded. These worms were transferred to mountages to spin the cocoons. After completion of the spinning process, the cocoons were harvested on the 6th day of mounting and then these cocoons were taken for the assessment of various economic parameters viz., cocoon weight, shell weight, shell ratio, filament length, non-breakable filament length, number of breaks and denier. The qualitative and quantitative parameters of cocoons were determined by following the methods as given by Sonwalkar (1993).

Matured larval weight

This character indicates the healthy and robust disposition of the larvae. It is a mean of the weights of 10 randomly selected fully matured larvae.

Cocoon weight

Cocoon weight indicates the approximate quantity of raw silk that could be reeled from the cocoons (Mahadevappa *et al.*, 2000). In the present experiment a sample is drawn from each replication comprising around 10 cocoons. The sample drawn represents the entire quality of each replication. Individual cocoon weight was taken from each sample of 10 cocoons and mean cocoon weight was calculated. The weights were taken in gram units.

Shell weight

This economic trait represents the total quantity of silk in a cocoon. Average single shell weight was calculated from 10 shells used for the assessment of cocoon weight.

Shell ratio

It denotes the total amount of silk available in a single cocoon and is expressed in percentage. It is calculated by using the following formula.

$$\text{Cocoon shell ratio} = \frac{\text{Weight of the cocoon shell}}{\text{Weight of the entire cocoon}} \times 100$$

Filament length

It is the total length of silk filament, unwound from a single cocoon measured in meters. In the present experiment a sample is drawn from each replication comprising around 10 cocoons. Ten cocoons were cooked and reeled on an eprouvette with a circumference of 1.125m and the mean value of filament length in meters was calculated as per the standard formula (Sonwalkar, 1993).

$$\text{Filament length (m)} = \text{Number of rotations} \times \text{Circumference of the wheel} (\chi)$$

$$\chi = 1.125\text{m}$$

Non-breakable filament length and Number of Breaks

It is the average length of filament that can be unwound from the cocoons without a break. Non-breakable filament length was calculated by using the formula as given by Sonwalkar (1993) and the number of breaks were recorded.

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

Denier

Denier represents the size of the yarn, i.e., the weight in grams of 9,000 meters of the filament. It was calculated using the formula as given by Sonwalkar (1993).

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

Variation in the denier of the cocoon silk filament will ultimately determine the uniformity and quality of raw silk yarn reeled.

Statistical analysis

All the experimental data recorded from the 4 replicates have been subjected to statistical analysis by following T-test.

Results and Discussion

Symptomological Changes

No conspicuous symptoms were noticed immediately after inoculation of fungal pathogen *Beauveria bassiana*. But gradually an infected silkworm became inactive, sluggish, stopped the feed and remained underneath the mulberry leaves. After 48 hours, the infected worms started to vomit digestive juice and later, the worms gradually became stiff and the movement of the worms was very much restricted and colour of the body changed to brown colour with oily specks. Initially the oily specks are in small in size with the advancement of the age, the size and number of the oil specks was enhanced (Photo. 3 & 5). Then the silkworm body became soft, pliable and later stiff and hard. Nearly on 7th or 8th day of the infection white efflorescence noticed near intersegment region, spiracles, then complete body was covered with the white mycelia and finally conidia developed on the body (Photo.4). The mummified cadaver became brittle and breaks into pieces when dropped from a certain height. Infected worms fail to spin the cocoons, but those which spin form flimsy cocoons (Photo.6). Cocoons formed by these infected worms were smaller and lighter in weight and the worms not emerged as moths.

Economical cocoon parameters

The overt changes observed in the economical traits of cocoon in *Beauveria bassiana* infected silkworm with reference to control are shown in Table-1 and Graphs-1-8. The results of the present study clearly indicated that *Beauveria bassiana* infestation influences the growth and development of silkworm and the economical cocoon parameters. Significant reduction of matured larval weight (2.08 g), cocoon weight (0.72 g), shell weight (0.09 g), shell ratio (12.8%), filament length (478.9 m), non-breakable filament length (120.8 m) and more number of breaks (4.1) and higher denier (2.62 d) was recorded in the experimental silkworm larvae. In contrast, to the experimental larvae significantly higher values were recorded in the majority of the economical characters such as matured larval weight (2.58 g), cocoon weight (1.44 g), shell weight (0.24 g), shell ratio (16.43%), filament length (671.6 m), non-breakable filament length (640.6 m) and less number of breaks (0.2) and lower denier (2.2 d) in control.

