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EFFECT OF DIFFERENT SOURCES OF HORIZONTAL TRANSMISSION OF BACTERIAL FLACHERIE ON ET_{50} FOR SYMPTOM EXPRESSION AND MORTALITY OF PM X CSR2

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ABSTRACT
Flacherie disease of silkworm *Bombyx mori* L. is also called as Thatte disease (Thatte roga), Benkiroga (Dhadevu roga) and it was first observed from a village in Malavalli taluk of Mandya district during summer months of 1990 (Doreswamy *et al*., 2001) cause flaccidity in larva, during which they become feeble, lethargic, vomit gut juice and extrude soft faeces with higher water content. Administration of 10^{-5} and 10^{-7} dilutions of horizontal sources of inoculum to fourth and fifth instar larvae exhibited significant results. It is very clearly indicated that, decreased bacterial dilution exhibited minimum ET_{50} value for symptom expression observed for contaminated faecal pellet (9.01. 9.18; 7.01, 7.35 days) for fourth and fifth instar at 10^{-5} and 10^{-7}, respectively followed by contaminated bed (9.09, 9.38; 7.18, 7.30 days) and (10.17, 10.25; 8.12, 8.30 days). The trend was found same in ET_{50} for larval mortality (9.97, 10.12; 8.01, 8.13 days). It was also concluded that, the horizontal transmission was found minimum in contaminated rearing equipment (9.42, 9.72; 11.03, 11.65 days) and (7.51, 7.67; 9.07 9.72 days) used for rearing compared to other sources of horizontal transmission. Which inturn recorded maximum number of days to express the disease in both the instars of PM x CSR2.

INTRODUCTION

Flacherie disease of silkworm is caused by different species of bacteria and viruses, individually or in combination. It is generally spread in rearing house by different means. Based on this hypothesis an effort has been made to know the different horizontal transmission which were found very common in the farmers rearing site. Selvakumar (2013) revealed that, the prevalence of both bacterial and viral flacherie were more during summer (5.0-20.00 %) followed by rainy (2.5- 15.00 %) and winter (0.00-7.50 %) seasons. Among several infectious diseases of silkworm, flacherie is known to cause huge cocoon crop loss of about 27 – 35 per cent with decrease in yield of cocoons to an extent of 11- 15 kg per 100DFL's (Selvakumar and Savithri, 2012).

Adverse environmental conditions such as high temperature and humidity and starvation, spacing in the rearing bed (Samson *et al*., 1981) are considered to be the most important pre-disposing factors for the spread of the disease within the bed and also in the rearing house. Further (Swathi, 2015) has studied the effect of different types of rearing structures on incidence of flacherie disease in silkworm. According to her, the highest incidence of flacherie disease was noticed in Ramanagara district during summer season in RCC house was found to be 20. 00 per cent followed by winter 12.00 and rainy 10.00 per cent. The same trend was observed in case of Chikkaballapur district. The highest incidence of flacherie disease was noticed due to unhygienic conditions followed in and around the rearing house.

Further (Samson, 1995) made observation on age of the silkworm, overcrowding, scanty feed, contaminated leaves, cross infectivity, etc are considered to be pre-disposing factors for the spread of the flacherie in the rearing bed. These factors not only weaken the worms and prone to many microbial infections. Particularly, bacterial flacherie coupled with viral infections are very frequent in silkworm rearing houses. These pre-disposing factors will influence on the multiplication and development of diseases during silkworm rearing (Balavenkatasubbaiah *et al*., 2006). Based on this objective different sources of horizontal transmission were administered to fourth and fifth instar larvae of PM x CSR2 to know the effective means of disease transmission in the rearing bed.

MATERIALS AND METHODS

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 (2013). 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 18º C for three hours. All growth medium and broth were sterilized in an autoclave at 15 lbs pressure for 20 min. Isolation, purification, inoculation and other microbiological works were carried out in laminar airflow chamber (Robert Pollock *et al*., 2002).

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 (Anusha, 2015). Usually for all per oral infection Koch postulatue technique was adopted. The same methodology was used in inoculating fourth and fifth instar larvae. Therefore, the common mode of horizontal transmission was assessed based on sources of inoculum (contaminated food, bed, rearing equipment, body surface, faecal pellet and floor area) (Pasteur, 1870).

