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Studies on the effect of *Bm*NPV stress on the larval and cocoon characters of silkworm, *Bombyx mori* L.

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Abstract— ***The stage of infection and dosage of BmNPV play an imperative role in determining the mortality of silkworm due to grasserie. A study was taken up to determine the induced effect of low, medium and higher doses of BmNPV during the fourth and fifth instars on bivoltine hybrid SK6 X SK7 and crossbreed N X (SK6 X SK7). The degree of susceptibility to BmNPV of both the breeds varied with respect to dosage and stage of administration. The degree of mortality due to grasserie in both the breeds was high in the batches that were infused with a higher dosage of BmNPV POB during the early stages of fourth instar. Larval and cocoon characters of both the breeds were also significantly affected in the batches that were inoculated with a higher dosage at an early stage when compared to the batches that were inoculated at the later stages. Bivoltine hybrids were found to be more susceptible to BmNPV infection in comparison to crossbreeds.***

Keywords— ***Silkworm, Grasserie, BmNPV, Larval and cocoon characters***

I. INTRODUCTION

Silkworm cocoon crops are extremely unpredictable considering the fact that the mulberry silkworm is susceptible to various diseases [1]. The most prevalent disease that causes considerable cocoon crop losses in India is grasserie caused by Bombyx mori Nuclear Polyhedrosis Virus (BmNPV) [2]. BmNPV is an occluded virus which belongs to the subgroup-A of Baculoviridae. Grasserie infected larvae are characterized by various symptoms which include inter segmental swelling, shiny integument, skin becoming fragile and exuding milk white haemolymph on bursting of body wall [3].

Different researchers have evaluated the factors responsible and extent of loss due to grasserie in different regions of India. Environmental factors like high temperature and humidity, persistence of BmNPV polyhedra in the rearing environment are the key factors that contribute to the crop losses due to nuclear polyhedrosis at farmers' level in India. Many studies were undertaken to evaluate different silkworm breeds/hybrids for their relative susceptibility or tolerance to various diseases [4]. The present study attempts to study the effect of different doses of BmNPV during the later instars on bivoltine hybrid SK6 X SK7 and crossbreed N X (SK6 X SK7).

II. METHODOLOGY

Silkworm bivoltine hybrid SK6 X SK7 and crossbreed N X (SK6 X SK7) was reared as per standard methods [5] during August-September, 2016. The leaves of mulberry S-1635 variety were fed. BmNPV inoculum was prepared as delineated by [6]. Different concentrations of BmNPV [(1x103 (low), 1x105 (medium) and 1x107 (high) polyhedral occlusion bodies (POB)/ml)] were prepared by diluting the stock solution in sterile milli-Q water and measuring the same with the help of a Neubauer haemocytometer. The different concentrations mentioned above were fed to the silkworm larvae per orally through mulberry leaves at five different stages (0 and 3rd day of IV instar and 0, 3rd and 5th day of V instar). 250 µl of inoculums was smeared homogeneously on a mulberry leaf disc, measuring 7.8 cm in diameter. Four such leaf discs were fed to each batch of hundred larvae [4].

Experimental as well as control groups had 3 replications consisting of 100 larvae each. The control group of *B. mori* larvae was fed with mulberry leaves treated with sterile distilled water. Various parameters including fourth and fifth instar larval duration, weight of mature larvae, larval and pupal mortality due to grasserie, pupation rate, single cocoon weight, single shell weight and shell percentage were assessed. The data was analysed statistically to study the effect.

III. RESULTS & DISCUSSION

The impact of inoculation of different doses of *Bm*NPV on the larval and cocoon characters of silkworm is depicted in Tables 1&2.

