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Changes in Protein Content in Virus and Bacterial Infected Fifth Instar Larvae of Silkworm, Bombyx Mori L. and Treatment with Antibiotics.

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ABSTRACT

Changes in protein level in Haemolymph, Fatbody and Silkgland of the 5th instar silkworm of hybrids, PM x CSR2, CSR4 x CSR2, and CSR2 x CSR4 were examined under inoculation with the viral and bacterial pathogen and treatment with antibiotics in healthy, pre-inoculated and post-inoculated conditions. The results indicate that the changes in protein content in infected larvae could be favourably reverted towards control levels by treatment with antibiotics, both in pre-and post-inoculated conditions. Protein content could be elevated by antibiotic supplementation in healthy larvae. Antibiotic treatment was more effective during post-inoculated conditions than pre-inoculated conditions. Pre-treatment with antibiotics seems to assist in the building up the resistance before the attack of the pathogen, thereby reducing the severity of infection.

Keywords: Bombyx mori, virus, bacterial, pre-and post-inoculated, antibiotics.

I. INTRODUCTION

The success and stability of silkworm cocoon production depends upon the protection of silkworms from the diseases caused by different pathogens. Among silkworm diseases, viral and bacterial infections are common during silkworm rearing. The most common diseases of silkworm are Grasserie caused by a virus nuclear polyhedrosis, Flacherie, caused by bacteria: Streptococcus and Staphylococcus in association with Flacherie virus, Muscardine, Aspergilosis, caused by fungal infection, and pebrine, a protozoan disease caused by a parasitic microsporidian, Nosema bombycis. The diseases prevail throughout the year, and in tropics they are significantly high (Srivastava and kumar, 2009). Various chemicals (antibiotics) are extensively employed to prevent the attack of diseases to silkworm, thereby, help in, increasing the productivity of silk. The beneficial action of the antibiotics has been attributed to the oral feeding of them along with mulberry leaves, which reduced significantly the incidence of the flacherie and grasserie (Radha, et al, 1980). The progress of multiplication of a pathogen in the host system is often reflected by specific metabolic variations along with gradual changes in the infected tissues. Susceptibility to a disease differs according to the physiological status of the host. Fortification of mulberry leaves is considered as one of the effective methods to enrich the silkworm diet. The biochemical parameters could be elevated by antibiotic supplementation in healthy larvae (Savithri and Murli, 2002). Knowledge of the biochemical changes during infection by pathogens in larvae of Bombyx mori would be useful for evolving suitable prophylactic and control measures against the disease.

Keeping this in view the present study was undertaken to understand the changes in protein content in haemolymph, fatbody and silkgland of Bombyx mori during infection by virus and bacterium, as also during treatment with antibiotics under pre and post inoculated conditions.

II. MATERIALS AND METHOD

Three silkworm races PM x CSR_2, CSR_4 x CSR_2 and CSR_2 x CSR_4 of Bombyx mori were used for present study. Newly moulted fourth instar larvae were selected from the rearing stock and were placed individually in

plastic cups. The doses of BmNPV and Bacillus sp. were prepared with the help of standard counting using Neaubur's chamber to count the NPV particles and spectrophotometer for Bacillus sp. The occlusion bodies (OBs) of nucleopolyhedrovirus were isolated and purified by diluting with distilled water and modified repeated centrifugation method and as suggested by cantwell (1970). The larvae were starved for about 10-12 h before inoculation then they were inoculated with BmNPV and Bacillus sp. Smear applied on $1cm^2$ piece mulberry leaf, air dried and fed to early fifth instar larvae. One set of larvae were treated with BmNPV suspension with sub lethal concentration ($1.5x10^6$OBs/ml @ 10 µl/100 worms. Similarly one set of larvae was treated with suspension of Bacillus sp. with concentration ($5x10^7$ particles/ml @ 2.5 µl/100 worms) and another set of larvae were treated with distilled water and used as control. The prologs of silkworm were clipped and haemolymph of control and treated larvae from all the three races were collected in eppendroff coated with phenylthiourea to avoid protein coagulation and kept in deep freezer at -20^0c until use. The silkgland and fatbody during fifth instar stage were dissected out, weighed and crushed in sample buffer (Arif, et. al., 2001) filtered and amount of protein was estimated and compared. Four antibiotics viz-Chloramphenicol, Streptomycin, Ampicillin, and Penicillin were used. Antibiotics treatment was given to the inoculated groups, prior to the antibiotic treatment the larvae were screened with four different dosages such as 100, 50, 10, and 4 mg/ml of which 4 mg/ml dose of antibiotics was effective hence preferred. Required solutions were prepared in sterile distilled water and 4 mg/ml dose of antibiotics solution was uniformly smeared on the mulberry leaves. Smeared leaves were dried under shade and fed to the silkworm larvae. For the purpose, the larvae were examined in three different conditions i.e. (1) Healthy larvae treated with antibiotics. (2) Larvae that were pre-inoculated with the virus and bacterial pathogen and then treated with antibiotics and (3) Larvae that were post inoculated with the pathogen while continuing treatment with antibiotics.

