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Research Article

INFLUENCE OF HORIZONTAL TRANSMISSION OF BACTERIAL FLACHERIE ON COCOON PARAMETERS IN SILKWORM *BOMBYX MORI* L.

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Abstract: Flacherie is a major disease of silkworm and cause flaccidity in larva. Adverse environmental conditions such as high temperature and humidity and starvation are considered to be the most important pre-disposing factors for the incidence of flacherie and cocoon crop loss (13). The horizontal transmission of bacterial flacherie affect the growth and cocoon parameters of PM x CSR_2. The single cocoon weight (g) was significantly lesser in the contaminated faecal pellet source of inoculum (0.851, 0.992 and 0.74, 0.79 g) and highest in contaminated floor area (0.951, 1.00 and 0.83, 0.84 g) at 10^{-5} and 10^{-7} respectively treated with different sources of bacterial flacherie for fourth and fifth instar worms. The lowest single shell weight was encountered in both fourth and fifth instar inoculated batch with contaminated faecal pellet (0.120, 0.134 and 0.105, 0.121 g) at 10^{-5} and 10^{-7}, respectively. Infection of silkworm with different sources of horizontal transmission of bacterial flacherie in both fourth and fifth instars inoculated at 10-5 and 10^{-7} is known to decrease the shell percentage of mulberry silkworm.

Keywords: *Bacterial flacherie, Cocoon parameters, Horizontal transmission, PM x CSR_2*

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Introduction

Flacherie is a major disease of silkworm and cause flaccidity in larva. The larvae become feeble and lethargic and possess transparent cephalothoracic region. They vomit gut juice and extrude soft faeces with higher water content. The disease affects the later instar silkworms and the loss due to disease was significant [1,2]. Bacterial flacherie is probably the most serious disease of silkworm causing cocoon crop loss to the tune of 40 percent [3]. The average flacherie disease incidences in Chikkaballapur area during summer season were found to be 5.00 to 15.00 percent, during rainy season 5.00 to 10.00 percent and during winter months 5.00 to 10.00 percent. The highest incidence was observed in summer season followed by rainy and winter seasons [4]. The silkworms infected with BmIFV survived upto spinning stage and spun flimsy and small cocoons. The weight of single cocoon was significantly reduced [5]. It is also confirmed that, the single cocoon weight reduced with decrease in dilution of *Kenchu virus* + *Staphylococcus aureus* (0.757g at 10-4, 0.611 to 0.684g at 10^{-3}) and the single shell weight reduced with decrease in dilution of *Kenchu virus* + Staphylococcus aureus (0.081 to 0.101g at 10-4, 0.080 to 0.095g at 10^{-3}). Further, the shell percentage was decreased with the increase in the percentage of release of *Kenchu virus* (4 %= 13.29 %; 20 %=12.74 % and 24 %=0.00 %) [6].

Material and Methods

Collection of samples

A survey was undertaken during the month of August, 2015 in Mallur village of Sidlaghatta taluk, Chikkaballapur district. The sources of inoculum were randomly selected from five sericulture farmers' in their commercial silkworm rearing houses as per the survey conducted on bacterial septecimia by Ashok Kumar and Ramakrishna [7]. They isolated and characterized the septicaemia causing bacterial species in silkworm rearing environment (soil, phylloplane, diseased silkworm and rearing house-silkworm rearing trays and culture from walls). The same procedure was adopted in collection of samples which are served as effective tool of transmission (contaminated food, bed, rearing equipment, body surface, faecal pellet and floor area) and used for inoculation for fourth and fifth instar silkworm larvae. All the sources of inoculum were collected with 9:1 proportion later subjected for 3000 rpm for 10 min followed by 5000 rpm for 5 min. The filtrate slowly decanted to conical flask. All glasswares were sterilized in a hot air oven at 180°C for three hours. Isolation, purification, inoculation and other microbiological works were carried out in laminar airflow chamber [8].

Inoculation of silkworms

Inoculation of silkworms was done on the fourth instar first day, fifth instar first day *i.e*., immediately after third and fourth moult, respectively. The spore dilution of 10^{-5} and 10^{-7} of different sources of inoculum were swabbed on mulberry leaf (10 x 15 sq. cm area) using sterilized cotton swab, air dried, made into small pieces and fed to the silkworms at the rate of 0.5ml per 50 worms. The results obtained in the present study are statistically analysed through complete randomized design and conclusions were drawn based on the observations recorded [9].

