



Effect of *Clavigralla gibbosa* 'spiny brown bug' infested mulberry leaf on the cocoon and post cocoon parameters of silkworm

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Abstract

Mulberry is the main food source for the silkworm *Bombyx mori* L. The spiky brown leaves that are infested with insects have a greater impact on the output of silkworms, affecting both the quantity and quality of the larvae and cocoons. There are several insects that may attack mulberry leaves, and while the term often refers to aphids or weevils on legume crops, mulberry plants can also be affected by similar sucking or boring pests. The nutritional quality of various mulberry leaves is severely degraded when silkworms (*Bombyx mori* L.) are fed mulberry leaves that are infested by sap-sucking insects such as mealy bugs (which cause 'Tukra' disease) or *Clavigralla* species, which in turn greatly hinders their growth and development. Leaf footed bugs or spiny brown bugs, which belong to the Coreidae family, are common names. This study was carried out using selected disease-free layings (DFLs) from commercially popular multivoltine race hybrid silkworms. The DFLs were split into two groups, each with one DFL per kind, and reared separately with healthy and infested selected popular four varieties of mulberry leaves. After installation, on the fifth day, 10 silkworm larvae were harvested by making sure they were fully grown, and the quantity was noted. Larval parameters, such as the number of cocoons collected, the weight of the cocoon, the weight of the cocoon shell, the shell ratio, the total length of the filament, and the length of the unbreakable filament, were evaluated by maintaining the larvae at a temperature of (26±1°C, 12±1 hour, and 80±5% RH) during the stop feeding process. The quality of silk after the cocoon phases was evaluated using the following cocoon parameters: The statistical computation was used to determine the variety feeding of randomly chosen Mulberry larvae, and the findings were documented. Varietal mulberry *Morus alba* the percentage of loss in the number of cocoons harvested was 41.90%, the weight of the cocoon was 35.10% gram, the weight of the cocoon shell was 24% gram, the weight of the cocoon shell was 27.80%, the total length of the filament was reduced by 26.40%, the length of the unbreakable filament was 40.80%, and the denier reeducation loss was 50% when compared to healthy and infested mulberry leaf feeding.

Keywords: Silkworm cocoon, cocoon shell weight, cocoon shell ratio, *Clavigralla gibbosa*, total filament length, non-breakable filament length, denier & varietal mulberry

Introduction

The silkworm, *Bombyx mori*, exclusively eats mulberry plants (*Morus* spp.). As a result, the quality of mulberry leaves has a direct bearing on the growth and development of silkworm larvae, as well as on the caliber of the cocoons they produce. Nevertheless, mulberry cultivation is often plagued by infestations of various insect pests, which can significantly reduce the quantity and quality of mulberry leaves. Only a small percentage of the numerous insect pests that attack mulberry trees actually inflict economic damage to the crop. *Clavigralla gibbosa*, sometimes known as the "Spiny Brown Bug," is a major pest. The majorities of them has a short life cycle and are present throughout the year. Delayed growth, malformed leaves, and other symptoms are seen. The pests diminish the quantity, quality, and yield of leaves. The *Clavigralla gibbosa* 'Spiny Brown Bug' is a more serious sap-sucking pest because of its frequent occurrence and widespread harm to mulberry plants in recent years. The young, tender leaves are eaten by the early-stage larvae, which reside in the apical and tender leaf portion of the mulberry shoot. In most cases, larvae live on leaves by constructing webs or folding them into a protective refuge. The larval web in which the apical leaves reside rolls and ties their margins. The web secreted from the larvae, which are still inside, occasionally rolls one or two leaves into the shape of a cup. Therefore, the Spiny

