

Post-infectional biochemical and physiological changes in mulberry

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Healthy growth and development of silkworm is largely dependent on the quality of mulberry leaves fed. Many diseases of mulberry are known to alter the biochemical composition and physiological processes in the host. Although the work done on these aspects is meagre, the findings support the fact that if silkworms are fed with diseased leaves which are obviously nutritionally inferior, it leads to prolonged larval duration and deterioration of cocoon characters. Many of the biochemical contents such as proteins, amino acids, carbohydrates, polyphenols, mineral contents, etc., analysed in the diseased leaves showed either increase or decrease, compared to healthy leaves. However, certain components remained unaltered. The enzymes—acid phosphatase, alkaline phosphatase, catalase, indoleacetic acid phosphatase, peroxidase and chlorophyllase showed significant increase in their activity due to pathogenesis. The photosynthetic rate, chlorophyll content, carotenoids, stomatal conductance and Hill reaction were found to be adversely affected by the foliar pathogens.

LIKE any other plant, mulberry is also liable to be diseased. The most common diseases found on mulberry are bacterial blight, leaf mosaic, powdery mildew, leaf-spot, leaf-rust, stem canker, violet root-rot, white root-rot, root-knot and dwarfing. Mulberry foliage is the sole food of silkworm *Bombyx mori* L., hence, the foliar diseases of mulberry, viz. powdery mildew, leaf-spot, leaf-rust, leaf-mosaic and bacterial blight are to be considered seriously as they cause direct damage to the foliage resulting in considerable loss of leaf yield. The incidence of these foliar diseases is quite common irrespective of the maturity of the leaves. The tender leaves are essential for 'chawki' (young age silkworms) rearing and medium coarse and coarse leaves for late age silkworm rearing. However, the powdery mildew and leaf-rust diseases are seen on medium coarse and coarse leaves, while leaf mosaic and spot diseases are seen on both tender and coarse leaves.

Mildew is caused by an ascomycetous fungus—*Phyllactinia corylea* (Pers.) Karst., which is an obligate parasite (Biotroph). It seems to be the most widespread disease of mulberry. The mulberry leaf-spot disease is caused by a deuteromycetous fungus—*Cercospora moricola* Cooke. Sikdar and Krishnaswami¹ have assessed the loss in leaf yield due to spot disease in the local mulberry and Kanva-2 or M-5 varieties, to be 9.9% and 11.5% respectively. The other leaf-spot diseases

caused by deuteromycetous genera (*Alternaria* sp. and *Myrothecium* sp.) are commonly seen on the Kanva-2 variety. The leaf-rust disease of mulberry is caused by basidiomycetous fungi, viz. *Aecidium mori* (Barclay) Sydow et Butler. and *Cerotelium fici* (Cast) Arth. These are obligate parasites. The loss of leaf yield due to rust is estimated to be 5–10% (ref. 2). The leaf mosaic disease is caused by a virus, which is named after the host and the symptom, as Mulberry Leaf Mosaic Virus (MLMV). It is also a biotroph. Dwarf disease is very common in Japan. It is caused by a mycoplasma.

Although there are several other root, stem and leaf, diseases caused by fungi, bacteria, viruses, mycoplasma and nematodes, much work has been done only on the above stated major foliar diseases. The common notion is that diseased leaves are always nutritionally inferior compared to healthy leaves and feeding silkworms with such leaves will lead to poor growth of silkworms which subsequently yield inferior cocoons. The feeding trials conducted so far also support this fact³⁻¹⁰ (also see ref. 32).

It was felt useful to find out what really happened in these infected leaves which made them unfit as silkworm feed. The preliminary investigations carried out regarding post-infectional changes in mulberry are briefly reviewed here.

Biochemical changes

Mulberry plant affected by a disease shows several post-infectional changes morphologically, anatomically, biochemically and physiologically. The biochemical and physiological changes are more directly related to each other, the consequent morphological and anatomical changes appear as symptoms. The change(s) may be qualitative and/or quantitative and vary depending on the pathogen as well as on the species or variety of mulberry. Many physiological processes of the diseased plant studied are also known to deviate from their normal course after infection.

