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PER ORAL INOCULATION OF *BACILLUS* SPECIES (SURFACE AND MIDGUT FLORA) ON LARVAL WEIGHT OF PM AND CSR_2

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ABSTRACT
The larval weight of third and fourth instar of PM and CSR_2 revealed, significantly mean larval weight of 5.65 and 10.77 g/10 was noticed due to Per oral inoculation of *Bacillus* species in the beginning of the healthy lots of third instar on contrary, the same instar with surface *Bacillus* infection recorded 4.27 and 9.84 g/10 larval weight at the beginning. However, the trend was same even in fourth instar inoculated batches 2.46 and 6.94 g/10, 13.83, 6.94 and 1.92 ,5.43 and 10.26 6.34 g/10 in PM and CSR2 larval weight. Further the mean larval weight of 5.68 and 10.52 g/10 was noticed in the beginning of the healthy lots of third instar on contrary, the same instar with midgut flora recorded 4.26 and 9.74 g/10 larval weights at the beginning. The mean weight of 1.58 to 1.18 and 1.26, to1.05 was recorded for third instar inoculated batch which was found to be lower than fourth and fifth instar lots in both PM and CSR_2 breeds of respectively. It is concluded that, per oral infection of surface and midgut flora of *Bacillus* resulted decreased larval weight both in beginning and end of the fourth instar inoculated batch.

INTRODUCTION

Silkworm diseases are the major problem in Sericulture, prevention of silkworm diseases is one of the most important aspects in the success of commercial silkworm rearing. In order, to obtain high and stable cocoon yield, it is necessary to make efforts first to decrease the pathogen load in the rearing environment so as to reduce the pathogenecity and secondly, to strengthen the larval health by increasing their disease resistance/tolerance level.

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 (Suparna *et al.,* 2011). Bacterial diseases are caused by different species of bacteria, viral diseases are caused by both occluded and non-occluded viruses, protozoan disease is caused by *Nosema bombycis*, and muscardine diseases are caused by fungus.

Flacherie disease of silkworm is caused by different species of bacteria and viruses, individually or in combination. Nataraju *et al.* (2004) reported that the silkworm crop loss to the tune of 22 to 43 per cent due to flacherie. In traditional districts of Tamil Nadu reported the flacherie incidence of 5.56, 4.64, 7.12 and 11.08 per cent in Coimbatore, Erode, Salem and Dharmapuri (Manimegalai and Chandramohan, 2005). During flacherie disease the growth and development of silkworm is altered due to infection.Therefore an attempt has been made to know the effect of infection in the larval weight of both third and fourth instar inoculated batches since they are vulnerable to this disease.

MATERIALS AND METHODS

The present study was carried during 2012-2013 in the Department of Sericulture, University of Agricultural Sciences, GKVK and Bangalore. The materials used and methodology employed is presented in this chapter.

All glass wares were sterilized in a hot air oven at 180C 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). A loopful of suspension was taken in inoculation needle and streaked on the NAmedium.These media are selected based on the nutritional requirement of microorganism and incubated at room temperature 24ºC-26ºC (Govindan *et al.*, 1998).

The design used was Completely Randomized Design. Rearing was carried out in individual compartments assigned with two treatment (bacillus isolated from surface and midgut) with five replications, for each treatment a quantum of 2.5mL of *Bacillus* inoculum (0.5 ml/replication) was smeared on 10×12 cm^2 leaf area and administered to 250 larvae of third, fourth larvae of both PM and CSR_2. A separate batch was also maintained as control (Dandin *et al.,* 2003). The treatments are given below.

PM inoculated with *Bacillus* isolated from the diseased silkworm body surface.

PM inoculated with *Bacillus* isolated from the diseaed silkworm midgut.

CSR_2 inoculated with *Bacillus* isolated from the diseased silkworm body surface

CSR_2 inoculated with *Bacillus* isolated from the diseaed

silkworm midgut.

Control (Healthy batch.)

RESULTS AND DISCUSSION

The statistical data on both the sources of inoculum on larval weight was assessed based on the sensitivity of PM and CSR_2 in third and fourth instar inoculated batches because these two breeds were commercially exploited in these districts. As per the laboratory data, the results revealed significant difference on larval weight (g/10) of PM and CSR_2 when they are administered with surface and midgut inoculums.The bacterial inoculum had profound influence on beginning and end of the instar.

