Mysore J. Agric. Sci., 51 (1) : 93-97, 2017

Relative Performance of Silkworm Breeds *Bombyx mori* L. to Late Larval Flacherie as Influenced by Light Intensity

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ABSTRACT

Flacherie is a syndrome associated with infectious flacherie, densonucleosis, cytoplasmic polyhedrosis, bacterial disease and *Thatteroga* in silkworm. Relative performance of different silkworm breeds *viz.*, Pure Mysore, CSR_2 and PM x CSR_2 showed higher variation in per cent larval weight reduction on 5th day of V instar (17.99, 27.13 and 23.00 %, respectively) at light intensity of 4.20 lux. Total larval mortality ranges from 44.00 - 65.00, 84.00 - 94.00 and 51.50 - 73.00 per cent, respectively with minimum being at 30.28 Lux and maximum being at 4.20 Lux. Deterioration in single cocoon weight was observed at the light intensity of 4.20 lux, when the silkworm were infected with pathogen. Among silkworm breed, CSR_2 found more susceptible to late larval flacherie infection compared to PM and PM x CSR_2 at different light intensities. Rate of mortality of silkworms decreases with an increase in intensity of light inside the rearing room.

MULBERRY silkworm *Bombyx mori* L. is susceptible to number of diseases like flacherie, grasserie, pebrine and muscardine. Among several infectious diseases, the flacherie disease causes an extensive damage to silkworm cocoon crop leading to economic loss to the sericulturists. All the silkworm breeds are susceptible to the virus and large difference exists among various breeds in their susceptibility to *Bm*IFV (Watanabe, 1986). The breed susceptibility of silkworm larvae depends upon the internal and external environmental factors. Several epidemiological and genetical studies demonstrated that the silkworm breeds have different levels of susceptibility to various pathogens (Sudhakara Rao *et al*., 2011).

The silkworm, *Bombyx mori* L. is affected by a number of biotic and abiotic factors. Among the biotic factors, pathogenic microorganisms cause immense loss to cocoon crops. Selvakumar *et al*. (2002) estimated the crop loss of about 11-15 kg cocoon loss per 100 DFLs which accounts for 27-35 per cent. Among abiotic factors light intensity plays a pivotal role causing flacherie at late larval stage and silkworm breeds exhibits varied susceptibility in those conditions (Doreswamy, 2002)

The symptoms of *Thatte* disease have been reported by Prasad *et al.* (1999), they described that the disease appear suddenly on fourth and fifth day of final instar. *Thatte* diseased worms look normal without showing unequality or any other morphological symptoms and they start to die. In begining a patch of 10 to 20 worms will be found dead in a tray and later it will spread to the worms of the entire tray, further which succumb to the disease within a day or two. The dead worms exhibited symptoms *viz*., flaccid body, blackening of the skin, vomiting and diarrhea prior to death.

Infectious flacherie virus multiplied in the midgut epithelium to the same extent in the larvae of both susceptible and resistance breeds of silkworm. In resistant breed, the infected goblet cells were discharged into the gut lumen at each moult and regenerative cells rapidly developed into new goblet cells (Inoue, 1974; Bhattacharya, 1990). Hence, an attempt has been made to evaluate the popular silkworm breeds for their performance when they were infected at their late stages for flacharie as influenced by light intensity.

MATERIAL AND METHODS

Collection of diseased samples : Fifth instar silkworms showing the symptoms of late larval flacherie disease *viz.*, vomiting fluid, shrinkage of body, worms feeding sparsely due to loss of appetite, inactive and suffering from disease were randomly selected from the rearing house of sericulture farmer at Muttagi village near Chamarajnagar district. The diseased samples were subjected for purification of pathogens and microscopic examination.

