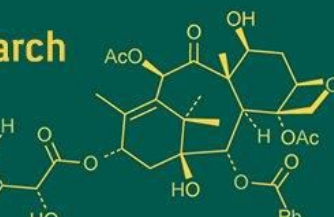
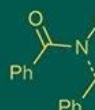


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Effects of feeding of *Myrothecium* Leaf spot infected mulberry leaves on the commercial characters of silkworm (*Bombyx mori* L)

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Abstract

Incidence of diseases in mulberry is a major constraint for successful sericulture in India, and *Myrothecium* leaf spot caused by *Myrothecium roridum* is particularly common in West Bengal. This study investigated the effects of feeding *Myrothecium* leaf-spot-infected mulberry leaves on silkworm growth, cocoon production, and silk quality. Larvae were fed leaves of different disease-severity grades, and their rearing parameters were recorded. Feeding infected leaves prolonged the larval duration by about three days compared to the 19-day control. The highest larval weight (3.42 g) was observed in the control, while Grade-IV infected leaves resulted in the lowest larval weight (1.09 g). No larvae survived to the fifth instar when fed Grade-V leaves. Larval length also decreased significantly, from 5.37 cm in the control to 2.26 cm in Grade-IV. Similar reductions were found in cocoon traits: single cocoon weight declined from 2.32 g to 0.65 g; shell weight from 0.55 g to 0.07 g; and shell ratio from 23.27% to 10.39%. Effective rate of rearing (ERR) also dropped sharply, from 21.27 kg to 1.51 kg in ERR weight and from 9733.33 to 1533.33 in ERR number between control and Grade-IV groups. Mortality was highest in Grade-IV larvae (84.66%) and lowest in the control (2.667%). Additionally, all major biochemical properties of mulberry leaves—moisture, protein, chlorophyll, and total sugar—declined drastically due to *Myrothecium* infection, negatively affecting larval growth and cocoon quality. This information is valuable for mulberry cultivators, helping them understand disease severity, seasonal occurrence, and its impact on silkworm production.

Keywords: *Bombyx mori*, *Myrothecium* leaf spot, *Morus* spp., economic parameters, crop loss, biochemical analysis, sericulture

1. Introduction

Sericulture is conceived as one of the important agro-based low capital investment labour intensive cottage industry having the potential to provide gainful employment of more than six million peoples of India. The quality and quantity of mulberry leaf used as a food source are known to affect the growth and development of silkworms (*Bombyx mori* L.), which is reflected in the commercial traits of the cocoon crop. Among the different biotic diseases of mulberry, *Myrothecium* leaf spot, *Pseudocercospora* Leaf spot, Bacterial leaf spot and Powdery mildew are very common diseases and causes huge losses to farmers of West Bengal. An attempt was made to find out the effects of feeding of mulberry diseased leaves on the quality and quantity of cocoon as well as silk quality. Larvae were fed with different graded of diseased leaf and their effects were measured with different silk worm rearing parameters (larval duration, larval length, larval weight, single cocoon weight, single shell weight, shell ratio, ERR weight, ERR number and mortality). Since the consumption of disease infected leaves by silkworm has metabolic impacts therefore possibility exists for total or partial failure cocoon production and/or production of inferior cocoons having poor market price. It is also true that reeling quality deteriorates from inferior quality of cocoons. Thus mulberry pathologists may consider it important to express crop loss in terms of cocoon crop loss and inferior cocoons produced at one point or total financial loss one the others. Qadri *et al.*, (1999) ^[19] estimated cocoon crop loss 54-58 percent at highest disease severity of 26 percent as well as poor quality cocoon. Tang *et al.*, (2005) ^[22] is of the opinion that for silkworm's growth nutrients e.g., protein, carbohydrates, vitamins, minerals etc. within the mulberry leaves are the sole source.

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Generally, researchers provide infected foliages only for one or two instars to find out the effects of the diseased foliage on cocoon production as well as other economy parameters. Cocoon production depends upon the activities of the larva and their moulting in all previous stages like Instar-I, Instar-II etc. Ito (1978) highlighted the importance of nutrition in production of silk in Japan. When a cultivar contains higher protein and low sugar, a better larval duration and decreased moulting ratio can be expected. Bakshi *et al.*, (1972) [2] considered mulberry infected leaves are unsuitable for silkworm feeding. Dutta *et al.*, (2011) [11] observed poor cocoon crop due to feeding of diseased leaves. Manimegalai *et al.*, (2006) [16] pointed out leaf quality is the major factor of higher productivity of quality cocoons. In second generation, there is a reduction in fecundity and hatching percentage of eggs laid by the moths when diseased mulberry leaves are fed. All other rearing and reeling parameters except ERR by number show significant negative correlation with disease severity (Quadri *et al.*, 1999) [19] opined that nutritive constituents of leaf control moulting response of silkworm. Only in the last three decades of the 20th century, Indian sericulture scientists have understood the problem of infected mulberry foliages in relation to quality cocoon production.