A. Larval duration

The larval duration varied with respect to the dosage and also the stage of inoculation. No significant differences with respect to larval duration were observed when the larvae were administered with a lower dose of *Bm*NPV POB whereas the larval duration increased in the batches that were treated with a higher dosage of *Bm*NPV POB. The larval batches that were inoculated during the early stages of fourth instar, displayed increased larval duration in comparison to the larvae that were inoculated during the later stages (Tables 1&2). The results obtained were in consonance with the ones reported by [7]. [8] reported that an augmented level in production of juvenile hormone and diminishing level of moulting hormone due to *Bm*NPV infection extends the larval period.

B. Larval weight

The larvae [SK6 X SK7 and N X (SK6 X SK7)] that were fed mulberry leaf discs with medium and higher dosages recorded less larval weight in comparison to those administered with lower dosage of *Bm*NPV POB. It was also observed that there was a significant reduction in larval weight in batches that were inoculated with the higher dose of *Bm*NPV POB during the earlier stages in comparison to the same fed during the later stages (Tables 1&2).

C. Mortality due to grasserie

It has been observed that the incidence of larval and pupal mortality due to grasserie in both the breeds SK6 X SK7 and N X (SK6 X SK7) was more in the batches that were administered a higher dose of $1x10^7$ POB/ml when compared to a lower dose of $1x10^3$ POB/ml. The larval and pupal mortality of the batches that were fed a medium dosage of inoculum was in the intermediate range (Tables 1&2). It has also been observed that the larval mortality was high in the batches that were inoculated at an early stage when compared to the batches that were inoculated at the later stages (Tables 1&2). Infection during the early stages of larval development enables the virus to complete many multiplication cycles and cause severe larval mortality [4]. Similar results were also reported by [4].

D. Pupation rate

It was observed that *Bm*NPV infection had adversely affected the pupation rate. Pupation rate of the treatment batches was significantly low in comparison to control. The results obtained corroborates with the results that were reported by [4].

E. Cocoon characters

Significant differences in the cocoon and shell weight were observed with respect to the dosage administered as well as the stage of administration. Larvae of the batches that were administered *Bm*NPV POB in the later stages exhibited more cocoon and shell weights in comparison to those that were inoculated during the earlier stages. Higher dosages also had a significant effect on the cocoon and shell weights. The results of shell percentage of control and the treatment batches are depicted in Tables 1&2. [9] also indicated that there will be a reduction in the economic traits when the infection takes place in the early stages of fifth instar, as the virus significantly affects the feeding and feed conversion efficiency of silkworm.

From the study, it was found that early stage infection of *Bm*NPV can cause considerable mortality in silkworm but when the infection is in the advanced larval stage, mortality reduces considerably. The dose of *Bm*NPV inoculums also plays a major role on the mortality, larval and cocoon characters of silkworm. Bivoltine hybrids were found to be more susceptible to *Bm*NPV infection in comparison to crossbreeds.

Table 1: Effect of *Bm*NPV inoculation on various characteristics of silkworm *Bombyx mori* SK6 X SK7