Chanturiya¹¹ reported that powdery mildew disease caused a decrease in the rate of oxidation process and a significant disruption in the process of carbohydrate(s) synthesis. Also, the nitrogenous matter was lowered and its ratio in diseased leaves was changed. This was conditioned because of the demands of feeding matter

by the pathogen itself from the infected tissues, due to greater intensity of the basic metabolism of the pathogen and also reduced synthesis by the plant due to infection. The diseased leaves had less moisture, more starch, cellulose, ash and SiO₂ contents compared to healthy leaves. Disruption of metabolic processes obviously decreased leaf yield and reduced their nutritive value greatly. As a result, the production and technological qualities of silkworm cocoons would be affected.

Shree *et al.*¹² analysed the protein content of healthy and powdery mildew leaves of mulberry using coomassie brilliant blue R-250, in two indigenous varieties (Kanva-2 and Mysore local) and four introduced varieties (Kosen, Papua, Harban and *Morus lembang*). Mildew infection significantly reduced the level of proteins in Kanva-2 by 52%, Mysore local by 40%, Kosen by 40% and *M. lembang* by 38.46%. However, in Papua and Harban, infection increased the protein level over the healthy leaves by 250% and 180% respectively. It could be that the proteins were synthesized at a faster rate and were more stable^{13,14} in the diseased leaves, or may be less utilized by the powdery mildew pathogen. Hence, it increases in Papua and Harban, while in the remaining four varieties, proteins were degraded faster and more of it was being utilized by the pathogen¹⁵, and, as a result, the protein level came down.

Shree and Sullia¹⁶ studied the effect of mildew and mosaic infection on the chlorophyll content of *Morus alba* L. var M-5; both the pathogens were biotrophs, one fungal and one viral. They found that, at the initial stages (15–20 days after infection) of mildew and mosaic disease, total chlorophyll, chl *a* and *b* contents in the infected leaves were lower when compared to those in healthy leaves (Figure 1). The healthier