The third instar lots of PM and CSR_2 was inoculated with bacteria isolated from the larval body surface revealed that, the larval weight recorded at the beginning and end of the instars was 0.62, 1.91 and 0.90,1.28 g/10 larvae which was lower than the healthy batch (0.65, 2.37 and 1.18, 1.24 g/10 larvae). Further, when same batch attain the 4th and 5th instar recorded less larval weight in the beginning of fourth instar inoculated batch (1.92 and 5.43., 10.26 and 6.34.,g/10). Further the trend was same in fifth instar of same batch did not show difference in the larval weight, that is (10.27 & 23.20., 18.30 & 25.43 g/10 larvae) one compared to healthy worms (Table 1).

The larval surface infection revealed, decreased larval weight from 4th instar inoculated larvae to 5th instar. In the beginning and end of the same instar, there was increase in the larval weight in healthy batch 2.55-13.80 and decreased in inoculated batch 1.95-13.40 g/10 in PM. The same trend was recorded for CSR_2 (6.38-22.00 and 5.40 22.50 g/10). In both the case larval surface infection decrease the larval weight of both the instar 36.12-23.25 g/10 recorded for end of the

Table 1: Effect of surface infection of *Bacillus* specieson larval weight (g/10) of PM and CSR_2 (third instar inoculated)

Instars	Pure Mysore						CSR_2					
	Healthy			Inoculated			Healthy			Inoculated		
	Beginning of instar	End of the instar	Mean	Beginning of instar	End of the instar	Mean	Beginning of instar	End of the instar	Mean	Beginning of instar	End of the instar	Mean
Third	0.65	2.37	1.51	0.62	1.91	1.26	1.18	1.24	1.21	0.9	1.28	1.09
Fourth	2.46	13.83	8.14	1.92	10.26	6.09	6.94	7	6.97	5.43	6.34	5.885
Fifth	13.85	24.85	19.35	10.27	18.3	14.28	24.2	28.32	26.26	23.2	25.34	24.42
Mean	5.65	13.68		4.27	10.15		10.77	12.18		9.84	10.98	
F-test	**	**		**	**		**	**		**	**	
S.Em.±	0.006	0.022		0.014	0.031		0.014	0.037		0.006	0.019	
CD@5%	0.025	0.097		0.059	0.134		0.063	0.158		0.026	0.84	

** Significant at 1 %, NS non significant

Table 2: Effect of larval surface (*Bacillus species)* infection on larval weight (g/10) of PM and CSR_2 (4th instar inoculated)

Instars	Pure Mysore						CSR_2					
	Healthy			Inoculated			Healthy			Inoculated		
	Beginning of instar	End of the instar	Mean	Beginning of instar	End of the instar	Mean	Beginning of instar	End of the instar	Mean	Beginning of instar	End of the instar	Mean
Fourth	2.55	13.8	8.175	1.95	13.4	7.67	6.38	22	14.19	5.4	22.5	13.95
Fifth	13.85	65.45	39.65	12.69	58.85	35.77	24	26.5	25.25	23	24	23.5
Mean	8.2	39.62		7.32	36.12		15.19	24.25		14.2	23.25	
F-test	**	**		**	**		**	**		**	**	
S.Em.±	0.006	0.028		0.069	0.138		0.045	0.05		0.045	0.05	
CD@1%	0.028	0.133		0.329	0.653		0.212	0.237		0.213	0.237	

** Significant at 1%, NS non significant

Table 3: Effect of midgut flora (*Bacillus species)* infection on larval weight (g/10) of PM and CSR_2 (third instar inoculated)