Appropriate matured larval weight is an indicator to measure the health of silkworm and in turn to obtain good quality of cocoon. Cocoon weight is an important commercial character used to determine the amount of raw silk that can be obtained. The cocoon price is determined based on the weight of the cocoon. The shell weight is more important than the cocoon weight since it is the shell that yields the silk for reeling. Thus, higher the weight of the shell, greater will be the silk yield. Shell percentage fairly indicates the quantity of raw silk that can be reeled from the cocoons and also helps in estimating renditta and thereby fixing a proper price for the cocoons. The silk filament length indicates the reelable length of silk filament from a cocoon. Reeling performance is better with cocoons having a larger filament length, which reduces the number of feeding ends. This results in increase of production, quality and also raw silk percentage of the cocoon. Generally, a longer non-breakable filament with less number of breaks, higher is the reelability. This indicates that higher the reelablity percentage higher is the raw silk quality. The fineness of cocoon filament is expressed by size i.e. denier (Sonwalkar, 1993; Mahadevappa *et al.,* 2000).

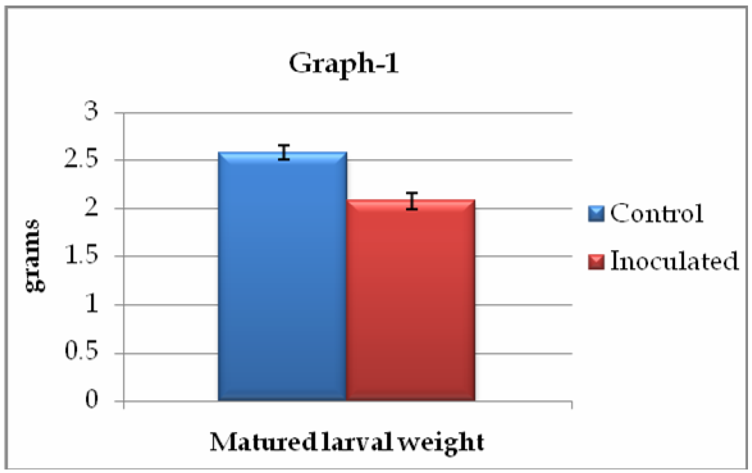
Reduction in the matured larval weight may be due to the consequence of fungal infection that leads to the decrease in food consumption, digestion, relative consumption rate, efficiency of conversion of ingested food in fifth instar of *Bombyx mori* infected with *Beauveria bassiana*. Cai (1989) analyzed the fungus growth and proliferation in silkworm body after 12 to 24 h post- inoculation of the *B. bassiana* conidium. The healthy growth and development of the silkworm is directly related to economical cocoon traits. It is well supported by Lakshmi Velide *et al.* (2013). She noticed poor cocoon characters from pebrine infected tropical tasar silkworm *Antheraea mylitta*.

