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Research Article

EFFECT OF DIFFERENT SOURCES OF HORIZONTAL TRANSMISSION OF BACTERIAL FLACHERIE ON DEFECTIVE COCOON AND PUPAL MORTALITY (%) OF PM X CSR_2

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Abstract: Flacherie disease of silkworm occurs in silkworm rearing and causes severe cocoon crop loss during summer and rainy seasons in all sericulture belts of Karnataka. The horizontal transmission of bacterial flacherie affect the growth and cocoon parameters of PM x CSR_2. However, the different sources of inoculum administered to both fourth and fifth instar inoculated batches exhibited significant difference among treatments compared to control. The highest percent defective cocoon was recorded in batch inoculated with contaminated faecal pellet (36.11, 32.59 and 52.85, 28.17 %). It is vivid from the data that, the horizontal transmission of bacterial flacherie was increased the defective cocoon percentage after spinning. In general, the bacteria multiplied in a faster rate after the death of the larva. The pupal mortality was found significantly different in different sources of inoculum among fourth and fifth instar batches and also at two dilutions (10^{-5} and 10^{-7}), respectively. Further, highest rate of pupal mortality was observed in contaminated faecal pellet (61.34, 54.88 and 59.29, 43.55 %) source of inoculum. It is attributed that, increased larval mortality and percent defective cocoons in the inoculated batch have resulted in increased pupal mortality in both the lots. As a secondary source of infection, bacteria within the cocoon cause pupal death before it is transforming to adult.

Keywords: *Bacterial flacherie, Defective cocoon percentage and pupal mortality, Horizontal transmission, PM x CSR_2*

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Introduction

Silkworm being a poikilothermic, it is sensitive to varied climatic conditions. Silkworms are affected by bacterial, viral, muscardine and protozoan pathogens and sometime combination of different pathogens. Flacherie disease of silkworm Bombyx mori L. is also called as "thatte disease" is caused by different species of bacteria and viruses, individually or in combination [1]. Bacterial flacherie has become the most serious disease of silkworm causing cocoon crop loss to the tune of 40 percent. 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 [2]. The symptoms of Thatte disease have been reported according to them, the disease appeared suddenly on fourth and fifth day of final instar. Thatte diseased worms look normal without showing unequal or any other morphological symptoms and they started dying. To begin with a patch of 10 to 20 worms died in a tray and later the worms of the entire tray succumbed to the disease within a day or two. The dead worms showed symptoms like flaccid body, blackening of the skin, vomiting and diarrhoea prior to death [3]. The 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 cocoons spun by infected larvae were lighter in weight, the pupa inside decompose, stain due to internal contents which is very common in bacterial infection (melting) before transform into adult and moth emergence could not be seen. If emerged, infected moths became sluggish with slightly crinkled wings and showed less interest in copulation [4].

Materials 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 commercial silkworm rearing houses.

Purification of inoculum

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.

Isolation of pathogenic bacteria

The pathogenic bacteria were isolated from contaminated food, bed, rearing equipment, body surface, faecal pellet and floor area. The bacteria present in different sources of inoculum were cultured on selective media for bacteria. A loopful of suspension was taken in inoculation needle and streaked on the Nutrient agar medium. These media are selected based on the nutritional requirement of microorganism and incubated at room temperature 24°C-26°C [5]. Later disease-causing bacterial colonies were isolated individually from large number of different bacteria were sub-cultured and isolated separately.

Purification of bacterial culture

To purify the organism, colony with distinct morphological characters was once again streaked on specific media by using streak plate method.

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.

