

## INFLUENCE OF A SAP-SUCKING INSECT (*PSEUDODENDROTHRIPS MORI*) ON MORUS FUNDAMENTAL ROLES

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### ABSTRACT

The thrips species *Pseudodendrothrips mori* significantly lowers the nutritional and fundamental role of mulberry leaves when it infects them, resulting in significant drops in total chlorophyll free amino acid, total soluble protein, total reducing sugar and total soluble sugar content. According to biochemical studies, there are reductions in free amino acids, total soluble proteins, reducing sugars, and soluble sugars. The silkworm *Bombyx mori* L. feeds primarily on mulberry (*Morus* spp.) leaves, and this highly polyphagous pest has a big impact on the leaves fundamental quality. To determine how *Pseudodendrothrips mori* infestation affects important fundamental biochemical elements and photosynthetic pigments in six popular, high-yielding Indian mulberry types of *Morus indica*, *Morus alba*, *Morus laevigata*, V1 (Victory), S36, and Sahana mulberry study was conducted. The findings revealed an overall decrease in these biochemical variables across all varieties. The chlorophyll concentration in the V1 (Victory) and S36 cultivar increased unexpectedly after a pest infestation, while photosynthetic fundamental pigments decreased significantly in the majority of other varieties. With the exception of V1 (Victory), all cultivars exhibited a reduction in Carotenoid levels. Changes in leaf chemistry like this are likely to negatively impact the health, growth, and development of silkworms, which would ultimately lead to a decrease in silk production. The presence of insect pests such as thrips interferes with the production of high quality mulberry, which in turn impairs the production of high quality silk. The study also looks at the population dynamics of *Pseudodendrothrips mori*, a major threat to mulberry production in sericulture contexts.

**Keywords:** Fundamental biochemical components, thrips (*Pseudodendrothrips mori*), Mulberry, photosynthetic pigments (Free amino acids, Total soluble Protein, Total Reducing Sugar, Total soluble Sugar).

### INTRODUCTION

The domesticated silkworm, *Bombyx mori* L., which is frequently preyed upon by a variety of insect pests during cultivation, especially thrips like *Pseudodendrothrips mori*, feeds exclusively on mulberry plants (*Morus* spp.). Because of their brief lifespan and year-round presence, thrips are among the most important of these pests. Infestation causes symptoms such as stunted growth and deformed leaves, which eventually lowers the quantity and quality of mulberry foliage. Particularly worrisome in recent years are sap-sucking insects, which often prey on mulberry plants and cause widespread harm. The soft shoot tips are where the early-stage larvae often reside, where they eat young, growing leaves (Agrios, G. N., 1969). They frequently create protective shelters by rolling the borders of apical leaves and attaching them together, folding leaves, or spinning webs. Sometimes, larvae use secreted silk to create cup-like structures by curling one or two leaves, where they stay hidden. Because of this behavior, the mulberry thrips is the common name for *Pseudodendrothrips mori* (Bray, H. G., *et al.*, 1954). Green mulberry leaves, which supply vital nutrients for silkworm growth, are the only food source for silkworms in commercial sericulture. *Bombyx mori* demonstrates this unique feeding behavior, called monophony. Without the yellow pigment attractant Morin, a kind of pentahydroxy flavones, larval feeding is suppressed, resulting in the host specificity. Since mulberry leaves are the main source of nourishment for

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silkworms on a large economic scale, their quality has a direct impact on the health of the larvae and the quality of the cocoons. Leaf quality is determined by both chemical and physical traits, the former of which are particularly important in establishing nutritional value. The proteins in mulberry leaves are essential for the creation of silk because silk is made up of two main proteins: fibroin and sericin. According to studies, the amino acids in mulberry proteins, such as total soluble protein, total reducing sugar, and total soluble sugar, make up around 70% of the silk proteins produced. Chlorophyll, xanthophylls, and Carotenoid are the photosynthetic pigments found in green leaves, and there are two forms of chlorophyll: bluish-green chlorophyll a and yellowish-green chlorophyll b. These pigments affect cocoon color, producing white (Bivoltine), dark golden yellow (Nistari), or light yellow (Multivoltine Pure Mysore) cocoons. Mulberry leaves may experience changes in their biochemical makeup as a result of sap-sucking pest infestations (Bose, PC., *et al.*, 1992), which can have a detrimental impact on the health, growth, and development of silkworms and, in the end, result in lower silk output. As a result, the purpose of this research was to examine how such infections affected photosynthetic pigments and significant biochemical constituents.

