



Rate Of Horizontal And Vertical Transmission Of *Bacillus Thuringiensis* Causing Flatcherie Disease And Its Effect On Commercial Characters Of Cocoons Of Mulberry Silkworm *Bombyx Mori* L.

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Introduction

Flatcherie disease is caused by *Bacillus thuringiensis* Var Sotto, pathogen by the ingestion of contaminated food plant leaves by Mulberry silkworm *Bombyx mori* L. It is often claimed that and Pasteur (1865) proved that "Nosema and Flacherie" is transmitted from generation to generation through the egg and contagious. On reviewing Pasteur's original work on "Flacherie" however, one is certainly not impressed with the fact that any evidence exists which proves that bacteria pass through the eggs of silkworms. Some evidence is advanced to show that the disease-producing bacillus is not transmitted from generation to generation through the egg. It is further shown that the *bacillus* is probably not very resistant to its environment and does not survive for any great length of time on the exterior of the eggs. The disease-producing bacillus was not recovered either from the exterior or from the interior of the eggs. One of the tests with 80 per cent alcohol yielded a growth, but the bacillus in question was not found. The broth tubes in which the eggs were crushed without the previous sterilization of the exterior yielded growths, but subsequent work involving a study of four organisms recovered did not demonstrate the presence of the disease producing bacillus. R. W. GLASER (1924).

Bacillus thuringiensis (Bt) is a Gram-positive spore-forming bacterium characterized by the formation of parasporal inclusions during sporulation (Aronson *et al.* 1986; Ohba 1996). After being ingested by the lepidopteran larvae, the parasporal inclusions are dissolved in larval midgut juice and release pro toxins. The activated toxins interact with the larval epithelial membrane and induce pore formation in the membrane, which ultimately leads to insect death (Gill *et al.* 1992). B.t is considered to be the causative agent for a special type of "Flacherie" disease of silkworms, known as "Sotto". Larvae affected by *Bacillus thuringiensis* lose their appetite, undergo convulsions and their bodies become stretched and cracked. The cadavers gradually become brown to black-brown and finally when rotting they turn black (Aruga 1994). The rate of transmission of *Nosema* sp. NIK-3h in mulberry silkworm was reported to be low. It transmits the infection to the progeny by only $1.80 \pm 0.4\%$, while the standard strains *N. bombycis* and *Nosema* sp. NIK-2r transmit the infection to an extent of 100% (Ananthalakshmi *et al.*, 1994; Nageswara Rao *et al.*, 2004). But in the case of silkworm *Bombyx mori*, limited studies were conducted on this *Bacillus* sp. regarding spore morphology, virulence and pathogenicity but no information is available on the rate of horizontal and vertical transmission. Hence, the present work was carried out to study the rate of horizontal and vertical transmission of *Bacillus thuringiensis* and its impact in economic cocoon characters of silkworm.

Materials and Methods:

The experiment of the study on rate of horizontal and vertical transmission of *Bacillus thuringiensis* causing Flatcherie disease in the Mulberry silkworm *Bombyx mori* L. conducted in, Dept of Sericulture, Sri Krishnadevaraya University, Anantapur, Silkworm breed CSR2 XCSR4 was reared as per the package practices up to the end of the experiment (Krishnaswami *et al.*, 1973).

Horizontal Transmission:

To determine the rate of horizontal transmission / rate of spread of *Bacillus thuringiensis* infection in healthy silkworm *Bombyx mori*, a specific number of infected carrier larvae were introduced into a healthy population of known number of individuals. Immediately after first moult (II instar) a few larvae were inoculated with *Bacillus thuringiensis* (1×10^{-6} cells/ml) and rearing was conducted. During second moult a few inoculated larvae were homogenized and examined under phase contrast microscope for the presence of pathogen and these larvae were considered as carriers. The faeces collected from the infected silkworm of *Bacillus thuringiensis* was also observed under phase contrast microscope and the infection was confirmed.

Specific number of *Bacillus thuringiensis* infected larval carriers was introduced (20 in 980 (2%), 40 in 960 (4%), 60 in 940 (6%), 80 in 920 (8%) and 100 in 900 (10%)] on the zero day of third instar silkworms. Control batch was also maintained and they did not carry any infected carriers. Each treatment had three replications of 1000 larvae each. After introduction of carriers in the healthy colony, the larvae were reared till spinning. All the larvae including the larvae died during rearing were examined for the presence of *Bacillus thuringiensis* and the result were analysed and the results presented in tables and discussed.