Single cocoon weight and shell weight (g)

After five days of spinning, ten cocoons were harvested from each replication treatment wise and mean cocoon weight and shell weight were recorded using electronic balance.

Shell ratio (%)

The shell ratio was calculated by using the formula.

Shell ratio (%) = [Weight of cocoon shell (g) / Weight of whole cocoon (g)] X 100

Table-1 *Influence of horizontal transmission of bacterial flacherie on single cocoon weight, shell weight and shell % of PM x CSR2*

Treatments	Single cocoon weight (g)		Single shell weight (g)		Shell %	
	10^{-5}	10^{-7}	10^{-5}	10^{-7}	10^{-5}	10^{-7}
T_1- Contaminated food	0.885	0.952	0.138	0.154	15.63	15.79
T_2- Contaminated bed	0.860	0.934	0.135	0.146	15.74	15.67
T_3- Contaminated rearing equipment	0.900	0.986	0.151	0.173	16.83	17.51
T_4- Contaminated body surface	0.894	0.974	0.142	0.160	15.90	16.42
T_5- Contaminated faecal pellet	0.851	0.922	0.120	0.134	14.13	14.49
T_6- Contaminated floor area	0.951	1.00	0.161	0.175	16.93	17.57
T7- Distilled water	1.32		0.243		18.47	
T8- Uninoculated	1.50		0.290		19.29	
'F' test	*	*	*	*	*	*
SEm ±	0.010	0.010	0.002	0.002	0.173	0.219
CD at 5 %	0.030	0.030	0.005	0.006	0.520	0.657

Table-2 *Deterioration of single cocoon weight, shell weight and shell % as influenced by different sources horizontal transmission of bacterial flacherie (5th instar inoculated batch, PM x CSR2) [* Significant]*

Treatments	Single cocoon weight (g)		Single shell weight (g)		Shell %	
	10^{-5}	10^{-7}	10^{-5}	10^{-7}	10^{-5}	10^{-7}
T_1- Contaminated food	0.76	0.83	0.113	0.131	14.86	15.89
T_2- Contaminated bed	0.75	0.8	0.112	0.124	14.91	15.4
T_3- Contaminated rearing equipment	0.78	0.84	0.131	0.14	16.77	16.2
T_4- Contaminated body surface	0.77	0.84	0.12	0.136	15.54	16.25
T_5- Contaminated faecal pellet	0.74	0.79	0.105	0.121	14.11	15.27
T_6- Contaminated floor area	0.83	0.84	0.135	0.15	16.18	17.21
T7- Distilled water	1.27		0.230		17.98	
T8- Uninoculated	1.36		0.253		18.63	
'F' test	*	*	*	*	*	*
SEm ±	0.005	0.005	0.001	0.002	0.132	0.129
CD at 5 %	0.015	0.016	0.004	0.005	0.396	0.387

Results and Discussion

The single cocoon weight (g) was significantly lesser in the contaminated faecal pellet source of inoculum (0.851, 0.992 and 0.74, 0.79 g) and highest in contaminated floor area (0.951, 1.00 and 0.83, 0.84 g) at 10^{-5} and 10^{-7} respectively treated with different sources of bacterial flacherie for fourth and fifth instar worms. Cocoon weight is influenced by the mature larval weight and since the infected resulted in reduced larval weight, the cocoon weight is also less. The earlier the infection in fifth instar, the greater was the reduction in economic traits [Table-1 & 2].

The lowest single shell weight was encountered in both fourth and fifth instar inoculated batch with contaminated faecal pellet (0.120, 0.134 and 0.105, 0.121 g) at 10^{-5} and 10^{-7}, respectively. The highest was recorded with contaminated floor area (0.161, 0.175 g and 0.135, 0.150 g). Lowest shell weight was recorded in the inoculation with contaminated faecal pellet, may be the chronic infection might have deteriorated the quantitative traits including shell weight [Table-1 & 2].

Infection of silkworm with different sources of horizontal transmission of bacterial flacherie in both fourth and fifth instars inoculated at 10^{-5} and 10^{-7} is known to decrease the shell percentage of mulberry silkworm. The cocoon shell percentage was lowest in inoculation with contaminated faecal pellet (14.13, 14.49 and 14.11, 15.27 %) and highest in contaminated floor area respectively. This is due to less shell weight and more pupal weight [Table-1 & 2].

