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INVESTIGATION ON CONVERSION EFFICIENCIES OF MULBERRY LEAVES IN SILKWORM *Bombyx mori* L. DURING THE PROGRESS OF ENTOMO-PATHOGENIC FUNGUS *Beauveria bassiana* (Bal) Vuill.

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Silkworm *Bombyx mori* is an economically important monophagous insect which is thoroughly domesticated by providing all the requisites for its growth and survival. Due to domestication over centuries, silkworms have become very gentle and delicate to the environmental conditions and different types of micro-pathological agents. The microorganisms such as virus, bacteria, fungi and protozoa cause diseases such as grasserie, flacherie, muscardine and pebrine which are the major threat to the sericulture industry. Chandrasekaran and Nataraju [1] stated that, out of the total crop loss due to different diseases, 10 to 40 per cent of crop loss occurs due to fungi, *Beauveria bassiana* in India. Nutrition is the basic unit of silkworm growth and development. Feeding and conversion efficiency of mulberry leaves such as leaf to cocoon ratio, leaf to shell ratio etc., in silkworm is one of the commercially important factors to obtain good dividends. Silkworm acquires its essential nutrients and water from the mulberry leaves it consumes. Various nutritional parameters such as ingestion of mulberry leaf, digestion, Efficiency of Conversion of Ingesta to Larva, Efficiency of Conversion of Digesta to Larva, Efficiency of Conversion of Ingesta to Cocoon, Efficiency of Conversion of Digesta to Cocoon, Efficiency of Conversion of Ingesta to Shell, Efficiency of Conversion of Digesta to Shell, I/g- Cocoon, D/g-Cocoon, I/g-Shell, D/g-Shell were estimated in the present investigation in *Beauveria bassiana* infected silkworms compared to untreated silkworms to understand the conversion efficiencies in infected silkworms. The results indicated a drop in ingesta, digesta, conversion efficiencies of ingesta and digesta to larva, cocoon and shell in the inoculated silkworms. On the contrary, ingesta and digesta required to produce one gram cocoon

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and one gram shell was higher in inoculated silkworms than in healthy silkworms. So, there is need to understand the various mechanisms involved and physiological changes occurring in the silkworm due to diseases which may be useful in developing prophylactic measures to overcome the attack of diseases.

Keywords: Ingesta; digesta; conversion efficiency of ingesta; conversion efficiency of digesta; I/g- Cocoon; D/g- Cocoon; I/g-Shell; D/g-Shell.

1. INTRODUCTION

The mulberry silkworm is an economically important insect that contributes to the economic development of the country. Silkworm is a very sensitive and monophagous insect that feeds only on mulberry and obtains all nutrients and water from the mulberry leaves to build its body, grow, sustain, synthesize silk proteins and spin cocoons. The success of silkworm rearing depends on the provision of nutritive mulberry leaves apart from other factors. Nourishment is a vital physiological factor that influences the growth and development of silkworms and silk production. The fifth instar is a voracious feeding stage of the silkworm, which warrants progressive changes in its biology and physiology. Biotic and abiotic factors influence the rate of consumption and utilization of mulberry leaf by the silkworm. Nutritional efficiency is considered as an important factor to assess the cost benefit ratio of cocoons and finally the sericulture industry. Infection influences the conversion efficiency of the ingested and digested mulberry leaf into the body biomass, silk synthesis and spinning of the cocoon.

Ueda [2] suggested that the studies on food consumption were confined to V instar as the maximum quantity (80-85%) of mulberry leaves are consumed in this stage of the silkworm life cycle and the silkworms are metabolically very active at this stage. Reynolds and Nottingham [3] reported that the food consumption and utilization are influenced by various biotic and abiotic factors, of which the most important are atmospheric temperature and humidity at the time of rearing according to Benchamin and Jolly [4]. Feed utilization efficiency of temperate strains of silkworm has been extensively studied by [5] and [6]. Later methodological changes were made by [7] and [8]. Scriber and Slansky [9] stated that understanding the food consumption pattern of insects is essential to gaining knowledge of their comprehensive metabolic and physiological activities. Muniraju et al. [10] studied the effect of temperature on leaf-silk conversion and reported higher silk conversion at low temperature of 26^0C throughout the rearing period. Therefore, in the present study, an attempt has been made to assess the various nutritional parameters and feed conversion efficiency of silkworms influenced by *Beauveria bassiana* infestation.

2. METHODOLOGY

The current investigation has been carried out on the commercially popular bivoltine double hybrid (CSR2 X CSR27) X (CSR6 X CSR26). The stock of silkworms has been maintained by following the standard protocol as suggested by [11]. Silkworms were reared by careful synchronization of several factors such as maintenance of a mulberry garden to provide suitable mulberry leaves for the silkworms, strict maintenance of hygiene in the rearing house and its surroundings, manipulation of required environmental conditions, procurement of good quality chawki worms etc. in the silkworm rearing laboratory of Department of Biosciences and Sericulture, Sri Padmavati Mahila Visvavidyalayam, Tirupati.