2. Material and Methods

Experiment was conducted at Mothabari (Latitude: 25.24145 and Longitude: 88.2509) Malda district of West Bengal during the year 2019. Composite leaf samples *i.e.* tender, medium and coarse leaf of S-1 variety meant for different instars of silkworm rearing (Chawki and Late age) was randomly collected from ten locations of a plot from each zone separately. The disease comprised of mainly *Myrothecium* leaf spot. Severity of disease was recorded from five plants of a plot where out of five plants; four from four corners and one from the centre of the plot were considered. Three branches are chosen at random and labelled on each plant. On the chosen branches, the total number of leaves and the number of diseased leaves were counted in order to determine the disease incidence. For measuring the percentage of disease index (PDI), all infected leaves were categorized into different grades of infection using the following 0-5 grading scale (FAO 1967). N X F1 crossbreed was chosen for this experiment. Throughout the raising process, which began in the larvae's second instar, the larvae were fed leaves infected with *Myrothecium* leaf spot (Grades I to V). Every treatment comprised fifty larvae and was carried out three times in duplicate. Important rearing, reeling, and parameter data were examined, and the average of three replications' worth of data is shown. The weight and length of the fully grown larvae, the weight of the cocoon, and the weight of a single shell, the shell ratio, and the mortality percentage were measured. Since there was 100% mortality before the larvae reached the fifth instar, observations in the case of the larvae fed with the contaminated leaves severity grade V could not

be documented. Five days following cocoon development, ten cocoons were chosen at random from each replication. The raising of the silkworms was carried out in accordance with Krishnaswami's (1970) [14] suggested protocol. Biochemical analysis such as, Leaf samples were collected from the naturally Grade-I and Grade-IV infected mulberry plants and healthy as control to mulberry plants of the same age and same field. Diseased leaves are categorized on the basis of the expression of infected on leaf lamina (1-5 grade). Leaf moisture followed by oven drying method, total chlorophyll followed by Arnon (1949). The total protein was estimated by the method of Lowry *et al.*, (1951) [15] and the total sugars were estimated by the method of Dubois *et al.*, (1956) [10] respectively.

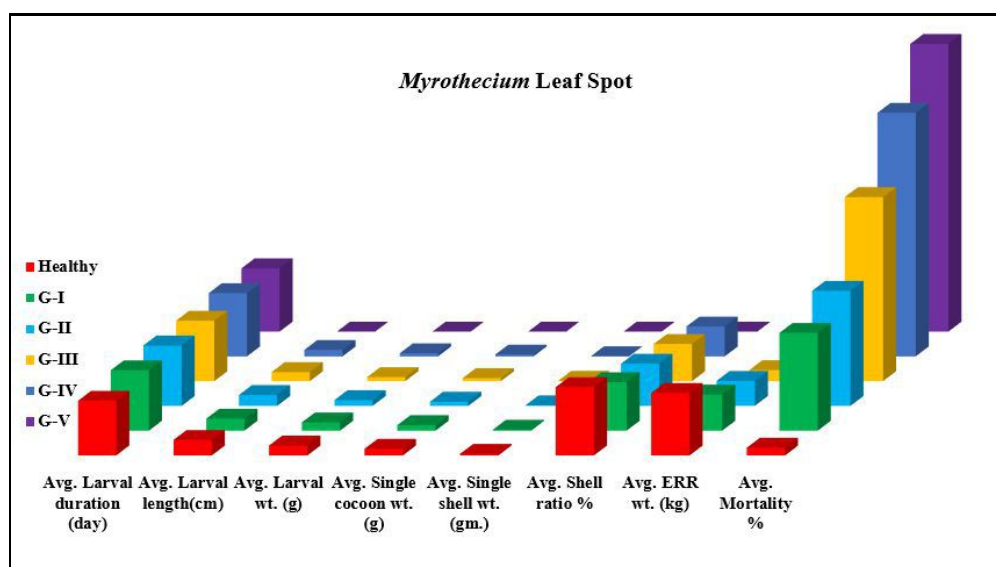
The rearing data was tested statistically with one way ANOVA (Completely Randomized design) with DMRT to determine significant differences among different treatments.