Treatment	Stage of inoculation	*Bm*NPV Dosage (POB/ ml)	IVth ILD (hr)	IVth moult Duration (hr)	Vth ILD (hr)	Wt. of 10 mature larvae (g)	Mortality due to grasserie (%)		Pupation Rate (%)	Cocoon characters		
							Larval	Pupal		SCW (g)	SSW (g)	Shell %
T0	Control	-	76	24	156	30.1	21.7	17.8	68.65	1.495	0.242	16.19
T1	0 day, IV instar	1×10^3	76	24	156	30.5	42.2	21.1	50.77	1.375	0.213	15.49
T2	0 day, IV instar	1×10^5	80	24	166	28.1	54.4	26.7	37.26	1.33	0.214	16.09
T3	0 day, IV instar	1×10^7	85	24	170	25.2	56.7	25	23.83	1.226	0.2	16.31
T4	3rd day, IV instar	1×10^3	76	24	156	30	51.1	21.1	55.35	1.379	0.233	16.90
T5	3rd day, IV instar	1×10^5	76	24	166	27.7	46.7	30	42.56	1.353	0.218	16.11
T6	3rd day, IV instar	1×10^7	76	24	170	27.7	54.4	26.7	43.81	1.321	0.214	16.20
T7	0 day, V instar	1×10^3	76	24	156	30.8	35.9	15.6	58.18	1.381	0.218	15.79
T8	0 day, V instar	1×10^5	76	24	160	27.9	40	30	43.42	1.36	0.201	14.78
T9	0 day, V instar	1×10^7	76	24	160	27.2	52.2	32.2	27.55	1.331	0.204	15.33
T10	3rd day, V instar	1×10^3	76	24	156	30.3	40	16.7	62.12	1.393	0.213	15.29
T11	3rd day, V instar	1×10^5	76	24	156	29	27.8	27.8	50.5	1.369	0.21	15.34
T12	3rd day, V instar	1×10^7	76	24	156	28	41.1	34.4	36.84	1.333	0.205	15.38
T13	5th day, V instar	1×10^3	76	24	156	28.4	31	18.9	64.44	1.448	0.238	16.44
T14	5th day, V instar	1×10^5	76	24	156	28.6	35	22.2	56.92	1.38	0.233	16.88
T15	5th day, V instar	1×10^7	76	24	156	28.3	33.3	27.6	43.88	1.353	0.208	15.37
		SE±	**0.6**	**0**	**1.15**	**0.35**	**2.61**	**1.64**	**3.21**	**0.02**	**0.003**	**0.24**
		CD	**1.80**	**0.00**	**3.49**	**1.06**	**7.92**	**4.97**	**9.74**	**0.06**	**0.01**	**0.73**

POB/ml: Polyhedral occlusion bodies per milliliter; ILD: Instar larval duration; SCW (g): Single cocoon weight in grams; SSW (g): Single shell weight in grams; SR%: Shell ratio in percentage; SE: Standard error; CD @ 5%: Critical difference @ 5%.

Table 2: Effect of *Bm*NPV inoculation on various characteristics of silkworm *Bombyx mori* N X (SK6 X SK7)

Treatment	Stage of inoculation	*Bm*NPV Dosage (POB/ ml)	IVth ILD (hr)	IVth moult Duration (hr)	Vth ILD (hr)	Wt. of 10 mature larvae (g)	Mortality due to grasserie (%)		Pupation Rate (%)	Cocoon characters		
							Larval	Pupal		SCW (g)	SSW (g)	Shell %
T0	Control	-	96	24	168	32.48	8.00	1.50	100	1.376	0.192	13.95
T1	0 day, IV instar	1×10^3	96	24	168	32.97	32.67	6.73	100	1.267	0.147	11.60
T2	0 day, IV instar	1×10^5	98	26	172	31.42	37.33	10.03	96.66	1.265	0.164	12.96
T3	0 day, IV instar	1×10^7	104	26	192	30.65	42.00	8.75	93.33	1.05	0.136	12.95
T4	3rd day, IV instar	1×10^3	96	24	168	32.74	24.67	6.38	96.66	1.313	0.187	14.24
T5	3rd day, IV instar	1×10^5	96	24	168	30.89	34.67	14.63	76.66	1.289	0.183	14.20
T6	3rd day, IV instar	1×10^7	96	24	192	30.08	41.33	5.45	93.33	1.115	0.14	12.56
T7	0 day, V instar	1×10^3	96	24	168	33.94	14.00	12.26	93.33	1.336	0.178	13.32
T8	0 day, V instar	1×10^5	96	24	168	31.15	24.00	16.58	83.33	1.296	0.175	13.50
T9	0 day, V instar	1×10^7	96	24	180	30.86	40.00	18.43	83.33	1.126	0.141	12.52
T10	3rd day, V instar	1×10^3	96	24	168	32.81	13.33	4.64	66.66	1.343	0.163	12.14
T11	3rd day, V instar	1×10^5	96	24	168	32.66	20.00	13.97	83.33	1.314	0.154	11.72
T12	3rd day, V instar	1×10^7	96	24	172	31.37	27.33	24.22	83.33	1.144	0.151	13.20
T13	5th day, V instar	1×10^3	96	24	168	31.80	9.33	6.00	86.66	1.346	0.187	13.89
T14	5th day, V instar	1×10^5	96	24	168	32.42	11.33	8.52	83.33	1.346	0.176	13.08
T15	5th day, V instar	1×10^7	96	24	168	31.67	14.67	12.42	83.33	1.155	0.161	13.94
		SE±	**0.51**	**0.17**	**2.68**	**0.26**	**3.03**	**1.48**	**2.27**	**0.03**	**0.005**	**0.18**
		CD	**1.55**	**0.52**	**8.13**	**0.79**	**9.19**	**4.49**	**6.89**	**0.09**	**0.02**	**0.55**