Table-1 *Influence of horizontal transmission of bacterial flacherie on defective cocoon percentage of silkworm Bombyx mori L. (4th and 5th instar inoculated batch)*

Treatments	Defective cocoon (%)			
	4th instar inoculated batch		5th instar inoculated batch	
	10^{-5}	10^{-7}	10^{-5}	10^{-7}
T_1- Contaminated food	27.64(31.72)	25.31(30.20)	41.24(39.96)	16.14(23.68)
T_2- Contaminated bed	31.75(34.30)	29.37(32.80)	50.50(45.29)	24.00(29.33)
T_3- Contaminated rearing equipment	15.27(23.00)	11.74(20.04)	21.40(27.55)	10.53(18.92)
T_4- Contaminated body surface	23.50(29.00)	20.95(27.24)	26.92(31.25)	13.88(21.87)
T_5- Contaminated faecal pellet	36.11(36.94)	32.59(34.81)	52.85(46.64)	28.17(32.05)
T_6- Contaminated floor area	10.55(18.95)	7.02(15.36)	14.05(21.99)	9.52(17.96)
T_7- Distilled water	5.50 (13.55)		8.36(16.72)	
T_8- Uninoculated	3.47(8.75)		4.96(12.79)	
'F' test	*	*	*	*
SEm ±	0.779	0.899	1.033	0.715
CD at 5 %	2.335	2.696	3.097	2.143

Table-2 *Influence of different sources of horizontal transmission of bacterial flacherie on pupal mortality of PM x CSR_2 (4th and 5th instar inoculated batch)*

Treatments	Pupal mortality %			
	4th instar inoculated batch		5th instar inoculated batch	
	10^{-5}	10^{-7}	10^{-5}	10^{-7}
T_1- Contaminated food	57.07(49.06)	52.02(46.16)	53.48(47.00)	36.78(37.33)
T_2- Contaminated bed	58.06(49.64)	53.80(47.18)	56.88(48.95)	41.70(40.22)
T_3- Contaminated rearing equipment	51.09(45.62)	47.79(43.73)	51.02(45.58)	27.50(31.63)
T_4- Contaminated body surface	55.05(47.90)	51.45(45.83)	52.23(46.28)	35.86(36.78)
T_5- Contaminated faecal pellet	61.34(51.55)	54.88(47.80)	59.29(50.35)	43.55(41.29)
T_6- Contaminated floor area	48.04(43.87)	46.33(42.90)	50.11(45.06)	26.48(30.97)
T_7- Distilled water	0.00(0.00)		0.00(0.00)	
T_8- Uninoculated	0.00(0.00)		0.00(0.00)	
'F' test	*	*	*	*
SEm ±	0.063	0.125	0.095	0.164
CD at 5 %	0.19	0.373	0.284	0.493

*() Values in Parenthesis are Arcsine transformed values, * Significant*

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 [6].

The percent defective cocoons formed due to disease was calculated as follows.

Percent defective cocoon = [No. of defective cocoon formed / Total no. of cocoons spun] X 100

The pupal mortality recorded based on the number of cocoons formed from inoculated lots and pupae died inside the cocoon due to disease was calculated.

Percent pupal mortality = [No. of pupae died / No. of pupae formed] x 100

Results and Discussion

The inoculated batches exhibited different types of defective cocoons administered due to different sources of horizontal transmission. The infected individuals (4th and 5th instar) constructed malformed cocoons, flimsy cocoons, melted cocoons and interior stained cocoons due to degeneration of pupal content. These cocoons are unable to harvest from the mountage.

The infected cocoons (4th instar) exerted significant influence on defective cocoon percentage by different types of horizontal transmission of bacterial flacherie in two dilutions (10-5 and 10-7). Among different sources, the highest percent defective cocoon was recorded in batch inoculated with contaminated faecal pellet (36.11 and 32.59 %) and lowest with contaminated floor area (10.55 and 7.02 %) when administered with 10-5 and 10-7, respectively. The same trend was seen in other sources viz., contaminated bed (31.75 and 29.37 %), contaminated food (27.64 and 25.31 %) contaminated body surface (23.50 and 20.95 %) and contaminated rearing equipment (15.27 and 11.74 %). However, significantly lowest percent defective cocoon was encountered in distilled water (5.50 %) and uninoculated control (3.47 %). Significant influence was exerted on percent defective cocoon (5th instar) by different sources of inoculum of bacterial flacherie in two dilutions. Among different sources, the highest percent defective cocoon was recorded in batch inoculated with contaminated faecal pellet (52.85 and 28.17 %) and lowest with contaminated floor area (14.05 and 9.52 %) when administered with 10-5 and 10-7, respectively [Table-1].