III. RESULTS AND DISCUSSION

Protein content estimated from haemolymph, fatbody and silkgland of PM x CSR2, CSR4 x CSR2 and CSR2 x CSR4 hybrids showed a decreasing trend in infected group. Treatment with the four antibiotics chosen effected over the control levels. This reversal was found to be most effective with Chloramphenicol and streptomycin for haemolymph, fatbody and silkgland in all the hybrids (table 1). Reversal of changes was effected under both pre and post inoculated conditions, and it was more pronounced in post inoculated silkworms. It was also interesting that the levels of protein content in haemolymph, fatbody and silkgland studied were elevated by all the four antibiotics even under control conditions wherein the worms were healthy and not subjected to infection by virus and bacterium (Table 2). The biochemical parameters could be elevated by antibiotics supplementation in healthy larvae (Savithri and Murli, 2003b). Manchev et. al., (1984), Rai and Devaiah (1988) and Sridhar et al., (2000) also reported that oral administration of antibiotics with mulberry leaves reduced the disease incidence. Sam Devadas (1991) reported Chloramphenicol was the most effective against Serratia marcescens, which was interpreted as due to fast multiplication of bacteria. The decrease in protein contents of haemolymph, fatbody and silkgland under the infection by virus and bacterium seems to be a reflection of stepped-up demand for energy in the host to combat the disease as a natural response. Sarma et al., (1994) reported a steady decrease in carbohydrate content as infection of NPV progressed up to the 5^{th} day of the 5^{th} instar, and they attributed this to the utilization of carbohydrates as an energy source required for the biosynthesis of viral constituents. Rajsekar and Pathak (1994) observed the significant reduction of total protein concentration in the silkglands of flacherie infected larvae; the decrease was suggested to be due to a decrease in the level of protein and aminoacids in the haemolymph of diseased worms which affects the formation of silk protein in the glands.

The fat body glycogen gets depleted with advancement of disease in B.mori (Ambica, 1990). This was suggested to indicate its utilization for deriving chemical energy for the synthesis of ATP that is essential for sustaining the life activities.

Kumar et al., (2011) observed quantitative and qualitative changes in protein profiles of various tissues of tropical tasar silkworm Antheraea mylitta and reported decreased protein trend in inoculated larvae and suggested that it may be due to drastic degradation of structural proteins.

Results of the present study indicate that the reduction in protein content in infected larvae favourably showed increasing trend in the protein level of control due to treatment with antibiotics, in both pre and post inoculated and treated conditions. The increase in protein level due to application of antibiotics supplementation also observed in healthy larvae. Antibiotics treatment was more effective during post-inoculated than pre-inoculated conditions. The results show that pre treatment with antibiotics seems to assist in the building up the resistance before the attack of the pathogen. The positive changes in haemolymph, fat body and silkgland protein level when diseased larvae inoculated with antibiotics.

