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Effect of Preservation of Mulberry Leaf on the Development of Bacterial Flacherie and on Larval and Cocoon Parameters of Silkworm *(Bombyx mori* L.)

I Paramasiva, P Rajendra Prasad and N P Eswara Reddy
Department of Entomology, S V Agricultural College, Tirupathi 517 502, Andhra Pradesh

ABSTRACT

Highest larval mortality (59.86%) was recorded when the bacterial flacherie infected larvae were reared with leaves without preservation and lowest mortality (49.71%) was recorded when the flacherie infected larvae were reared with leaves preserved for 24 h. Maximum larval (1.22g), cocoon (1.02g) and shell weights (0.14g) were recorded when infected larvae reared with leaves without preservation and minimum larval (0.89g), cocoon (0.84g) and shell weights (0.07g) were recorded when the larvae were reared with 24 h preserved leaves. Highest shell ratio of 14.20 per cent was recorded when infected larvae were reared with fresh leaves. Lowest shell ratio of 8.3 per cent was recorded when the infected larvae were reared with 24 h preserved leaves.

Key words : *Bacillus thuringiensis* var. *kurstaki,* Bacterial Flacherie, *Bombyx mori,* Sericulture, Silkworm

Sericulture is an agrobased industry generating enough employment potential and fetching remunerative returns to the silkworm rearers and other entrepreneurs engaged in various sectors of the industry. It also earns considerable foreign exchange to the county. In the world, India ranks second in raw silk production i.e., next to China. Silkworms are affected by a number of diseases due to various biological, chemical, physical, nutritional and environmental causes. Being poikilothermic in nature silkworms respond very quickly to the environmental changes, particularly temperature and relative humidity. Higher or lower temperature, humidity, ventilation and feed, adversely affect the physiological function of the silkworms. As result a they become susceptible to diseases. About 34-40 per cent of the total crop in a year is lost due to diseases in India (Vaidya, 1960). However, with the advent of improved silkworm rearing technology, cocoon production has increased in recent years. Still there is about 20 per cent crop loss due to diseases (Baig and Pradeep Kumar, 1987).

The chief diseases affecting mulberry silkworm, *Bombyx mori* L. are flacherie, grasserie, muscardine and pebrine (Dasgupta, 1950). Both bacteria and viruses cause microbial flacherie. Bacterial flacherie is caused by *Bacillus thuringiensis.* In India, flacherie prevails to the extent of 57.2 per cent among the diseases of silkworm (Samson *et al.,* 1990). Degradation of biochemical constituents during post harvest preservation that influences the feed value of preserved leaves is inevitable at times during which period the leaves lose moisture and undergo biochemical changes (Govindan *et al*., 1988) which are further influenced by environment (Krishmaswami *et al*., 1971). Hence, the present investigation was carried out to know the effect of preserved leaves on larval and cocoon parameters of flacherie infected silkworm.

MATERIAL AND METHODS

Disinfection of rearing room and equipment

The rearing room and equipment were disinfected with five per cent formalin, prior to the commencement of silkworm rearing. Formalin was sprayed at the rate of 800 ml per 10m^2 area and the room was kept closed for two days for the effective diffusion of formaldehyde gas (Krishnaswami *et al.*, 1973)

Silkworm rearing

The disease free layings of PMxCSR$_2$ race were procured from the Government Grainage, Palamaner. For all the experiments, the eggs were incubated at room temperature by adopting standard incubation and dark treatment practices (Krishnaswami *et al.,* 1973). Silkworms were reared on the leaves of mulberry variety M$_5$.

Collection and maintenance of bacterial culture

The culture of *Bacillus thuringiensis* var. *kurstaki* obtained from the Biotech International Limited, Bengaluru, was further sub-cultured on nutrient agar medium. The pure culture was used for infection. Twenty ml of sterilized medium was

used for each petriplate. The plates were inoculated by streaking with spores of the bacteria and incubated at room temperature for about two days. The spores from the culture plate were harvested in to distilled water and the culture of the bacteria was maintained by sub-culturing. The spore load obtained was diluted with distilled water by following the serial dilution technique. All the culture operations were carried out in an inoculation chamber under aseptic conditions. The spore counts were recorded by using "Neubauer" haemocytometer.

To study the effect of leaf preservation on the incidence of flacherie disease the leaves were preserved for 0, 6, 12, 18 and 24 h and fed to the silkworms in both inoculated (bacterial spore suspension containing $1x10^6$ spores per ml was used for inducing the flacherie disease) and untreated batch. Each treatment with four replications containing 50 worms were maintained. Then the observations on larval mortality, larval weight and cocoon parameters were recorded.

RESULTS AND DICUSSION

Larval mortality

The preserved leaves had a significant influence on the larval mortality. Significantly higher mortality (41.21%) was recorded in both the bacteria infected and without bacteria infected batches fed with leaves without preservation. This was followed by 40.26 per cent mortality recorded in the larvae fed with 6h preserved leaves. Low mortality (35.31%) was recorded with 24 h preserved leaves both in bacteria infected and without bacteria infected batches. The larval mortalities were 36.71 and 34.42 per cent with 12 and 18 h preserved leaves, respectively. Highest mortality (54.84%) was recorded in the larvae infected with bacteria compared to the normal untreated check (19.53%). In the interaction effect, highest larval mortality (59.86%) was recorded when the infected larvae were reared with leaves without preservation and lowest mortality (49.71%) was recorded when the larvae were reared with leaves preserved for 24 h. The results are in conformity with the findings of Barman (1992) who reported that one-day preserved leaves had showed lowest mortality due to virus infection than tender leaves (Table).