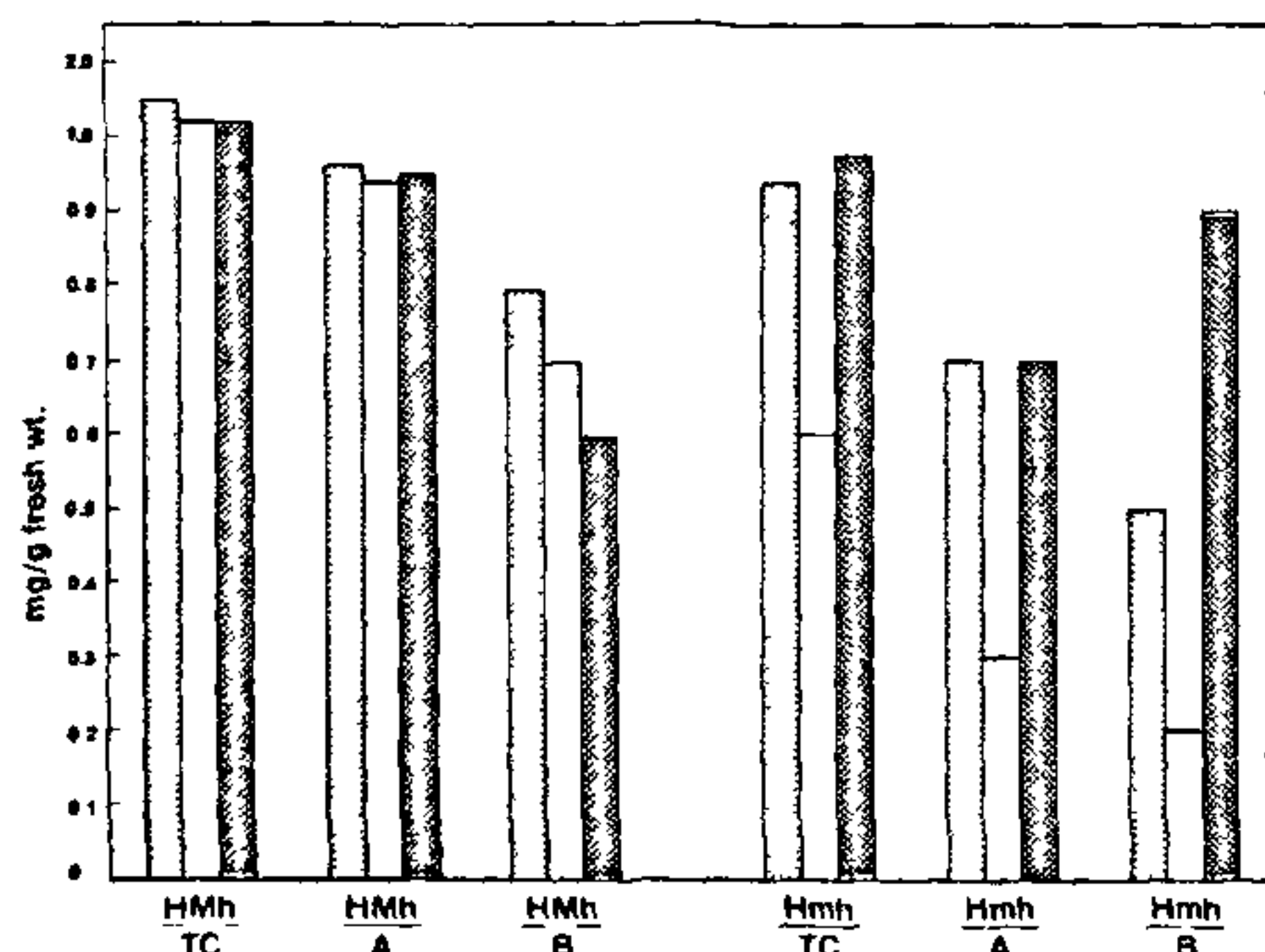


Figure 1. Effect of mildew and mosaic infection at initial stages on the total chlorophyll, chl *a* and *b* content of healthy and diseased mulberry leaves. H, healthy (uninfected) leaves; M, mildew-affected; h, healthy portions of infected leaves; m, mosaic-affected; TC, total chlorophyll; A, chl *a*; B, chl *b*. Source: Shree and Sullia¹⁶.

portions of the mildewed leaves showed more reduction in the chl *b* content compared to that in totally uninfected leaves. In the mosaic leaves, drastic reduction of chlorophyll content was recorded. However, compared to healthy leaves, the healthy portions of the infected leaves contained more of total chlorophyll and chl *b* content. During the later stages of mosaic infection, greater reduction in the total chlorophyll, chl *a* and *b* contents was observed (Figure 2). Also, in both mildewed and mosaic-affected leaves, the chl *a* content showed a greater decrease compared to healthy leaves. The viral infection caused greater reduction in the total chlorophyll content compared to that due to fungal infection. The fungal pathogens might have influenced the photosynthetic process either by affecting the chloroplasts or the chlorophyll pigments. It is well known that chlorosis is the characteristic symptom of viral disease in plants. The intracellular manifestation of chlorosis is either because of the loss of chlorophyll or the breakdown of chloroplast or both¹⁷. Padma¹⁰ studied the effect of mildew infection on the total chlorophyll content of mulberry leaves of *M. alba* var. M-5 and found a negligible decrease.