IV. CONCLUSION

Protein concentration in the haemolymph, fatbody and silkgland estimated from three hybrid races indicated that, the disease could be managed by using chloramphenicol and streptomycin application which improved the range of protein concentration in all the races with highest in PM x CSR2. Further studies on protein concentration in larva, fat body and silk gland tissue were performed in PM x CSR2 revealed that amount of proteins present in haemolymph of controls are higher than in infected larvae, however the protein concentration and amount of proteins recovered with the use of chloramphenicol and streptomycin and near to control group. So as a prophylactic measure chloramphenicol and streptomycin can be used to treat the mulberry leaves and before silkworm feeding to manage the viral and bacterial infection in order to harvest better cocoon crops.

Thus the results obtained from present study it can be concluded that, out of the three multi x bivoltine (PM x CSR2) and bivoltine x bivoltine (CSR2 x CSR4 and CSR4 x CSR2) hybrid races, PM x CSR2 hybrid race is most suited to this region even at varied condition. During the adverse condition, the diseased larvae if treated with Chloramphenicol at early stages of infection the menace due to disease could be controlled and cocoon crop loss may be avoided, which might attract more farmers to practice sericulture and prevent the suicidal attempt by deprived farmer in Vidarbha.

Table 1. Protein content in pre and post inoculated larvae treated with antibiotics

Silkworm races			**control**	**Infected**		**Chloramphenicol**	**Streptomycin**	**Ampicillin**	**Penicillin**
				Virus	Bacterial				
PMxCSR2	Haemolymph	Pre	30.23	21.15	19.01	24.15	20.10	22.13	22.00
		Post	32.19	23.16	22.21	26.40	24.05	23.03	23.50
	Fatbody	Pre	310.1	283.0	213.0	304.0	280.3	281.0	270.0
		Post	316.0	287.4	218.0	310.7	301.2	290.0	280.0
	Silkgland	Pre	85.01	68.92	60.30	78.15	70.6	67.5	70.0
		Post	90.03	70.90	62.50	80.15	72.5	70.6	72.0
CSR4xCSR2	Haemolymph	Pre	27.05	18.05	17.15	25.02	23.15	20.3	20.07
		Post	29.96	20.10	18.17	27.40	25.2	21.01	22.50
	Fatbody	Pre	310.3	255.1	200.2	303.1	280.5	270.5	246.6
		Post	319.5	260.2	205.0	305.2	300.1	268.4	250.5
	Silkgland	Pre	90.05	57.50	50.30	65.05	64.6	60.2	60.66
		Post	92.07	62.5	60.50	70.05	68.5	63.01	60.30
CSR2xCSR4	Haemolymph	Pre	23.05	15.23	14.15	22.05	18.07	18.15	19.31
		Post	28.02	17.50	16.07	23.05	20.5	19.05	20.50
	Fatbody	Pre	310.5	231.3	203.5	280.1	260.5	241.5	246.3
		Post	315.3	241.2	209.6	290.3	270.6	250.2	253.8
	Silkgland	Pre	60.3	49.5	40.3	51.3	49.3	44.5	48.30
		Post	65.02	50.7	42.4	56.05	52.0	50.75	50.40

Pre= Larvae inoculated with BmNPV and Bacillus sp. before antibiotic treatment.
Post=Larvae inoculated with BmNPV and Bacillus sp. after antibiotic treatment

Table 2. Changes in protein content in Healthy larvae treated with antibiotics.

Silkworm races		Control	Chloramphenicol	Streptomycin	Ampicillin	Penicillin
PMxCSR2	Haemolymph	35.25	38.47	36.79	35.35	35.55
	Fatbody	321.6	338.6	331.3	334.3	329.6
	Silkgland	95.15	98.00	96.89	96.01	96.30
CSR4xCSR2	Haemolymph	30.81	33.53	32.15	32.01	31.50
	Fatbody	315.7	389.1	355.6	330.3	347.1
	Silkgland	93.00	95.01	94.30	94.15	93.12
CSR2xCSR4	Haemolymph	29.89	32.04	32.30	30.60	30.01
	Fatbody	310.2	334.8	326.4	320.2	313.2
	Silkgland	65.66	70.40	70.20	67.40	65.50

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