RELATIVE RESISTANCE OF DIFFERENT STRAINS OF SILKWORM *BOMBYX MORI* L. TO MICROBIAL DISEASES

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Abstract

Four strains of silkworm *Bombyx mori* L., i.e. J-101, 206MKD, C-102 and 207-PO were evaluated for their resistance to microbial diseases. For each strain 225 larvae of 4th instar were separated from mass rearing lot and divided into three replications. The rearing of silkworm was carried out at 25±2°C and 70±5%rH. The results revealed that bacterial flacherie caused overall 20.52, 18.5, 16.82 and 5.53 percent mortality in C-102, J-101, 206-MKD and 207-PO, respectively. The mortality did not differ significantly among C-102, J-101 and 206-MKD. Viral grasserie resulted overall 16.65 6.95, 5.52 and 1.87 percent mortality in J-101, C-102, 206-MKD and 207-PO, respectively. There was non-significant difference of mortality between 206-MKD and C-102. White muscardine was not found in 5th instar larvae as well as in spinning larvae, however it caused maximum 2.99% mortality in J-101 during pupal stage. Cumulative mortality because of these three diseases was in the array of J-101 (38.59%) > C-102 (29.22%) > 206-MKD (23.67%) > 207-PO (9.93%). Thus 207-PO was relatively the most resistant strain to flacherie and grasserie while J-101 was relatively the most susceptible strain to these microbial diseases. 206-MKD and C-102 were intermediate between 207-PO and J-101, however they were relatively more resistant to grasserie than flacherie.

Key words: Resistance, silkworms *Bombyx mori*, Microbial diseases, J-101, C-102, 206-MKD, 207-PO, Flacherie, Grasserie, White muscardine, Nuclear polyhedrosis virus, Bacteria, *Bacillus* spp. *Streptococcus* spp. *Beauveria bassiana.*

Introduction

Silkworms, *Bombyx mori* L. are affected by various diseases caused by microorganisms. Flacherie disease of the silkworm is a major factor leading to

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serious losses to silkworm production in Japan (Inoue and Tanada, 1977; Hashimoto and Kawase, 1983). Chitra, et al., (1974) and Shyamala et al., (1987) have reported about 20-40% and 17-27% loss of silkworm production due to flacherie caused by bacteria and infectious nuclear polyhedrosis virus, in India, respectively. Similarly, white muscardine disease caused by *Beauveria bassiana* is one of the major constraints in mulberry silk production (Raghavaiah and Jayaramaiah, 1987).

The incidence and lethality of diseases depend along other factors upon silkworm rearing temperature (Aruga and Tanaka, 1968; Kobayashi et al., 1981), rearing of susceptible silkworm strains and infestation of mulberry used for food by infected *Glyphodes pyloalis* Walk. (Watanabe, 1981), different mulberry varieties being fed (Basavarajappa and Savanurmath, 1996), and moisture on leaves & maturity stage of mulberry leaves (Sivaprakasam, et al., 1996).

Watanabe and Maeda, (1981) found that certain silkworm strains were highly susceptible to virus while those of other strains were highly resistant to the virus though they were fed on high concentration of the virus inoculum. They discovered a single gene that determined either the susceptibility or the nonsusceptibility of the silkworm to DNV infection. Watanabe (1967) also developed resistance in the silkworm, *Bombyx mori* L. in the laboratory to cytoplasmic polyhedrosis virus by selecting survivors from virus fed larvae for eight generations. Thus resisrance in silkworms to microbial diseases is one of the vital factor for abating losses to silk production. In present studies, therefore four strains of silkworm were evaluated for their relative resistance to different microbial diseases.

Materials and Methods

Four bivoltine strains of *Bombyx mori* L. (lepidoptera; Bombycidae), namely J-101, 206-MKD, C-102 and 207-PO were screened for their resistance to microbial diseases during the rearing season of the Autumn. J-101 and 206-MKD were of japanese origin while C-102 and 207-PO were that of chinese. For each strain 225 full grown 4th instar larvae were separated from mass rearing lot at random and divided into three replications. The larvae were kept in wooden trays 75x60x6.5cm and reared upto adults at 25±2°C and 70±5% rH. The larvae were fed five times daily. Occurrence of diseases was recorded in 5th instar larvae, spinning larvae and pupae/cocoon. Preliminary epizootic micro-

organisms were classified on the basis of general etiological symptoms appeared on silkworm bodies (Krishnaswami, et al., 1973). The causal agents were finally determined after the death of the silkworm with the help of light microscope. The mortality recorded during 5th larval instar, spinning and pupal/cocoon stages was converted into percentage and the mortality data were analyzed statistically by applying Analysis of Variance (ANOVA), Least Significant Difference (LSD) and t-test.