Shree and Umesh Kumar¹⁸ investigated the changes in the elemental composition of leaves of indigenous mulberry (*M. alba* var. S-54) attacked by mildew, rust and spot pathogens. Of the 22 elements (Cu, Pb, Zn, Ni, Co, Mo, Sn, W, Zr, Ag, Sb, As, Bi, Cr, V, Ba, Sr, Ga, Be, Y, La and Nb) analysed, 13 (Ba, Cr, Cu, Ga, Pb, Mo, Ni, Ag, Nb, Sn, V, Y and Zr) were found to be affected by fungal infections, while the contents of the other nine elements remained unaltered. All the three infections increased the V content and decreased the

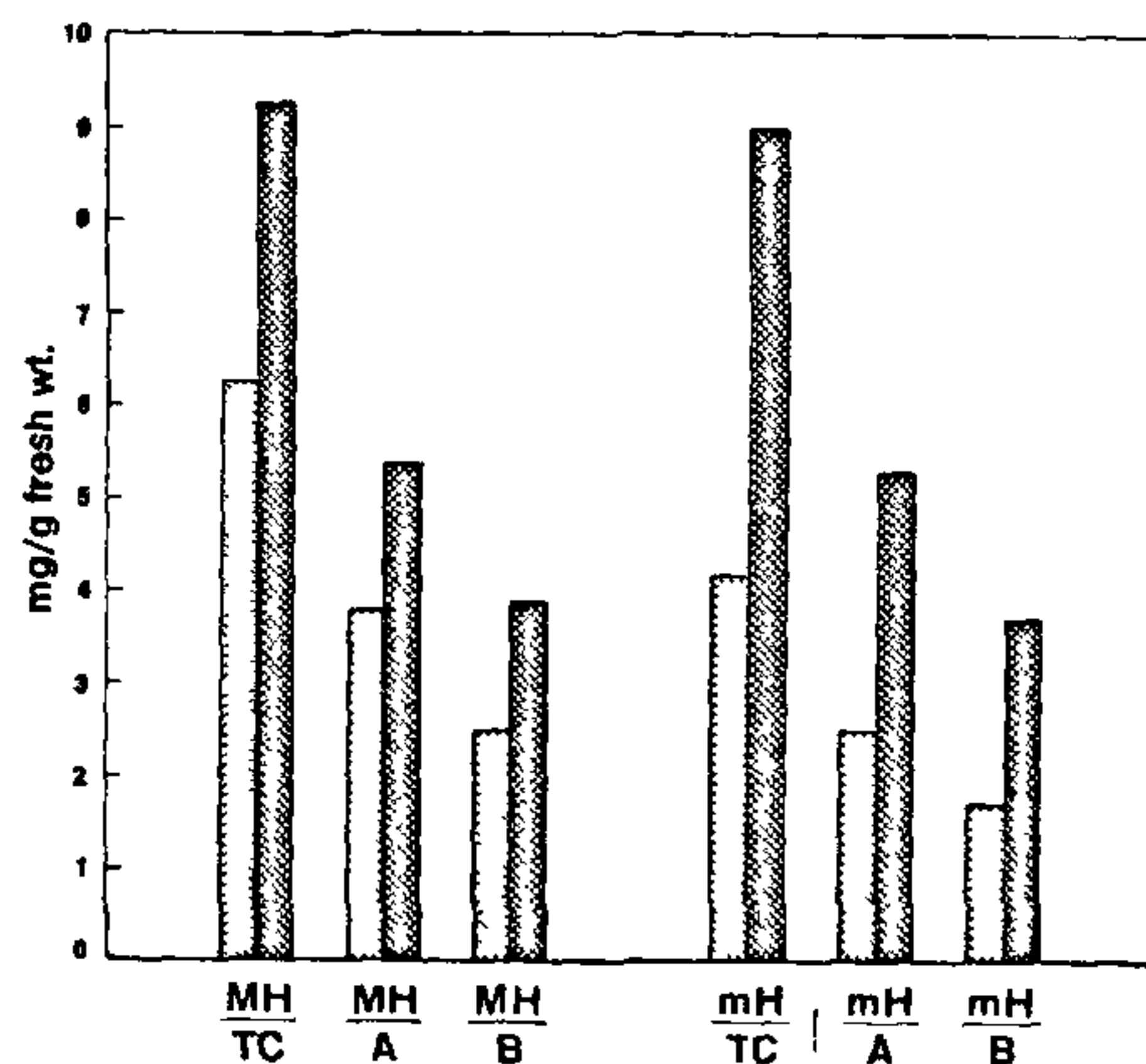


Figure 2. Effect of mildew and mosaic infection at advanced stages on the total chlorophyll, chl *a* and *b* content of healthy and diseased mulberry leaves. M, mildew affected; m, mosaic affected; H, healthy; TC, total chlorophyll; A, chl *a*; B, chl *b*.