### **MATERIALS AND METHODS**

The healthy and leaf roller infested leaves of six popular indigenous mulberry varieties viz., *Morus indica*, *Morus alba*, *Morus laevigata*, Vi (victory) and Sahana mulberry were collected from plantations in and around India. The leaves were oven dried and processed to analyze the fresh mulberry leaves were utilized to estimate the photosynthetic pigments (Free amino acids, Total soluble Protein, Total Reducing Sugar, Total soluble Sugar). The photosynthetic pigments 100 mg of fresh mulberry leaf tissue was placed in a vial containing 7ml of dimethyl sulphoxide (DMSO) and chlorophyll was extracted into the fluid without grinding at 65°C, incubated for three hours. Liquid was transferred to graduated tube and made up to a total volume of 10ml with DMSO and absorption spectra were recorded at 663 and 645 nm using DU-40 spectrophotometer immediately. The content of total chlorophyll, chlorophyll-a chlorophyll-b chlorophyll-a/b and Carotenoid were estimated using the method suggested by (Arnon. 1949).

**Estimation of total free amino acids:** Total free amino acids were determined by Ninhydrin method (Moore and Stein, 1948). 50 mg of dried mulberry leaf powder was ground in 5 ml of 80% methanol. The methanol layer (2 ml) was taken in a test tube and 1 ml of ninhydrin reagent (4% ninhydrin in methyl cellosolve and 0.2 M acetate buffer in the ratio of (1:1)) was added to it. The samples were boiled for 20 min and cooled; the volume was made up to 10 ml with distilled water. Absorbance was noted at 570 nm.

**Total soluble proteins:** Protein content was determined using the method of Lowry *et al.* (1951). To 1 ml of sample (leaf extract), 5ml of alkaline copper reagent (1% CuSO<sub>4</sub>+1% Na-K-tartrate + 2% Na<sub>2</sub>CO<sub>3</sub> in 1N NaOH) was added and incubated at room temperature for 10 min. For this, 0.5 ml of folin-phenol reagent was added and allowed to stand for 30 min. The OD was measured at 750 nm by UV-spectrophotometer.

**The total soluble sugars contents** were determined as described by Hodge and Hofreiter (1962) (Anthrone method). 100 mg of leaf powder was ground in 20 ml of 80% ethanol and incubated at 95°C for 10 min. To 1 ml of the supernatant sample, 4ml of anthrone reagent was added. The reaction mixture was shaken gently and kept over a boiling water bath for 10 min and allowed to cool down. The OD of blue green solution was measured at 625 nm. Glucose was used for plotting a standard curve.

**The reducing sugars:** The reducing sugars were estimated by Dinitro salicylic acid (DNS) method explained by Miller (1972). 500 mg of dried leaf powder was ground in 10 ml of 80% methanol. To 3 ml aliquot of the extract, 3 ml of di-nitrosalicylic acid (DNS) reagent was added and the mixture was boiled for 5 min in a water bath. 1ml of 40% sodium potassium tartarate was added and OD was measured at 575 nm. Glucose was used as a standard.

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Data obtained were analyzed by Statistically **Two Way ANOVA and Post-hoc test and Turkey Test** online by Microsoft Excel. Each value three replicate estimated the values. Significant differences were established at **P<0.05 and P<0.01** levels.

