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Studies on the Impact of Leaf Webber *Diaphania pulverulentalis* on Different Mulberry Varieties and Silkworm *Bombyx mori* L.

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ABSTRACT

Background: Leaf webber *Diaphania pulverulentalis* is identified as one of the major pest which infests mulberry gardens and cause damage to mulberry growth and development and reduced nutritive levels in leaves. As mulberry is the prime food source of silkworm *Bombyx mori* L. the leaf webber infested leaves fed to the silkworm shows greater impact on the yield of, cocoon both quantity and quality. Hence, the present investigation was carried out to understand the impact of leaf webber infestation on various popular mulberry varieties and on silkworm growth and cocoon yield.

Methods: The study was conducted in selected ten mandals of Anantapuramu district, Andhra Pradesh during 2016-2019. The infested leaves were collected randomly form each mulberry garden and the same were analysed for various parameters like growth and development and biochemical parameters and silkworm bioassay. And analysed as per standard operating proceures.

Result: Photosynthetic pigments of leaf webber infested mulberry leaves. *i.e* total chlorophylls in selected three mulberry varieties are decreased by an average of 40.17% and followed by carotenoids with a average decrease of 40.85%. Biomolecule components mainly proteins in selected varieties found to be decreased with the average percentage of 24.62 and carbohydrates and starch decreased with the average percentage of 47.09% 16.09% respectively. but in the phenols are increased with the average percentage of 57.39%, when compared with control (7.61%).

Key words: Biochemical parameters, Cocoons, Leaf webber, Mulberry, Silkworm.

INTRODUCTION

Morus sps are only source of food for the silkworms, *Bombyx mori* L. which is attacked by a number of insect pests during cultivation. The important pests are Leaf webber, Mealy bug, *etc*. Majority of them are found throughout the year due to short life cycle. The symptoms are stunted growth,deformed leaves, *etc*. The pests cause reduction in leaf yield, quality and quantity of leaves. Among defoliators, the leaf roller is of more concern due to its regular incidence and extensive damage to mulberry in recent years. The early stage larvae inhabit the apical part of the mulberry shoot and feed on the young, tender leaves. Generally, larvae reside on the leaves by forming web or folding the leaves and making a shelter. The leaf margins of apical leaves are rolled and tied by the larval web wherein they live. Sometimes one or two leaves are rolled into cup shape with the web secreted from the larvae, which remains inside. Hence, the pest is commonly called as “leaf roller/leaf webber”.

The infested plants show stunted growth. The incidence is severe from October to January. Since, leaf webber infestation is becoming severe year by year especially in mulberry gardens of Ananthapur district which is one of the major sericulture traditional area. And the leaf webber incidence is becoming a major concern in recent years due to heavy incidence in mulberry as well as some of the vegetable crops. Hence, the present investigation is aimed to know the impact of leaf webber on three important mulberry varieties namely V_1, S_{36} and Mysore local and also on silkworm growth and cocoon yield.

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MATERIALS AND METHODS

The study was conducted in selected ten mandals namely Agali, Amarapuram, Gorantla, Gudibanda, Hindupur, Madakasira, Penukonda, Parigi, Roddam, Rolla in Madakasira, Penukonda and Hindupur divisions of Anantapuramu district, Andhra Pradesh during 2016-2019. The infested leaves were collected randomly form each mandal and the same were analysed for various parameters like growth and development and biochemical parameters and silkworm bioassay. Biochemical parameters such as proteins, carbohydrates, phenols and chlorophylls contents were analyzed. Bioassay study was conducted on silk worm growth and development and as the parameters observed were larval weight (gm), number of cocoons harvested, total

breakable filament length (m), denier by feeding both infested and healthy mulberry plants. The study was conducted in the gardens planted in randomized block design and data was collected from three replications, The statistical data was analyzed in two way anova using SPSS software.