Isolation of pathogens : The silkworms exhibiting the symptoms of late larval flacherie were collected and surface sterilized by 70-95 per cent Ethanol for 2 minutes. The mid gut juice was collected and homogenized aseptically with sterilized distilled water and filtered through double layered muslin cloth. The stock suspension was prepared and from which serial dilutions were prepared (10^{-1},10^{-2},10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}) using 9 ml sterile water blanks. In the same way haemolymph was also collected by cutting the front pair of prolegs and homogenated with sterile distilled water and filtered through filter paper to obtain the stock suspension from serial dilutions (10^{-1},10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}) using 9 ml sterile water blanks. The infected and dead larvae showing the symptoms of late larval flacherie disease were dissected and midgut was collected. The midguts were homogenized in distilled water. The homogenated gut was further filtered and the filtrate was centrifuged at 3000 rpm for 10 minutes. The supernatant collected was again centrifuged at 15000 rpm for 20 minutes and the purified virus particles were identified as *Bm*IFV and *Bm*DNV (Nataraju *et al*., 1998; Siromani *et al*., 1994; Patil, 1990 and Chitra *et al*., 1975).

Treatments detail : Different coloured cloths *viz.,* black cloth (T_1), white cloth (T_2), yellow cloth (T_3) and gunny cloth (T_4) were used to cover the rearing cage to create different light intensity inside the rearing cage, uncovered with inoculated (T_5) and uncovered un-inoculated (T_6) controls were also maintained to study the effect of light intensity on late larval flacherie. No. of treatments and replication used were six and four, respectively and 50 worms were used per replication.

The susceptibility of silkworm breeds *viz.,* Pure Mysore, CSR_2 and PM x CSR_2 were inoculated with purified inoculums of late larval flacherie. The inoculated V instar larvae of all the silkworm breeds were exposed to different light intensity against late larval flacherie of silkworm. Both inoculated and respective control batches were reared. Ripened worms in each case were mounted and cocoons were harvested separately. The light intensity was measured by using lux meter. The required observations were recorded and compared.

Results and Discussion

Per cent reduction in larval weight (5th day of V instar) : The larval weight reduction in response of different breeds as influenced by light intensity and late larval flacherie showed significant results (Table I). In case of Pure Mysore, the per cent reduction in larval weight was significantly more (17.99 %) at the light intensity of 4.20 lux (black cloth) followed by 12.51, 12.02 and 10.08 per cent recorded at the light intensities of 10.25 lux (white cloth), 18.34 lux (yellow cloth) and 20.23 lux (gunny cloth), respectively. Whereas, it was significantly less (7.65 %) at the light intensity of 30.28 lux (uncovered with inoculated). In case of CSR_2 breed, the per cent reduction in larval weight was significantly more (27.13 %) at the light intensity of 4.20 lux (black cloth) followed by 22.58, 19.42 and 17.80 per cent recorded at the light intensities of 10.25 lux (white cloth), 18.34 lux (yellow cloth) and 20.23 lux (gunny cloth), respectively. However, it was significantly lower (14.79 %) at the light intensity of 30.28 lux (uncovered

Table I

Response of different silkworm breeds Bombyx mori *L. to late larval flacherie as influenced by light intensity in relation to per cent reduction in larval weight*

Treatments	Light intensity (Lux)	Per cent reduction in larval weight		
		PM	CSR_2	PM x CSR_2
White cloth	10.25	12.51 (20.71)	22.58 (28.31)	17.21 (24.46)
Black cloth	4.20	17.99 (25.09)	27.13 (31.39)	23.00 (28.64)
Yellow cloth	18.34	12.02 (20.26)	19.42 (26.14)	13.60 (21.57)
Gunny cloth	20.23	10.08 (18.50)	17.80 (24.95)	13.78 (21.77)
Uncovered with inoculated	30.28	7.65 (16.00)	14.79 (22.60)	12.12 (20.30)
Uncovered uninoculated	32.24	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
F.test		*	*	*
SEm ±		0.542	0.406	1.001
CD at 5 %		1.610	1.207	2.975

Values in Parenthesis arc Arcsine transformed values

* Significant

with inoculated). In case of PM x CSR_2, the per cent reduction in larval weight was significantly more (23.00 %) at the light intensity of 4.20 lux (black cloth) followed by 17.21, 13.60 and 13.78 per cent were noticed at the light intensities of 10.25 lux (white cloth), 18.34 lux (yellow cloth) and 20.23 lux (gunny cloth), respectively (Table I). The lowest of larval weight was recorded as12.12 per cent at the light intensity of 30.28 lux (uncovered with inoculated).