Brown insect is generally referred to as *Clavigralla gibbosa* 'Spiny Brown Bug'. Plants that are infested exhibit stunted growth. From October through January, the incidence is high. The 'Spiny Brown Bug' infestation of *Clavigralla gibbosa* is getting worse every year, particularly in mulberry gardens, which are one of the main traditional sericulture regions. Additionally, the prevalence of the *Clavigralla gibbosa* 'Spiny Brown Bug' on some of the vegetable and mulberry crops, as well as on mulberry, has grown to be a significant issue in recent years. Therefore, the purpose of the current study is to ascertain the effect of leaf-footed bugs on the aforementioned four significant mulberry types *Morus alba*, *Morus indica*, *Morus nigra*, and *Morus serrata* as well as on the whole developmental stage of mulberry larvae, which feed silkworms. Mulberry seedlings, petioles, and leaf undersides are colonized by pests, which feed on the sap. Honeydew causes mulberry leaves to curl, wrinkle, and turn a glossy black before developing sooty mold. Mulberry leaves are twisted by leaf footed bug and twisted mulberry leaf, resulting in shorter internodes and stunt shoots (Akinmoladun, C., *et al.*, 2018 and Biabani, MR, *et al.*, 2005) ^[1, 2]. *Bombyx mori* silkworms are monophagous, meaning that they only eat soft, clean mulberry leaves. Pests decrease digestibility by lowering leaf nitrogen, causing sooty mold, or spreading pathogens. Mulberry larvae may reject damaged leaves, resulting in uneven development and

inadequate silk protein production. In practice, rearers report lower cocoon production because to severe pest infestations. The quality of mulberry leaves has a direct impact on the success of silkworm culture in sericulture, with leaf quality accounting for around 60–70% of the total cost of cocoon manufacturing. On the other hand, the mulberry ecosystem is susceptible to infestation by pests that have a major

impact on the productivity and quality of mulberry leaves. A physiological shift occurs in the foliage as a result of pest infestations, such as sap-sucking insects and leaf eaters. This includes a decrease in nutrients and a reduction in photosynthesis. This study looks at how these pest-infected leaves affect the larval development, diet, and later financial attributes (cocoon production) of *Bombyx mori*.

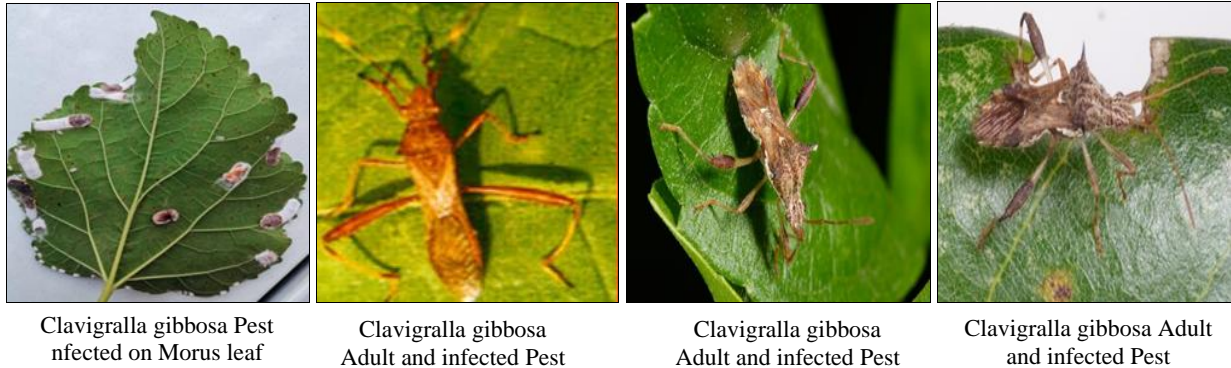


Fig 1: Shows *Clavigralla gibbosa* Adult and infected Pest and infected *Morus* leaf.
(Ref. All Photographs viewed from Google site)

Materials and methods

The study was conducted in selected on Tarai Belt of Uttar Pradesh Sericulture garden. The infested varietal mulberry leaves were collected randomly forms each Sericulture garden and the same were analyzed for various parameters like growth and development of silkworm bioassay. Bioassay study was conducted on silk worm growth and development and as the parameters observed were larval weight (gm) of 3rd 4th 5th instar larvae, larval duration and survivability of larvae harvested, by feeding both infested and healthy four varietal mulberry plants. The study was conducted in the gardens planted in randomized block design and data was collected from three replications (Krishanaswamy, S., 1978), the statistical data was analyzed in Two Way ANOVA using Post-hoc test by Microsoft excel online software.

