Original Research Article

Response of Silkworm (*Bombyx mori* L.) Breeds to Temperature and *Bm*NPV Stress

ABSTRACT

BmNPV (*Bombyx mori* nuclear polyhedrosis virus) causes nuclear polyhedrosis in silkworms. NPV can affect *B. mori* in different stages of its life cycle but the infected silkworm expresses disease symptoms during the final stage of larval growth and dies without producing a cocoon resulting in the wastage of time and labour. This paper reports on the larval and pupal mortality rate of silkworm breeds under temperature and *Bm*NPV stress condition. Infection with nuclear polyhedrosis virus after temperature variation at fourth and fifth instar of silkworm causes increase in mortality at 30 ± 1^0 C compared to 25 ± 1^0 C. However, the highest total mortality was recorded in breed PO_1 and least in breed U-3 at both temperatures. Thus, silkworm breed U-3 may have the ability to tolerate BmNPV incidence and can be exploit for evolving disease-tolerant silkworm hybrids.

Keywords: *BmNPV, Silkworm, Breeds, Larval, Mortality*

1. INTRODUCTION

Silkworm, (*Bombyx mori* L.) is sensitive to environmental, nutritional and microbial factors which results in outbreak of various diseases leading to silkworm mortality and cocoon crop loss. The most prevalent and serious diseases in the silkworm are grasserie, flacherie, muscardine and pebrine caused by virus, bacteria, fungi and microsporidia, respectively Jiang and Xia [1]. Among the viral diseases, nuclear polyhedrosis is most severe and contagious. NPV can affect *B. mori* in different stages of its life cycle but the infected silkworm expresses disease symptoms during the final stage of larval growth and dies without producing a cocoon resulting in the wastage of time and labour for the farmers thereby causing significant financial losses thus posing a serious threat to the global sericulture Brancalhao [2]. In India, more than 50 per cent of silk cocoon crop losses are attributed to *Bm*NPV infection Khurad *et al*. [3]. The temperature fluctuations directly influence the incidence of viral diseases. The optimum conditions of temperature (25±1ºC) and humidity (75%) favours the best production and least incidence of nuclear polyhedrosis although with the increase in both factors, the incidence of nuclear polyhedrosis also increases even in uncontaminated batches Basavarajappa and Savanurmath [4].

Among many measures of silkworm disease control and prevention, the utilization of disease tolerant silkworm breeds along with the disinfection would be the most effective step in the direction of the disease prevention Sivaprasad and Chandrashekharaiah [5]. In order to obtain high and stable cocoon yield it is necessary to reduce the incidence which in turn decrease pathogen quantity and pathogenicity and inturn strengthen the larval health by increasing their disease resistance ability Singh *et al*. [6]. The production and use of relatively tolerant silkworm breeds can be a best approach to prevent an infectious disease such as nuclear polyhedrosis. The screening of breeds for their relative tolerance for *Bm*NPV would be helpful in identifying the silkworm breeds that are less susceptible and

they might be exploited commercially to evolve tolerant breeds through breeding plans for increased silk productivity.

The cocoon crop loss due to disease incidence in silkworm breeds is utmost, thus in order to minimize the loss there is a need to evolve tolerant breeds which can perform well even under adverse eco-climatic conditions to get sustainable cocoon yield. Therefore, the present experiment was undertaken to screen the available breeds for their relative tolerance to *Bm*NPV and to identify and utilize the comparatively tolerant breeds for evolving certain crosses for use in future breeding programme.

2. MATERIALS AND METHOD

Twelve bivoltine silkworm breeds namely; WM, ND_5, NB_4D_2, U-4, PO_1, ND_3, U-6, CSR_2, SH_6, SPO, U-3 and NSP maintained at Division of Sericulture, SKUAST-J, Chatha, Jammu were utilized for conducting experiment. The silkworm eggs of above said breeds were incubated at optimum temperature (25±1ºC) and 80±5 per cent relative humidity, followed by black boxing, until hatching in the laboratory. The newly hatched worms were then shifted to rearing beds and reared as per rearing method suggested by Krishnaswami (1978) [7].

2.1 Isolation and purification of polyhedra

Silkworm larvae showing symptoms of grasserie disease like intersegmental swelling and shiny body were collected from the rearing room. Then the haemolymph was collected from each diseased larvae in sterilized glass tubes by outward bending the worm in left hand and cutting the first pair of prolegs followed by gentle press. Test tubes containing the haemolymph subjected for bacterial degradation for 24 hours and centrifuged at 5000 rpm for 10 minutes for pelleting the *Bm*NPV polyhedra. The polyhedra were washed thrice with distilled water and then with 1M NaCl by centrifugation at 5000 rpm for 10 minutes. The polyhedra were then suspended in distilled water and stored at 4ºC in the refrigerator. The polyhedral concentration was determined using the Neubauer's haemocytometer.