Simultaneously pure *Beauveria bassiana* culture was maintained by using Potato Dextrose Agar (PDA) medium. Conidia were collected from the 3-week-old *Beauveria bassiana* pure culture and sterile inoculum was prepared in a beaker that contained 50 ml of sterile double distilled water and a drop of tween-20 was added. LD50 value was calculated for the 5^{th} instar silkworms by following the Probit analysis (Leora software, 1987). Immediately after passing the fourth moult, the silkworms were inoculated with sub-lethal concentration (2.15 X 10^4 conidia/ml) and for the control batch, healthy silkworms were treated with double distilled water. For both control and experimental batches, four replications were maintained with hundred silkworms in each replication.

After induction of the myco-pathogen, *Beauveria bassiana*, the larvae were taken for experimentation to evaluate the dynamic daily changes in ingesta, digesta and conversion efficiencies. A specific quantity of mulberry leaf was weighed and fed to the silkworms from the 1^{st} day to the 6^{th} day of the 5^{th} instar, the quantity of leftover leaves and excreta were weighed every day for calculating the ingesta, digesta, excreta, conversion of ingesta to larva, conversion of digesta to larva, conversion of ingesta to cocoon, conversion of digesta to cocoon, conversion of ingesta to shell, conversion of digesta to shell, I/g-cocoon, D/g-cocoon, I/g-shell and D/g-shell by following standard protocols as recommended by [12], and [13].

2.1 Ingesta

Ingesta refers to consumption of mulberry leaves by silkworm from the total mulberry leaf provided. Fresh mulberry leaves were fed to the silkworms and simultaneously, the same quantity of dry leaf weight was noted. The leftover mulberry leaf was kept in an oven at 80°C until it reached a constant weight, which was then recorded. Using the following formula, the total dry weight of mulberry leaves consumed by silkworm on each day from the first day to the sixth day during the 5^{th} phase of its lifecycle was computed and expressed in grams per larva.

Ingesta = (Total leaf provided – Leaf leftover) / Number of larvae

2.2 Digesta

Digesta refers to the entire amount of food digested and absorbed out of the total amount of mulberry leaves given to it. The dry weight of the leaf supplied and consumed everyday was taken into account. It was calculated by the following formula and expressed in grams per larva.

Digesta = (Dry weight of leaf ingested – Dry weight of litter) / Number of larvae

2.3 Conversion Efficiencies

2.3.1 Efficiency of conversion of ingesta (ECI) to larva (%)

Conversion efficiency of ingested food to larval body matter is known as the efficiency of conversion of ingesta to larva and was calculated by employing the below given formula in both healthy and inoculated silkworms.

ECI to Larva = (Dry weight gained by Larva / Dry weight of food Ingested) X 100

2.3.2 Efficiency conversion of digesta (ECD) to larva (%)

The conversion efficiency of digested food to larval biomass is known as the efficiency of conversion of digesta to larva and was calculated by using the formula mentioned below.

ECD to Larva = (Dry weight gained by Larva /Dry weight of food Digested) X 100

2.3.3 Efficiency of conversion of ingesta (ECI) to cocoon (%)

The efficiency of conversion of ingesta to cocoon is an important parameter to assess the healthiness of silkworms. It indicates the total intake of mulberry leaf required for the production of 1 g of a cocoon and it was calculated by using the formula given below.

ECI to Cocoon = (Dry weight of Cocoon / Dry weight of food Ingested) X 100

2.3.4 Efficiency conversion of digesta (ECD) to cocoon (%)

The efficiency of conversion of the digested food to cocoon is called efficiency of conversion of digesta to cocoon and was estimated by using the formula.

ECD to Cocoon =(Dry weight of Cocoon / Dry weight of food Digested) X 100

2.3.5 Efficiency of conversion of ingesta (ECI) to shell (%)

The efficiency of conversion of ingesta to shell is referred to as the leaf-shell conversion rate and ultimately indicates the robustness of silkworm. It was estimated by using the below given formula.

ECI to Shell = (Dry weight of Shell / Dry weight of food Ingested) X 100

2.3.6 Efficiency of conversion of digesta (ECD) to shell (%)

Efficiency conversion of digesta into shell was calculated by using the below given formula.

ECD to Shell = (Dry weight of Shell / Dry weight of food Digested) X 100

2.3.7 Ingesta per gram cocoon

Ingesta per gram cocoon indicates the amount of ingested food required to produce one gram of cocoon and is computed using the formula given below. The ingested food converted to one gram of cocoon was calculated by using the formula given below.

Ingesta I/g. cocoon = Ingesta /Cocoon weight

2.3.8 Digesta per gram cocoon

Digesta per gram cocoon indicates the amount of digested food required to produce one gram of cocoon and is computed using the formula given below. The digested food converted to one gram of cocoon was calculated by using the formulas given below.

Digesta D/g. cocoon = Digesta /Cocoon weight

2.3.9 Ingesta per gram shell

The ingested food converted to one gram of shell was worked out using the formula mentioned below.

Ingesta I/g. shell = Ingesta /Shell weight

2.3.10 Digesta per gram shell

The digested food converted to one gram of shell was worked out using the formula mentioned below.

Digesta D/g. shell = Digesta /Shell weight

3. RESULTS AND DISCUSSION

Nutritional indices like ingesta and digesta were calculated every day and presented in Tables and Graphs 1 and 2. The nutritional efficiency parameters were studied and tabulated in Table 3 and Graphs 3 to12.