Effect of Spiny Brown Bug or leaf footed Bug (*Clavigralla gibbosa*) infested mulberry leaf on the growth and development of silkworm: This study was conducted by using selected disease free layings (DFLs) of commercially popular Pure Mysore (PM) x NB4D2 and Nistari x NB4D2 silkworms and they were divided into two batches of one DFLs for each variety and reared separately with healthy and infested selected popular four varieties of mulberry leaves.

Number of cocoons harvested: Cocoons were harvested on fifth day after mounting by ensuring complete development of pupae and the number was recorded.

Assessment of post cocoon parameters: Cocoons were stifled by keeping them in hot air oven for three days at 65±1°C. The following cocoon parameters were studied to assess the quality.

Cocoon, shell weight (gm): Ten cocoons were collected randomly and weight was recorded then pupae were removed and shell weight was recorded.

Cocoon shell percentage: Ten cocoons were selected at randomly and shell percentage was calculated by using the formula.

$$\text{Cocoon Shell percentage of Mulberry silkworm} = \frac{\text{Weight of shell}}{\text{Weight of Cocoon}} \times 100$$

Reeling parameters: Total filament length (m), non-breakable filament length (m) and denier (μ) was recorded and calculated as per standard procedure/formula.

Result and Discussion

Significant variations were discovered in the effect of healthy and pest-infested leaves of four species of mulberry on the growth and development of silkworm larvae and cocoon, as shown in the Table, Graphs, and Fig. Effect of mulberry leaves infected with leaf footed bugs (*Clavigralla gibbosa*) on the development of silkworms' cocoon and post cocoon during silkworm rearing, significant alterations in the cocoon and post cocoon characteristics of silkworms were seen when grown on specific mulberry kinds that were either healthy or infected with leaf footed bugs. Parameters for the cocoon post (*Morus alba*, *Morus indica*, *Morus nigra*, and *Morus serrata*) The number of cocoons harvested from the *Morus alba* variety on the fifth day of mounting was recorded separately in infested and control, and the number of cocoons produced by one DFLs larva of each infested and control was 150 infested and 258 control, for a reduction rate of 41.90%. The number of cocoons from a single DFLs larva of each infested and control were 140 in the infested and 250 in the control, with a reduction rate of 44.00 percent. The percentage decrease in the quantity of cocoons from one DFLs larva of each infested and controls; *Morus nigra* was 50.00%, with 120 infested and 240 controls. The percentage decrease between the infested and control groups were 52.40, with 100 cocoons produced by each DFLs larvae of *Morus serrata* in the infested and 210 in the control. Weight of the cocoon (g) *Alba Morus* Infested cocoon weight was 1.20 g, whereas healthy cocoon weight was 1.85 g. The reduction rate was 35.10 percent. The weight of the cocoon from afflicted *Morus indica* was 1.10 g, while the weight of the cocoon from healthy *Morus indica* was 1.5 g. 37.10 percent of the total decrease was accounted for. The weight of the cocoon from infested *Morus nigra*