The information was also subjected to percentage of changes (decrease/increase) in the infested and healthy leaves and was calculated as:

$$\text{Percentage (\%) Decrease/ Increase} = \frac{\text{Values of healthy leaves} - \text{values of infested leaves}}{\text{values of healthy leaves}} \times 100$$

## RESULT AND DISCUSSIONS

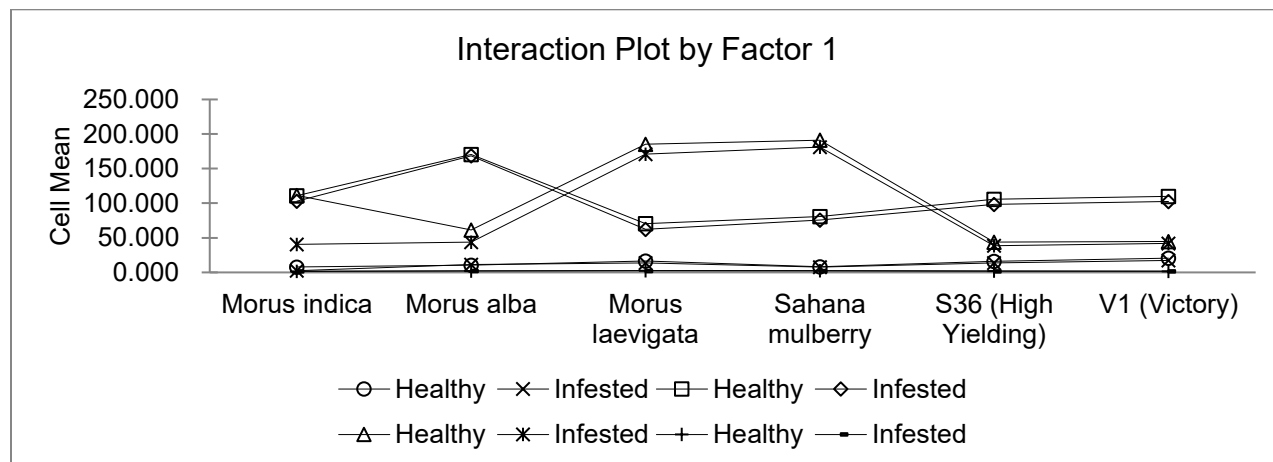
In mulberry leaves infected by leaf-rolling thrips, significant alterations were seen in photosynthetic pigments and essential biochemical elements such as free amino acids, total soluble protein, total reducing sugar, and total soluble sugar across six native kinds: *Morus indica*, *Morus alba*, *Morus laevigata*, Sahana, and V1 (Victory). In all contaminated cultivars, there was a consistent drop in these biochemical components when compared to healthy ones. The greatest decreases were seen in the high-yielding S-36 variety: In infested leaves, the free amino acids dropped from 20.84 mg/gm to 17.11 g/g, the soluble protein fell from 110.12 mg/mg to 102.55 mg/mg, the total reducing sugar decreased from 45.20 mg/g to 42.12 mg/g, and the total soluble sugar decreased from 1.76 mg/g to 1.53 mg/g. Other kinds exhibited similar patterns, with *Morus indica* exhibiting a decrease in protein from 110.55 mg/mg to 102.55 mg/mg, sugars from 111.22 mg/g to 40.55 mg/g, and free amino acids in chlorophyll dropping precipitously from 7.88 µg/mg to 2.44 µg/mg. These results demonstrate that a thrips infestation causes a considerable reduction in the amount of nitrogenous and photosynthetic chemicals found in mulberry leaves. Even cultivars with initially higher nutrient content, like *Morus laevigata*, suffered significant proportional losses, highlighting the vulnerability of current varieties and the need for timely pest management or the development of resistant strains against *Pseudodendrothrips mori*. The decrease in photosynthetic pigments is directly related to pest damage, a pattern also seen in mulberry plants that have been attacked by giant African snails (Sri Padmavathi Mahila *et al.*, 1997), thrips (Uritani, 1961; Shree *et al.*, 1989), and mealybugs (Umesh *et al.*, 1989). The severity of the infestation, the amount of tissue damage, and the particular mulberry genotype all influence the degree of change. Modified chlorophyll levels have a negative impact on photosynthetic efficiency (Shree *et al.*, 2005; Sathya *et al.*, 2000b), which in turn lowers protein production and plant productivity (Chandraamohan *et al.*, 2002). Consequently, the leaves lack nutrition. Infestations of pests cause sophisticated metabolic disturbances in the host plant, not simply biochemical alterations. The nutritional value of pest-damaged leaves is low, and they are not suitable for silkworm farming since they have a detrimental impact on the quantity and quality of silk production (Latha, 1999). Since silkworms only eat mulberry leaves, it's essential to have good pest and disease control. Most animals, including insects like *Pseudodendrothrips mori*, with the exception of a few aphids and mites, must consume Carotenoid, which are essential organic compounds with a variety of biological functions (Mahadeva *et al.*, 2015). During its larval stage, this common pest consumes a variety of organic substrates, which may have varying Carotenoid levels. Carotenoid is chemicals that are closely related to chlorophyll in mulberry leaves (Mahadeva, 2004b). The Carotenoid profile of adult thrips was hypothesized to be a reflection of their larval food. Studies involving raising larvae on substrates with varied Carotenoid compositions showed that although the natal substrate has an impact on adult profiles; it is not an exact replica of them (Naraswamy *et al.*, 2003). In conclusion, mulberry leaves undergo substantial changes in their biochemical, nutritional, and photosynthetic characteristics as a result of pest infestation, which produces lower quality foliage (Muthgowda *et al.*, 1990; Raman, 1994; Sathya *et al.*, 2000a). These modifications have the potential to be used as markers for identifying *Pseudodendrothrips* infestations. It is also recommended to use caution when choosing rearing substrates

