IN VIVO USE OF STREPTOMYCIN SULFATE FOR BACTERIAL DISEASE CONTROL IN A*NTHERAEA ASSAMENSIS* HELFER THROUGH LEAF FRESHNESS TECHNIQUE

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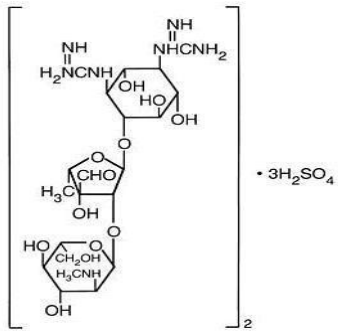
ABSTRACT: Among all biotic and abiotic constrains in Muga silkworm culture, disease is the most consistent and devastating one. Bacterial flacherie is such a silkworm disease that inflects more than 42% crop loss. Antibacterial drugs like streptomycin sulfate can be used successfully through leaf freshness technique useable in indoor rearing of Muga culture. Drug solution is mixed with leaf sap as absorbent by Som plant twigs that larvae feed on and, targeted disease causing bacteria in larval gut are exposed to streptomycin action. *In vivo* use of streptomycin sulfate resulted complete control of flacherie disease caused by Bacillus bacteria up to fourth instars. Being bacteria free in gut, larval feeding was more that was directly reflected in larval body weight and indirectly increases vurvivality of larvae. In this experiment with *in vivo* use of streptomycin sulfate 42% more survivality up to fifth instars of Muga larvae was achieved over 92% mortality in control rearing.

KEY WORDS: Flacherie, streptomycin sulfate, leaf freshness, survivality, body weight, mortality, *in vivo*.

Antheraea assamensis Helfer, the golden-yellow silk producer silk moth, is semi-domesticated sericigenous insect species endemic to North East India particularly Brahmaputra valley of Assam. This silk moth is semi-domesticated owing to the fact that only cocooning and grainage operations are conducted indoor and, reared on outdoor host plant trees. It is polyphagous, multivoltine reared in six different seasons throughout the year. Out of these six seasons two seasons *viz*., May-June and October-November are commercial crop season, whereas other seasons are seed crop season. Again, the seed crops during December-January and June –July are called pre-seed crop. Thus, each commercial crop is preceded by one pre-seed crop and one seed crop. Since this pattern of Muga silkworm cultivation has been an age old practice, it is obviously environment controlled and the rearing performance is quite different in each season. Being outdoor in nature Muga silkworm are exposed to various natural vagaries and thus prone to numbers of diseases and pests leading to heavy crop loss (Choudhury, 1981; Samson, 1987 and Thangavelu et al. 1988). Of all constrains, silkworm diseases being the most important that inflect heavy loss to the crop. The 'Flacherie' disease caused by bacteria is most important, causing serious damage to the Muga silkworm (Chakravorty et al. 2007). The chief disease affecting silkworm is flacherie caused by Bacillus bacteria. One casual bacillus of silkworm flacherie is *B. thuringiensis* which is widely distributed facultative entomogenous bacterium with as many as 34 varieties. It is a garm-positive spore forming bacterium widely distributed in the soils of various regions of the world. The endotoxin of *B. thuringiensis* is known to destroy the gut lining, causing paralysis and death in many insect species belonging to orders, Diptera and Lepidoptera including economically important insects such as silkworm, *Bombyx mori* (Aizawa, 1971; Nataraju et al., 1991). *B. thuringiensis* infected