Among all breeds CSR_2 showed highest per cent reduction in larval weight this may be due late larval flacherie coupled with less intensity of light has resulted in less synthesis of red fluroscent protein which is essential in combating with flacherie. Narayanaswamy *et al*. (1985) reported that, the lethal time required for 50 per cent weight reduction in *kenchu* virus stock culture was 9 days in PM, 11 days:12 hours in Hosa Mysore and 17 days:7 hours in NB_4D_2, while, no weight reduction was noticed in NB_7 and NB_{18} bivoltine breeds.

The present results can be corroborated with the outcome of research conducted by Rajanna (1986). According to him the fifth instar larvae reared at light duration (LD) (16:8 hr) of photoperiod exhibited maximum weight. The minimum larval weight has been reported at photoperiod of LD 24:0 hr followed by LD 20:4 hr. The weight of larvae reared under natural condition has been equalent larval weight at LD 12:12 hr. The larval maturity has been delayed by 1 to 1.5 days when reared at continuous darkness as compared to larvae reared at continuous light in which larvae matures early.

Total larval mortality (%) : The total larval mortality as influenced by light intensity during late larval flacherie intensities exhibited significant results. In case of Pure Mysore silkworm breed, the larval mortality was significantly more (65.00%) at the light intensity of 4.20 lux (black cloth) followed by to 52.00, 46.50, 45.50 and 44.00 per cent were noticed at the light of 10.25 lux (white cloth), 18.34 lux (yellow cloth), 20.23 lux (gunny cloth) and 30.28 lux (uncovered with inoculated), respectively (Table II) (Fig 1). Total larval mortality was significantly less (11.00%) when the light intensity was 32.24 lux (uncovered uninoculated). In case of CSR_2, the larval mortality was significantly more (94.00%) at the light intensities of 4.20 lux (black cloth) followed by 86.00, 85.50, 85.00 and 84.00 per cent were noticed at the light intensities of 10.25 lux (white cloth), 18.34 lux (yellow cloth), 20.23 lux (gunny cloth) and 30.28 lux (uncovered with inoculated), respectively. Whereas, it was significantly lower (31.50 %) at the light intensity of 32.24 lux which is uncovered and uninoculated. In case of cross breed, PM x CSR_2

TABLE II

Effect of light intensity and late larval flacherie on total larval mortality of different breeds of silkworm Bombyx mori *L.*

Treatments	Light intensity (Lux)	Total larval mortality (%)		
		PM	CSR_2	PM x CSR_2
White cloth	10.25	52.00	86.00	58.00
Black cloth	4.20	65.00	94.00	73.00
Yellow cloth	18.34	46.50	85.50	55.00
Gunny cloth	20.23	45.50	85.00	53.00
Uncovered with inoculated	30.28	44.00	84.00	51.50
Uncovered uninoculated	32.24	11.00	31.50	15.00
F.test -	-	*	*	*
SEm± -	-	1.323	1.184	1.074
CD at 5 %	-	3.930	3.519	3.190

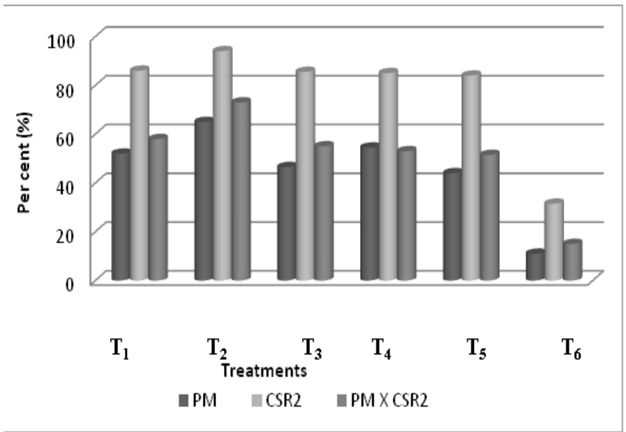

Fig. 1: Effect of light intensity and late larval flacherie on total larval mortality of different breeds of silkworm *Bombyx mori* L.