3.1 Ingesta

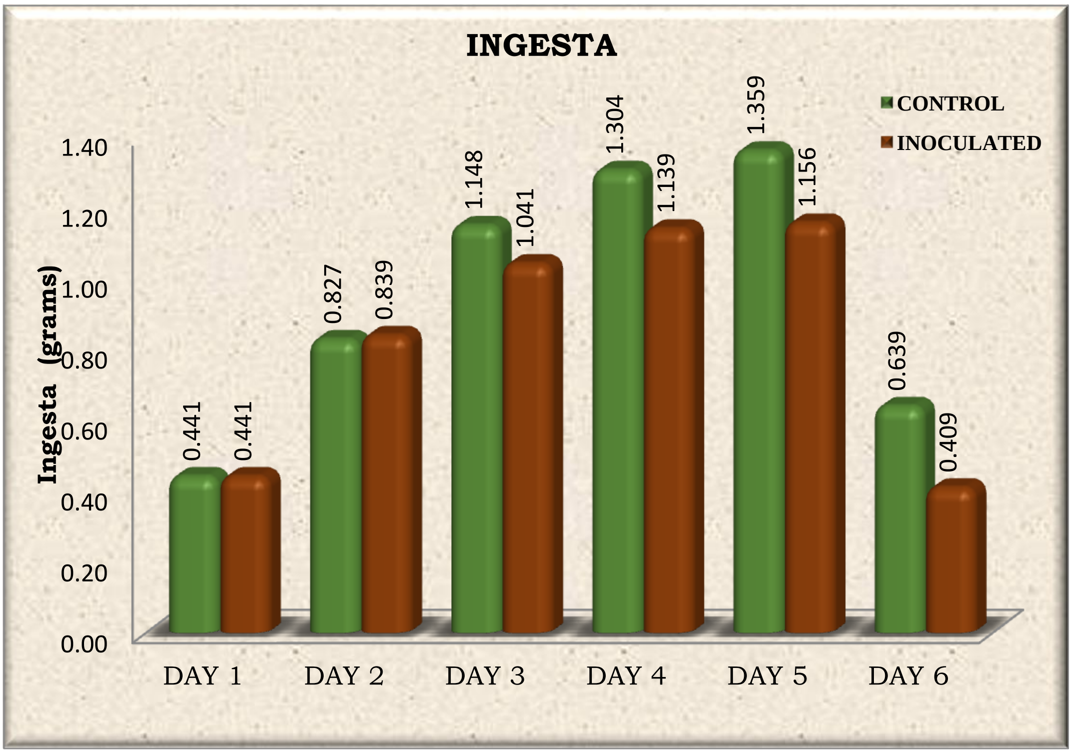
The data recorded on ingestion of the silkworms are presented in (Table 1 and Graph 1). The mean ingesta from day 1 to day 6 of inoculated larvae ranged from 0.441g to 0.409g. Maximum ingesta was observed on the 5th day whereas minimum ingesta was observed on the 6th day. The ingesta from day 1 to day 6 of control i.e., in healthy larvae ranged from 0.441g to 0.639g. Maximum ingesta was observed on the 5th day whereas minimum ingesta was observed on the 1st day. A highly significant reduction of ingestion was noticed in inoculated compared to control from the third day to the sixth day. In both inoculated (0.441g/larva to 1.156g/larva) and healthy silkworms (0.441g/larva to 1.359g/larva), steady enhancement of ingestion of the mulberry leaf was recorded up to fifth day. However, as compared to healthy silkworms, there was no significant difference in mulberry leaf consumption on the first and second day of the instar. Infected silkworms consumed more feed on the second day compared to untreated silkworms. Food consumption in experimental animals decreased dramatically after the third day and remained low until the conclusion of the instar compared to healthy.

The food intake was high after 24 hrs of infection. Higher consumption of mulberry leaf in the inoculated worm is to meet the energy requirement for the enhanced metabolism as a result of infection by the fungal pathogen *Beauveria bassiana* and to meet the nutritional demands of the pathogen. The decrease in mulberry leaf intake from the third day onwards could be attributable to an increase in the severity of the pathogen *Beauveria bassiana* infection. Zhang YH, et al. [14] studied the epigenetic regulation of response in silkworms due to *Bombyx mori*cypovirus infection and stated that when the host is invaded by pathogens, gene expression is regulated because of the changes in DNA methylation levels which may activate or suppress the relevant signaling pathways and trigger a series of immune responses against viral invasion. Song et al. [15] identified the expression of UGT46A1 gene and its effect on silkworm feeding and stated that silkworm *Bombyx mori* uses a complex olfactory system to detect its food. Due to the production of endotoxins by *Beauveria bassiana* the expression of UGT46A1 gene might have been suppressed, so the diseased worms consume fewer amounts of mulberry leaves. Silkworm feeds on mulberry leaves depending on its olfactory and gustatory stimuli. Qiu et al. [16] noticed that the insect olfactory system is a highly specific and sensitive chemical detector, which plays a significant role in feeding. It is mainly responsible for the sense of smell. Nagata and Nagasawa [17] reported that the insect's frontal ganglia promote feeding behaviour through the contraction of the foregut and regulation of another neural network. The pathological condition of the inoculated worms might have impaired the olfactory senses of the silkworms and so they consumed less quantity of leaves.