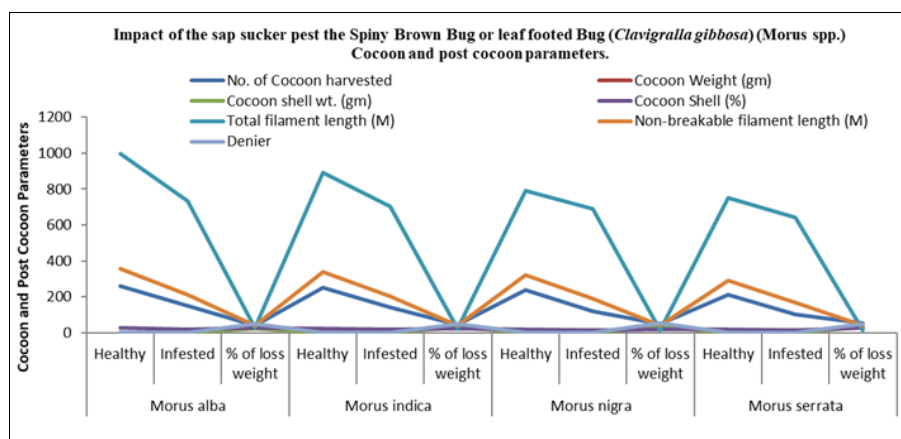
was 1.01g, whereas the weight of the cocoon from healthy *Morus nigra* was 1.60g. The percentage of reduction was 36.90, and the cocoon weight of *Morus serrata* from infected plants was 0.91 g, while the weight of healthy plants was 1.54 g. 40.90% was the amount of the decrease. Weight of the cocoon shell (in grams) The shell weight of *Morus alba* was 0.19 gram in infested and 0.25 gram in healthy cocoons. The reduction rate was 24.00%. Infected *Morus indica* had a weight of 0.14 gm, but healthy cocoon had a weight of 0.20 gm. There was a 30.00% decrease. The shell weight of an infested *Morus nigra* type was found to be 0.12 g, while the shell weight of a healthy cocoon was 0.18 g. The reduction was 33.30%. The shell weight of the infested *Morus serrata* variety was found to be 0.10 grams, while that of the healthy cocoon shell was found to be 0.15 grams. The decrease rate was 33.30%. (%) of cocoon shell the proportion of the cocoon shell in infested *Morus Alba* was 20.44%, while it was 28.32% in healthy plants. Over the healthy, the drop rate was 27.80%. Infested mulberry plants had a cocoon shell percentage of 18.44%, while healthy ones had a cocoon shell percentage of 24.31%. Over the healthy, the percentage of decrease was 24.20. In infested areas, the percentage of cocoon shell made from *Morus nigra* was 20.11%. In contrast, it was 24.20 in good health. The decrease over the healthy was 16.42 percent. Parameters for reeling (m): The total length of the filament (m) was measured as 732 m in infested cocoons and 995 m in healthy cocoons, representing a 26.40% decrease. The filament length was 702 m in infested cocoons of *Morus indica* and 890 m in healthy cocoons, which represents a 21.10% drop. The percentage reduction was 12.70, as measured by the 690m of *Morus nigra* in contaminated cocoons vs. the 790m of *Morus nigra* in healthy cocoons. The non-breakable filament length (in meters) was 210 meters in the infested *Morus alba* and *Morus indica*, but only 355 meters and 201 meters in the healthy ones. The observed reduction percentage was 40.80 and 40.90, respectively. This infestation's *Morus nigra* and *Morus serrata* were measured at 190m and 170m, respectively, whereas the healthy ones were measured at 320m and 170m, respectively. The percentage decreases that were seen were 40.60 and 41.40, respectively. The denier of silk filament in infested *Morus alba* and *Morus indica* was 1.20 and 1.10, respectively, while in healthy it was 2.40 and 2.20. The difference 50 was noted respectively. In infested and healthy trees, the denier of silk filament was 1.01 and 0.99 for *Morus nigra* and *Morus serrata*, respectively, and 2.10 and 1.99 for the latter. Varietal mulberry leaves had a difference of 51.90 and 50.30, respectively. In the fifth instar worms, feeding on afflicted leaves caused complete death. Because low-quality leaves lack antibacterial and antiviral compounds, the stress state caused by eating them may have made the larva more susceptible to illness. (Chandrasekharan, K *et al.*, 2006; Fraenkel, GS., 1959 and Gad, AA., 2010) [3, 5, 10] previously hypothesized that the primary cause of flacherie and grasserie was the consumption of inappropriate leaves. The current discovery lends credence to this notion. In order to maximize output, this research emphasizes the necessity of giving silk worms healthy, disease-free mulberry leaves, for which farmers should use appropriate disease management methods (Karnavar, GK *et al.*, 1973; Keit Sekou, et. a., 2017 and Kona, VS, *et al.*, 2012) [7, 9]. The quality of silk worm growth and development as well as cocoon production