3. Results

The experiment revealed that the quality of mulberry leaves has a predominating influence on the development of silk worm larvae and infection of *Myrothecium* leaf spot adversely affected quality of cocoons. Feeding of quality mulberry leaves has a predominating influence on the development of silk worm larvae, cocoon quality and yield. An attempt was made to find out effects of feeding of *Myrothecium* leaf spot infected leaves on the quality and quantity of cocoon and silk quality. The respective data have been presented in table 1. It was observed from the study that the total larval duration was increased by 3 more days due to the feeding of different graded infected leaves in comparison to control (22 days). Feeding of diseased leaves significantly reduced the silk worm rearing parameters in comparison to control. The highest larval length and weight (5.37cm and 3.42 g) were recorded in the larvae fed with healthy leaves (control). The larval length and weight of silk worm decreased with the increased level of disease grades of leaves and found lowest larvae length 2.26 cm and weight 1.09 g with the feeding of Grade-IV diseased leaves of mulberry. While none of the silkworms survived till 5th instars when Grade-V leaves were used for feeding. Similar trends were also observed between healthy and different grades of diseased leaves in relation to other parameters with minimum values in Grade-IV diseased leaves. It was decreased from 2.32 g to 0.65 g for single cocoon weight; from 0.55 g to 0.07 g for single shell weight; from 23.73% and 10.39% for shell ratio percentage, from 21.73 kg to 1.51 kg for effective rate of rearing weight, and from 9733.33 to 1533.33 for effective rate of rearing number in respect of healthy and Grade-IV diseased leaves. The mortality percentage was found maximum in Grade-IV fed larvae (84.67%) while it was minimum 2.67% among healthy leaf fed larvae. Significant differences were noticed among the different grades of diseased and healthy leaves in relation to different silk worm rearing parameters.

Table 1: Effects of the feeding of *Myrothecium* Leaf Spot infected mulberry leaves on the commercially important cocoon characters of the silk worm.

Treatment	Healthy	G-I	G-II	G-III	G-IV	G-V	CD1%	CD 5%
Avg. Larval duration (day)	19	21	21	21	22	22	-	-
Avg. Larval length(cm)	5.37	4.16	3.78	3.15	2.26	0.00	0.29	0.20
Avg. Larval wt. (g)	3.42	2.88	2.02	1.42	1.09	0.00	0.58	0.41
Avg. Single cocoon wt. (g)	2.32	1.94	1.47	1.07	0.65	0.00	0.23	0.16
Avg. Single shell wt. (gm.)	0.55	0.33	0.22	0.14	0.07	0.00	0.08	0.06
Avg. Shell ratio%	23.73	16.87	14.68	12.92	10.39	0.00	1.87	1.31
Avg. ERR wt. (kg)	21.73	12.54	8.69	3.85	1.51	0.00	1.89	1.33
Avg. ERR (No)	9733.3	6600	6000	3600	1533.3	0.000	505.8	355.6
Avg. Mortality%	2.67	34.00	40.00	64.00	84.67	100.0	5.05	3.55

**Fig 1:** Effects of the feeding of *Myrothecium* Leaf Spot infected mulberry leaves on the commercially important cocoon characters of the silk worm.

To assess the loss in quality due to the different diseases in mulberry leaves several biochemical attributes *i.e.* total moisture contents, total chlorophyll contents, total protein contents and total sugar contents were measured through standard method. The popular mulberry variety (S1) was selected for the study. The data obtained through different methods against disease. The total moisture content in mulberry leaves was found to be influenced greatly with the infection of pathogens and the percentage of reduction increased with the higher grades of diseased leaves. The reduction in moisture content of disease leaves were compared with the moisture content of healthy leaves. Maximum reduction in moisture content 9.11% in Grade-I

and 16.16% in Grade-IV. The chlorophyll content of diseased leaves was also varied with the infection of pathogens. Maximum reduction 43.40% was observed in Grade-IV in *Myrothecium* leaf spot infected diseased leaves. The effects of pathogens invasion in the form of diseases in mulberry leaves were also noticed in total protein of leaves. Reduction of 23.74% to 42.46% reduction in total protein were observed in Grade-I and Grade-IV diseased leaves in comparison to healthy leaves.

Total sugar content of diseased leaves was reduced substantially due to the infection of pathogens. Maximum reduction in total sugar 37.80% was observed in Grade-IV. (Table 2 and Fig 2.)

Table 2: Effects on the biochemical attributes of mulberry leaves due to *Myrothecium* leaf spot disease.