(A) Biochemical parameters

Determination of total carbohydrates

The total carbohydrate was estimated by Anthrone method (Hedge and Hofreiter, 1962). Carbohydrate was calculated in relation to fresh weight basis and was expressed as mg/g tissue, the calculation was done by using following formula

$$\text{Amount of carbohydrate} = \frac{\text{Mg of glucose}}{\text{Volume of test sample}} \times 100$$

Estimation of proteins

Protein content of leaves was estimated by Lowry's method (Lowry *et al.* 1951). Protein percentage was calculated in relation to fresh weight basis. The amount of protein was expressed as mg/g.

Estimation of starch

The starch content was estimated by following the method prescribed by Hedge and Hofreiter (1962). D-glucose was used as standard and the value was multiplied by a factor 0.9 to get starch content and it was expressed as mg/g of mulberry leaves.

(B) Photosynthetic pigments

Chlorophylls

Total chlorophyll content in leaves was estimated by using the method of Arnon (1949) by using the formula and recorded in mg/g weight of leaf.

Chlorophyll-a (mg/g tissue)=12.7(A663)-2.69(A645)V/1000 × w

Chlorophylls-b (mg/g tissue)=22.9 (A645)-4.68(A663)V/1000 × w

Total chlorophyll (mg/g tissue)=20.2(A645)-8.02(A663)V/1000 × w

Estimation of carotenoids (mg/g)

Kirk and Allen (1965) formula was used by estimation of carotenoids.

Total carotenoids (µg/ml)=

O.D. 480 + (0.114 × O.D. 663 - 0.638 × O.D. 645)

Estimation of phenols (µg/g)

Phenols were estimated following the method of Malik[7] and Singh (1980), by using different concetration of catechol, concetration of phenols was expressed in µg/g fresh weight material, equivalent to catechol.

(C) Effect of leaf webber infested mulberry leaf on the growth and development of silkworm

This study was conducted by using selected disease free layings (dfls) of commercially popular double hybrid silk worms and they were divided into two batches of one dfl for each variety and reared separately with healthy and infested selected popular three varieties of mulberry leaves.

Larval weight (g)

Larval weight was recorded every day after first feeding in all the three instars starting from 3rd instar till mounting. For this study ten larvae were randomly selected from control as well as infested.

Number of cocoons harvestd

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

Assessment of post cocoon parametes

Cocoons were stifled by keeping them in hot air oven for three days at 70°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 cocoon were selected at randomly and shell percentage was calculated by using the formula.

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

Reeling parametes

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

RESULTS AND DISCUSSION

The present investigation revealed that the healthy and pest infested leaves of V_1, S_{36} and Mysore local mulberry variety showed significant changes on biochemical parameters and impact on silkworm development which were indicated in Table 1 to 2, Graphs 1 to 2 and Fig 1 to Fig 2.

Biochemical parameters

Protein (mg/g)

In this study, the protein content in healthy leaves of V_1 variety was observed as 86.32 mg and the infested plants it was 60.06 mg, with a reduction percentage of 30.42. And in S_{36} variety the protein content in healthy leaves was observed as 69.13 mg, infested it was 55.36 mg, with a percentage reduction of 19.91. Similarly in Mysore local, healthy leaves was observed as 62.03 mg, infested it was 47.42 mg, with the percentage reduction of 23.55.

Starch (mg/g)

The healthy V_1 shown starch content of 15.01 mg and in leaf webber infested it was observed as 12.32 mg and the reduction percentage was observed as 17.92. And in S_{36} variety healthy leaves, the starch content was noted as 14.26 mg and in infested leaves it was 11.21 mg. Then comparision of healthy and infested leaves the percentage reduction was

21.38. And in Mysore local, mulberry healthy leaves was observed as 13.23 mg and in infested leaves it was 12.04 mg, the reduction percentage was 8.99.