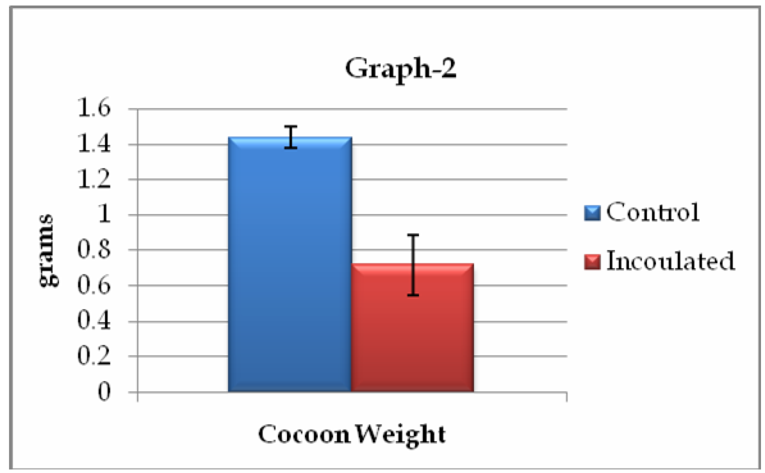
Economical Cocoon Parameters

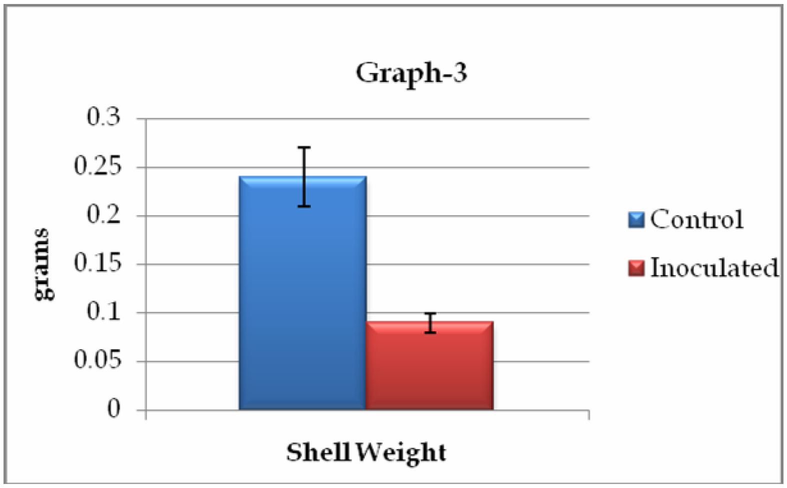
Table.1 Changes in economical cocoon parameters of silkworm *Bombyx mori* L. inoculated with fungal pathogen *Beauveria bassiana* (Bals.) Vuill. with reference to control

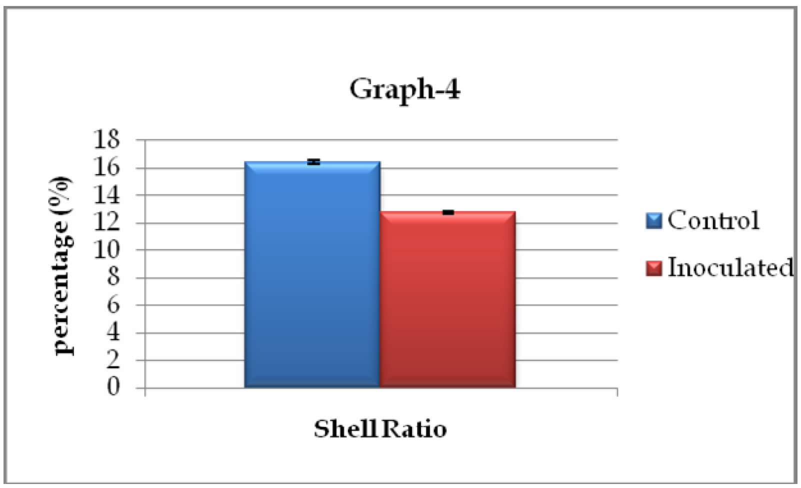
Treatment	Matured larval Weight (g)	Cocoon weight (g)	Shell Weight (g)	Shell Ratio (%)	Filament Length (m)	Non-Breakable Filament Length (m)	Number of Breaks	Denier (d)
Control	2.58 ± 0.07	1.44 ±0.06	0.24 ± 0.03	16.43 ±0.12	671.6 ± 18.33	640.6 ± 14.08	0.2 ± 0.42	2.20 ± 0.12
Inoculated	2.08 ± 0.09 ****	0.72 ± 0.17 ****	0.09 ± 0.01 ****	12.80 ±0.10 ****	478.9 ± 15.74 ****	120.8 ± 22.42 ****	4.1 ± 2.42 ****	2.62 ± 0.07 ****