Table 1. Every day changes in ingesta of silkworm *Bombyx mori* L. inoculated with fungal pathogen *Beauveria bassiana* (Bals.) Vuill. with reference to control during 5th instar

Ingesta/ larva (g)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Control	0.4405±0.030	0.827 ± 0.008	1.148±0.019	1.304 ± 0.013	1.359 ± 0.011	0.639 ± 0.025
Inoculated	0.441±0.029	0.839 ± 0.017	1.041 ± 0.011	1.139 ± 0.015	1.156 ± 0.015	0.409 ± 0.018
P value	1.000	0.154	0.000	0.000	0.000	0.000
Sig	NS	NS	***	***	***	***

*NS = Not Significant *= p< 0.05: ** = p<0.01: *** = p< 0.001 (Highly Significant)*



Graph 1.

3.2 Digesta

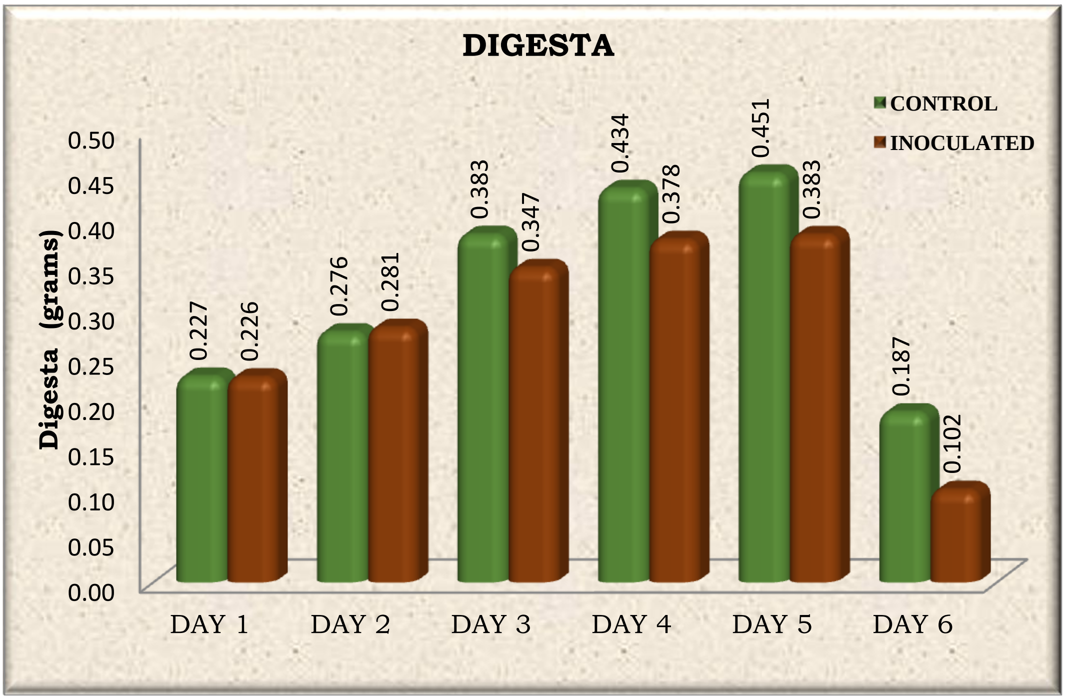
The digesta from day 1 to day 6 of inoculated larvae (Table 2 and Graph 2) ranged from 0.227g to 0.102g. Maximum digesta (0.383 g/larva) was observed on the 5th day whereas minimum digesta (0.102 g/larva) was observed on the 6th day. The mean digesta from day 1 to day 6 of control, healthy larvae ranged from 0.227g to 0.187g. Maximum digesta (0.451 g/larva) was observed on the 5th day whereas minimum digesta (0.187 g/larva) was observed on the 6th day. A highly significant variation in digesta was noticed in inoculated silkworms from the third day to the sixth day with a modest increase on the second with reference to control. From the 1st to the 5th day of the 5th instar, a steady improvement in food digestion was observed in both treated (0.226g/larva to 0.383g/larva) and untreated (0.227g/larva to 0.451g/larva) batches, but on the 6th day, a drastic decline in digestion was observed in both treated (0.102g/larva) and untreated (0.187g/larva) batches.

The digestion of food was high after 24 hrs of infection in the infected silkworms and is to meet the energy requirement for the amplified metabolism as a result of inoculation by the fungal pathogen *Beauveria bassiana* to overcome the stress created by the pathogen. The decrease in digesta from the third day onwards may be due to less consumption of food by the infested silkworm and increase in the severity of the pathogen *Beauveria bassiana* infection. The gut microbiota present in silkworm may secrete enzymes important in digestion. Raman et al. [18] observed the relationship between gut bacteria and digestion and reported that the gut microbiota directly influences the nutrient uptake and metabolism in *Bombyx mori*.

Table 2. Day to day changes in digesta of silkworm *Bombyx mori* L. inoculated with fungal pathogen *Beauveria bassiana* (Bals.) Vuill. with reference to control during 5th instar

Digesta/ larva (g)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Control	0.227 ± 0.011	0.276 ± 0.007	0.383 ± 0.008	0.434 ± 0.011	0.451 ± 0.011	0.187 ± 0.009
Inoculated	0.226 ± 0.022	0.281 ± 0.008	0.347 ± 0.018	0.378 ± 0.010	0.383 ± 0.009	0.102 ± 0.014
P value	0.936	0.263	0.001	0.000	0.000	0.000
Sig	NS	NS	***	***	***	***

*NS = Not Significant *= p<0.05 :** = p<0.01 : *** = p<= 0.001 (Highly Significant)*



Graph 2.