larvae lost appetite and became sluggish from 5 to 6 hours of infection, larvae vomited the green fluid, excreta were soft and stick to the rearing bed. During molting, skin was not shed properly. The infection also led to diarrhea. As the disease advanced, the larvae became extremely sluggish, showed irritability to touch as if in pain. Later the colour of the larvae started to change into dark colour and larvae became almost inactive and unable to spin cocoons. The larval body started to shrink and the larvae became completely paralyzed. Finally, larvae completely turned to brown colour. Within 30 minutes after the larvae ingested the spores of *B. thuringiensis*, the mid gut epithelial cells became disorganized compared to healthy larvae. Some of the epithelial cells became detached from the wall of the mid gut. There exist 34 different varieties of *B. thuringiensis*. Among the 23 varieties of *Bt* tested, only eight were reported to be pathogenic to silkworms and the rest as non-pathogenic (Selvakumar et al. 1999). Disease incidence may be due to the lowering of pH by the introduction of bacteria, which provides congeniality and could lead to degeneration of peritropic membrane, which blocks the absorption of nutrients that is reflected by cessation of feeding. From flacherie infected mulberry silkworm, Sridhar et al. (2000) isolated bacteria belonging to genus *Streptococcus.*

The biological defense against pathogens in insects included the innate *physical* barriers *viz.,* integument and intestinal wall and humeral responses such as the activation of prophenol oxidase cascade and induction of immune proteins namely, lysozymes, lectins, antibacterial proteins and antifungal proteins primarily by the fat bodies. Intestine harbor a great diversity of native microbes which promote gut maturation, and integrity, antagonism against pathogens by producing antimicrobial proteins and immune modulation (Girishkumar et al., 2005). An countable number of researchers made effort to control 'Flacherie' in Muga silkworm by various biological and non-biological agents as spray, including streptomycin, an antibacterial drug, and reported as effective. Streptomycin sulfate is a bactericidal antibiotic and is a water-soluble amino glycoside derived from *Streptomyces griseus*. It is marketed as the sulfate salt of Streptomycin. The chemical name of Streptomycin sulfate is D-Strep amine, O - 2 - dioxin - 2 - (methyl amino) - α - L - glucopyranosyl - (1→2) - O - 5 - dioxin - 3 - C - formyl - α - L - lyxofuranosyl - (1→4) - N,N1-bis(aminoiminomethyl)-,sulfate (2:3) (salt). The molecular formula for Streptomycin Sulfate is (C21H39N7O12)2 - 3H2SO4 and the molecular weight is 1457.41. It acts by interfering with normal protein synthesis. Streptomycin is considered a second-line agent for the treatment of gram-negative bacillary bacteria. In this present context of study, effort has been made to test the efficacy of this drug in vivo in controlling bacterial diseases under indoor reared Muga silkworm through a new leaf freshness technique.



MATERIALS AND METHOD

Eggs of Muga silkworm dfls were placed in plastic container covered with wet foam pad below the lid. The foam pad was wetted and squeezed two times in a day to maintain optimum humidity (80 – 85%) inside the container. Tender leafy twigs of Som plant (*Persea bombycina* Kost) were collected in bucket with water. Collected twigs were treated in clean water mixed with certain chemical solutions for three hours for freshness (proofed to keep twigs fresh). Clean and blank water was poured in two bottles in one of which, two treated twigs were inserted (C). In the other bottle, a solution of streptomycin sulfate @ 5% / liter was added to make the water in the bottle 0.5% strength (T). Another two Som plant twigs already treated were inserted into the second bottle. Now, these two bottles were placed in plastic buckets separately so as to collect dead larvae as well as litters. On 02-02-2010 day, 50 nos. hatched out larvae were brushed in each treatment (T & C), which were allowed to grow inside room. Closed watch was maintained not to crawl out from these. Each day dead larvae and litters were collected for record. Leafy twigs were replaced with newly treated one as and when necessary. Fresh solution in T bottle and water in C bottle were added as the leafs absorbed it. Collected litters of each instar were weighted and recorded. Data were tabulated for discussion.