2.2 Infectivity technique

Silkworm larvae of each breed were separated into two batches after third moult and fourth moult. Each batch consists of three replications with 50 larvae in each replication. In the first batch- silkworms were kept at temperature 25 ± 1^0 C followed by inoculation and in second batch- silkworms were kept at temperature 30 ± 1^0 C and then inoculated. The infection was carried out per orally by feeding silkworm with virus suspension smeared leaves. First of all, the leaves were washed in running water, shade dried and then sterilized. These mulberry leaves were smeared evenly with the virus suspension at 1×10^3 POB per ml by using non-absorbent cotton. For subsequent feeding inoculum free leaves were used for treated batches. The observations on larval and pupal mortality were recorded in three batches till spinning.

2.3 Observation recorded

The observations were recorded on two parameters as follows.

2.3.1 Larval Mortality (%): Larval mortality was recorded after per oral infection of treated polyhedra of *Bm*NPV. The daily observation of diseased symptoms along with counting of dead larvae was made till the end of spinning Lakshmi *et al.* [8].

2.3.2 Pupal Mortality (%): Pupal mortality was recorded by counting dead pupae from each batch of silkworm breeds Lakshmi *et al.* [8].

3. RESULTS AND DISCUSSION

3.1 Temperature variation and *Bm*NPV inoculum after third moult

Exposure of silkworm breeds to room temperature (25 ± 1^0 C) and high temperature (30 ± 1^0 C) for six hours followed by *Bm*NPV (1×10^3 POB/ml) inoculation on first day of fourth instar recorded significant variation in larval and pupal mortality. At 25 ± 1^0 C temperature, the significant variations were observed in larval and mortality and the maximum larval mortality was recorded in breed PO_1 (27.33±1.45) followed by breeds SPO (23.00±1.73) and NSP (22.00±1.15), while maximum pupal mortality was recorded in breed PO_1 (13.00±1.00) followed by SPO (12.66±0.66), ND_3 (12.33±1.20) and NSP (11.66±0.66). The lowest larval mortality was observed in U-3 (10.66±1.20) that was statistically at par with breeds U-4 (11.00±1.15), ND_5 (12.33±1.45) and U-6 (13.00±1.15) and the least pupal mortality was recorded in breed U-3 (6.66±1.20) followed by breed U-4 i.e. 7.00±0.57 **(Table 1).**

At 30 ± 1^0 C temperature, the larval and pupal mortality varied significantly and the highest larval mortality was recorded in breed PO_1 (34.33±1.45) followed by breed NSP (31.00±1.15) and SPO (30.66±0.67), whereas lowest was recorded in breed U-3 (11.33±0.88) followed by breed U-4 (14.66±1.20) and ND_5 (15.66±1.45). However, the maximum pupal mortality was recorded in breed SPO (18.00±0.57) that was found statistically at par with breeds PO_1 (17.66±0.88) and NB_4D_2 (17.33±0.33), while minimum pupal mortality was observed in breed U-3 (9.66±0.88) followed by breed U-4 i.e. 10.33±0.88 **(Table 1).**

Table 1: Mortality percentage of silkworm breeds due temperature exposures and *Bm*NPV inoculum (1×10^3 POB/ml) after third moult

Breeds	25±1˚C temperature		30±1˚C temperature	
	Larval	Pupal	Larval	Pupal
WM	15.00±1.15^{ab}	10.33±0.33^{abcd}	20.00±1.15^{bcd}	16.00±0.57^{cd}
ND_5	12.33±1.45^{a}	8.33±0.88^{abc}	15.66±1.45^{ab}	11.66±0.88^{abc}
NB_4D_2	15.33±1.20^{abc}	11.33±0.88^{bcd}	22.00±1.15^{cde}	17.33±0.33^{d}
U-4	11.00±1.15^{a}	7.00±0.57^{ab}	14.66±1.20^{ab}	10.33±0.88^{ab}
PO_1	27.33±1.45^{e}	13.00±1.00^{d}	34.33±1.45^{g}	17.66±0.88^{d}
ND_3	20.00±0.57^{bcd}	12.33±1.20^{cd}	24.00±1.15^{de}	16.33±1.45^{cd}
U-6	13.00±1.15^{a}	10.00±0.57^{abcd}	18.00±0.57^{bc}	12.66±1.20^{abcd}
CSR_2	21.66±0.88^{cde}	11.33±0.88^{bcd}	25.66±0.88^{ef}	14.00±0.57^{abcd}
SH_6	21.33±1.45^{bcde}	10.66±1.20^{abcd}	25.66±0.88^{ef}	15.33±1.85^{bcd}
SPO	23.00±1.73^{de}	12.66±0.66^{cd}	30.66±0.67^{fg}	18.00±0.57^{d}
U-3	10.66±1.20^{a}	6.66±1.20^{a}	11.33±0.88^{a}	9.66±0.88^{a}
NSP	22.00±1.15^{de}	11.66±0.66^{cd}	31.00±1.15^{fg}	16.66±1.76^{cd}

