The Pharma Innovation Journal 2015; 4(6): 101-103

www.ThePharmaJournal.com

The Pharma Innovation

ISSN: 2277- 7695
TPI 2015; 4(6): 101-103

www.thepharmajournal.com
Received: 28-06-2015
Accepted: 30-07-2015

Jansi rani L
PG & Research, Department of Zoology, Muslim Arts College, Thiruvithancode, Kanyakumari District, Tamil Nadu, South India 629174 Kanyakumari District 629174.

Venkatesh B
PG & Research, Department of Zoology, Muslim Arts College, Thiruvithancode, Kanyakumari District, Tamil Nadu, South India 629174 Kanyakumari District 629174.

Kumaran T
PG & Research, Department of Zoology, Muslim Arts College, Thiruvithancode, Kanyakumari District, Tamil Nadu, South India 629174 Kanyakumari District 629174.

Correspondence:
Jansi rani L
PG & Research, Department of Zoology, Muslim Arts College, Thiruvithancode, Kanyakumari District, Tamil Nadu, South Kanyakumari-629174, India.

Sensitivity and Antibiogram Studies on Pathogenic Microbes Isolated From the Silkworm *Bombyx Mori* L.

Jansi rani L, Venkatesh B, Kumaran T

Abstract
Sericulture is an agro-based income generative venture. Developing countries, the loss due to diseases in sericulture is to the tune at 10% of the total crop loss. In the present study the diseased silkworm were collected, and immobilized by chloroform and surface sterilized by methanol. The larvae were dissected under aseptic condition to remove foregut, silkgand, reproductive system and a piece of skin. Each observed colony was purified and subjected to morphological, physiological and biochemical tests. As a result the organisms identified were Staphylococuss aureus, Staphylococcus epidermis, Staphylococcus albus, Bacillus thuriensis and Bacillus Orpheus. Sentivity to antibiotics was tested for selected pathogens isolated from disease infected worms. The antibiotics viz. streptomycin, ampicillin and penicillin were used. Control was maintained. *In vitro* screening of the pathogen for antibiotic sensitivity revealed differential sensitivity microbes for different antibiotics. Streptomycin was found to be the most effective antibiotics to control the pathogen.

Keywords: silkworm, pathogen, agar medium, biochemical test, antibiotics

1. Introduction
Sericulture is an agro-based rural income generative venture. Silk from the mulberry silkworm earns a lot of foreign exchange. Countries like Japan and china are dominating the sericulture profession. In India, sericulture practice is often affected by disease outbreak. In developed countries, the loss due to diseases in sericulture is to the tune at 10% of the total crop loss(Yokoyama, 1971).While in less developed countries like India the loss is to an extent at 30-40 percent (Nanavathy, 1965) [9].

Bombyx mori L. is attacked by several disease and predators. The chief disease affecting silkworm are flacherie, grasserie, muscardine and pebrine (Chira *et al.*, 1975) [2]. Flacherie may be caused by microbial and antimicrobial agents. Since silkworm is reared year after year, this may help the preparation of the disease producing organisms, pest and parasites of silkworm. Disease and pests cause great loss and trouble to the silk industry. The silk yield or the production of silk depends upon the requirements of temperature, humidity and leaf quality and it is different for varieties of worms in the Tirunelveli district of Tamilnadu. Sidhu and Singh (1968 a) had reported that the pathogenic microbial infection on Bombyx mori induces shift in metabolic profiles and the activities of enzymes amylase, invertase, trehalase and protease were affected. Flacherie disease of silkworm was considered to be caused by the abnormal multiplication of bacteria in the larval gut lumen.

Bacteria are known to play an important role in causing flacherie of silkworm as individual pathogen or in combination with viruses. Their in rolement in causing disease further leads to the spoilage of cocoon and silk quality and reduction in fecundity (Savithiri and murali 2003) [8] Various workers have reported disease prevalence in sericultural countries and attempt have been made to save the cocoon crop by application of different antibiotics, disinfectants and chemical (Sam devadass, 1999, Nahar,1995) [5]. But the *in vitro* screening of antibiotics was different by different workers. Savithiri and Murali (2003) [8] reported 6 antibiotics to control the bacterial flacherie caused by Bacillus coagulans. The reported tetracycline to be the most sensitive antibiotics. Inhibition zone of 10mm was observed for 24hrsexposure to tetracycline at a dose of 1% concentration. The sensitivity of B. coagulans to six antibiotics was in the order. Tetracycline<amoxicillin<ampicillin<Chloramphenicol<Cephalexin (Savithri and Murali 2003) [8].

In the Tirunelveli district of Tamil Nadu sericulture activities are carried out in selected places like Tenkasi, Sencottai and Ambasamudram Taluks. However, the production of silk is not up to the expected standard. The sericulture farmers often report the disease as the

Causative factors behind the poor production of silk. Our preliminary studies also reported the incidence of bacterial flacherie in these area. Hence, the present study is planned to find out the bacterial pathogens that limits silk production in Tirunelveli District. Also it has been planned to study the sensitivity of different drugs to the common pathogens so as to develop suitable therapeutic agent to protect the silkworm from pathogenic attack.