Cu, Mo, Ga and Y contents. Spot and rust pathogens caused significant increase in the Ag content of leaves while the mildew drastically increased the Ni content.

Umesh Kumar and Shree¹⁹ conducted studies on the mineral composition of leaves of exotic mulberry varieties *Morus cathyana* Hemsl. and *Morus australis* Poir. both infected by the fungal biotrophs, viz. *P. corylea* and *A. mori*. It was found that Ca, K, Mg, Fe, Na, Mn, Cu, Zn, Ba, Cr, Ga, Pb, Mo, Ni, Nb, Ag, Sn, V, Y and Zr content(s) either increased significantly ($P < 0.001$) or decreased ($P < 0.001$). Irrespective of the infection by the two pathogens, these post-infectious biochemical changes adversely affected silkworm growth and development and consequently the cocoon crop.

Wang²⁰ reported lower nutritive value of the rust-infected leaves of mulberry; Sundareswaran *et al.*²¹ have reported the effect of leaf-rust disease caused by *C. fici* on the nutritive quality of leaves of six high-yielding mulberry varieties, viz. K2, S30, S36, S41, S54 and Mysore local. There was a statistically significant reduction in moisture, crude protein, reducing sugar and total sugars in the rust-affected leaves compared to healthy leaves. However, the total mineral content increased (Table 1). The loss in moisture was found to be maximum in S36 and minimum in K2. This loss may be attributed to the disruption of leaf tissue due to growth of the fungus resulting in excessive evaporation of water²². The reduction in crude protein was maximum in local and minimum in S30. The decrease of reducing sugars was maximum in K2 and minimum in S41. In total sugars, reduction was maximum in S30 while the minimum was recorded in K2. Increase in the total mineral content was maximum in K2 and minimum in S30. This could be attributed to the diversion of mineral contents to the site of infection by pathogen-induced stimulation²³. The leaf-rust causes decrease in the moisture, proteins and sugar contents and makes the leaves less nutritive for silkworms.

Padma¹⁰ studied the effect of rust infection on the chlorophyll content of mulberry leaves of *M. alba* var. M-5. The infection caused a decrease in the contents of chl *a*, chl *b* and total chlorophyll, by 43%.

Siddaramaiah and Hegde⁹ found that, increase in the intensity of infection by *C. moricola* resulted in higher

nitrogen and phosphorus but lower potash level in mulberry leaves. They²⁴ also found that *Cercospora* infection induces changes in the biochemical constituents like total amino acids, phenols and sugars which may affect the quality of mulberry leaves. The total chlorophyll, chl *a* and chl *b* decreased with the increase in disease intensity whereas carotenoids, total amino acids and total phenols increased. The reducing sugar content gradually increased in diseased leaves, while total sugar content decreased.

Padma¹⁰ estimated the chlorophyll content in the spotted leaves of mulberry *M. alba* var. M-5 infected by *Myrothecium mori* Sullia *et* Padma and *Alternaria solani* Sorauer. The infections caused more drastic reduction (> 50%) in the total chlorophyll content of leaves as the pathogens were necrotrophic in nature.