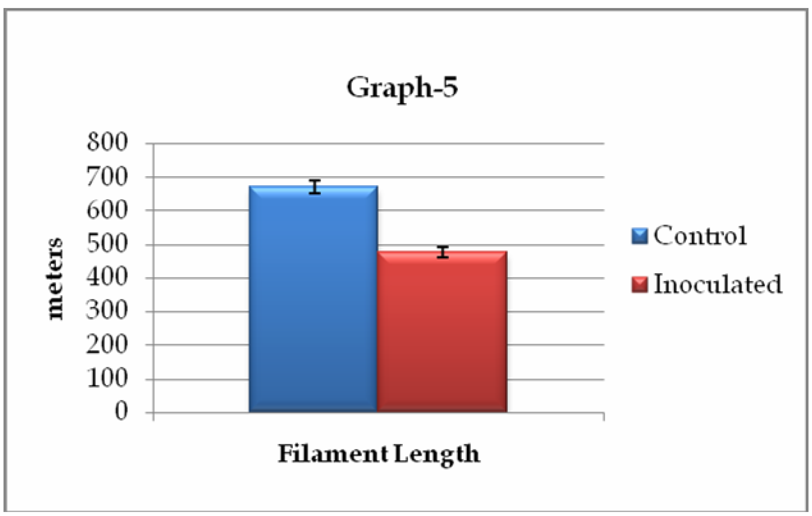
Mean±Standard Deviation; ****P≤0.001

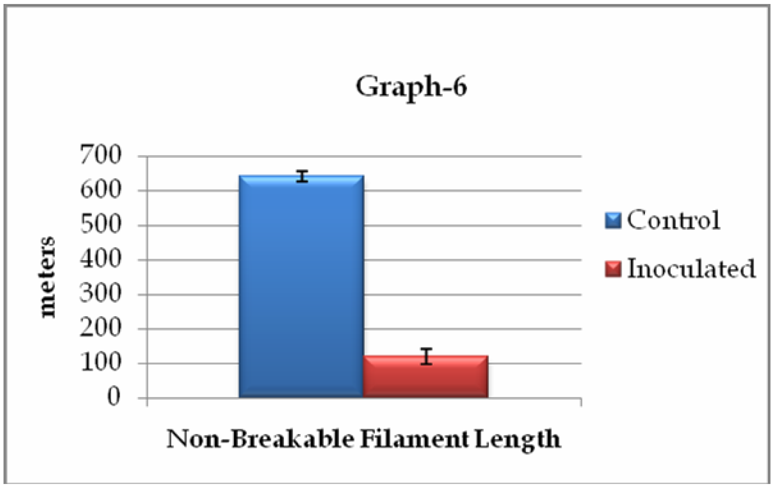


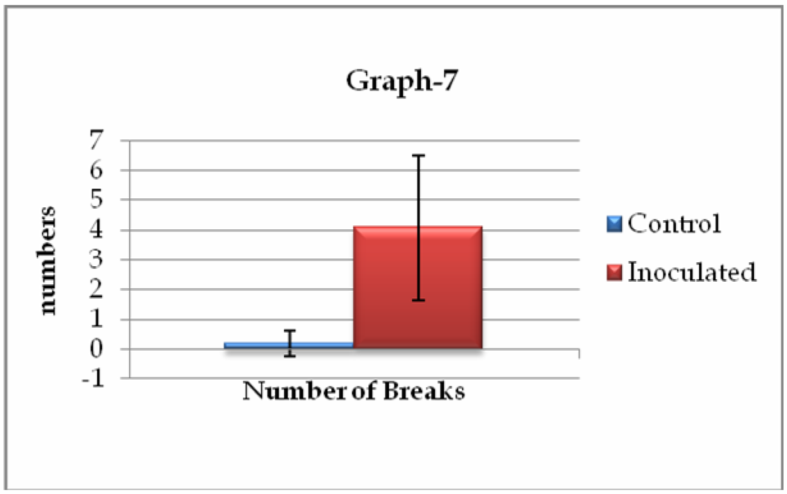



Graph-3
0.3
0.25
0.2
0.15
0.1
0.05
0
grams
Control
Inoculated
Shell Weight


Graph-4
18
16
14
12
10
8
6
4
2
0
percentage (%)
Control
Inoculated
Shell Ratio


Graph-5
800
700
600
500
400
300
200
100
0
meters
Control
Inoculated
Filament Length