Materials and Methods

Collection of Sample and Identification of Bacteria

From the sericulture farms diseased worms were collected, the samples were immobilized by chloroform and surface sterilized by methanol. The larvae was dissected under aseptic conditions to remove the foregut, silkgland, reproductive system and a piece of skin. These above portions were weighted and homogenised separately in 5ml peptone broth and 1ml of each suspension was plated on nutrient agar medium, Rose Bengal agar medium and saboroud's dextrose agar medium. The plates were incubated at 37 ^{0}c for 48hrs. Each observed colony was purified and subjected to morphological, physiological and biochemical tests and identified through microbiological test. (Govindhan *et al.,* 1998) [3].

Antibiogram Studies

Sensitivity to antibiotics was tested for selected pathogens isolated from disease infected worms. The antibiotics Streptomycin, Ampicillin, and Penicillin were prepared in 3 concentrations 1:25, 1:50 and 1:100 and loaded in sterile discs. The air dried nutrient agar plates were taken and 0.1ml test organisms were swabbed different concentration of antibiotics, Impregnated disc were placed in centre of plate, sterile water was used as control. The plates were incubated at room temperature for 2 days. After incubation the zone of inhibition was measured.

Result

From the gut of the disease affected IV instar, Different species of bacteria were isolated viz., *Staphylococcus aureus, S. epidermis, S. albus, Bacillus thuringiensis* and *B. orphans*. Five predominant colonies isolated and identified using various biochemical tests were tabulated in table 1. Bacterial infection was high in the gut of IV instar stages so fourth instar larval gut was isolated and the pathogens were isolated. Of the three organs tested, gut, skin and silkgland, the bacterial population was high in the gut. The total heterohphie population in the gut tissue was $16x10^{-7}$ per 0.1 mg and gut tissue whereas it was $16x10^{-3}$ per 0.1mg of tissue in the skin and $16x10^{-2}$ in the silkgland. The study clearly suggests that the bacterial infection starts from the gut and gut is the main target organ for bacteria.

Table 1: Morphological and biochemical confirmation of pathogenic bacteria isolated from the diseased Bombyx mori.L

Sl. No	Test performed	Staphylococcus aureus	Staphylococcus epidermis	Staphylococcus albus	Bacillus thuriensis	Bacillus orpheus
1.	Catalase Test	+	+	+	-	+
2.	Coagulase Test	+	-	-	+	+
3.	Oxidase Test	-	-	+	+	+
4.	Methyl red Test	-	-	+	+	+
5.	Voges proscauer Test	+	+	+	+	+
6.	Indole Test	+	+	+	-	+
7.	Citrate utilization	+	+	+	+	+
8.	Starch Hydrolysis	+	+	+	+	+
9.	Gelatin Hydrolysis	+	+	+	+	+
10.	Casein Hydrolysis	+	+	+	+	+
11.	Manital Salt	+	-	-	+	+
12.	Growth on Nacl agar	+	-	+	+	+
13.	Growth temperature	+	+	+	+	+
14.	Acid from Carbohydrates	-	-	+	+	+

As the gut is infected with the the bacteria the voraciously feeding IV instar larvae takind mulberry leaves were much affected and from the gut, the infection reaches haemolymph. The most common symptoms observed in the infected larvae were softened, transparent body, oozing haemolymph and retarded movement. Of the different specific bacteria isolated, the total count of *S. albus* was higher in the gut than the other organisms. The entry of the pathogens is mainly through the mulberry leaves. The disease infected worm fails to metamorphose and moult in to V th instar is gradually reduced.

The antibiogram studies mortality was heavier when the pathogenic microbes were treated gut silkworms. The highest mortality rate (51%) was observed in Penicilium sp. At $30x10^{-5}$ dilution followed by (32%) at $50x10^{-7}$ dilution and Aspergillus flavus (44%) at $30x10^{-5}$ dilution. The lowest mortality rate was observed in control. In order to control the bacterial infection, the sensitivity of isolated microbes from the silkworm *Bombyx mori* was tested against different concentrations of standared antibiotics (Table 2 and Fig. 1)

Table 2: Inhibitory effect of certain Antibiotics against bacterial species isolated from the diseased *Bombyx mori.L*

Sl. No	Name of the Antibiotics	Microorganisms	Zone formation (mm)s			
			Con 1	Con 2	Con 3	Control
1.	Ampicillin	*Staphylococcus Epidermis* *Bacillus thuriensis* *Bacillus orpheus*	3mm 5mm 5mm	6mm 6mm 6mm	10mm 6mm 6mm	-
2.	Penicillin	*Staphylococcus Epidermis* *Bacillus thuriensis* *Bacillus orpheus*	4mm 5mm 4mm	5mm 6mm 4mm	7mm 5mm 9mm	-
3.	Streptomycin	*Staphylococcus Epidermis* *Bacillus thuriensis* *Bacillus orpheus*	14mm 13mm 12mm	15mm 15mm 14mm	14mm 18mm16mm	-