Umesh Kumar and Shree²⁵ analysed the total chlorophyll, chl *a* and *b*, chl *a/b* (ratio), total soluble proteins, polyphenols, nitrogen, proline, ascorbic acid, reducing sugars, total sugars, starch and titrable acid number (TAN) in the leaves of mulberry *M. alba* var. M-5, infected by *Myrothecium roridum* Tode. Ex. Fr. (Table 2). Polyphenols, nitrogen, proline, reducing sugars, total sugars, starch and TAN were found to increase. On the contrary, total soluble proteins and ascorbic acid contents got reduced due to infection. The reduction in the chlorophyll content might be due to the loss of structural integrity of chloroplasts²⁶. The significant increase in titrable acidity indicated stimulation of organic acid synthesis²⁷. The decrease in total soluble proteins could be due to their breakdown by proteolytic enzymes secreted by the pathogen²⁸. Polyphenols increased to considerable extent, probably due to the activity of polyphenol oxidase²⁹. The increase in the nitrogen content could be due to the pathogen-induced uptake. Free proline accumulation was perhaps because of blockage of protein synthesis or accelerated proteolytic activity²⁷. The ascorbic acid which is reported to play an important role in disease resistance²⁹ was also found to decrease. Starch, reducing sugars and total sugar contents increased in the infected mulberry leaves. Alabi and Naqvi³⁰ suggested that starch was not utilized for the growth of the pathogen, hence it accumulated in the leaf tissue.

Table 1. Percentage of nutritive loss/increase in mulberry due to rust infection (mean values of 1981-83)

Variety	% of infected leaves	Loss in moisture	Increase in total minerals	Loss in crude protein	Loss in reducing sugars	Loss in total sugars
Local	29.2*	12.3*	10.2*	10.2*	0.6*	3.7*
K2	43.5*	5.9*	10.8*	8.4*	0.8*	2.2*
S30	25.2	7.6*	8.3*	6.2*	0.8*	5.2*
S36	24.9	12.4*	9.8*	6.7*	0.4*	4.6*
S41	25.1	9.7*	4.3*	6.8*	0.3	2.4*
S54	25.2	11.1*	4.5*	7.1*	0.8*	2.8*

* Significant at 5%. Source Sundareswaran *et al.*²¹.

Table 2. Some parameters of *M. alba* var. M-5 leaves infected by *M. roridum*.^{††}

Parameter	Healthy	Infected
Total chlorophyll*	66.56	56.91
Chl a*	40.82	24.66
Chl b*	25.86	22.28
Chl a/b ratio*	1.57	1.55
Total soluble proteins**	141.16	131.28
Polyphenols**	62.14	81.16
Nitrogen**	0.85	0.92
Proline**	1.32	1.47
Ascorbic acid**	106.00	98.00
Reducing sugars**	22.50	32.16
Total sugars**	119.38	121.39
Starch**	53.00	54.65
TAN [†]	13.38	19.62

Expressed as: *mg/g of fresh tissue; **mg/g of dry tissue and [†]0.1N NaOH required to neutralize the acid in 100 g fresh tissue; ^{††}Significant at 5%.

Source: Umesh Kumar and Shree²⁵.

The enzymatic changes in the healthy and *M. roridum*-infected leaves of mulberry (*M. alba* var. M-5) were investigated by Umesh Kumar and Prithviraje Urs³¹. They found a significant increase in the activity of acid phosphatase, catalase, indoleacetic acid oxidase and peroxidase due to pathogenesis.

Umesh Kumar³² studied the biochemical changes in mulberry varieties (both exotic and indigenous) infected by foliar fungal pathogens. The diseases accordingly were the powdery mildew, leaf spot and rust. The indigenous mulberry varieties chosen were S36, S41, S54, M5, Berhampore local, Channapatna local and Mysore local. The exotic mulberry plants were *M. australis*, *M. cathyana*, *M. lebang*, *M. macroura* Mig., *M. multicaulis* Pers., *M. nigra* L., Okinawa-2, Farmer's field and Goshoyerami. He has made an extensive study on the change in organic and inorganic constituents, photosynthetic pigments, enzyme activities and finally their effect on the commercial characters of silkworm cocoon. His findings are very briefly outlined here. Most of the mulberry varieties infected by the three foliar fungal pathogens, viz. *P. corylea*, *C. moricola* and *A. mori*, showed decrease in reducing sugar content whereas the total soluble sugars and starch content(s) increased. The total amino acid content increased quantitatively in all the mulberry plants analysed. The total soluble proteins showed both increase as well as decrease. The qualitative analysis of amino acids and sugars also showed both increase and decrease. The macronutrients (N and K), micronutrients (Fe, Mg, Cu and Zn), secondary nutrients (Ca, Mn and Na), and the total mineral content showed slight increase or decrease in their content(s). Of the 20 trace elements analysed, 12 of them (Ba, Cr, Pb, Mo, Nb, Ag, Sn, V, Ni, Ga, Y and Zr) were found to vary depending on the pathogen(s) while the contents of other 8 elements (An, Ar, Be, Bi, Co, La, St and Tn) remained unaltered. The foliar pathogens decreased the chl a, chl b and total chloro-