Results

Mainly two microbial diseases, i.e. flacherie and grasserie were found in 5th instar larvae, spinning larvae and pupae/cocoon. These were caused by a complex of bacteria *Bacillus* spp.& *Streptococcus* spp. and nuclear polyhedrosis virus. Few number of pupae were also found infected with white muscardine (*Beauveria bassiana*) but the fungal infection did not appear in larval and spinning stadia.

i. Incidence of flacherie (bacteria)

Severe bacterial infection was prevailed in 5th instar larvae, spinning larvae and pupae. The mortality caused by bacteria is presented in Table 1.

Table 1. Mean percent mortality caused by bacteria in four strains of *B. mori*

Silkworm strains	% mortality in developmental stages			Overall Mortality (%)
	5th instar	spinning	pupa/cocoon	
J-101	8.00** a	4.89* a	6.06* a	18.95** a
206-MKD	5.78 b	4.00 ab	7.04 a	16.82 a
C-102	7.56 ab	5.33 a	7.63 a	20.52 a
207-PO	1.78 c	1.78 b	1.97 b	5.53 b
LSD	2.12	2.39	3.81	4.23

* Significant at 95% level; ** - significant at 99% level
Figures sharing same alphabets in the same column are non-significant

The mortality due to bacteria during 5th larval instar was highly significant (P99%) where maximum 8% and minimum 1.76% mortality was recorded in J-101 and 207-PO, respectively. The difference between J-101 & C-102 and C-102 & 206-MKD was statistically non-significant. On the other hand the mortality in 207-PO was significantly low than J-101, 206-MKD and C-102. In spinning larvae and pupae mortality was significant (P95%) with maximum 5.33%, 7.63% and minimum 1.78%, 1.97% in C-102 and 207-PO, respectively. The difference among J-101, 206-MKD and C-102 was non-significant while 207-PO differed significantly except at spinning stage when it was non-significant with 206-MKD. The overall mortality because of bacteria was highly significant(P99%) and invariably same pattern was observed, i.e. maximum 20.52% mortality was recorded in C-102 which was non-significant with that of J-101 and 206-MKD. Whereas, minimum (5.53%) mortality was incurred in 207-PO which differed significantly with J-101, 206-MKD and C-102.

ii. Incidence of grasserie (NPV)

The second most destructive disease was grasserie which was caused by NPV; the consequential mortality is displaced in Table 2.

Table 2. Mean percent mortality caused by NPV in four strains of *B. mori*

Silkworm strains	% mortality in developmental stages			Overall Mortality (%)
	5th instar	spinning	pupa/cocoon	
J-101	6.55** a	2.67* a	7.31** a	16.65** a
206-MKD	2.67 b	0.88 b	1.96 b	5.52 b
C-102	1.78 b	2.22 ab	2.38 b	6.95 b
207-PO	0.44 b	0.44 b	0.99 b	1.87 c
LSD	2.34	1.47	2.22	2.31

* = significant at 95% level
** = significant at 99% level
Figures sharing same alphabets in the same column are non-significant among themselves

The mortality due to NPV was highly significant (P99%) except in spinning lravae where it was significant (P95%). During 5th larval instar maximum 6.55% mortality was observed in J-101 and it differed significantly

with that of 206-MKD, C-102 and 207-PO. The difference among 206-MKD, C-102 and 207-PO was statistically non-significant. Almost similar trend of mortality among the four strains of silkworm was observed in spinning larvae and pupae with exception that J-101 and C-102 differed non-significantly in spinning larvae. Overall maximum 16.65% and minimum 1.87% mortality by virtue of NPV was observed in J-101 and 207-PO. But the difference between 206-MKD and C-102 was statistically non-significant.

iii. Incidence of white muscardine (*Beauveria bassiana*)

White muscardine was only observed in pupae of all four silkworm strains which was statistically non-significant. Maximum 2.99% and minimum 1.33% mortality was conduced to *B.bassiana* in J-101 and 206-MKD, respectively (Table 3).

To work out the most susceptible stage among 5th instar larvae, spinning larvae and pupae to flacherie, grasserie and white muscardine, the mortality recorded in theses stages was compared and the results are showed in Table 3.