RESULTS AND DISCUSSION

Data of the experiment in contrast between treatment and control over different parameters related to silkworm rearing have been shown in Table-1 and statistically analyzed. All these parameters are related to the growth and development of Muga silkworm which in turn, influence directly or indirectly by bacterial diseases. In the T rearing, symptoms of flacherie disease were not seen up to IVth instars to cause larval death. The death of larvae was found to be due to some other causes like failure to molt out into next instar, loss of griping power resulting fall down from bait, stunted growth with slow feeding rate and ultimate death. However, in fifth instar two cases of dead larvae hanging from twig branch were recorded indicating flacherie incidence. Contrary to T rearing, most of the larval death occurred in C rearing due to bacterial flacherie exhibiting the typical symptom hanging with its head downward from twig's branch and body turning into shrinkage to black-brown colour. Data in the table-1 profoundly indicate low mortality and high survivality throughout all the instars in T rearing than recorded in case of C rearing (Fig.1). Trend value of survivality in T is much higher (408) than C (298). This firmly establishes a changing positive trend in larval survivality as result of flacherie control by streptomycin sulfate. This also supported high mortality trend (156.64) among C larvae without streptomycin treatment in contrast to lower mortality trend (61.02) in T. At the end of fifth instar, 50% well grown larvae survived for spinning in T rearing against only 8% larval survivality in control rearing (C). A difference of 42% was found in aggregate larval mortality of all instars in these two treatments. Thus, bacterial flacherie caused 42% larval mortality as calculated from the mortality data of T and C rearing. Larval growth measured as larval weight (body tissue growth), although indicates differences between the treatments, the differences were within the scope of limitation and not distant. Therefore, body tissue growth in T rearing and C rearing, also statistically analyzed as trend value 14.5868 and 13.531 respectively, was not significantly different. Earlier worker like Venkatachalamurthi et al. (1951) reported that streptomycin significantly

increased the body weight of silkworm larvae. The litter weights of each instar in these two rearing, exhibited a marked differences in size of population that indirectly support the changed survivality of Muga larvae through *in vivo* use of antibacterial agent (streptomycin sulfate) in treatment rearing (T).

Streptomycin sulfate solution in the bottle did find its way into the leaves through twig's stem of which lower ends were kept immersed in the solution. So, as a physiological principle the solution of streptomycin was pulled by leaves triggered by transpiration. The leaves were ingested by the larvae in T rearing. Streptomycin sulfate along with leaf feed entered into the gut of larvae, and then into gut wall tissue system. A fall in gut pH (8.0-8.2 to 7.0-7.2) due to bacterial infection has been reported (Ono et al., 1968; Chitra et al., 1974). Inside the targeted bacterial cells present in tissue system and gut, streptomycin sulfate interfere in protein synthetic mechanism. The mode of action how streptomycin inhibits or kills the bacteria has been explained by Villanova (1990) and Stenfors Arnesen et al., 2008. As this antibacterial drug entered inside the bodies of larvae in T rearing, there was almost no dead report due to typical flacherie disease. Whereas in control rearing (C) through leaf freshness technique, 42% mortality was recorded due to bacterial flacherie out of 92% total larval mortality. Aruga et al., 1971 reported that bacterial flacherie is the most important disease affecting silkworm, causing 70% crop loss in Japan. Thus, the antibacterial action of streptomycin sulfate inside larval body has been proofed against flacherie disease causing bacteria in Muga silkworm, *A. assamensis* Helfer. Similar result was also reported by Savithri et al. (2003b). They reported that antibiotics streptomycin @500 ppm reduced satisfactorily disease incidence in the worms. Dutta et al. (2010) reported *in vitro* use of streptomycin sulfate @1000 ppm and successfully controlled bacterial diseases in Muga silkworm up to 52.37%. Besides streptomycin sulfate, other antibacterial agents have also been reported to control bacterial diseases in silkworm. Intestinal microbial environment can be fortified by supplementing probiotic microbes. This could lead to higher survival, fewer incidences of diseases and reduced mortality along with low feed conversion ratio and increased production (Tannock, 1997). The inhibitory potential of Ampicillin, Amoxicillin, Chloramphenicol and Tetracycline in different concentration has been reported positive (*in vitro*) against *Bacillus* coagulans, a bacterium that cause silkworm disease (Savithri et al., 2003a,b).