Vertical transmission:

The larvae of *Bombyx mori* were brushed and reared up to IV moult. Immediately after IV moult silkworm larvae were fed with Mulberry leaves containing an inoculation of 1×10^5 cells /ml of *Bacillus thuringiensis* per os. Another set of larvae were kept without inoculation which serves as control batch. All the batches were reared till spinning and allowed to spin the cocoons. Three replications were maintained for each treatment with 1000 silkworms for each replication. All the cocoons spun from inoculated and control batches were kept for moth emergence. Moth obtained from the inoculated larvae was provisionally regarded as infected and were allow to pair and lay eggs. Moths obtained from batch without inoculation were provisionally regarded as healthy and were allowed to pair and lay eggs. The pairing of moths was as follows.

In treatment (T1) one set of infected female moths (IF) were allowed to pair with healthy male moths (HM). In another treatment (T2) a set of infected female moths (IF) were paired with infected male moths (IM). In third treatment (T3), healthy female moths (HF) were allowed to mate with infected male moths (IM). Yet in another treatment (T4) a set of experiment healthy female moths (HF) were allowed to pair with healthy male moths (HM). After mating, female moths were allowed to lay eggs and layings were prepared from individual mother moth. The male and female moths were macerated separately after egg laying and the wet mount were examined for the spore of *Bacillus thuringiensis* and the observations were recorded. The layings obtained from the different treatments were surface sterilized by formaline for 10 min at room temperature. The layings were incubated for normal embryonic development at $25 \pm 1^\circ \text{C}$. The newly hatched larvae from each laying were reared up to II moult and larvae were homogenized individually and examined for *Bacillus thuringiensis*. One hundred larvae/batch or treatment were picked randomly and examined under phase contrast microscope for the presence of *Bacillus thuringiensis*. The percent of transmission with respect to treatment was calculated.

Impact on cocoon characters:

The larvae of *Bombyx mori* were brushed and reared up to II moult. Immediately after II moult, larvae were inoculated with inoculum of 1×10^5 cells / ml of *Bacillus thuringiensis*. A control batch without inoculation of *Bacillus thuringiensis* was also maintained for comparison. Three replications were

maintained for each treatment with 1000 silkworms for each replication. Rearing was conducted up to spinning. The mature larvae were allowed to spin their cocoons, harvested and economic characters of cocoon viz., Single cocoon weight, Single shell weight and Silk Ratio % were assessed and analyzed statistically.

Results

The result of horizontal transmission of *Bacillus thuringiensis* infection in the healthy silkworm population is presented in Table 1. It is observed that 20, 40, 60, 80 and 100 carriers of *Bacillus thuringiensis* infected silkworms spread the infection to an extent ranging from 52.68 to 92.24%. The rate of spread of infection *Bacillus thuringiensis* was high, where as the infection in control batch was nil (Table 1).

The result of mode of vertical transmission of *Bacillus thuringiensis* in *Bombyx mori* L. presented in Fig. 1. In case of progeny larvae hatched from eggs obtained from infected female X healthy male (IF X HM) as well as infected female X infected male (IF X IM) viz., T1, T2 revealed zero infection. The larvae hatched from the eggs obtained from healthy female moths X infected males (HF x IM) (Fig 1: T3) also did not reveal the infection in their progenies. The rate of transmission of *Bacillus thuringiensis* to the F1 progeny in T1 (IF x HM) and T2 (IF x IM) treatment batches was 0%. The progeny of the control batch (HF x HM)-T4 with no inoculation of *Bacillus thuringiensis* also didn't reveal any infection and the rate of transmission was nil (Table 2)

The impact of *Bacillus thuringiensis* infection on cocoon characters of silkworm in *Bombyx mori* L. was calculated and presented in Table 3 and the result was compared with control batch. The average single cocoon wt., shell wt. and shell ratio (%) was 1.76 gr, 0.396 gr, and 22.45% respectively in control batch. In case of *Bacillus thuringiensis* infected batch, the cocoon characters of silkworm were 1.57 gr, 0.378 gr, 20.79% respectively. It is observed that these characters were significantly reduced compared to control batch (Table 3).

Table 1: Rate of horizontal transmission of *Bacillus thuringiensis* in *Bombyx mori* L.

S.No.	Batch	Sample Size	% of carriers introduced	% of infection <i>Bacillus thuringiensis</i>
1	A	1000	2	52.68
2	B	1000	4	67.18
3	C	1000	6	73.88
4	D	1000	8	80.66
5	E	1000	10	92.24
6	Control	1000	0	0

Table 2: Rate of Vertical transmission of *Bacillus thuringiensis* in *Bombyx mori* L.