Carbohydrates (mg/g)

Carbohydrates content in V_1 healthy mulberry leaves noted as 57.64 mg and in leaf webber infested mulberry leaves it was observed as 31.36 mg, with a reduction percentage of 45.59. And in S_{36} variety the carbohydrates content was 53.56 mg in healthy and was 27.66 mg, in infested and reduction percentage of 48.35. In Mysore local variety the carbohydrate content was observed as 55.18 mg, were as in infested it was 29.05 mg, the percentage of reduction it was 47.35.

Phenols (µg/g)

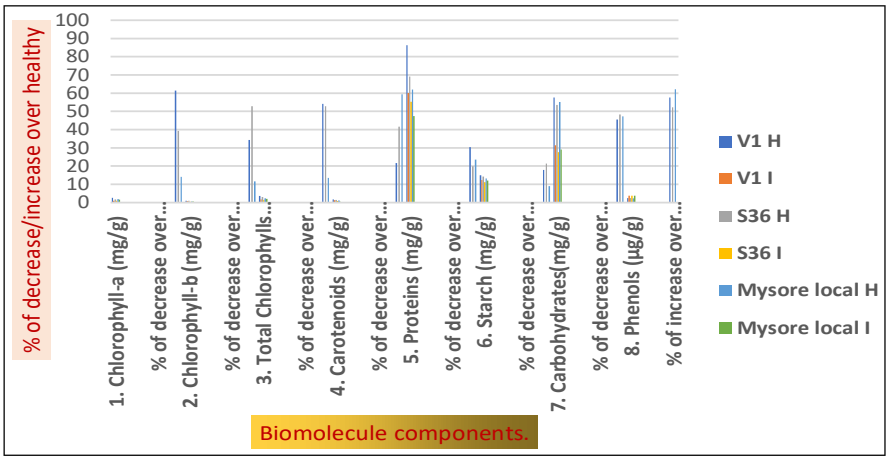
Phenols in the mulberry infested leaves was observed as 3.69 µg and the healthy mulberry leaves it was 2.34 µg and the increase of phenols was 57.69% in V_1 variety. Where as in S_{36} variety, the phenol content in infested leaves, it was

Table 1: Effect of leaf webber on biomolecular coposition of mulberry varieties.

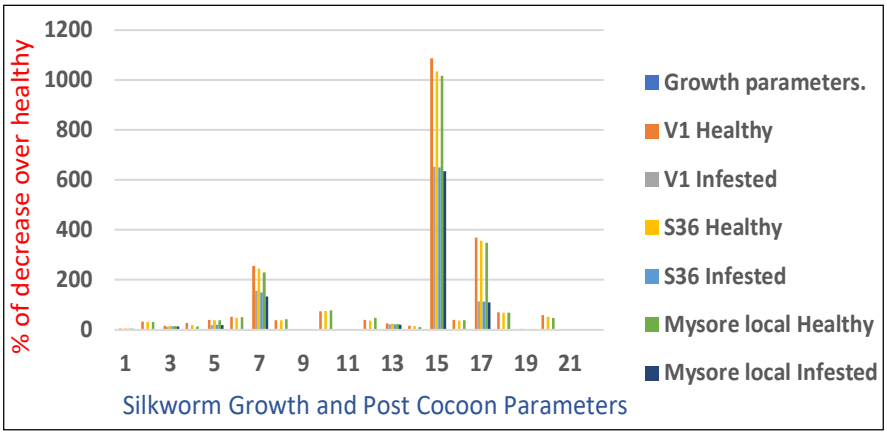
Biomolecule components	V_1		S_{36}		Mysore local	
	Healthy	Infested	Healthy	Infested	Healthy	Infested
Chlorophyll-a (mg/g)	2.62	1.01	1.68	0.96	1.78	1.53
		61.45		39.28		14.04
Chlorophyll-b (mg/g)	0.96	0.63	0.89	0.42	0.52	0.46
		34.37		52.80		11.53
Total Chlorophylls (mg/g)	3.58	1.64	2.95	1.39	2.30	1.99
		54.18		52.88		13.47
Carotenoids (mg/g)	1.62	1.27	1.42	0.83	1.06	0.43
		21.60		41.54		59.43
Proteins (mg/g)	86.32	60.06	69.13	55.36	62.03	47.42
% of decrease over control		30.42		19.91		23.55
Starch (mg/g)	15.01	12.32	14.26	11.21	13.23	12.04
		17.92		21.38		8.99
Carbohydrates (mg/g)	57.64	31.36	53.56	27.66	55.18	29.05
		45.59		48.35		47.35
Phenols (µg/g)	2.34	3.69	2.41	3.67	2.28	3.70
		57.69		52.28		62.20