depend on biochemical elements. The presence of chlorophylls, carbohydrates, and proteins in the leaf, which determines its quality (Murugan, K. *et al.*, 1998; Nagashetti, SM, *et al.*, 2013 and Nagavelli, U. *et al.*, 2012) [11, 12, 13]. Proteins are essential for producing silk. Because pathogens use the proteins in infected leaves as food, the protein levels in those leaves must have decreased more quickly than in healthy leaves. The health of silkworms and the quantity of cocoons produced are directly impacted by a carbohydrate found in mulberry leaves (Ram, R *et al.*, 2000; Samson, MV *et al.*, 1998 and Saxena, RN, 1990) [14, 15, 16]. A decrease in carbohydrate metabolism might be brought about by a decline in photosynthetic ability. The defense mechanism of plants against infection is phenols, which are also responsible for plants' disease resistance (Shilpashree, PS *et al.*, 2013 and Thomas, S., 2013) [17, 23]. In our study, the phenol concentration in infected plants was considerably higher than in healthy ones. Higher amounts of phenols aid in the development of an excessively sensitive response to leaf webber pest infection. The current research found that silkworm development and cocoon production were significantly impacted by feeding mulberry leaves that were infested with leaf webber (Thomas, S., 2012; Subramanyam, B, 1990) [21, 22]. The leaf nutritional condition is reflected by the lower economic characteristics of these silkworms. Mulberry leaves infested with leaf webber pests were used to raise silkworms, resulting in a very low quality of cocoons with lower density, more breaks during reeling, and less continuity with a shorter filament length. When silk worms were fed mulberry plant leaves that were infested, their silk output was significantly reduced (Sivaprakasam, N, *et al.*, 1996) [20]. When compared to healthy leaves of all tested mulberry types, the cocoon weight, shell weight, shell ratio, fiber length, and denier of larvae fed mite-infested leaves were found to be considerably impacted. The larvae that were fed healthy leaves had a considerably shorter larval stage (Singla, S *et al.*, 2013) [19]. Feeding silkworm larvae leaves that are infested with mites has a negative impact on the larval weight as well as all of the financial cocoon characteristics that were examined, including cocoon weight, shell weight, shell ratio, filament length, and denier. Due to detrimental impacts on leaf nutrients (protein, carbohydrate, chlorophyll content, and possibly additional micronutrients, vitamins, and minerals), the larvae grown on mite-infested leaves were unable to obtain adequate nutrition. As a result, the larvae are undernourished and develop more slowly. Their body weight stayed less than that of the control larvae, who were raised on nutritious leaves, even though they gained the necessary weight and were now prepared to pupate. At the same time, the other productivity indicators, including filament length, denier, shell ratio, shell weight, and cocoon weight, were also negatively impacted. The impact of mulberry cultivars afflicted by the spider mite *Tetranychus ludeni* on the cocoon characteristics of the silkworm race, NB-18, was also noted by (Shubha, K., 2005) [18]. In contrast to the mite-infested leaves, which were no longer suitable for silkworm feeding/rearing, healthy leaves had higher levels of moisture, total protein, total mineral, total fibre, total sugar, and total chlorophyll. From the above studies it could be concluded that the mite infestation is one of the limiting factors in getting better quality of mulberry leaf which is major contributing factor in producing bender cocoon crop and silk. It is also felt that the impact of mite damage on the

performance of young age silkworms needs to studied in depth to accretion the damage potential.