The results obtained in the present study are statistically analysed through complete randomized design and conclusions were drawn based on the observations recorded (Sundar Raj *et al*., 1979).

RESULTS AND DISCUSSION

After administration of different sources of inoculum, the infected larvae (4th and 5th instar) exhibited following symptoms *viz*., the larvae become weak, vomiting yellow body fluid followed by the anterior portion of the infected larval midgut bulged in between thorax and abdomen made the larval skin tapery, the flow of blood in the body of the silkworm was more compared to uninoculated batches and started wriggling in the bed in the form of 'C' shaped larvae. While wriggling the entire larval midgut contents were pushed back as a result worms were unable to pass excreta due to sealing of anal plap. Before death, the corpse body become hard and after the death the larval body start become blackening posterior to anterior. After 24 hours it has become fragile, hang down oozing of brown colour fluid from the body and emits foul smell (Plate 1).

Paramasiva and Rajendra Prasad (2009) reported that, insects infected with pathogenic bacteria exhibit symptoms such as loss of appetite, diarrhoea, vomiting, larvae softening and foul odour upon death. Cocoons spun by the infected worms did not exhibit any external symptoms. The size of the cocoon was drastically reduced, compared to normal cocoons. When cocoons were cut open, the pupae were found dead or malformed. The infected cocoons were lighter in weight and moth emergence could not be seen. Infected moths became sluggish with slightly crinkled wings and showed less interest in copulation. The same type of symptoms were observed in both fourth and fifth instar larvae of PM x CSR2 in the present investigation.

The fourth instar inoculated batch of PM x CSR2 with different sources of horizontal transmission caused variation in ET_{50} for symptom expression and larval mortality. As per the data, the lesser number of days of 9.01, 9.18 days and 9.97, 10.12 days (contaminated faecal pellet) was noticed for 10-5 and 10-7 inoculum administered batches, respectively. However, the increased trend of symptom expression and larval mortality was noticed from contaminated bed (9.09, 9.38 and 10.17, 10.25 days) followed by contaminated food (9.17, 9.59 and 10.31, 10.40 days), contaminated body surface (9.30, 9.68 and 10.97, 11.54 days), contaminated rearing equipment (9.42, 9.72 and 11.03, 11.65 days) and contaminated floor area (9.51, 9.84 and 11.25, 11.84 days) in both the inoculated batches (Table 1).

The fifth instar inoculated batch of PM x CSR2 with different sources of inoculum caused variation in ET_{50} for symptom expression and larval mortality. As per the data, the minimum days of 7.01, 7.35 days and 8.01, 8.13 days (contaminated faecal pellet) was noticed for 10-5 and 10-7 inoculum administered batches, respectively. The remaining treatments are in increasing order followed by contaminated bed (7.18, 7.30; 8.12, 8.30days), contaminated food(7.30, 7.42; 8.25,

Table 1: Effect of different sources of horizontal transmission of bacterial flacherie on rearing parameters of silkworm, *Bombyx mori* L. (4th instar inoculated batch, PM x CSR2)

Treatments	ET_{50} for symptom expression(days)		ET_{50} for larval mortality (days)	
	10^{-5}	10^{-7}	10^{-5}	10^{-7}
T_1- Contaminated food	9.17	9.59	10.31	10.4
T_2- Contaminated bed	9.09	9.38	10.17	10.25
T_3- Contaminated rearing equipment	9.42	9.72	11.03	11.65
T_4- Contaminated body surface	9.3	9.68	10.97	11.54
T_5- Contaminated faecal pellet	9.01	9.18	9.97	10.12
T_6- Contaminated floor area	9.51	9.84	11.25	11.84
T_7- Distilled water	-	-		
T_8- Uninoculated	-	-		
'F' test	*	*	*	*
SEm ±	0.004	0.005	0.01	0.009
CD at 5 %	0.013	0.014	0.031	0.026