revealed significantly higher total larval mortality (73.00 %) at the light intensities of 4.20 lux (black cloth) followed by 58.00, 55.00, 53.00 and 51.50 per cent were noticed at the light intensities of 10.25 lux (white cloth), 18.34 lux (yellow cloth), 20.23 lux (gunny cloth) and 30.28 lux (uncovered with inoculated), respectively. Significantly lower (15.00 %) mortality was observed at the light intensity of 32.24 lux (uncovered uninoculated). The results vindicates multivotines are comparatively tolerant to late larval flacherie than bivoltine which are sensitive to late larval flacherie pathogens.

Lower light intensity may favour the multiplication of pathogen as it results in making worms to become weak and prolonged larval duration due to infection of *Bm*DNV and *Bm*IFV causing *Thatte* disease. However, high intensity of light favoured the regeneration of goblet cells and RFP which improves the growth and development of silkworm and completes larval duration normally (Bhattacharya, 1992).

Among 25 breeds screened against *Bm*IFV, the bivoltine breed *viz*., 5N, CSR_{19}, H304 and B218 and multivotine breeds *viz*., Mysore Princes, Kollegal Jawan, C-Nichi and BL69 were found more tolerant to *Bm*IFV. The bivoltine breeds 61N and A210, multivoltine breeds 96A, BL65 and 96E were found moderately tolerant. Further it is confirmed that CSR_2 and CSR_4 were found more susceptible to *Bm*IFV infection (Selvakumar *et al*., 2011). The present outcome confirm with these results.

Single cocoon weight (g) : The maximum single cocoon weight as influenced by light intensity and late larval flacherie was found significant and recorded 1.05, 1.25 and 1.33 g per single cocoon in PM, CSR_2 and PM x CSR_2 breeds, respectively at the light intensity of 32.24 lux (uncovered uninoculated control). However, it was least (1.00, 1.11 and 1.17 g/) in PM, CSR_2 and PM x CSR_2 at the light intensity of 4.20 lux (black cloth), respectively (Table III).

Hema *et al.* (2011) revealed that the single cocoon weight is ranging from 0.98 (Pure Mysore) to 1.41 g (ND_7) with cocoon shell ratio lowest of 11.31 per cent in Nistari and highest of 18.83 for ND_7 when some of the multivotine and bivotine breeds are inoculated with *Bm*IFV.

TABLE III

Effect of light intensity and late larval flacherie on single cocoon weight of different breeds of silkworm Bombyx mori *L.*

Treatments	Light intensity (Lux)	Single cocoon weight (g)		
		PM	CSR_2	PM x CSR_2
White cloth	10.25	1.01	1.12	1.22
Black cloth	4.20	1.00	1.11	1.17
Yellow cloth	18.34	1.01	1.17	1.27
Gunny cloth	20.23	1.02	1.17	1.28
Uncovered with inoculated	30.28	1.02	1.18	1.27
Uncovered uninoculated	32.24	1.05	1.25	1.33
F.test		*	*	*
SEm		0.003	0.004	0.012
CD at 5 %		0.008	0.013	0.035

Faruki and Kundu (2005) studied the sensitivity of the silkworm larvae to UV-radiation on some commercially relevant traits when they exposed first three instars of the two multivotine strains, Nistari- M and Urboshi-1 of the silkworm, *Bombyx mori* L. They found that UV-rays reduced the weight of larvae, pupae and cocoon of both the strains and sexes of *B. mori,* also an increased larval mortality was recorded at all the doses of UV-rays.

The present research communicates that when the silkworms were provided varied light intensity during their rearing period will definitely affects the larval growth and imparts their mortality. Infection at the beginning of larval stage especially with reference to flacherie disease, will tend to have more negative effect when they exposed varied larval intensity of light. Among the different breeds subjected for the study, multivotine (PM) and multivotine based cross breed (PM X CSR2) shows relative resistance against pathogens even under lower intensities of light when compared to bivoltine (BV).

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(*Received :* November, 2016 *Accepted :* January, 2017)