Table 1: mpact of the sap sucker pest the Spiny Brown Bug or leaf footed Bug (*Clavigralla gibbosa*) (*Morus* spp.) Cocoon and post cocoon parameters

Growth/ Cocoon Parameter	<i>Morus alba</i>			<i>Morus indica</i>			<i>Morus nigra</i>			<i>Morus serrata</i>		
	Healthy	Infested	% of loss weight	Healthy	Infested	% of loss weight	Healthy	Infested	% of loss weight	Healthy	Infested	% of loss weight
No. of Cocoon harvested	258	150	41.90	250	140	44.00	240	120	50.00	210	100	52.40
Cocoon Weight (gm)	1.85	1.20	35.10	1.75	1.10	37.10	1.60	1.01	36.90	1.54	0.91	40.90
Cocoon shell wt. (gm)	0.25	0.19	24.00	0.20	0.14	30.00	0.18	0.12	33.30	0.15	0.10	33.30
Cocoon Shell (%)	28.32	20.44	27.80	24.31	18.44	24.20	20.11	16.42	18.30	19.12	14.22	25.60
Total filament length (M)	995	732	26.40	890	702	21.10	790	690	12.70	750	640	14.70
Non-breakable filament length (M)	355	210	40.80	340	201	40.90	320	190	40.60	290	170	41.40
Denier	2.40	1.20	50.00	2.20	1.10	50.00	2.10	1.01	51.90	1.99	0.99	50.30



Two factor ANOVA Factor 2

	Means	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested	
	No of Cocoon Harvested	258.0	152.0	252.0	142.0	242.3	123.0	212.7	103.0	185.6
	Cocoon Weight	1.8	1.2	1.8	1.1	1.6	1.0	1.6	0.9	1.4
	Cocoon Shell Weight	0.3	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.2
Factor 1	Cocoon Shell %	28.4	20.5	24.4	18.4	20.2	16.5	19.4	14.5	20.3
	Total filament length	996.3	734.7	890.0	705.3	797.3	695.0	755.0	644.3	777.3
	Non-Breakable filament length	357.7	215.0	345.0	204.3	325.7	192.0	294.0	172.7	263.3
	Denier	2.4	1.2	2.2	1.1	2.1	1.0	2.0	1.0	1.6
		235.0	160.7	216.5	153.2	198.5	146.9	183.5	133.8	178.5

ANOVA table

Source	SS	df	MS	F	P-value				
Factor 1	11,645,278.03	6	1,940,879.671	260678.72	1.24E-229				
Factor 2	189,238.11	7	27,034.016	3630.93	6.92E-129				
Interaction	290,933.34	42	6,926.984	930.36	3.57E-125				
Error	833.89	112	7.445						
Total	12,126,283.37	167							

Post hoc analysis for Factor 1

Tukey simultaneous comparison t-values (d.f. = 112)

		Cocoon Shell Weight	Cocoon Weight	Denier	Cocoon Shell %	No of Cocoon Harvested	Non-Breakable filament length	Total filament length
		0.2	1.4	1.6	20.3	185.6	263.3	777.3
Cocoon Shell Weight	0.2							
Cocoon Weight	1.4	1.55						
Denier	1.6	1.87	0.32					
Cocoon Shell %	20.3	25.53	23.98	23.66				

