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Effect of some antibiotics on larval mortality of Oak Tasar Silkworm (*Antheraea proylei* Jolly) due to bacterial infection

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Three antibiotics viz., Streptomycin, Gentamicin and Tetracycline were employed for their efficacy in reducing larval mortality due to infection of four potential bacterial pathogens (*Bacillus thuringiensis*, *Escherichia coli*, *Proteus* sp. and *Streptococcus faecalis*) of Oak Tasar silkworm *Antheraea proylei*larvae. *In vitro* susceptibility, sensitivity and resistance tests were also conducted for the three antibiotics at four different concentrations against the four pathogenic bacteria. All the three antibiotics were found to have reduced the larval mortality due to bacterial infection. Minimum larval mortality following infection of *B. thuringiensis* was recorded with the treatment of Gentamicin and Tetracycline. Gentamicin was found to be most effective to all the four bacterial pathogens studied.

Key words: Oak Tasar silkworm, *Antheraea proylei*, antibiotics, larval mortality, bacterial disease

INTRODUCTION

Rearing of Oak Tasar silkworm is of a great potential in the State of Manipur as there are abundant natural resources and manpower. Despite having vast natural resources and manpower Oak Tasar sericulture is not encouraging due to diseases and predators. During the course of silkworm rearing, larvae came into contact with pathogens and under the influence of environment a complex process of injury and resistance took place reducing the quality as well as quantity of silk production. Important biological agents eliciting infectious silkworm diseases are viruses, bacteria, fungi and protozoa. Poor quality food leaves fail to provide sufficient quantity of essential nutrient requirement resulting to higher rate of multiplication of infectious bacteria and development of flacherie disease (Nataraju *et. al,* 2005). Bacterial diseases are one of the important factors for reducing the quality and quantity of silk production in silk industry.Bacterial diseases of silkworm are infectious, and therefore, the basic requirement for getting a stable crop is to control bacterial pathogens causing infectious diseases in and around therearing site.

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The important pathogenic bacteria of *Antheraea proylei* larvae are *Bacillus thuringiensis*, *Escherichia coli*, *Serratia* sp., *Micrococcus* sp., *Staphylococcus* sp., *Proteus* sp., *Pseudomonas* sp., *Pantoeaagglomerans* etc. (Chandra and Singh, 2004). Only comprehensive and integrated steps can result in prevention of silkworm diseases. Studies on screening of suitable disinfectants and antibiotics have been conducted by previous workers on other silk producing worms (Shan *et. al*. 2017). In the present study three antibiotics were tested *in vitro* for their sensitivity against four important bacterial pathogens of *Antheraea proylei* larvae and were further tested for their efficacy in controlling diseases.

MATERIALS AND METHODS

In vitro evaluation of antibiotics against pathogenic bacteria

Three antibiotics were tested in vitro for their sensitivity and resistance against four pathogenic bacteria viz., *Bacillus thuringiensis, Escherichia coli, Proteus* sp. and *Streptococcus faecalis* isolated from diseased *Antheraea proylei* larvae. Antibiotic sensitivity and resistance tests were conducted for

Streptomycin, Gentamicin and Tetracycline in four concentrations (0.025, 0.05, 0.1 and 0.15 % w/v) following Bauer - Kirby's Single Disc Method (Bauer *et. al,* 1966). Antibiotic sensitivity and resistance were obtained by interpreting the inhibition zone following Bauer - Kirby antibiotic sensitivity interpretation (Hudzicki, 2016).

Effect of antibiotics on larval mortality

The antibiotics screened *in vitro* against pathogenic bacteria of *Antheraea proylei* larvae were further studied for their efficacy in controlling larval mortality. Healthy *Antheraea proylei* larvae were shifted in well disinfected room for indoor rearing during their mid IV-instar larval stage and fed on oak (*Quercus serrata*Thunb.) leaves coated with *E. coli*.

Oak leaves were sprayed with suspensions of antibiotics in two concentrations (0.1 and 0.15% w/v) and dried for 30 minutes in the rearing room. The leaves were then fed to *Antheraea proylei* larvae once, a day after final moult or on the first appearance of disease symptoms whichever was earlier. The larvae were fed with untreated leaves during the remaining period of their life cycle. Controls for each treatment were also maintained separately by feeding bacteria treated leaves but avoiding treatment of antibiotics.

Three other bacterial types viz., *Bacillus thuringiensis*, *Proteus* sp. and *Streptococcus faecalis* were also tested as above. Batches of 100 larvae each were tested for each antibiotic treatment following infection of the four bacterial pathogens.

Observations, on larval mortality, were made daily throughout the rearing period. Larval mortality due to bacterial infection was confirmed on the basis of appearance of symptoms, examinations of smears of gut content and haemolymph and post mortem changes in the larval body. The percentage larval mortality incidence and mean percentage mortality reduction over control were calculated.