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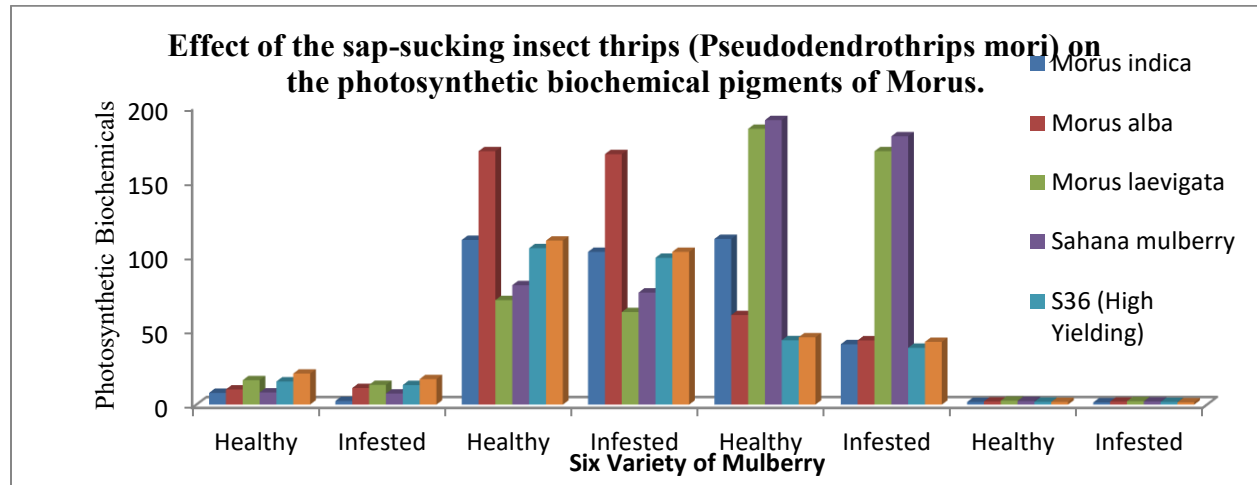
for mulberry pests (Narayanaswamy, 2003). Feeding silkworms with leaves that are so damaged impairs their growth and development, resulting in lower yield and poorer quality of natural silk fiber.