S.No.	Batch	Sample Size	% of infection <i>Bacillus thuringiensis</i>
1	A	1000	0
2	B	1000	0
3	C	1000	0
4	D	1000	0
5	E	1000	0
6	Control	1000	0

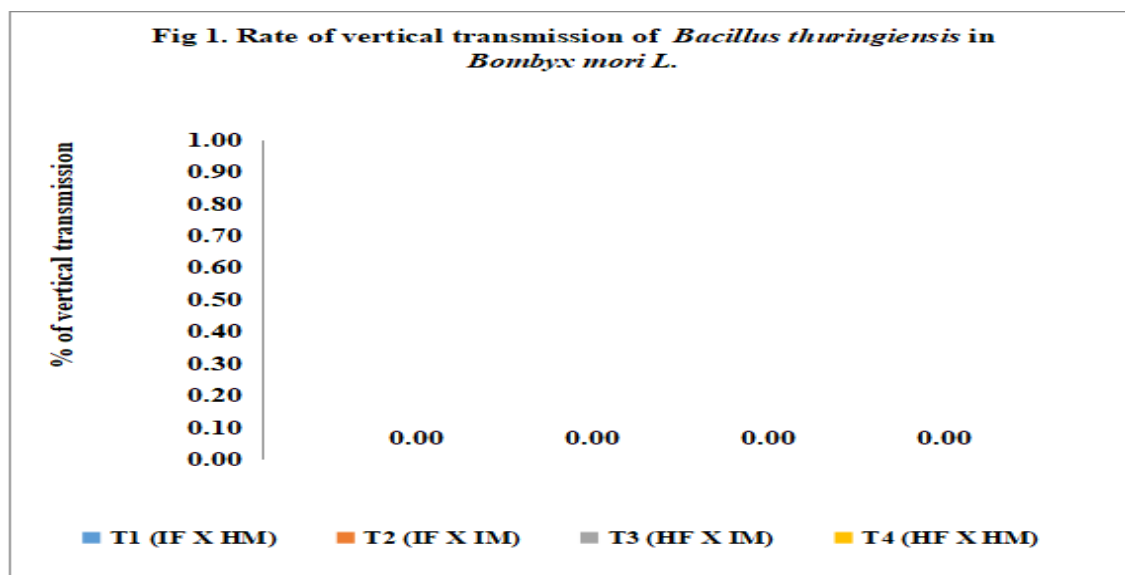


Table 3: Effect of *Bacillus thuringiensis* infection on economic characters of *Bombyx mori* silkworm cocoon

S.No.	Batch	Sample Size	Single cocoon wt.	Single shell wt.	Shell Ratio %
1	A	1000	1.61	0.384	21.22
2	B	1000	1.68	0.381	21.10
3	C	1000	1.64	0.379	20.80
4	D	1000	1.60	0.375	20.66
5	E	1000	1.68	0.373	20.18
6	Average	1000	1.57	0.378	20.79
7	Control	1000	1.76	0.396	22.45

Discussion

Bacillus thuringiensis transmits infection to other healthy silkworm larvae and the results of the experiment quantify and affirm the fast spreading nature of the disease through secondary contamination. The extent of contamination was in proportion to the number of infected larvae introduced and it reached to as high as 92.24% level when 10% infected larvae be introduced. In the case of control the infection rate was 0.00%. In similar study of Pebrine, Ishihara and Fujiwara (1965) also confirmed the above findings and reported that the change of the epizootic pattern corresponded to the change in number of larvae excreting spores of *N. bombycis* in mulberry silkworm, *Bombyx mori* L.

Investigation on the mode of transmission indicated that the *Bacillus thuringiensis* no transmission is noticed in the progeny. Zero transmission is recorded (transovarial) in *Bacillus thuringiensis*. But in the case of microsporidia such as *Nosema kingi* in drosophilids (Armstrong, 1976) and *Thelohania* species in mosquitoes (Kellen *et al.*, 1965) transmission was recorded. In *Bombyx mori* L silkworm, *N.bombycis* is reported to transmit infection by transovum (Masera, 1938) and by transovarial transmission (Han and Watanabe, 1988). In case of the rate of *Bacillus thuringiensis* transmission in progeny from female moth was 0%. This supports study of R. W. Glaser (1924)

Apart from high infectivity in horizontal and zero infectivity in vertical transmission, the infection of *Bacillus thuringiensis* its rate of transmission in the *Bombyx mori* L. resulted in significant impact on the economic parameters of silkworm. A comparison in single cocoon wt., single shell wt. and SR% with respect to healthy control (1.76gr, 0.396 gr and 22.45%,) indicated that *Bacillus thuringiensis* infected batches were (1.57gr, 0.378 gr, 20.79%) significantly lower, respectively.

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