Table 2: Effect of leaf webber infested leaf on silkworm growth and post cocoon parameters.

Growth parameters.	V_1		S_{36}		Mysore local	
	Healthy	Infested	Healthy	Infested	Healthy	Infested
Wt. of larvae in 3rd instar	4.86	3.27	4.66	3.22	4.58	3.18
% of decrease over healthy		32.70		30.90		30.56
Wt. of larvae in 4th instar	15.68	11.5	15.44	12.63	14.67	12.58
% of decrease over healthy		26.40		18.19		13.83
Wt of larvae in 5th instar	39.41	18.76	38.31	19.96	37.34	18.51
% of decrease over healthy		52.39		47.89		50.42
No of cocoons harvested	256	157	245	149	229	133
% of decrease over control		38.67		38.68		41.92
Cocoon weight (g)	1.9	0.45	1.7	0.41	1.6	0.35
% of decrease over control		73.52		75.88		78.12
Cocoon shell wt (g)	0.216	0.132	0.19	0.12	0.19	0.10
% of decrease over control		38.88		36.84		47.36
Cocoonshell (%)	25.32	21.43	24.32	20.81	22.94	20.69
% of decrease over control		15.36		14.43		9.80
Total filament length (m)	1087.36	653.42	1034.17	649.85	1017.32	635.18
% of decrease over control		39.90		37.16		37.56
Non-breakable-filament length (m)	369	114	356	112	348	109
% of decrease over control		69.05		68.53		68.67
Denier	2.9	1.2	2.7	1.3	2.3	1.2
% of decrease over control		58.62		51.85		47.82



Graph 1: Effect of leaf webber on biomolecular coposition of mulberry varieties.



Graph 2: Effect of leaf webber infested leaf on silkworm growth and post cocoon parameters.

Fig 1: Infested and healthy mulberry and silkworm rearing.

Fig 2: Biochemical analysis in different mulberry varieties selected mulberry gardens.

observed as 3.67 µg and the control it was 2.41 µg, there was increase in 52.28 per cent. In Mysore local variety, infested it was observed as 3.70 µg, were as in control it was 2.41 µg. The per cent of increase over healthy it was 62.20.

Photosynthetic pigments-(mg/g)

In the present study, chlorophylls and carotenoids are decreased in leaf webber infested mulberry leaves. In V_1 mulberry healthy leaves, chl-a, chl-b, total chlorophylls and carotenoids were observed as 2.62 mg, 0.96 mg and 3.58 mg and 1.62 mg respectively were as in infested it was recorded as 1.01 mg, 0.63 mg, 1.64 mg and 1.27 mg and with the percentage of reduction of 61.45, 34.37 and 54.18 and 21.60 respectively.

In S_{36} variety healthy mulberry leaves it was observed as 1.68 mg, 0.89 mg and 2.95 mg (chlorophyll a,b and total chlorophyll) and 1.42 mg (carotenoids) and in infested mulberry leaves it is recorded as 0.96 mg, 0.42 mg and 1.39 mg and 0.83 mg (carotenoids). And the percentage of reduction was 39.28, 52.80 and 52.88 and 41.54 respectively.

In Mysore local variety healthy leaves it was observed as 1.78 mg, 0.52 mg and 2.30 mg, (chlorophyll a,b and total chlorophyll) and 1.06 mg (carotenoids) were as in infested mulberry leaves it was recorded as 1.53 mg, 0.46 mg and 1.99 mg and 0.43 mg (carotenoids). The percentage of reduction was 14.04, 11.53 and 13.47 and 59.43 (carotenoids) respectively.