These experiment results are in confirmative with findings of Chitra *et al*. [10] according to them different bacterial isolates *viz*., *Aerobacter cloacae*, *Achromobacter superficialis, Achromobacter delmarvae, Staphylococcus albus, Escherichia freundii* and *Pseudomonas ovalis* varied in their pathogenicity to silkworms and stage of growth of larvae affected their virulence. Invasion of pathogens into larval body caused considerable reduction in cocoon weight from 57.1 to 92.00 percent in V instar of silkworm *Bombyx mori* L. and deleterious effect increased with age of infection. The older larvae at the time of infection the lower was their net gain in weight. The same line of observation was reported in the present study.

The above results are in accordance with the observations of Sanakal *et al*. who reported that the worms infected with BmIFV survived upto spinning stage and spun flimsy and small cocoons. The weight of single cocoon was significantly reduced. It is also confirmed that, the single cocoon weight reduced with decrease in dilution of *Kenchu virus* + *Staphylococcus aureus* (0.757g at 10^{-4}, 0.611 to 0.684g at 10^{-3}) and the single shell weight reduced with decrease in dilution of *Kenchu virus* + *Staphylococcus aureus* (0.081 to 0.101g at 10^{-4}, 0.080 to 0.095g at 10^{-3}). Further, the shell percentage was decreased with the increase in the percentage of release of *Kenchu virus* (4 %= 13.29 %; 20 %=12.74 % and 24 %=0.00 %) [11]. The more pupal weight and less shell weight might be the reason for less shell ratio in combined infection with *Bacillus sp.* + *Streptococcus faecalis* (9.56 to 10.80%). The shell weight was significantly higher in uninoculated control (0.260 g) compared to shell weight 0.139, 0.152, 0.184, 0.249, 0.256 and 0.185 g at 10^{-2}, 10^{-4}, 10^{-8}, 10^{-16} and 10^{-32} and average of six dilutions, respectively in case of infection with *Staphylococcus aureus* which was found on par with that of *Streptococcus faecalis*. With reduced rate of assimilation in infection, the physiology of silk synthesis might have been affected drastically resulting in less silk protein synthesis that resulted in less cocoon shell weight [12].

The fourth instar silkworms fed with mixture of IFV + *Streptococcus faecalis* and also in simultaneous per oral infection of fourth instar silkworms with *Kenchu virus* + *Staphylococcus aureus* resulted in deterioration of cocoon quantitative traits [13]. The infection with BmNPV from first to third day of fifth instar resulted in greater reduction in cocoon weight [14] and Ravikumar [15] observed the similar reduction in the cocoon weight of CSR4 (40.93 %) and in KSO^{-1} (16.25 %) [16].

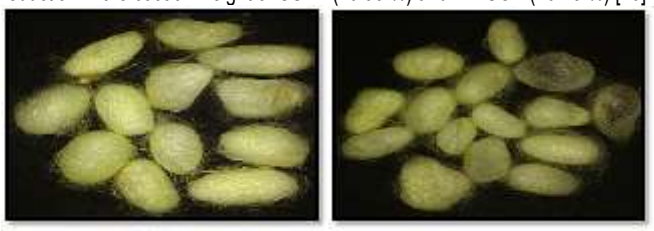

Fig-1 Defective cocoons

Conclusion

The cocoon parameters *viz*., single cocoon weight, single shell weight and shell percentage have registered significant results among all the treatments (contaminated food, bed, rearing equipment, body surface, faecal pellet and floor area) inoculated with bacterial flacherie inoculum. However, contaminated faecal pellet inoculum administered batch deteriorated all the cocoon parameters at 10^{-5} and 10^{-7} dilutions by showing higher infection compared to other sources of horizontal transmission of bacterial flacherie and deteorated the cocoon parameters.

Application of Research: It facilitates the information about flacherie which affects the cocoon parameters and taking precautionary measures in managing the disease at silkworm rearing site to get good cocoon yield

Research Category: Agricultural Entomology

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Research project name or number: Effect of different sources of Horizontal Transmission of Bacterial Flacherie on Rearing and Cocoon Parameters of Silkworm (*Bombyx mori* L.)

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area: Department of Sericulture, University of Agriculture Sciences, GKVK, Bengaluru, 560065

Sample Collection: Mallur village, Shidlghatta Taluk, Chikkabballapur District.

Cultivar / Variety name: Mulberry – *Morus indica* L. Victory – 1(V-1)
Silkworm – *Bombyx mori* L. PM x CSR_2

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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