Instars	Pure Mysore						CSR_2					
	Healthy			Inoculated			Healthy			Inoculated		
	Beginning of instar	End of the instar	Mean	Beginning of instar	End of the instar	Mean	Beginning of instar	End of the instar	Mean	Beginning of instar	End of the instar	Mean
Third	0.7	2.47	1.58	0.65	1.87	1.26	1.16	1.2	1.18	0.84	1.26	1.05
Fourth	2.55	13.8	8.17	1.95	10.28	6.11	6.4	23	14.7	5.39	22	13.69
Fifth	13.81	65.4	39.6	10.2	58.8	30	24	26	25	23	24.34	23.67
Mean	5.68	27.22		4,26	23.65		10.52	16.73		9.74	15.86	
F-test	**	**		**	**		**	**		**	**	
S.Em.±	0.008	0.022		0.04	0.063		0.037	0.052		0.037	0.037	
CD@5%	0.036	0.097		0.009	0.27		0.159	0.224		0.158	0.158	

** Significant at 1 %, NS non significant

Table 4: Effect of larval midgut flora (*Bacillus species)* infection on larval weight (g/10) of PM and CSR_2 (4^{th} instar inoculated)

Instars	Pure Mysore Healthy			Inoculated			CSR_2 Healthy			Inoculaed		
	Beginning of instar	End of the instar	Mean	Beginning of instar	End of the instar	Mean	Beginning of instar	End of the instar	Mean	Beginning of instar	End of the instar	Mean
Fourth	2.57	13.07	7.82	2.29	12.02	7.15	6.35	21.5	13.92	5.40	22.6	14
Fifth	13.73	65.36	39.54	13.12	55.62	34.37	25.5	26.5	26	23.0	24.6	23.8
Mean	8.15	39.21		7.70	33.82		45.42	24		14.2	23.6	
F-test	**	**		**	**		**	**		**	**	
S.Em.±	0.012	0.22		0.009	0.041		0.022	0.032		0.045	0.006	
CD@5%	0.057	0.104		0.045	0.195		0.107	0.150		0.213	0.03	

** Significant at 1 %, NS non significant

inoculated batches of PM and CSR_2 respectively (Table 2).

The midgut flora had profound influence on the larval weight of PM and CSR_2 and found significant results. The 3^{rd} instar healthy and inoculated batches noticed mean value of 1.58 and 1.26, 1.18 and 1.05 g/ 10 larvae. Out of the lot, maximum mean value of 39.60 and 30.00, 25.00 and 23.67g/10 recorded for 5^{th} instar batches of both. As per the data, infected batch with midgut flora decreased the larval weight compared to healthy lots of all the instars (Table 3).

The cultured inoculums from midgut flora administered to fourth instar larvae of PM and CSR_2 revealed significant results. In the beginning of the 4^{th} instar, the larvae recorded mean value of 2.29 and 5.40 g/10 in inoculated batches compared to 5^{th} instar *i.e.*13.12-23 in PM and CSR_2 respectively. Even though there was a decrease in the larval weight due to *Bacillus sp.* maximum mean value of 33.82 and 23.60 noticed for inoculated batches of the same. It is very much vivid from the data, as the larval age increased there was decreased in larval weight from 7.82-7.15 and 39.54-34.37, 21.50-22.60 and 26.50 -24.60 larval weights was noticed in the inoculated batches (Table 4).

This may be due to later instars of both the breeds are less sensitive to surface infection of *Bacillus*.These observations are confirmed by many authors who worked on flacherie disease of silkworm. These findings are discussed critically by Doreswamy *et al.* (2004) who studied the seasonal incidence of flacherie disease and its influence on silkworm *Bombyx mori*. He reported that, the incidence of flacherie disease was more during summer season followed by winter and rainy season and recorded the least larval weight of 26.45 and 26.43 g/10 larvae during April and May. The highest larval weight was recorded during December (34.88 g/10) and January (34.50 g/10).

Manjunath (2007) also observed application of plant extracts to PM X CSR_2 worms coupled with 10^{-1}, 10^{-2}, 10^{-3} *Bacillus* spore suspension resulted in significant effect on third and fourth instar larval weight and was found significant and ranged from 1.00 to 1.02 g. Further maximum and minimum larval weight of 1.17, 1.15 and 1.14g was recorded at 1:1 and 1.16, 1.09 and 1.09g for 1:3 found which were decreasing order for 10^{-1} 10^{-2} and 10^{-3} dilutions respectively. Among the bacterial spore dilutions, higher larval weight was observed in 10^{-1} dilutions (1.17 and 1.16g) compared to10^{-3} (1.14 and 1.09g).

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