Chlorophylls are green pigments present in all plants. It absorbs light energy and also transfers into other parts of the photosystem, it is mainly useful in photosynthesis. In the present study the reduction of chlorophyll content was observed high in V_1 and S_{36} mulberry and Mysore local respectively.

Effect of leaf webber infested mulberry leaf on the growth and development of Silkworm

During rearing of silkworm significant changes were observed in the growth and development of silkworms reared on selected mulberry varieties infested with leaf webber as well as healthy.

Larval weight

V_1 mulbery

In all the days of 3rd instar, larval weight was shown significant reduction in the infested compared to healthy with the larval weight of 3.27 g and 4.86 g respectively. The percentage of reduction was 32.71. In 4th instar, larvae weight was observed as 11.54 g when larva was fed with infested leaves and in healthy leaves, the larval weight was recorded as 15.68 g and with the percentage reduction was 26.40. In all the days of 5th instar, larval weight has shown significant reduction in the infested compared to healthy with the larval weight of 18.76 g and 39.41 g respectively. The percentage of reduction was 52.39.

S_{36} mulbery

In all the days of 3rd instar, larval weight was shown significant reductions in the infested compared to healthy 3.22 g and 4.66 g respectively. The percentage of reduction was 30.90. In 4th instar larvae weight was 12.63 g in infested leaves and in healthy leaves the larval weight was 15.44 g, the percentage reduction was 18.19. In all the days of 5th instar, larval weight has shown significant reduction in the infested compared to healthy 19.96 g and 38.31 g respectively. The percentage of reduction was 47.89.

Mysore local

In all the days of 3rd instar larval weight was shown significant reductions in the infested compared to healthy 3.18 g and 4.58 g respectively. The percentage of reduction was 30.56. In 4th instar the infested larvae weight was 12.58 g, in healthy 14.67 g, the percentage reduction was13.83 observed infested larval weight. In all the days of 5th instar larval weight has shown significant reduction in the infested compared to healthy 18.51 g and 37.34 g respectively. The percentage of reduction was 50.42.

Post cocoon parameters (V_1, S_{36} and M.L)-Number of cocoons harvested

V_1 variety- On the 5th day of mounting, the number of cocoons spun was recorded separately in infested and control and the number of cocoons from one dfl larvae of each infested and control were 157 infested and 256 control, the percentage of reduction was 38.67. S_{36}-The number of cocoons from one dfl larvae of each infested and control were 149 in infested and 245 in control, the percentage of reduction was 38.68. Mysore Local -The number of cocoons from one dfl larvae of each infested and control were 133 infested and 229 control respectively, the percentage reduction was 41.92.

Cocoon weight (g)

V_1 - Cocoon weight from infested was 0.45 g and healthy was 1.9 g. The percentage of reduction was 73.52. In S_{36} - Cocoon weight from infested was 0.41 g and healthy was 1.7 g. The percentage of reduction was 75.88. M.L - Cocoon weight from infested was 0.35 g and healthy was 1.6g. The percentage of reduction was 78.12.

Cocoon shell weight (g)

V_1 - Shell weight was 0.132 g in infested and in healthy the cocoon weight was 0.216 g. The percentage of reduction was 38.88. S_{36}- It was 0.12 g in infested, where as in healthy cocoon weight was 0.196 g. The percentage of reduction was 36. In infested Mysore Local variety, the shell weight was observed as 0.10 g, where as in healthy cocoon shell weight was 0.196 g. The percentage of reduction was 47.36.

Cocoon shell percentage (%)

V_1-The cocoon shell percentage in infested was 21.43 where as in healthy it was 25.32. The percentage of reduction was 15.36 over the healthy. S_{36}- The cocoon shell percentage in infested was 20.81 where as in healthy it was 24.32. The percentage of reduction was 14.43 over the healthy. M.L-The cocoon shell percentage in infested was 20.69. Where as in healthy it was 22.94. The percentage of reduction was 9.80 over the healthy.