The endotoxins released by Beauveria bassiana in the diseased silkworms may affect the gut microbiota and may influence the digestion in the silkworms.

According to [19] and [20], mulberry leaf consumption and digestion is dependent on the silkworm's ability to assimilate and to transform digested material into body mass. Nath et al. [21] made similar observations with silkworms infested by Uzi flies. This observation is consistent with observations of [22-24], who found that control batches consumed more food and assimilated more nutrients than *Bm*NPV infected batches. Food consumption was higher in *Bm*NPV infected larvae in the study carried out by [25], who reported that food digestion was also higher in *Bm*NPV infected larvae.

3.3 Conversion Efficiencies

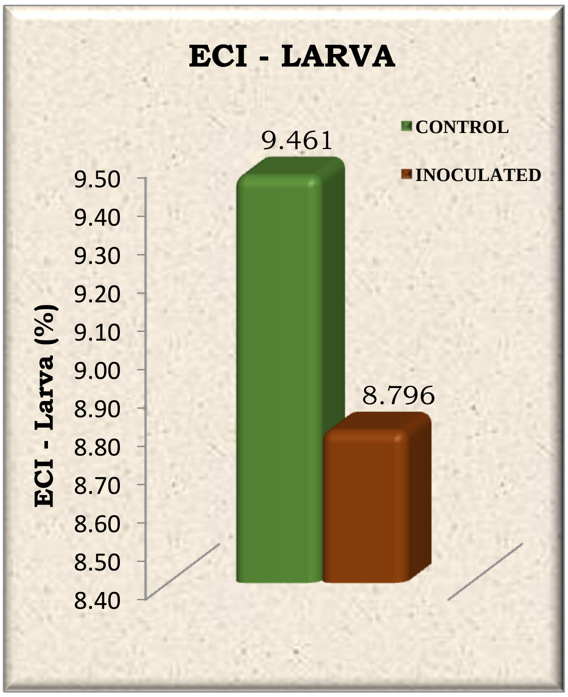
The data obtained for the conversion efficiency of ingesta and digesta to larval biomass, cocoon, shell and ingesta/digesta required to produce one gram of cocoon and shell are tabulated in Table 3 and Graphs 3 to 12.

Efficiency conversion of ingesta and digesta to larva was less in diseased (8.796, 25.028) compared to control (9.461, 27.254). Similarly, efficiency of conversion of ingesta and digesta to cocoon in infected silkworms (21.095, 60.023) was observed to be less compared to control (31.130, 89.673). Likewise, efficiency of conversion of ingesta and digesta to shell in parasitized silkworms (4.398, 12.514) was reduced compared to control (6.558, 18.892). Efficiency of conversion of ingesta and digesta to matured larval weight, cocoon and shell are greatly influenced by the nutritional status and physiological state of the silkworms. The diseased silkworms showed the decreased efficiency of conversion of both ingesta and digesta into larval biomass, cocoon and shell. So, the larval weight, cocoon weight and shell weight were less. Feed conversion efficiency contributes directly or indirectly to the major chunk of the cost benefit ratio of silkworm farming and is considered as an important physiological criterion to evaluate the superiority of silkworm breeds. But, due to the pathological stress induced by the pathogen, the infected silkworms were unable to convert ingesta and digesta into larval biomass, cocoon and shell efficiently. As a result, it can be stated that the endotoxin beauvericin released by the pathogen *Beauveria bassiana* during the progression of the disease white muscardine induced a decrease in the conversion efficiency of ingesta and digesta to larval weight, cocoon and shell. Ding et al. [26,27] and [28] stated that the efficiency conversion of ingesta to cocoon (ECI-C) and shell (ECI-S) which are referred as leaf-cocoon and leaf-shell conversion rate are the ultimate indices to assess nutritional efficiency of the silkworms in terms of quality production of cocoon and shell. Nath et al. [21] and [29] suggested that the less choice of feed leads to some physiological adaptations to withstand nutritional stress conditions. Abiotic factors also influence the conversion efficiencies of mulberry leaves by the silkworms. Paul et al. [30] observed poor ECI and ECD in silkworm fed with mulberry leaves having less moisture content. Singh and Ninagi [31] noticed that less food ingested silkworms had high ECI and ECD to cocoon and shell. Anantharaman et al. [32] noticed significant variation of ECI and ECD to larva due to different variety of mulberry leaves. Rahmathulla and Himantharaj [33] noticed that the conversion efficiency varied among different breeds. Rahmathulla and Suresh [34] concluded that conversion efficiency of ingesta was high in summer season indicating that abiotic factor like temperature also influences the efficiency.