**Table: Effect of the sap-sucking insect thrips (*Pseudodendrothrips mori*) on the photosynthetic biochemical pigments of *Morus*.**

Biochemical's in Photosynthetic Pigments	Free Amino acid ( $\mu\text{g/gm}$ )		Total soluble Protein (mg/mg)		Total Reducing Sugar (mg/gm)		Total soluble Sugar (mg/gm)	
	Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested
Indian mulberry Variety								
<b>Morus indica</b>	7.88	2.44	110.55	102.55	111.22	40.55	1.77	1.5
<b>Morus alba</b>	10.16	11.12	170.15	168.15	60.11	43.12	2.12	2.01
<b>Morus laevigata</b>	16.36	13.24	70.15	62.12	185.11	170.12	2.62	2.31
<b>Sahana mulberry</b>	8.15	7.45	80.15	75.22	191.02	180.12	2.4	2.12
<b>S36 (High Yielding)</b>	15.55	13.12	105.01	98.55	43.2	38.12	1.9	1.8
<b>V1 (Victory)</b>	20.84	17.11	110.12	102.55	45.2	42.12	1.76	1.53



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Two Way ANOVA (ANALYSIS OF VARIANCE)							
Source	SS	df	MS	F	p-value		
Factor 1	17,716.6729	5	3,543.33459	14901.31	4.69E-137		
Factor 2	321,834.5576	7	45,976.36537	193351.20	3.62E-196		
Interaction	163,840.8154	35	4,681.16615	19686.40	7.80E-171		
Error	22.8275	96	0.23779				
Total	503,414.8734	143					
Post hoc analysis for Factor- 1 Turkey simultaneous comparison t-values (d.f. = 96)							
		S36 High Yielding)	V1 (Victory)	Morus indica	Morus alba	Morus laevigata	Sahana mulberry
		39.982	42.539	47.447	58.719	65.506	68.593
S36 (High Yielding)	39.982						
V1 (Victory)	42.539	18.17					
Morus indica	47.447	53.03	34.87				
Morus alba	58.719	133.11	114.94	80.07			
Morus laevigata	65.506	181.32	163.16	128.29	48.22		
Sahana mulberry	68.593	203.25	185.09	150.22	70.15	21.93	
Critical values for experiment wise error rate:							
		0.05	2.92				
		0.01	3.48				

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<b>p-values for pair wise t-tests</b>									
		S36 (High Yielding)	V1 (Victory)	Morus indica	Morus alba	Morus laevigata	Sahana mulberry		
		39.982	42.539	47.447	58.719	65.506	68.593		
S36 (High Yielding)	39.982								
V1 (Victory)	42.539	7.99E-33							
Morus indica	47.447	6.45E-73	2.61E-56						
Morus alba	58.719	1.06E-110	1.27E-104	1.04E-89					
Morus laevigata	65.506	1.54E-123	3.75E-119	3.56E-109	4.33E-69				
Sahana mulberry	68.593	2.75E-128	2.15E-124	1.01E-115	2.77E-84	3.66E-39			
<b>Post hoc analysis for Factor- 2 Turkey simultaneous comparison t-values (d.f. = 96)</b>									
		Infested	Healthy	Infested	Healthy	Infested	Infested	Healthy	Healthy
		1.905	2.132	10.952	13.227	86.183	101.728	106.262	107.993
Infested	1.905								
Healthy	2.132	1.39							
Infested	10.952	55.66	54.26						
Healthy	13.227	69.66	68.26	14.00					
Infested	86.183	518.49	517.10	462.83	448.83				
Infested	101.728	614.13	612.73	558.47	544.47	95.64			
Healthy	106.262	642.02	640.62	586.36	572.36	123.53	27.89		
Healthy	107.993	652.67	651.28	597.02	583.02	134.18	38.55	10.65	
<b>Critical values for experiment wise error rate:</b>									
		0.05	3.10						
		0.01	3.66						
<b>p-values for pair wise t-tests</b>									
		Infested	Healthy	Infested	Healthy	Infested	Infested	Healthy	Healthy
		1.905	2.132	10.952	13.227	86.183	101.728	106.262	107.993