Reeling parameters (m): Length of total filament (m)

V_1- Total reelable length of silk filament in infested cocoons recorded as 653.42 m, where as in healthy cocoons 1087.36 m, the percentage of reduction was 39.90. S_{36}- In infested cocoons the filaments length was 649.85 m, where as in healthy cocoons 1034.17 m, the percentage of reduction was 37.16. Mysore Local - in infested cocoons recorded as 635.18 m, where as in healthy cocoons 1017.32 m, the percentage of reduction was 37.56 observed.

Non breakable filament length (m)

V_1- It was in infested 114.2 m, where as in healthy there was 369 m. The percentage of reduction was 69.05 observed. S_{36} - In this infested it as112 m, where as in healthy there was 356 m. The percentage of reduction was 68.53 observed. M.L- Infested as 109 m, where as healthy there was 348 m. The percentage of reduction was 68.67 observed.

Denier (µ)

V_1- The denier of silk filament in infested was 1.2 and in healthy it was 2.9. The difference 58.62 was observed. S_{36} - The denier of silk filament in infested was 1.3 and in healthy it was 2.7. The difference 51.85 was observed. M.L- The denier of silk filament in infested was 1.2 and in healthy it was 2.3. The difference 47.82 was observed.

Biochemical components are important for growth and development of silk worm and quality of cocoon production. The quality of leaf derived by the presence of proteins, carbohydrates and chlorophylls contents. Proteins are important for silk production. The proteins were degraded more faster in infectious leaves than healthy leaves due to utilization by pathogen as a result, the protein levels must have shown reduction in infested leaves.

Carbohydrates in mulberry leaves directly influence the health of silkworm and cocoon yield. Decrease of carbohydrate metabolic activity could be due to reduced photosynthetic capacity.

Phenols are the defense mechanism of the plants and responsible for disease resistance in plants against infection. In our study the phenol content was significantly increased in infested plants over healthy. Increase in phenols levels help in the formation of hyper sensitive reaction towards the leaf webber pest infection.

In the present study, it was observed that feeding with leaf webber infested mulberry leaves caused significant changes on silkworm growth and cocoon formation. The reduced economic characters obtained in these silkworms are indicative of the leaf nutritive status. Silkworms reared with leaf webber pest infested mulberry leaves produced very poor quality of cocoons with less compactness, more number of breaks while reeling and less continuity with reduced filament length. The silk worms fed with infested leaves of mulberry plant suffered a significant reduction in silk production Anonymous, (1996a), Narayanaswamy *et al*., (2003); Umeshkumar, (1991).

CONCLUSION

For this study three varieties have been selected namely Mysore Local, S36 and V_1 Variety and among the three varieties the severity of leaf webber was more on V_1 variety., Among the physiological parameters studied in three varieties the reduction, in photosynthetic pigments by leaf webber in V_1 variety was more in infested over healthy, (54.18%) followed by S_{36} (52.88) and Mysore Local (11.53%). In case of Carotenoids reduction was more in Mysore Local (59.43%), followed by S36 (41.54%) and V_1 (21.60%). The biomolecule components mainly proteins severely decreased in V_1 variety. but phenols content was found more in Mysore Local (62.20%), followed by V_1 (57.69) and S_{36} (52.28%). And all the selected three varieties were significantly effected by leaf webber.

The present study concluded that the leaf webber pest *Diaphania pulverulentalis* has shown significant impact on mulberry which altered growth parameters, nutritive and metabolic activities in all the three varities *viz*. V_1 mulberry, Mysore local followed by S_{36} varieties. This infestation caused reduction in the leaf yield and quantity, quality reduction in mulberry varieties of leaves and its effects on silkworm growth and quality of cocoon production, its leads to economic loss in sericulture farmers with silk industry.

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