Larval weight

Maximum larval weight of (1.38g) was recorded in the normal untreated check batch, compared to infected larvae (1.06g). The larval weight was maximum when the larvae were reared with leaves without preservation. The larval weight was minimum when the larvae reared with leaves preserved for 24 h. In the interaction effect, maximum larval weight of 1.22g was recorded with infected larvae were reared with leaves without preservation and minimum (0.89g) when the larvae were reared with 24 h preserved leaves (Table).

Cocoon weight

Highest cocoon weight of 1.17g was recorded when larvae were reared without infection. Lowest cocoon weight of 0.94g was recorded with infected batch. Maximum cocoon weight of 1.15g was recorded when the larvae were reared with leaves without preservation. Minimum cocoon weight of 0.98g was recorded when larvae were reared with leaves preserved of 24 h. Highest cocoon weight (1.02g) was recorded by infected larvae reared with fresh leaves. Lowest cocoon weight of 0.84g was recorded when the infected larvae were reared with 24 h preserved leaves (Table).

Pupal weight

Highest pupal weight 0.94g was recorded when the larvae reared with fresh leaves. Lowest pupal weight of 0.83g was recorded when the larvae were reared with 24 h preserved leaves. Highest pupal weight of 0.88g was recorded when the infected larvae reared with leaves without preservation. Lowest pupal weight of 0.77g was recorded when the infected larvae reared with 24 h preserved leaves (Table).

Shell weight

The shell weight was maximum of 0.23g in normal untreated check batch and was minimum of 0.10g in infected batch. Maximum shell weight (0.22g) was recorded in both the bacteria infected and without bacteria infected batches fed with leaves without preservation. Minimum shell weight of 0.13g was recorded with 24 h preserved leaves both in bacteria infected and without bacteria infected batches. In the interaction effect, maximum shell weight of 0.14g was recorded when the infected larvae were reared with leaves without preservation and minimum shell weight of 0.07g was recorded when infected larvae were reared with leaves preserved for 24 h (Table).

Shell ratio

The shell ratio was highest (20.10%) when the larvae were reared without infection compared to infected larvae (10.66%). Independent effect of preserved leaves was significant. Shell ratio of 18.40 per cent was recorded when the larvae were reared on leaves without preservation. Minimum shell ratio of 13.40 per cent was recorded when the larvae were reared with 24 h preserved leaves.

Table1. Influence of leaf preservation on the larval mortality, larval weight, cocoon weight, pupal weight, shell weight and shell ratio of *Bombyx mori* L. infected with bacterial flacherie.

Duration of preservation (h)	Larval mortality (%)			Larval weight (g)		
	With bacterial infection	Without bacterial infection	Mean	With bacterial infection	Without bacterial infection	Mean
0	59.86(50.71)	22.57(28.39)	41.21(39.93)	1.22	1.53	1.37
6	59.81(50.65)	20.72(27.06)	40.26(39.45)	1.13	1.44	1.28
12	53.61(47.06)	19.82(26.42)	36.71(37.29)	1.09	1.40	1.24
18	51.23(45.69)	17.62(24.80)	34.42(35.91)	0.98	1.31	1.14
24	49.71(44.83)	16.92(24.27)	35.31(35.24)	0.89	1.22	1.05
Mean	54.84(47.75)	19.53(26.21)		1.06	1.38	
	F-ratio	SEM+	CD	F-ratio	SEM+	CD
Effect of bacteria	**	2.2119	6.2875	**	0.0402	0.1142
Effect of treatments	NS	1.8060	5.1338	**	0.0328	0.0933
Interaction effect	NS	4.0384	11.4794	NS	0.0734	0.2086

Cocoon weight (g)			Pupal weight (g)		
With bacterial infection	Without bacterial infection	Mean	With bacterial infection	Without bacterial infection	Mean
1.02	1.28	1.15	0.88	0.99	0.94
1.00	1.19	1.10	0.87	0.96	0.92
0.95	1.17	1.06	0.83	0.94	0.89
0.90	1.14	1.02	0.81	0.92	0.87
0.84	1.09	0.98	0.77	0.89	0.83
0.94	1.17		0.84	0.94	
F-ratio	SEM+	CD	F-ratio	SEM+	CD
**	0.0122	0.0348	NS	0.0541	0.1538
**	0.0100	0.0284	NS	0.0442	0.1256
NS	0.0223	0.0635	NS	0.0988	0.2806

Shell weight (g)			Shell ratio (g)		
With bacterial infection	Without bacterial infection	Mean	With bacterial infection	Without bacterial infection	Mean
0.14	0.29	0.22	14.20(22.14)	22.60(28.39)	18.40(25.40)
0.13	0.23	0.18	12.90(21.05)	20.70(27.06)	16.80(24.20)
0.12	0.23	0.17	9.10(17.56)	19.50(26.21)	14.30(22.22)
0.09	0.22	0.15	8.80(17.26)	19.20(25.99)	14.00(21.97)
0.07	0.20	0.13	8.30(16.74)	18.50(25.47)	13.40(21.47)
0.10	0.23		10.66(19.09)	20.10(26.64)	
F-ratio	SEM+	CD	F-ratio	SEM+	CD
**	0.0072	0.0204	**	0.6850	1.9471
**	0.0059	0.0167	**	0.5593	1.5898
NS	0.0131	0.0373	NS	1.2506	3.5549

Highest shell ratio of 14.20 per cent was recorded when infected larvae were reared with fresh leaves. Lowest shell ratio of 8.3 per cent was recorded when the infected larvae were reared with 24 h preserved leaves (Table). The reason for highest larval and cocoon parameters might be due to the preservation of the leaf, some of the carbohydrates were used in the metabolic process and these are not properly utilized by the larvae when such preserved leaves were given to the larvae.

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