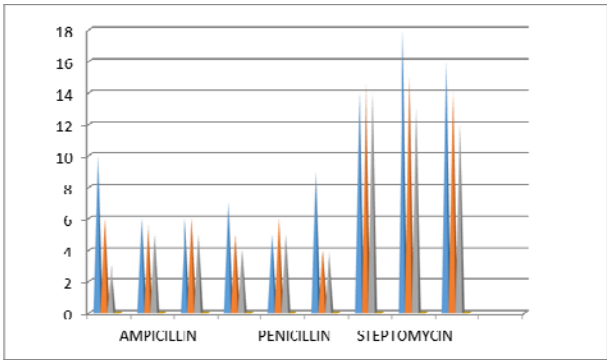

Fig 1: Graphical representation of antibiotics against bacteria in different concentration

Discussion

In the present investigation the pathogenic microbes isolated from the gut of the disease affected larvae, 5 species of bacteria were isolated. The hot and humid climate accompanied with handling problems could have increased the bacterial infection in silkworm B. mori. Anitha 1994 [1], has reported that the bacteria as the etiological agent of flacherie in silkworms as early as 1870. The rise in temperature and humidity in rearing place leads to dysfunction of alimentary canal which encourages flacherie. Nataraju *et al.,* 2005 [6]. Rearing condition is followed by mulberry leaves of poor quality Manimegalai and Chandramohan (2005) [4].

The impact was high in silkworm larvae with B. thuriensis. Yungen and Bharathi (2001) [10] had reported that the bacteria B. thuriensis produce delta endotoxin which primarily acts on the gut epithelial surface membrane of the larvae and cause histo pathological changes the midgut B. thuriensis cause damage to the epithelial cells of midgut through crystalline parasporal body which could release the toxic parasporal crystals under the alkaline conditions in the intestinal juice. Yungen and Bharthi (2001) [10] also reported that certain bacteria like S. marcescens was found to infect the haemocoel causing the pathological changes in the blood and has less effect on the midgut of silkworm larvae.

The present study also reported differential sensitivity of bacteria for antibiotics as reported earlier (Savithri and Murali 2003) [8] Various workers have reported disease prevalence in sericultured countries and attempts have been made to save the cocoon crop by application of different antibiotics, disinfectant and chemical (Sam Devadass,1999; Nehar,1995) [5] The sensitivity of *B. coagulans* to six antibiotics was in the order Tetracyclin< amoxylin< ampicillin<chloramphenicol< cephelexin (Savithri and Murali 2003) [8] The use of antibiotics could reduce the pathogenicity of the bacteria in the silkworm there by protecting the cocoon crops against the bacterium.

Bacteria are known to play an important role in causing flacherie of silkworm as individual pathogen or in combination with viruses. There in movement in causing disease further leads to the spoilage of cocoon and silk quality and reduction in fecundity (Savithiri and murali, 2004) [8] in my investigation the sensitivity of bacterial isolates to three antibiotics was in order Streptomycin, Penicillin and Ampicillin respectively. The use of antibiotics could present or reduce the pathogenicity of bacteria in the silkworm thereby protecting the cocoon crops against the bacterium.

References

1. Anitha T, Sironmani P, Meena P, Vanitha R. Sericologia 1994; 34(1):97-102.
2. Chira C, Karanth NGK, Vasantha Rajan VN. Diseases of the mulberry silkworm, Bombyx mori LJ Sci Indust. Res 1975; 34:386-401.
3. Govindhan R, Narayanaswamy TK, Devaiah MC. Principles of silkworm pathology. Seri. Scientific publishers, Bangalore 1998, 420.
4. Manimegalai S, Chandramohan N. Sericologia 2005; 45(1): 51-58.
5. Nahar A. *In vitro* screening of some disinfectants and antibiotics against Serratia marcesans. Bill. Seric. Res 1995; 6:79-83.
6. Nataraju B, Sathyaprasad K, Manjunath D, Aswani Kumar C. Silkworm crop protection. Central silk board, 2005, 61-85.
7. Samdevadass C. Some studies on the pathology of Serratia marcesans in silkworm Bombyx mori. Dissertation ICTRTS, Mysore, India, 1991.
8. Savithri G, Murali. *In vitro* screening of antibiotics against Bacillus thurinensis. Indian J seric. 2003; 42(1):25-27.
9. Nanavathy MM. Silkworm from Grub to Glamour, paramount Publishing House, Bombay, 1965, 227.
10. Yungen M, Bharati D. Effect of methoprene, MH-III larval, adult moth characters, cocoon quality and silk proteins of silkworm, Bombyx mori L. feed on mulberry leaf and artificial diet. Philippine Journal of Science. 2001; 130:45-52.
11. Yokoyama T. Silkworm Genetics Illustrated: Japan society for Promotion of Science, Tokyo, 1971.