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Deterioration of Cocoon Traits of Silkworm, *Bombyx mori* L. by the Synergistic Infection with Late Larval Flacherie Pathogens

Since, the silkworm, *Bombyx mori* L. is susceptible for many infectious agents, mixed infections are very common. It is needless to stress the importance of mixed or combined infections in productive insect like B. mori as the mixed infections play major role in reducing the cocoon crop yields. Mixed infections may occur simultaneously or one after the other and usually the interaction between the two or more involved pathogens results in synergism thereby the cocoon productivity both quantitatively and qualitatively.

Simultaneous per oral infection of B.mori (pure mysore) in fourth instar with Kenchu virus (BmDNV-2) and the bacterium *Staphylococus aureus* resulted in higher mortality rate as well as deterioration in cocoon quantitative traits as compared to Kenchu virus or bacterium alone. Melting of cocoons also occurred due to the late larval flacherie (Govindan *et al*., 1990). Sanakal *et al*. (1996) reported that the infectious flacherie virus caused death in silkworms after 7-10 days and the cocoons produced by the survivors had the deteriorated economic characters. The cocoons were flimsy and small. There was 2-3 days delay in spinning. There was reduction in most of the economic characters.

The worms suffering from the disease, known much as thatte roga among the sericulturists of Karnataka did not form the cocoons (Samson *et al*., 1975) whereas Yamatani (1977) reported that the IFV infected fifth instar larvae were strong enough and spun the cocoons eventhough affected.

Mulberry silkworm larvae exhibiting specific symptoms of thatte roga were collected from traditional sericultural villages of Chikkaballapur, Siddlaghatta and Chintamani taluks in Kolar districts of Karnataka State were surface disinfected and the midgut juice was collected. The stock suspension was made from which serial dilutions were prepared (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}) using sterile water blanks. Haemolymph was also collected by cutting the front pair of prolegs and homogenised with sterile distilled water and filtered through filter paper to obtain the stock suspension from which serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}) were prepared using 9ml sterile water blanks (Nataraju *et al*., 1999: Sironmani *et al*., 1994: Patil, 1990 and Chitra *et al*., 1973). 0.5 ml of each dilution of both midgut juice and haemolymph was transferred to separate petridishes containing nutrient agar medium and spread thoroughly and the culture plates were incubated at 37^0C for 3 days. The bacterial colonies that developed on the culture plates were picked and purified by routine methods. Pathogenisity of the individual bacteria confirming the principle of Koch, s postulates in causing the disease were identified as *Streptococcus faecalis*, *Staphylococcus aureus* and *Bacillus* SP.

The midguts of the infected and dead larvae were homogenized in 1:1 (w/v) 0.05 M phosphate buffer of P^H 7.2. The gut homogenate was further filtered and the filtrate was centrifuged at 3000 rpm for 10 minutes. The supernatant was again centrifuged at 15000 rpm for 20 minutes and the purified virus particles were identified as BmIFV and BmDNV.

Mulberry leaves of Kanva-2 variety were washed with tap water and surface sterilized with 70 per cent alcohol using sterile cotton wad. Two ml suspension of mixture of the pathogens was uniformly smeared and fed to silkworms (PM X

NB_4D_2) after passing third moult. The observations viz., cocoon weight, shell weight, pupal weight, Shell ratio per centage; single filament length and denier were recorded.

The data on cocoon traits of the present investigation revealed that the cocoon weight was significantly reduced with the inoculation of BmIFV + BmDNV (11.73 g/10 cocoons), *Bacillus sp.*+ *S. faecalis* +*S. aureus* (11.45 g/10 cocoons), BmIFV + BmDNV + *Bacillus sp.* (11.20 g/10 cocoons), BmIFV + BmDNV + *S. faecalis* (11.34 g/10 cocoons) and BmIFV + BmDNV + *S. aureus* (11.66 g/10 cocoons) whereas the cocoon weight was significantly higher in distilled water control (17.00 g/ cocoons), untreated control (16.92 g/10 cocoons). Cocoon shell weight was significantly less (1.62, 1.70, 1.60, 1.64, 1.76, 1.78, 1.84, 1.73, 1.81, 1.73, 1.82 and 1.88 g/10 shells, respectively) in inoculation with BmIFV + BmDNV, *Bacillus sp.* + *S. faecalis* + *S. aureus*, BmIFV + BmDNV + *Bacillus sp.*, BmIFV + BmDNV + *S. faecalis*, BmIFV + BmDNV + *S. aureus*, BmIFV + *S. aureus*, BmIFV + *S. faecalis*, BmIFV + *Bacillus sp.*, BmDNV + *S. faecalis*, BmDNV + *S. aureus* and *Bacillus sp.*+ *S. faecalis*. The pupal weight was significantly less in BmIFV + BmDNV (10.07g/10), *Bacillus sp.* + *S. faecalis* + *S. aureus* (9.72), BmIFV + BmDNV + *Bacillus sp.* (9.60), BmIFV + BmDNV + *S. faecalis* (9.690 and BmIFV + BmDNV + S. aureus (9.88 g/10 pupae) and it was significantly higher in distilled water control (14.350, untreated control (14.30).

The shell ratio per centage was significantly less in inoculation with BmIFV + BmDNV (13.50), BmIFV + *S. aureus* (13.59), BmIFV + *S. faecalis* (13.31), BmIFV + *Bacillus sp.* (12.32), BmDNV + *Bacillus sp.* (12.41), BmDNV + *S. faecalis* (11.66), BmDNV + *S. aureus* (12.34) *Bacillus sp.* + *S. faecalis* (11.64) *Bacillus sp.* + *S. aureus* (11.92) and *S. faecalis* + *S. aureus* (12.02). Whereas it was significantly more in respect of distilled water control (15.58), untreated control (15.38).