Table 3. Mean percent mortality during three developmental stages of *B.mori* caused by Bacteria, NPV & *B.bassiana*

D. Stages	% mortality in silkworm strains			
	J-101	206-MKD	C-102	207-PO
		A-FLACHERIE		
5th instar	8.00*a	5.78^{ns}	7.56^{ns}	1.78^{ns}
Spinning	4.89 b	4.00	5.33	1.78
Pupa	6.06 b	7.04	7.63	1.97
		B-GRASSERIE		
5th instar	6.55*a	2.67*a	1.78^{ns}	0.44^{ns}
spinning	2.67 b	0.89 b	2.22	0.44
pupa	7.31 a	1.96 a	2.95	0.99
		C-WHITE MUSCARDINE		
5th instar	-	-	-	-
spinning	-	-	-	-
pupa	2.99	1.33	1.75	2.52

* = significant at 95% level
ns = non-significant;
Figures in the same column having same alphabets are non-significant among themselves

There was non-significant difference in mortality caused by bacteria in 5th instar larvae, spinning larvae and pupae in 206-MKD, C-102 and 207-PO but it was significant in J-101 where mortality in 5th instar lravae was significantly high than spinning larvae and pupae; the latter two were non-significant between themselves. Likewise, mortality conduced by NPV in 5th instar larvae, spinning larvae and pupae varied significantly in J-101 and 206-MKD contrarily to C-102 and 207-PO. Both in J-101 and 206-MKD, mortality was significantly less in spinning larvae than 5th instar larvae and pupae. The variation in mortality during 5th larval instar and pupal stage was non-significant. White muscardine was only appeared in pupae.

Discussion

The flacherie and grasserie diseases caused by Bacillus spp., Streptococcus spp. and nuclear polyhedrosis virus are the two major microbial diseases found in the four strains of silkworm. Whereas, the white muscardine (*B.bassiana*) is comparatively negligible. These three diseases cause 9.93-38.59% mortality from 5th larval instar to pupal stage (Fig.1).

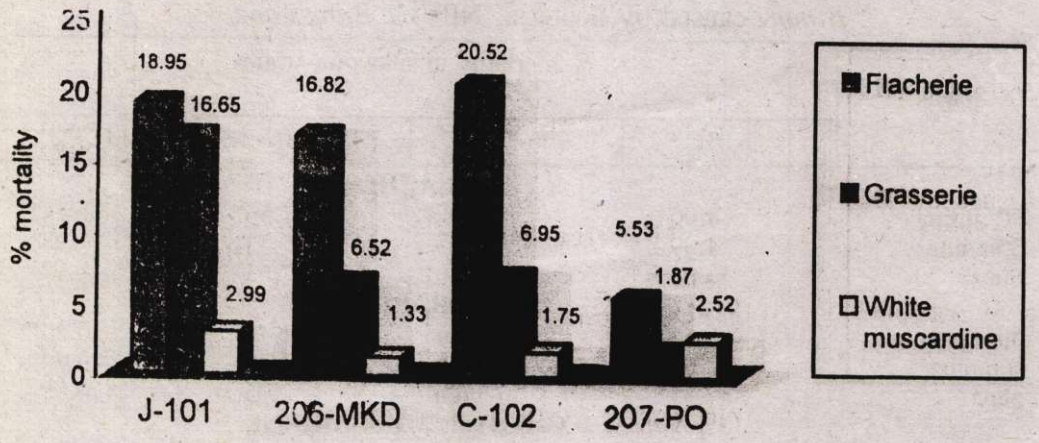

Fig.1: Cumulative percent mortality in four strains of Bombyx mori

207-PO is relatively the most resistant strain with 9.93% cumulative mortality, the resistance is significantly more to grasserie than flacherie ($t_{cal.}$ 3.59 > $t_{tab.}$ 2.120; P95%). On the other side J-101 is relatively the most susceptible strain with 38.59% cumulative mortality and invariably same

degree of susceptibility is found against grasserie and flacherie ($t_{cal.}$ 0.817 < $t_{tab.}$ 2.120; P95%). 206-MKD and C-102 are intermediate between J-101 and 207-PO with no great degree of difference between themselves. Furthermore, 206-MKD ($t_{cal.}$ 4.72 > $t_{tab.}$ 2.120; P95%), C-102 ($t_{cal.}$ 6.93 > $t_{tab.}$ 2.120; P95%) and are significantly more resistant to the grasserie than flacherie. Nearly all the four strains are relatively resistant to white muscardine. This variation in susceptibility towards microbial diseases of different strains of silkworm have also described by Watanabe and Maeda (1981), where they reported J-124, a suseptible strain and Daizo, a non-susceptible strain to denoso nuclear virus (DNV). They also found a single gene that determines either the susceptibility or the non-susceptibility of the insect. Similarly, Shyamala et al. (1987) have reported too that silkworm breed Pure Mysore was more susceptible than the bivoltine breeds NB4D2, NB7 and NB18. Hosa Mysore was intermediate between Pure Mysore and the bivoltines.