* Significant

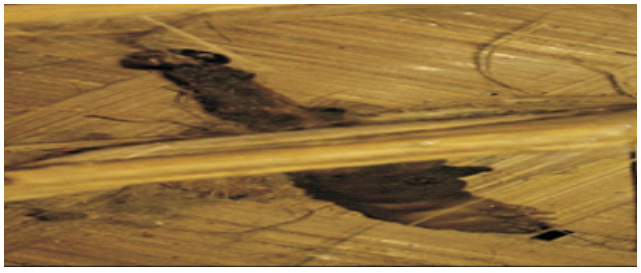
Table 2 : Effect of different sources of horizontal transmission of bacterial flacherie on ET_{50} for symptom expression and ET_{50} for larval mortality of silkworm *Bombyx mori L.* (5th instar inoculated batch)

Treatments	ET_{50} for symptom expression (days)		ET_{50} for larval mortality (days)	
	10^{-5}	10^{-7}	10^{-5}	10^{-7}
T_1- Contaminated food	7.3	7.42	8.25	8.43
T_2- Contaminated bed	7.18	7.3	8.12	8.3
T_3- Contaminated rearing equipment	7.51	7.67	9.07	9.72
T_4- Contaminated body surface	7.43	7.59	8.39	8.7
T_5- Contaminated faecal pellet	7.01	7.35	8.01	8.13
T_6- Contaminated floor area	7.63	7.76	9.39	9.96
T_7- Distilled water	-		-	
T_8- Uninoculated	-		-	
'F' test	*	*	*	*
SEm ±	0.004	0.097	0.008	0.005
CD at 5 %	0.013	0.29	0.025	0.015

* Significant

Larvae showing symptoms of bacterial flacherie

After death larvae turns into black colour



Hanging dead larvae

Plate1: Symptoms of bacterial flacherie

8.43 days). The remaining sources of inoculum contaminated body surface (7.43, 7.59; 8.39, 8.70days) and contaminated floor area(7.63, 7.76; 9.39, 9.96 days) of ET_{50} for symptom expression and larval mortality recorded in fifty instar larval batch (Table 2).

This experimental data supported by Anusha and Bhaskar (2016) reported that, when third and fourth instar larvae inoculated with *Bacillus* sp. as surface inoculation recorded decreased larval weight from 1.95 to 13.40 g/10 in PM and 6.38 to 22 g/10 in CSR2 at end of both the instars registering decrease in their development during bacillus infection as observed in the present experiment.

The same observations were confirmed with Siromani *et al*. (1994), bacterial flacherie had comaparitively a long period of lethal infection of about 7 to 14 days. Death occurred within 10 to 24 hour in case of bactrerial flacherie. Further, Anitha *et al*. (1994) further reported that, when the silkworm larvae were fed with 106 cells of *Bacillus*, Staphylococcus and Serratia, no mortality was observed till 48 h. when 107 and 108 cells were fed, mortality was observed within 48 h, which was 100 per cent in Bacillus and Staphylococcus infections and 80 per cent in Serratia infection. The LD50 was 5.9 x 107 for Bacillus and Staphylococcus infections and 2.49 x 107 in Serratia infection as revealed in ET_{50} values of fourth and fifth instar larvae of PM x CSR2 in the present study.

ET_{50} for symptom expression value reduced significantly in *Streptococcus faecalis* (166.33h), *Staphylococcus aureus* (171.25h) in the mean of six dilutions (stock, 10^{-2}, 10^{-4}, 10^{-8}, 10^{-16} and 10^{-32}). The values increased with increase in bacterial dilutions. The ET_{50} value for larval mortality was observed in *Bacillus* sp. (186.00h) and *Staphylococcus aureus* for stock, 10^{-2}, 10^{-4}, 10^{-8} and mean of dilutions. Death occurred within 10 to 24 h during bacterial flacherie (Doreswamy *et al*., 2001).

It was further supported by Chitra *et al*. (1973) the infection

with several bacteria caused severe mortality and majority of the deaths occurred during moult and when the worms were about to spin. Mortality was maximum in fifth instar irrespective of the age of the silkworm. The per cent mortality due to different bacterial species was found to be 37.50 (*Achromobacter superficialis*) to 65.00 (*Achromobacter delmarvae*, *Pseudomonas ovalis*, *P. boreopolis*) per cent on eighth day of fifth instar. As it is revealed in the present experiment.

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