Significantly shorter filament was obtained in inoculation with BmIFV + BmDNV (420.27), BmIFV + BmDNV + *Bacillus* sp. (424.44), BmIFV + BmDNV + *S faecalis* (426.21) BmIFV + BmDNV + *S. aureus* (445.55), BmIFV + *S. aureus* (447.16), BmIFV + *S. faecalis* (450.44), BmIFV + *Bacillus* sp. (446.10), BmDNV + *Bacillus* sp. (442.99) and BmDNV + S. faecalis (452.21) whereas it was significantly longer in inoculation with distilled water control (730.00), untreated control (725.00). Significantly thinner denier was obtained in inoculation with BmIFV + BmDNV + Bacillus sp. (1.75) and BmIFV + BmDNV (1.80) whereas thicker denier was obtained in distilled water control (2.40) and untreated control (2.30).

It is well known that the interaction of two or more pathogens results in synergism thereby affecting the cocoon productivity both quantitatively and qualitatively. The above results are in accordance with the observations of Matsumoto *et al.* (1986) who observed that the fourth instar silkworms fed with mixture of IFV + *S. faecalis* and also in simultaneous per oral infection of fourth instar silkworms with kenchu virus + *S. aureus* (Govindan *et al.*, 1990) resulted in deterioration of cocoon quantitative traits as compared to infection with kenchu virus or *S. aureus* alone. Lowest shell weight recorded in the inoculation with BmIFV + BmDNV may be due to the chronic infection which might have deteriorated the quantitative traits including shell weight. The lowest pupal weight recorded in BmIFV + BmDNV + *Bacillus* sp. may be due to the associative effect of *Bacillus* sp. with BmIFV and BmDNV that caused retarded growth of larvae thus contributing for reduced pupal weight, Nevertheless, the highest pupal weight recorded in the inoculation of *Bacillus* sp. + *S. aureus* may be probably due to the negligible or weak

Table 1. Deterioration of cocoon parameters by synergistic infection of late larval flacherie pathogens in silkworm, *Bombyx mori*

Treatments	Ten cocoon weight (g)	Ten shell weight (g)	Ten pupal weight (g)	Shell ratio (%)	Single filament length (m)	Single filament Denier
T_1	11.73	1.62	10.07	13.50	420.27	1.80
T_2	11.45	1.70	9.72	14.83	45.71	1.94
T_3	11.20	1.60	9.60	15.69	424.44	1.75
T_4	11.34	1.64	9.69	14.16	426.21	1.92
T_5	11.66	1.76	9.88	14.97	445.55	1.99
T_6	13.17	1.78	11.37	13.59	447.16	2.01
T_7	13.81	1.84	11.96	13.31	450.44	2.04
T_8	14.06	1.73	12.34	12.32	446.10	1.96
T_9	14.57	1.81	12.74	12.41	442.99	1.99
T_{10}	14.83	1.73	13.09	11.66	452.21	2.07
T_{11}	14.76	1.82	12.93	12.34	462.94	2.13
T_{12}	16.20	1.88	14.28	11.64	495.16	2.20
T_{13}	16.59	2.05	14.59	11.92	583.05	2.24
T_{14}	16.50	2.05	14.48	12.02	549.16	2.22
T_{15}	16.92	2.60	14.30	15.38	725.00	2.30
T_{16}	17.00	2.65	14.35	15.58	730.00	2.40
F- test	*	*	*	*	*	*
S.Em±	0.43	0.13	0.33	0.61	11.86	0.05
CD at 5%	1.22	0.38	0.93	1.74	33.41	0.15

Treatment details: T_1 (BmIFV + BmDNV), T_2 (Bacillus sp.+ *S. faecalis* + S. aureus), T_3 (BmIFV + BmDNV + Bacillus sp.), T_4 (BmIFV + BmDNV + S. faecalis), T_5 (BmIFV + BmDNV + S. aureus), T_6 (BmIFV + S. aureus),

T_7 (BmIFV + S. faecalis), T_8 (BmIFV + Bacillus sp.), T_9 (BmDNV + Bacillus sp.), T_{10} (BmDNV + *S. faecalis*), T_{11} (BmDNV + S. aureus), T_{12} (Bacillus sp.+ S. faecalis), T_{13} (Bacillus + S. aureus), T_{14} (S. faecalis + S. aureus), T_{15} (Untreated control) and T_{16} (Distilled water control).

synergism. Further more, both *Bacillus* sp. and *S. aureus* were less pathogenic than BmIFV and BmDNV. The shorter filament with co-infection of non-occluded viruses BmIFV + BmDNV might be due to the fact that the infected fifth instar worms survived up to spinning stage and spun flimsy and small cocoons resulting in reduced cocoon filament length. The BmIFV and BmDNV inoculated worms were able to spin cocoons, which were flimsy and small resulting in deterioration of most of the economic characters. The lowest denier was due to the small and flimsy cocoons spun by the worms infected with BmIFV + BmDNV + *Bacillus* SP. The deterioration observed in denier may be as noticed in other metric traits (Sanakal *et al.,* 1996).

Department of Sericulture
Sericulture College
Chintamani - 563125

C.DORESWAMY
R.GOVINDAN
M.C.DEVAIAH
M.V.MUNISWAMAPPA

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