Content	Healthy Leaf	Grade-I		Grade-IV	
		Total	Reduction (%)	Total	Reduction (%)
Total Moisture	72.00	65.44	9.11	60.36	16.16
Total Chlorophyll	4.23	3.31	21.75	2.06	43.40
Total Protein	21.62	17.25	23.74	12.44	42.46
Total Sugar	42.45	34.36	19.05	26.34	37.80

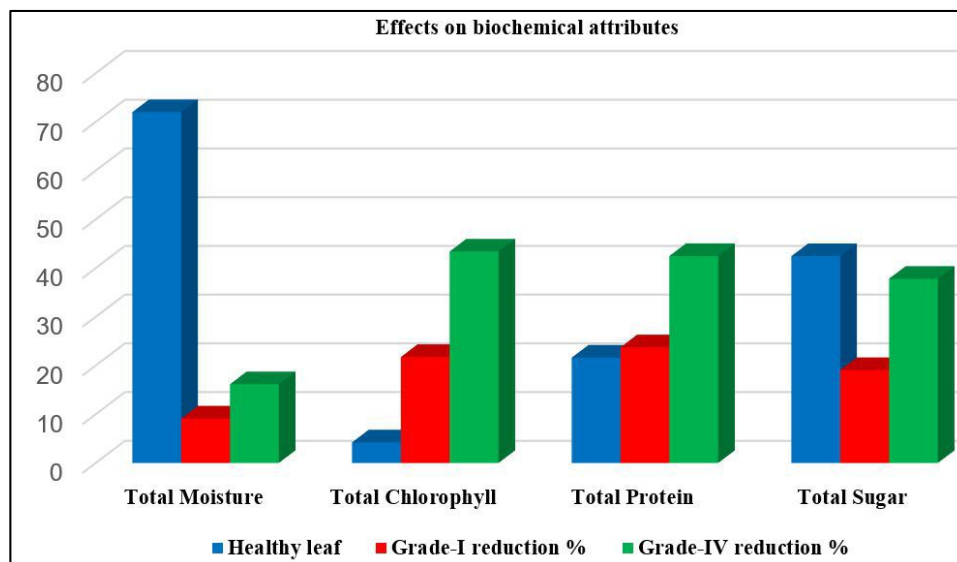


Fig 2: Effects on the biochemical attributes of mulberry leaves due to *Myrothecium* leaf spot disease.

4. Discussion

Several silk worm rearing parameters (larval duration, larval length, larval weight, single cocoon weight, single shell weight, shell ratio, ERR weight, ERR number and mortality) were considered in the study and the experiment confirmed that reduction in quality and yield during rearing of silkworm was proportional to the level of disease severity in the leaves used for feeding. In India, it is a well-established fact that the cost of mulberry production is rather high (60%) as compared to the rearing of silkworm for cocoon production (40%). Chaudhary *et al.*, (1987) [7] highlighted about the need of both qualitative (thickness, wax content, nutritional parameters etc.) and quantitative (leaf yield, shoot biomass etc.) improvement of mulberry among researchers and farmers. The nutritional quality of foliage is governed by various factors like varietal behaviour (Arug *et al.*, 1994, Ravikumar *et al.*, 1988) [1, 21], soil status (Das *et al.*, 1993 and Mishra and Sharma 2004) [9, 17], disease intensity (Das *et al.*, 2001; Govindaiah and Naik 2005) [8, 12] etc. Monir *et al.*, (2017) [18] reported that Incidence of different diseases in mulberry is one of the major problems for successful sericulture in India. Since the basic food of silkworm is mulberry leaf, and hence quality of mulberry leaf is considered as prime importance criterion for development of quality cocoon and silk (Bose *et al.*, 1989) [5] as growth of larva followed by cocoon production is dependent upon nutritional quality. Nagaraju *et al.*, (2002) depended the importance of tender, succulent and nutritious leaves for the better growth and development of young silkworms (Instar I & II) and 'progressively mature leaves with less moisture content' for late age silkworms. Many researchers considered and analysed leaf moisture, moisture retention (immediately after harvest and after six hours of harvest), protein, nitrogen, chlorophyll, and carbohydrate and amino acid contents as leaf quality characters (Bongale *et al.*, 1991; Bose and Bindroo 2001; Chaluvachari and Bongale 1995) [3, 6].

5. Conclusion

Based on this study it has been concluded that *Myrothecium roridum* is very common and causes significant losses to the farmers. Feeding of diseased leaves resulted in poor cocoon crop and causes reduction in the income of the rearers, therefore timely management of diseases is the prerequisite

for harvesting healthy and nutritious leaves. S-1 variety leaves contain high protein. Higher protein content was measured in mature healthy leaves but protein content in mulberry leaves decreased with the intensity of severity of diseased leaves as higher reduction in protein content were noticed in grade IV diseased leaves than grade-I. Sugars play an important role in silkworm growth, and it acts as one of the essential biting factors of larvae. As a result, sugar is an essential biochemical attribute for mulberry genotype selection for silkworm rearing. Future perspectives may include conducting awareness programs to promote the use of healthy leaves and the adoption of recommended packages and practices among farmers.

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