J-101 is more susceptible in 5th instar larvae and pupae than spinning larvae. This may be inasmuch to the short span of the latter stadium. But this may also be due to age of the silkworm as Chitra et al., (1974) enumerated that the reaction of the larvae to the pathogen was influenced by its age. The effect of age on resistance and/or susceptibility in two Chinese strains is not profound.

Conclusion

The flacherie and grasserie caused heavy mortality to J-101, while 206-MKD and C-102 are equally prone to flacherie but resistant to grasserie. However, 207-PO is resistant strain to these major diseases. Mortality is comparatively high in 5th instar larvae than spinning larvae and pupae. On the basis of these findings it is recommended that hybrids of resistant strains of silkworm like 207-PO should be encouraged at the sericulture farms to avoid losses to silkworm production. Further, it is argued that resistance of silkworms to microbial diseases, should be considered in breeding programmes along with other economical characteristics.

References

Aruga, H. and S. Tanaka. 1968. Effect of high temperature on resistance of silkworm, *Bombyx mori* L., to flacherie virus disease. Jour. Sericult. Sci. Jap., 37: 441-444.

Basavarajappa, S. and C. J. Savanurmath. 1996. Effect of different mulberry varieties late-age silkworm *Bombyx mori* on incidence of grasserie and the cocoon characters. Jour. Zool., Uttar Pradesh (India). 16(2): 104-108.

Chitra, C., N. G. K. Karanth and V. N. Vasantharajan. 1974. Studies on "Sappe" disease of the silkworm, *Bombyx mori* II. Effect of age of larvae on the manifestation of the disease. Jour. Invert. Path. 24: 248-252.

Hashimoto, Y. and S. Kawase. 1983. Characteristics of structural proteins of infectious flacherie virus from the silkworm, *Bombyx mori*. Jour. Invert. Path. 41: 68-76.

Inoue, H. and Y. Tanada. 1977. Thermal therapy of the flacherie virus disease in the silkworm, *Bombyx mori*. Jour. Invert. Path. 29: 63-68.

Kobayashi, M., S. Inagaki and S. Kawase. 1981. Effect of high temperature on the development of nuclear polyhidrosis virus in the silkworm, *Bombyx mori*. Jour. Invert. Path. 38: 386-394.

Krishnaswami, S., M. N. Narasimhanna, S. K. Suryanarayan and S. Kumararaj. 1973. Sericulture manual; 2-silkworm rearing. FAO Agri. Serv. Bull. 15/2. pp. 131.

Raghavaiah, G. and M. Jayaramaiah. 1987. Antifungal activity of selected plant extracts against the white muscardine fungus. Curr. Res., Uni. Agri. Sci. Bangalore. 17(5): 62-64.

Shyamala, M. B., R. Govindan, A. K. Hadimani, T. K. Narayanaswamy and S. Eswarapp. 1987. Kenchu - a silkworm flacherie in Karnataka. UAS-Tech. Seri. Uni. Agri. Sci. India. 49: pp. 146.

Sivaprakasam, N., S. Jayarani and R. J. Rabindra. 1996. Influence of certain physical factors on the grasserie disease of silkworm, *Bombyx mori* L. Ind. Jour. Sericult. 35: 69-70.

Watanabe, H. 1967. Development of resistance in the silkworm, *Bombyx mori*, to peroral infection of a cytoplasmic polyhidrosis virus. Jour. Invert.Path. 9:474-479.

Watanabe, H. 1981. Characteristics of densonucleosis in the silkworm, *Bombyx mori*. JARQ, 15(2):133-136.

Watanabe, H. and S. Maeda. 1981. Genetically determined nonsusceptibility of the silkworm *Bombyx mori*, to infection with a densonucleosis virus (Densovirus). Jour. Invert. Path. 38: 370-373.