No of Cocoon Harvested	185.6	235.44	233.89	233.57	209.91				
Non-Breakable filament length	263.3	334.04	332.49	332.17	308.51	98.60			
Total filament length	777.3	986.52	984.98	984.66	960.99	751.09	652.49		
critical values for experimentwise error rate:									
		0.05	3.01						
		0.01	3.55						
p-values for pair wise t-tests									
		Cocoon Shell Weight	Cocoon Weight	Denier	Cocoon Shell %	No of Cocoon Harvested	Non-Breakable filament length	Total filament length	
		0.2	1.4	1.6	20.3	185.6	263.3	777.3	
Cocoon Shell Weight	0.2								
Cocoon Weight	1.4	.1249							
Denier	1.6	.0643	.7475						
Cocoon Shell %	20.3	1.66E-48	6.20E-46	2.21E-45					
No of Cocoon Harvested	185.6	8.59E-153	1.79E-152	2.09E-152	3.19E-147				
Non-Breakable filament length	263.3	8.78E-170	1.47E-169	1.64E-169	6.40E-166	1.10E-110			
Total filament length	777.3	1.95E-222	2.32E-222	2.41E-222	3.67E-221	3.55E-209	2.48E-202		
Post hoc analysis for Factor 2									
Tukey simultaneous comparison t-values (d.f. = 112)									
		Infested	Infested	Infested	Infested	Healthy	Healthy	Healthy	Healthy
		133.8	146.9	153.2	160.7	183.5	198.5	216.5	235.0
Infested	133.8								
Infested	146.9	15.62							
Infested	153.2	23.06	7.44						
Infested	160.7	31.93	16.31	8.87					
Healthy	183.5	59.08	43.46	36.02	27.15				
Healthy	198.5	76.84	61.22	53.77	44.91	17.75			
Healthy	216.5	98.23	82.61	75.17	66.30	39.15	21.39		
Healthy	235.0	120.16	104.54	97.10	88.23	61.08	43.33	21.93	
critical values for experiment wise error rate:									
		0.05	3.09						
		0.01	3.63						
p-values for pair wise t-tests									
		Infested	Infested	Infested	Infested	Healthy	Healthy	Healthy	Healthy
		133.8	146.9	153.2	160.7	183.5	198.5	216.5	235.0
Infested	133.8								
Infested	146.9	6.72E-30							
Infested	153.2	2.38E-44	2.18E-11						
Infested	160.7	4.45E-58	2.35E-31	1.35E-14					
Healthy	183.5	2.93E-86	5.96E-72	2.01E-63	4.31E-51				
Healthy	198.5	9.89E-99	6.23E-88	7.81E-82	1.86E-73	2.42E-34			
Healthy	216.5	1.67E-110	3.41E-102	1.11E-97	1.04E-91	3.55E-67	2.31E-41		
Healthy	235.0	3.25E-120	1.68E-113	6.02E-110	2.38E-105	7.93E-88	8.30E-72	2.42E-42	

Statistical analysis table states that mulberry infestation severely cuts cocoon yield and quality fewer cocoon lower weight, poor shell percentage, shorter filament. We have next Turkey analysis and Post hoc to rank which traits suffer most and estimate percentage loss per traits, helping to judgment economic damage of sericulture as low yield of mulberry which affect the silk industry and rearers.

Conclusion

With its effects on silkworm cocoon and post-cocoon production, this leaf infestation resulted in a decline in the quantity and quality of leaves from mulberry cultivars, which led to financial losses for sericulture farmers who work in the silk business. The main location of silkworm synthesis is the Spiny Brown Bug cell in the mulberry leaf pest. For raising silkworms, it is forbidden to use protein-rich mulberry cultivars to infected leaves. Due to its higher

protein and carbohydrate content, the mulberry type was ideal for feeding silkworms when compared to the other four mulberry varieties. The current research found that the leaf-footed bug has had a noticeable effect on mulberry, changing its growth, cocoon, and post-cocoon characteristics, as well as its nutritional and metabolic processes across all four varieties, with *Morus mulberry* being followed by the other mulberry varieties. This infestation has resulted in a decrease in leaf yield, a decline in the quality of mulberry leaf varieties, and its impact on silkworm cocoon and post-cocoon cocoon production, all of which have caused financial losses to silkworm rearers who are involved in the silk industry.

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