RESULTS AND DISCUSSION

Among the antibiotics tested *in vitro* against four pathogenic bacteria, Gentamicin (0.1% and 0.15% w/v) was found to be effective against all the four bacterial types studied whereas Gentamicin (0.025% w/v) was resistant and Gentamicin (0.05% w/v) showed intermediate effect (Table 1). Streptomycin (0.025% w/v) was found to be resistant to all the four bacteria tested whereas 0.05% w/v showed intermediate effect on *E. coli*, *Proteus* sp. and *Streptococcus faecalis* and resistant to *Bacillus thuringiensis*. Streptomycin (0.10 & 0.15 % w/v) was sensitive to all the bacteria studied except *Bacillus thuringiensis*, which showed intermediate effect. All the four bacterial types were found to be resistant to Tetracycline (0.025% w/v). Tetracycline (0.05% w/v) showed intermediate effect on *Bacillus thuringiensis* while *E. coli*, *Streptococcus faecalis* and *Proteus* sp. were resistant. *Bacillus thuringiensis* was sensitive to Tetracycline (0.10% w/v) but *Streptococcus faecalis* and *Proteus* showed intermediate effect and resistant to *E. coli*. Tetracycline (0.15% w/v) was found to be sensitive to all bacterial types studied except *E. coli* that showed intermediate effect.

The effect of three antibiotics on the larval mortality due to bacterial diseases. of *A. proylei* larvae are well known (Table 2). The lowest larval mortality of *A. proylei* larvae infected with *Bacillus thuringiensis* (20.30%) was observed with the treatments of Gentamicin and Tetracycline (0.15% w/v) against the control (48.30%). 20.70% mortality of larva was recorded following the treatment of Gentamicin at 0.10 w/v concentration. Highest larval mortality 28.30% was recorded with the treatment of Streptomycin (0.10% w/v).

In case of *E. coli* infection, lowest mortality (18.30%) of *A. proylei* larvae was observed in Gentamicin (0.15% w/v) treatments followed by (20.30%) with treatments of Streptomycin (0.15% w/v) against the larval mortality of control (36.3%). Highest larval mortality (27.30%) was recorded with the treatment of Tetracycline (0.10% w/v).In case of *Proteus* sp. infection the lowest mortality (20.3%) was observed in Gentamicin (0.15% w/v) treatment as compared to the mortality of the control (56%). Highest larval mortality (34.30%) even following antibiotic treatment was recorded for *Proteus* sp. infection following the treatment of Streptomycin (0.10%w/v).

Treatments of Gentamicin and Tetracycline at 0.15% w/v concentrations lowered the mortality of *A. proylei* larvae to 16% and 16.7% respectively due to infection of *Streptococcus faecalis* against the control (41.7%).The mean mortality of *A. proylei* larvae due to feeding of bacteria coated leaves was

Table 1: Sensitivity and resistance of bacteria against antibiotics

Antibiotics	Conc. %w/v	*B. thuringiensis*	*E. coli*	*Proteus* sp.	*S. faecalis*
Streptomycin	0.025	-	-	-	-
	0.05	-	±	±	±
	0.10	±	+	+	+
	0.15	+	+	+	+
Gentamicin	0.025	-	-	-	-
	0.05	±	±	±	±
	0.10	+	+	+	+
	0.15	+	+	+	+
Tetracycline	0.025	-	-	-	-
	0.05	±	-	-	±
	0.10	+	-	±	±
	0.15	+	±	+	+

\+ Sensitive ± Intermediate - Resistant

Table 2: Effect of antibiotics on % larval mortality following bacterial infections and mean mortality (%) over control

Antibiotics	Conc. (%) w/v	Mortality (%) due to bacterial infections					Mean mortality (%) reduction over control
		B. thuringiensis	*E. coli*	*Proteus* sp.	*S. faecalis*	Mean	
Streptomycin	0.10	28.30±1.20	22.30±1.20	34.30±3.48	24.00±1.15	27.25±1.64	40.22
	0.15	24.30±0.88	20.30±1.86	26.30±2.33	20.30±1.86	22.83±1.10	49.91
Gentamicin	0.10	22.70±0.88	24.70±1.20	28.00±2.31	20.30±0.33	23.92±1.03	47.53
	0.15	20.30±0.88	18.30±0.33	20.30±1.20	16.00±2.08	18.75±0.77	58.87
Tetracycline	0.10	24.30±1.45	27.30±1.45	29.70±1.20	19.70±1.20	25.25±1.26	44.61
	0.15	20.30±1.20	22.70±1.20	28.70±2.03	16.70±1.86	22.08±1.48	51.55
Control	-	48.30±0.88	36.30±0.88	56.00±3.21	41.70±3.71	45.58±2.47	-

45.58% during the whole period of the study. The least mean larval mortality (18.75%) was recorded with Gentamicin (0.15 w/v) treatment. The percentage reduction of mortality over the mean mortality of the control was highest with the treatment of Gentamicin, 0.15% w/v (58.87%) followed by Tetracycline, 0.15% w/v (51.55%), Streptomycin 0.15% w/v (49.91%) and Gentamicin, 0.10% w/v

(47.53%).Data indicated that all the treatments tested in the present study reduced the larval mortality due to bacterial infections.

CONCLUSION

Antibiotics used in clinical treatments were found to be effective in reducing larval mortality of *Antheraea proylei* larvae. Among all the three antibiotics studied Gentamicin was found to be most effective to all four bacterial pathogens used. Antibiotics can also be used successfully as a component in the control and management of bacterial diseases of Oak Tasar silkworm.

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