POB/ml: Polyhedral occlusion bodies per milliliter; ILD: Instar larval duration; SCW (g): Single cocoon weight in grams; SSW (g): Single shell weight in grams; SR%: Shell ratio in percentage; SE: Standard error; CD @ 5%: Critical difference @ 5%.

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REFERENCES

[1] K.P. Kiran Kumar and S. Sankar Naik, "Development of Polyvoltine X Bivoltine hybrids of mulberry silkworm, *Bombyx mori* L. tolerant to *Bm*NPV," *Int Jou of Zoo res*, Vol. 7(4), pp. 300-309, 2011.

[2] K. Chandrasekharan, B. Nataraju, S.D. Sharma, T. Selvakumar, M. Balavenkatasubbaiah and S.B. Dandin, "Grasserie and post-cocoon mortality in silkworm," *Indian silk*, Vol. 45(4), pp. 12-13, 2006.

[3] Z. Hossain, K. Rahul and S. Chanda, *A Practical Handbook on Silkworm Diseases & Pests*, 1st ed., N.B. Kar., Ed. India: Central Silk Board, 2018.

[4] K. Chandrasekharan, B. Nataraju, M. Balavenkatasubbaiah, S.D. Sharma, T. Selvakumar and S.B. Dandin, "Effect of *Bm*NPV infection during the later instars on the larval and cocoon characters of silkworm, *Bombyx mori* L.," *Ind Jou of sericulture*, Vol. 45(2), pp. 104-109, 2006.

[5] S. Krishnaswami, "*Sericulture Manual, Vol. 2: Silkworm rearing*". FAO Bulletin, Rome, 1978.

[6] H. Sugimori, T. Nagamine and M. Kobayashi, "Analysis of structural polypeptides of *Bombyx mori* (Lepidoptera:Bombycidae) Nuclear polyhedrosis virus," *Appl Entomol Zool*, Vol. 25, pp. 67-77, 1990.

[7] K.M. Vijaya Kumari, M. Balavenkatasubbaiah, R.K. Rajan, M.T. Himantharaj, B. Nataraju and M. Rekha, "Influence of temperature and relative humidity on the rearing performance and disease influence in CSR hybrid silkworm, *Bombyx mori* L." *Int J Indust Entomol*, Vol 3(2), pp. 113-116, 2001.

[8] V.S. Mikhailov, E.A. Zemskov and E.B. Abramova, "Protein synthesis in pupae of the silkworm, *Bombyx mori* after infection with nuclear polyhedrosis virus: resistance to viral infection acquired during pupal period," *J Gen Virol*, Vol. 73(12), pp. 1195-1202, 1992.

[9] G. Satish, R.Govindan, T.K. Narayanaswamy, J. Ashoka and C. Bheemanna, "Instar susceptibility of silkworm, *Bombyx mori* L. to nuclear polyherdrosis virus," *Environ and Ecol*, Vol 7(2), pp. 289-292, 1989.