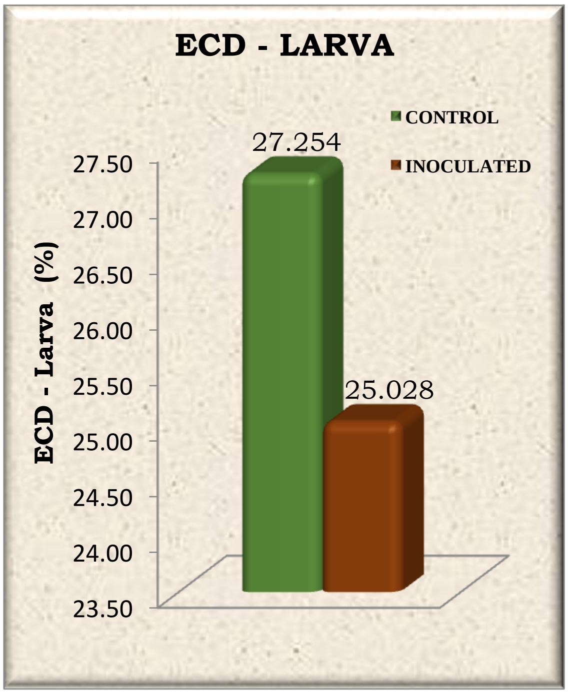
Table 3 and Graphs 3 – 12.

	Batches	Mean	Std. deviation	t-value	p value	Sig
ECI-Larva	CONTROL	9.461	0.090	17.866	0.000	***
	INOCULATED	8.796	0.009			
ECD-Larva	CONTROL	27.254	0.183	29.713	0.000	***
	INOCULATED	25.028	0.010			
ECI-Cocoon	CONTROL	31.130	0.189	127.560	0.000	***
	INOCULATED	21.095	0.033			
ECD-Cocoon	CONTROL	89.673	0.729	99.547	0.000	***
	INOCULATED	60.023	0.009			
ECI-Shell	CONTROL	6.558	0.171	29.729	0.000	***
	INOCULATED	4.398	0.046			
ECD-Shell	CONTROL	18.892	0.054	278.129	0.000	***
	INOCULATED	12.514	0.015			
I/g- Cocoon	CONTROL	3.410	0.085	16.288	0.000	***
	INOCULATED	3.988	0.017			
D/g- Cocoon	CONTROL	1.154	0.005	41.257	0.000	***
	INOCULATED	1.402	0.014			
I/g- Shell	CONTROL	15.640	0.091	222.554	0.000	***
	INOCULATED	22.738	0.012			
D/g- Shell	CONTROL	5.293	0.009	131.181	0.000	***
	INOCULATED	7.991	0.050			

*NS = Not Significant : * = p< 0.05 : **=p<0.01 : *** = p< 0.001*



Graph 3.



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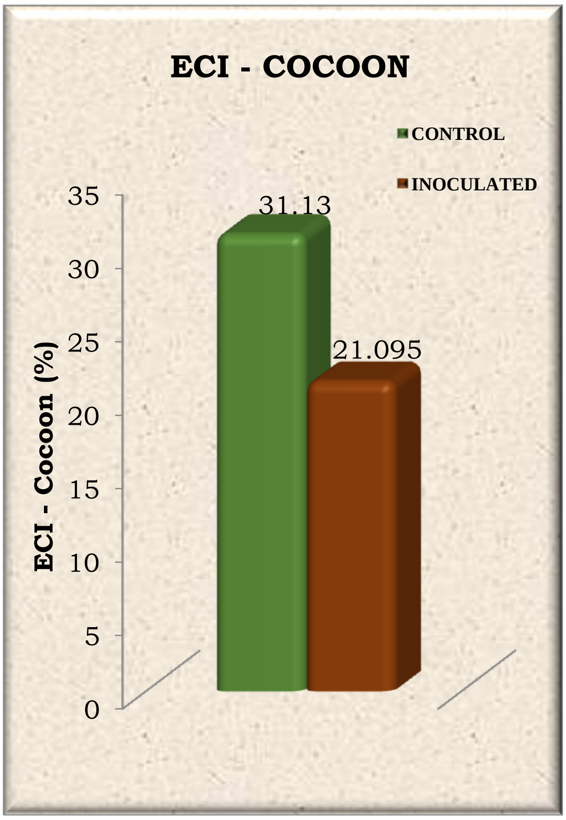

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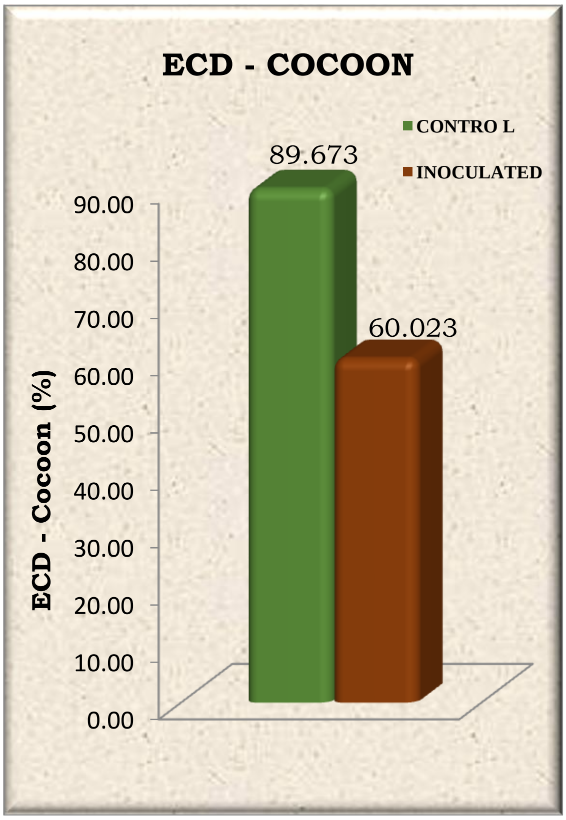