Graph-6
meters
700
600
500
400
300
200
100
0
Control
Inoculated
Non-Breakable Filament Length


Graph-7
numbers
7
6
5
4
3
2
1
0
-1
Control
Inoculated
Number of Breaks

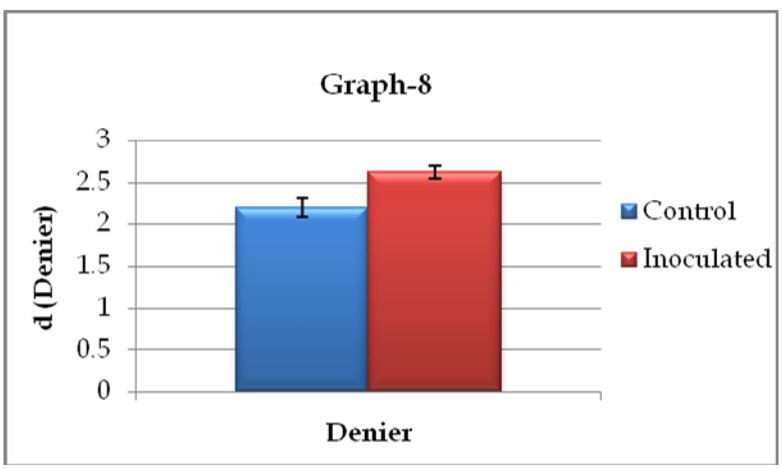

Graph-8
d (Denier)
3
2.5
2
1.5
1
0.5
0
Control
Inoculated
Denier

Photo.1 Healthy 5th Instar Silkworm Larvae

Photo.2 *Beauveria bassiana* Pure Culture

Photo.3 Oily specks on infected silkworm Larvae

Photo.4 Fully grown mycelia on the infected silkworm

Photo.5 Gradual changes in silkworm *Bombyx mori* L inoculated with *Beauveria bassiana*

Photo.6 Experimental Cocoons

Photo.7 Control Cocoons

The reduction in economical cocoon characters could be attributed to loss of appetite, lethargic conditions and physiological stress induced by the fungal pathogen *Beauveria bassiana* in 5th instar silkworm larvae. Jhansi Lakshmi (2003) observed the reduction of economical parameters due to *Beauveria bassiana* infection and attributed that it may be due to the decline in the synthesis of silk proteins and the direct or indirect effect of *Beauveria bassiana* on the growth and development of silkgland of the silkworm *Bombyx mori.* More number of breakages and higher denier were noticed in experimental cocoons with reference to healthy ones. It may be assumed that the physiological and biochemical stress induced by a fungal pathogen caused to exude uneven amounts of silk fluid in lumps. Rath *et al* (2003) have reported the decrease in shell weight in *Antheraea mylitta* larvae infected with *Nosema* sp. Rath and Sinha (2005) studied on the parasitization of fifth instar larvae of *Antheraea mylitta* by Uzifly have reported the decrease in cocoon weight and shell weight in the infected larvae.

Silkworm *Bombyx mori* is completely domesticated economic insect and *Beauveria bassiana* is an aggressive parasite. Therefore, the disease in silkworm results in a drastic reduction in the growth and development and qualitative and quantitative cocoon yield parameters. This ultimately has a direct effect on the sericulture community of the country due to reduced returns, in turn affecting the economy of the country, since silk as the end product of sericulture industry is a good source of foreign exchange for the country. Therefore, it is the prime responsibility of sericulture scientists and sericulture farmers to explore many more strategies to prevent/control the occurrence of the disease to enhance the qualitative and quantitative parameters of cocoon crops.

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