Bacterial flacherie has been found to be a major constrain in Muga cultivation in Assam that cause heavy loss in each season. The prevention or management of this disease is a vital component for successful rearing for higher yield and quality cocoons. Thus, *in vivo* application of streptomycin sulfate through leaf freshness technology may through ray of hope in controlling bacterial flacherie in Muga silkworm indoor cultivation for bumper harvest and quality cocoons.

LITERATURE CITED

Aizawa, K. 1971. Strain improvement and preservation of virulence of pathogens. In. Microbial control of insects and mites, H. D. Burges and N. W. Hussy (Eds.), Academic press London. pp. 655-672.

Aruga, H. & Tanada, Y. 1971. The cytoplasm Polyhedrosis virus of the silkworm. University of Tokyo Press, Japan, p. 234.

Chakravorty, R., Das, R., Neog, K., Das, K. & Sahu, M. 2007. A diagnostic manual for diseases and pest of Muga silkworm and their host plants. Published by CMER&TI, Central Silk Board, Lahdoigarh, Jorhat, Assam. pp 1-47.

Chitra, C., Karanth, N. G. K. & Vasantharajan, V. N. 1974. Studies on 'Sappe' disease of the silkworm *Bombyx mori* L. II. Effect of age of larva on the manifestation of the disease. J. Invertebr. Pathol., 24; 218-232.

Choudhury, S. N. 1981. Disease, pest and parasites. In: Choudhury, S. N. (Eds.). Muga silk industry. Directorate of Sericulture and Weaving. Govt. of Assam, Guwahati, India. pp. 74-81.

Dutta, P., Neog, K., Das, R., Das, K., Handique, P. K. & Chakravorty, R. 2010. Evaluation of some botanicals, antibiotics, carbon source and carrier against the bacterial disease of Muga silkworm, *Antheraea assamensis*. Scrocologia, 50 (1); 113-122.

Girishkumar, C. P., Thangam Menon & Anitha Devi, C. P. 2005. Bacteria for breakfast: Probiotics for good health. Advanced Biotech., March, pp. 15-20.

Nataraju, B., Balavenkatasubbaiah, M., Baig, M., Singh, D. B. & Sengupta, K. 1991. A report on the distribution of *Bacillus thuringiensis* in Seri cultural areas of Karnataka. Indian Jn. Seric., 30 (1): 56-58.

Ono, M. & Kato, S. 1968. Studies on the dissolution of peritropic membrane of the silkworm *Bombyx mori*. Scric. Expt. Stn. Jpn., 23: 9-16.

Samson, M. V. 1987. Bacterial diseases of silkworm and their control. Ph. D thesis, CSR&TI, Mysore; 1-137.

Savithri, G. & Murali Mohan, P. 2003a. Pathogenocity of the bacterium *Bacillus coagulans* in silkworm Bombyx mori L. Indian J. Seric., 42 (1): 4-8.

Savithri, G. & Murali Mohan, P. 2003b. *In vitro* screening of antibiotics against *Bacillus coagulans*. Indian J. Seric., 4231: 25-28.

Selvakumar, T., Nataraju, B. & Datta, R. K. 1999. Characterization of *Bacillus thuringiensis* varieties in relation to pathogenicity to silkworm, *Bombyx mori*. Indian J. Seric., 1: 75-78.

Sridhar, R., Subramanian, A. & Chandramohan, N. 2000. Efficacy of two antibiotics against bacterial flacherie of silkworm, *Bombyx mori* L., 39 (2): 176-177.

Stenfors Arnesen, L. P. & Fagerlund, A. 2008. From soil to gut: Bacillus cereus and its food poisoning toxins. Granum PEFEMS Microbiol Rev. Jul, 32 (4): 579-606. E-pub 2008 Apr 15.

Tannock, G. W. 1997. Modification of the normal micro biota by diet, stress, antimicrobial agents and probiotics. In. Microbiology Series, Mackie R. L., B. A. Issacson, (Eds.), Chapman and Hall, International Thomson publishing, New York, pp. 435-465.