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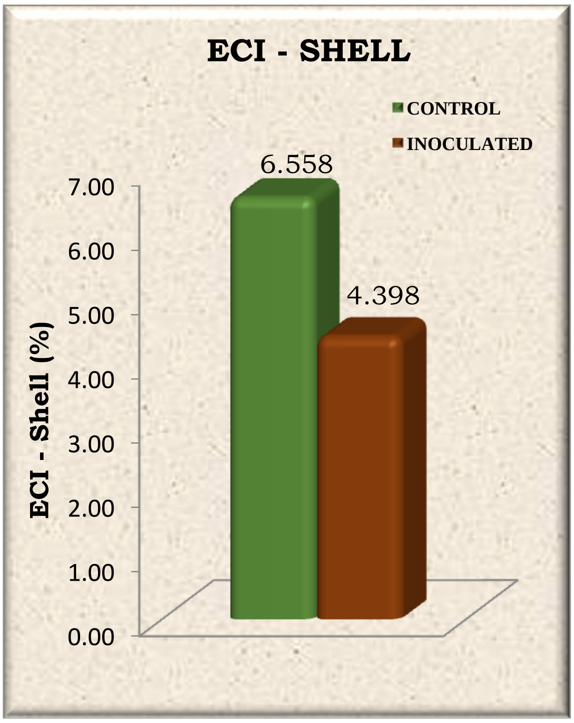

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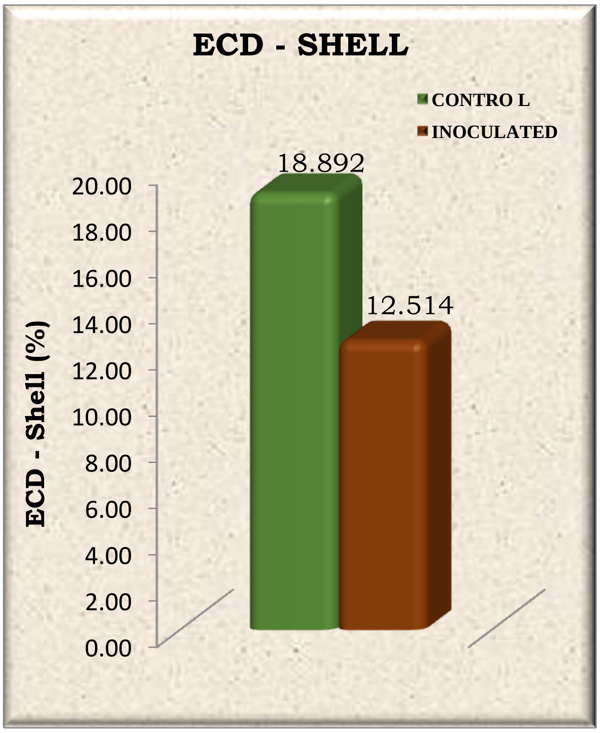
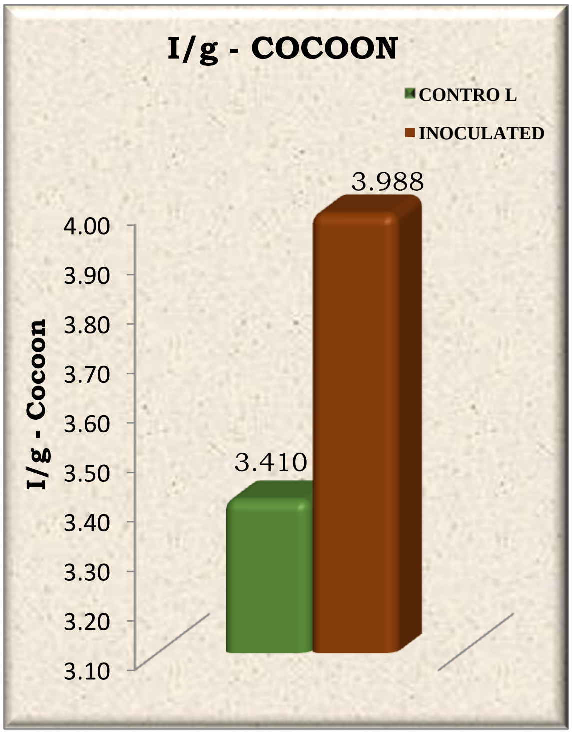


Graph 8.