phyll content in most of the leaves of indigenous, and interestingly, in all the exotic mulberry plants studied. The chl a/b (ratio) increased in most of the mulberry varieties. The total carotenoid content and chlorophyllase activity significantly increased. The respiratory enzyme activity (catalase and peroxidase) increased in all the diseased plants.

Ishizaka³³ analysed the electrophoretic patterns of proteins in the immature leaves from dwarf diseased mulberry which revealed a marked decrease in the levels of 'B' and 'C' fractions. Depending on the progress of the disease, the pattern of proteins in immature leaves from diseased plants was quite comparable to that of the healthy plants.

Physiological changes

During pathogenesis, various metabolic processes of the host are known to undergo deviations from the normal course. One such process is photosynthesis. The effect of infection by facultative (*M. mori*, *A. solani*) and obligate (*P. corylea*, *A. mori*) parasites on photosynthesis in mulberry plant (*M. alba* var. M-5) was studied by Padma¹⁰. In general, irrespective of the nature of the pathogens, the host photosynthetic rate was significantly reduced. The greatest reduction was noticed in *A. solani*-infected leaves. This may be because the fungus causes necrosis of leaves leading to leaf spot or blight. The chloroplast is disrupted extensively in necrotic tissues. *A. mori*, *A. solani* and *M. mori* caused 38, 42 and 50% reduction in Hill reaction respectively, but it was drastic by *P. corylea* (72%). In mildewed leaves, stomatal conductance increased (0.14 cm s^{-1}) over control, whereas in others (*A. mori*, *A. solani* and *M. mori*-infected leaves), it was marginally reduced.

Conclusion

The importance of quality of mulberry leaves on healthy growth and development of silkworm and silk production is stressed by many scientists. It is desirable to know the effect of different diseases on the various biochemical and physiological changes in mulberry, which in turn decides the nutritional status of leaves. Many of the diseases whether caused by fungi or mycoplasma or bacteria or virus are known to alter the biochemistry and physiology of the host. The productivity is brought down as the photosynthetic rate is adversely affected during pathogenesis. The extent of utilization of biochemical content(s) of the host by the pathogen(s) is decided by their level of parasitism. Biotrophs cause much damage compared to facultative parasites/saprophytes. It is well known now, that feeding silkworms with diseased leaves will result in

prolonged larval period and deterioration of cocoon characters^{3-10,32}.

Sericulturists, knowingly or unknowingly, feed the silkworms with the diseased leaves which get mixed up with the healthy leaves while plucking from the plant. The farmers hardly care to protect the plants from the pathogens. They would rather uproot the entire diseased plant and replant another sapling in its place, than spraying any chemical(s) to control or prevent the disease. Those who feed the worms do not bother to separate out the diseased leaves. This can be partly attributed to their ignorance and lack of awareness. However, in large scale rearings, it is also not practical because of time and labour consumption. We know that silk produced in our country is not of good enough quality to compete in the international market. Possibly our farmers who carelessly feed the diseased leaves to silkworms are partly responsible for this low quality silk production although quantity-wise we have improved. To overcome this flaw, firstly, mulberry plants must and should be protected from diseases; secondly, care and precaution must be taken not to feed the diseased leaves during silk-worm rearing.

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