Thangavelu, K., Chakravorty, A. K.., Bhagowati, A. K. & Isa, Md. 1988. Handbook of muga culture, Central Silk Board, Bangalore.

Venkatachalamurthi, M. R., Shankarappa, B. S. & Sreenivasaya, M. 1951. Influence of antibiotics on the growth of silkworm *Bombyx mori* L.. Curr. Sci., 20: 269-270.

Villanova, P. A. 1990. National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Disk Susceptibility Tests-Fourth Edition. Approved Standard NCCLS Document M2-A4.Vol.10, No.7, NCCLS.

Table 1. Tabulation of data showing comparison between treatment and control on different parameters and their statistical analysis.

Larval Instars	Nos. of larvae brushed		Treatment with streptomycin (T)				Control without treatment (C)			
	T	C	Larval weight in g (5 nos.)	Mortality (%)	Survivability (%)	Litters Weight (g)	Larval weight in g (5 nos.)	Mortality (%)	Survivability (%)	Litters weight (g)
I instar	50	50	0.0332	0	100	1.41	0.0218	4	96	1.409
II instar			0.0906	0	100	5.907	0.0892	6.25	90	5.893
III instar			0.414	12	88	20.972	0.4	28.89	64	17.851
IV instar			2.229	20.45	70	42.299	2.153	37.5	40	30.14
V instar			11.82	28.57	50	256.8	10.867	80	8	81.645
TREND			14.5868	61.02	408	327.388	13.531	156.64	298	136.938

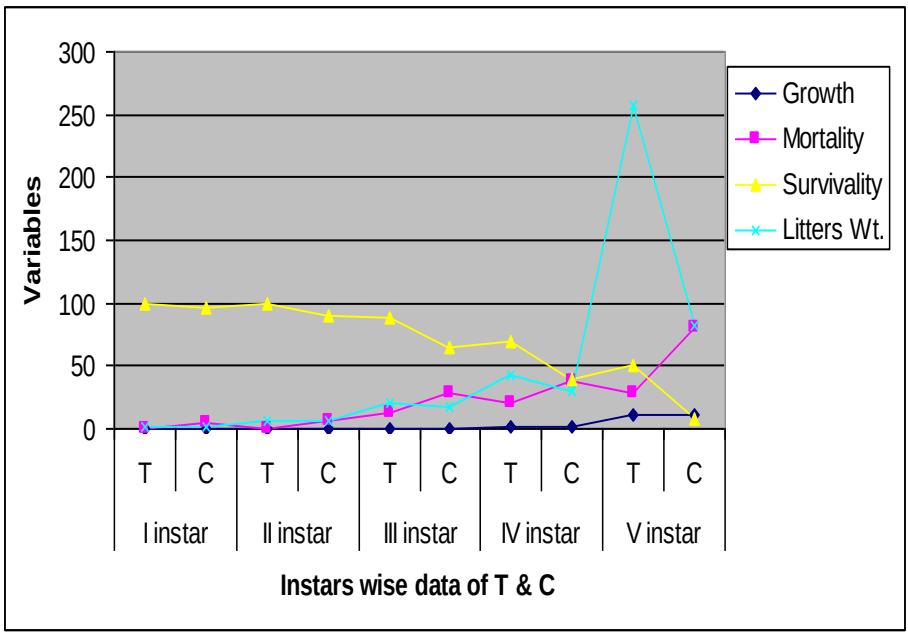


Figure 1. Graphical presentation of data of treatment and control on larval growth, mortality, survivality and litter weight.

A – Control (II nd instars)

B – Control (V th instars)

C – Treatment (II nd instars)

D – Treatment (V th instars)

E – Leaf treated in chemicals for freshness

Figure 2. Photographs of Control rearing (A, B), Treatment rearing (C, D) and Treatment of leaf for freshness (E) before use as feed.