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Infested	1.905								
Healthy	2.132	.1664							
Infested	10.952	7.23E-75	7.67E-74						
Healthy	13.227	5.37E-84	3.61E-83	6.39E-25					
Infested	86.183	2.73E-167	3.54E-167	1.47E-162	2.81E-161				
Infested	101.728	2.40E-174	2.99E-174	2.19E-170	2.50E-169	5.06E-97			
Healthy	106.262	3.38E-176	4.17E-176	2.04E-172	2.07E-171	1.32E-107	7.76E-48		
Healthy	107.993	6.97E-177	8.56E-177	3.62E-173	3.53E-172	4.90E-111	3.25E-60	5.91E-18	

Statistically the above table shows that the ANOVA table lays out the statistical backbone for comparing six mulberry varietal leaf under at least two conditions healthy vs infested, and three measured trait, giving Factor 2 = 7 df. Pos-hoc Turkey mean values the top row rank the genotype S36=39.98 V1=42.54, Morus indica = 47.45, Morus alba = 58.72, Morus laevigata = 65.51, Sahana = 68.59. The pair wise t-values are huge e.g. S36 vs Sahana = 203.25. Since the critical Turkey t at  $\alpha = 0.05$  is only 2.92, every pair exceeds it confirming each genotype differs significantly from every other. Statically summarized that the six genotypes are distinct from the traits. The infested condition shifts size depends on genotype interaction. Sahana mulberry and Morus laevigata have the highest over all means, V1 and S36 the lowest in this particular metric depending on weather higher values are good or bad, we do not pick accordingly. Because interaction is significant we should not just quote overall genotype means we need to look at healthy vs infested within each genotype to decide which holds up best under pest pressure. In practice we should next plot each genotypes healthy and infested means side by side calculated percentage loss and flag the one with the smallest loss and acceptable absolute performance. At the bottom line genotype all distinct with Sahana and Morus laevigata at top, S36 at the bottom for the reported metric. Healthy vs infested every traits shows a massive statistically significant drop under pest infestation. Because both main effects and interaction were significant in ANOVA and we should now calculate percentage loss per genotype to decide which one tolerates infestation best likely the ones with highest absolute healthy value and smallest relative drops. That means every healthy infested comparison even the closest ones like 1.90 vs 2.12 are statistically distinct. In plain terms infestation has real measurable effect on each trait, and the magnitude difference between healthy and infested leaves are huge. So we can confidently say that the pest infestation disturb the photosynthetic activity and biochemical constituent of the mulberry leaf which impact on the feeding of the silkworm.

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### CONCLUSION

The study demonstrated occurrence of free amino acids biochemical changes due to Pseudodendrothrips mulberry infestation in mulberry plants. Mulberry plants responded to biochemical changes associated with feeding of thrips, through the accumulation of phenol and protein contents as defensive mechanism. Losses of the photosynthetic pigments are in response to Pseudodendrothrips mulberry suggested a feeding induced stress response in the host plants. Thus, the present study provides a better understanding of defensive mechanism by biochemical changes due to the impacts of Pseudodendrothrips mulberry infestation in mulberry. Leaf thrips pest is a serious sap sucking polyphagous pest of mulberry (*Morus Alba L.*), which is the sole food for silkworm *Bombyx mori L.* It infested the tender leaves of the host causing considerable damage which alters the leaf quality. An attempt was made to know the changes in the biochemical components photosynthetic pigments in six popular high yielding and nutritive indigenous mulberry varieties (*Morus indica*, *Morus Alba*, *Morus laevigata*, S36 (High yielding), Sahana mulberry and V1 (victory) variety under infestation by thrips pest. The alterations in biochemical components of mulberry foliage will adversely influence the health, growth and development of silkworm. This in turn results in the production of low quality silk. This in turn results in the production of low quality silk. The present study analyses the population dynamics of Pseudodendrothrips mulberry, a serious sap sucking pest of mulberry in a sericulture farm. Hence, the present study is carried out to know the rapid pest proliferation incidence of the pest on mulberry and its intensity level for the production of natural fiber silk.

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