It is vivid from the data that, the horizontal transmission of bacterial flacherie was increased the defective cocoon percentage after spinning. In general the bacteria multiplied in a faster rate after the death of the larva. It is in accordance with who reported that, worms infected with BmIFV survived up to spinning and spun flimsy, small and malformed cocoons [7]. The earlier the infection in fifth instar, the greater was the reduction in economic traits.

In the infected lots (4th and 5th instar) pupal mortality was observed in all the sources of inoculum administered. The infected silkworms did not spun any cocoons. But, transformed into pupae showing bulged body, blackening of pupal segments from posterior to anterior [Fig-1 & 2]. These characteristics are significantly influenced by different sources of horizontal transmission of bacterial flacherie wherein highest pupal mortality of 61.34 and 54.88 percent was noticed in contaminated faecal pellet inoculum administered followed by contaminated bed (58.06 and 53.80 %), contaminated food (57.07 and 52.02 %), contaminated body surface (55.05 and 51.45 %) and contaminated rearing equipment (51.09 and 47.79 %) and lowest was observed in contaminated floor area (48.04 and 46.33 %) among the sources. The different horizontal sources of inoculum administered to fifth instar larvae caused variation in pupal mortality even though the inoculum was administered in the beginning of the fifth instar which was significantly influenced and highest pupal mortality of 59.29 and 43.55 percent was noticed in contaminated faecal pellet [Table-2].

Fig-1 Infected individuals not spinned cocoons but transformed into pupae showing bulged body, blackening of pupal segments

This might be due to increased larval mortality and percent defective cocoons has resulted in increased pupal mortality in both the lots. As a secondary source of infection these bacteria within the cocoon cause pupal death before it is transforming to adult.

It is further confirmed that, the bacteria separated from the body surface adhered to the inside of cocoon shells and the bacteria on exuvia serve as infection source [8]. These bacteria multiply easily under humid conditions as revealed in the present study the contaminated faecal pellet has more moisture percentage than contaminated food, bed, rearing equipment and least disease transmission was observe in contaminated floor area.

Fig-2 Defective cocoons

Conclusion

The defective cocoons and pupal mortality (%) of both the instars inoculated with bacterial flacherie inoculum recorded significant results. However, maximum of 36.11, 32.59; 52.85, 28.17 percent defective cocoons and 61.54, 54.88; 59.29, 43.55 percent pupal mortality were recorded for contaminated faecal pellet inoculum at both the dilutions 10-5 and 10-7, respectively. The increased bacterial dilution decreases the defective cocoon and pupal mortality (%) significantly.

Application of Research: It shows the different sources of horizontal transmission of bacterial flacherie and its effect on cocoon and pupal parameters.

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 CSR2

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

References

[1] Chakrabarty S., Saha A. K., Manna B. And Bindroo B. B. (2012) *Applied Biol. Res*., 14, 1-14.
[2] Swathi H. C. (2015) *Ph.D. (Seri.) Thesis, University of Agricultural Sciences, Bengaluru, 560 065, India*. pp. 61-69.
[3] Prasad N. R., Keshawa Reddy K. S. And Govindaraju S. T. (1999) *Indian Silk*, 38(4), 13-14.
[4] Paramasiva I. And Rajendra Prasad P. (2009) *Indian J. Seric*., 48(1), 81-84.
[5] Govindan R., Narayanaswamy T. K. And Devaiah M. C. (1998) *Principles of Silkworm Pathology. Seri. Scientific Publishers, Bangalore*, p. 420.
[6] Sundarraj N., Nagaraju S., Venkataramu M. N. And Jagannath M. K. (1972) *Design and Analysis of field experiments. Directorate of research, UAS, Bangalore*, p. 419.
[7] Sanakal R. D., Ingalhalli S. S., Singh K. K., Basavarajappa S. Hinchigeri S. B. And Savanurmath C. J. (1996) *Indian. J. Seric*., 35, 90-94.
[8] Enomoto S., Moriyama H. And Iwanami S. (1987) *J. A. R. Q*., 21, 117-121.