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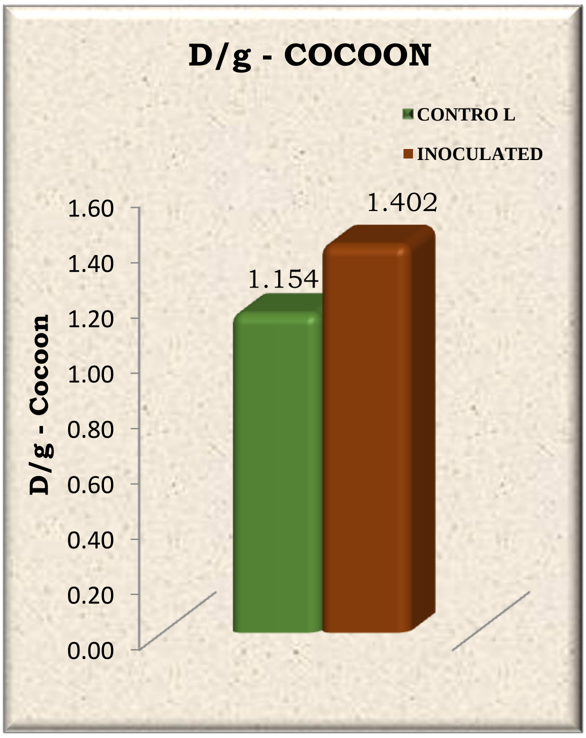

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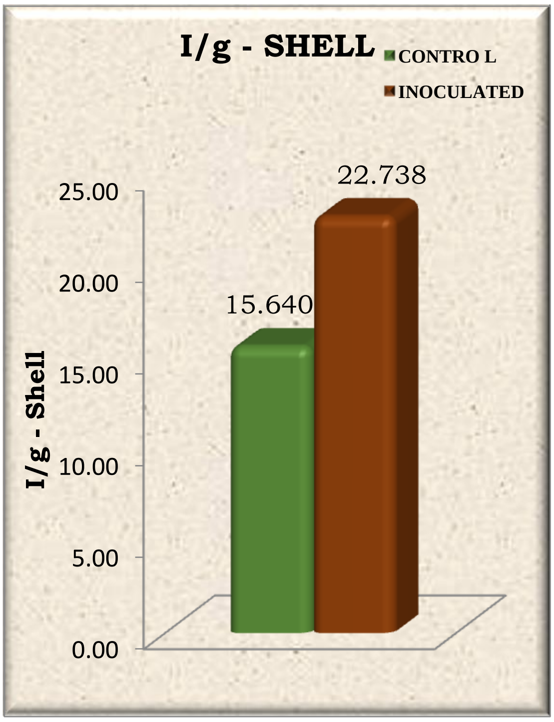
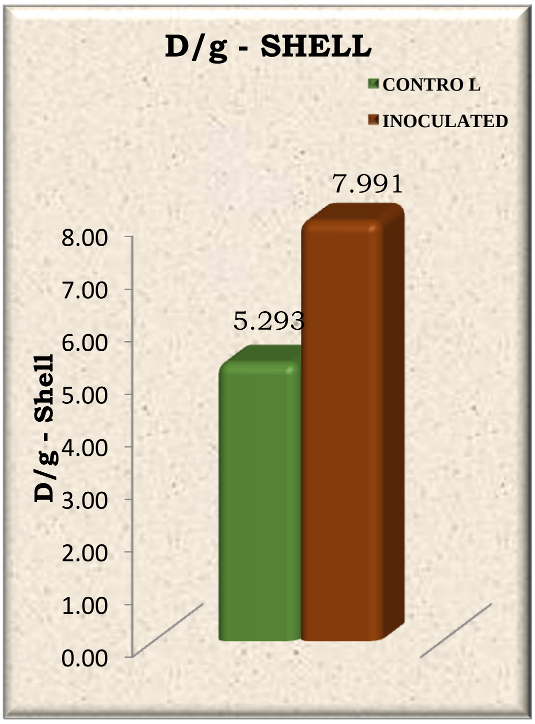


Graph 11.



Graph 12.

However, the amount of ingesta required to produce one gram cocoon (I/g-C) and shell (I/g-S) in infested silkworms was recorded (3.988, 22.738) to be higher than in control (3.410,15.640). Even the amount of digesta required to produce one gram of cocoon (D/g-C) and shell (D/g-S) was higher in inoculated silkworms (1.402, 7.991) than in healthy silkworms (1.154, 5.293). These observations are in contrary with the observations made by [35] who experimented on silkworms exposed to various stress factors and reported less value of ingesta required to produce one gram of cocoon under stress conditions. Takano and Arai [19] and [35] opined that nutritional efficiencies in the larval stage significantly influence the growth and development of pupa, adult and silk produce. The efficiency with which food is ingested and converted to larval body mass influences all the economic parameters of silkworm cocoons. Anantharaman et al. [32] observed that in summer reared batches less ingesta and digesta was required to produce 1 gram of cocoon and shell indicating its conversion efficiency under stress. Rahmathulla and Suresh [34] reported that in rainy and winter season more food was required to produce 1 g of shell than in summer season due to physiological stress in summer.

4. CONCLUSION

The present study revealed significant variations in ingesta and digesta in *Beauveria bassiana* infected silkworms. The conversion efficiencies of inoculated silkworms in terms of ingesta and digesta to larval weight, cocoon and shell decreased due to the progression of the disease caused by *Beauveria bassiana*. Conversely, ingesta and digesta required to produce one gram of cocoon and shell was more in the infected silkworms. This study provides an insight for the researchers to understand the underlying nutritional and physiological mechanisms of interaction between the host and the pathogen and its effect on the host as well as to study its effect on the gustatory, olfactory system and molecular mechanism of feeding in silkworms and its influence on conversion efficiencies and helps in devising the prophylactic measures to combat the disease to improve the growth and development of silkworms, in turn to enhance the economic characters of cocoon crops.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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