Each value is a mean±standard error of three replications.
Figures followed by same letters in column are non-significant by Tukey HSD test

3.2 Temperature variation and *Bm*NPV inoculum after fourth moult

Larvae of Silkworm breeds exposed to room temperature (25±1^0 C) and high temperature (30±1^0 C) for six hours followed by *Bm*NPV (1×10^3 POB/ml) inoculation on first day of fifth instar recorded significant variation in larval and pupal mortality. At 25±1^0 C temperature, significant variations were observed in larval and pupal mortality, the highest larval mortality was recorded in breeds NSP (21.00±1.15) that was statistically at par with PO_1 (20.00±1.15) and SPO (20.00±0.57). The highest pupal mortality was recorded in PO_1 (11.66±0.66) that was statistically at par with CSR_2 (11.00±1.15), SPO (11.00±1.15) and NSP (10.66±1.20). However, the lowest larval and pupal mortality was found in breed U-3 i.e., 7.33±0.88 and 4.66±0.88, respectively. At 30±1^0 C temperature, the larval and pupal mortality varied significantly and the maximum larval mortality was observed in breed PO_1 (41.66±1.20) followed by breed SPO (38.00±1.15). However, the highest pupal mortality was observed in breed PO_1 (18.33±0.88) followed by breed SPO (17.66±1.20), while the least larval mortality and pupal mortality was observed in U-3 (16.00±1.15 and 11.00±1.15), respectively **(Table 2).**

Table 2: Mortality percentage of silkworm breeds due temperature exposures and *Bm*NPV inoculum (1×10^3 POB/ml) after fourth moult

Breeds	25±1˚C		30±1˚C	
	Larval	Pupal	Larval	Pupal
WM	13.00±1.15^{bcd}	7.66±0.88^{abc}	30.33±0.88^{c}	15.66±1.20^{abc}
ND_5	10.66±0.66^{abc}	6.00±0.57^{ab}	22.00±1.15^{ab}	12.33±0.88^{ab}
NB_4D_2	12.33±1.45^{abcd}	9.33±0.88^{bc}	31.00±1.15^{c}	14.33±1.45^{abc}
U-4	9.00±0.57^{ab}	6.00±0.57^{ab}	19.66±1.45^{ab}	11.66±0.88^{a}
PO_1	20.00±1.15^{e}	11.66±0.66^{c}	41.66±1.20^{e}	18.33±0.88^{c}
ND_3	16.66±0.88^{de}	10.00±0.57^{bc}	30.00±0.57^{c}	15.33±1.20^{abc}
U-6	10.00±1.15^{ab}	8.66±0.33^{abc}	23.00±1.15^{b}	13.00±1.15^{abc}
CSR_2	16.66±1.20^{de}	11.00±1.15^{c}	32.33±1.45^{cd}	16.66±1.20^{abc}
SH_6	15.66±1.45^{cde}	9.66±0.88^{bc}	34.00±1.73^{cd}	16.00±1.15^{abc}
SPO	20.00±0.57^{e}	11.00±1.15^{c}	38.00±1.15^{de}	17.66±1.20^{bc}
U-3	7.33±0.88^{a}	4.66±0.88^{a}	16.00±1.15^{a}	11.00±1.15^{a}
NSP	21.00±1.15^{e}	10.66±1.20^{c}	34.33±1.45^{cd}	15.00±1.15^{abc}

Each value is a mean±standard error of three replications.
Figures followed by same letters in column are non-significant by Tukey HSD test.

These findings on variation on mortality rate at different temperature after inoculation of *Bm*NPV are in accordance with the earlier studies of Lakshmi *et al*. [8]. Aruga *et al.* [9] found that temperature higher or lower than 25^0 C act as a stress and tends to increase the larval susceptibility to viral infections. Himeno *et al.* [10] concluded that virus multiplication at 25^0 C temperature in fifth instar larvae went hidden but were reactivated by cold temperature treatment and observed that the incidence of nuclear polyhedrosis virus was higher in sudden changes from room temperature to low temperature. Matsubara [11] studied the

changes in the resistance to NPV by subjecting fourth and fifth instar larvae to high temperature (33, 35 and 37^0 C) treatments at various periods of time immediately after the ecdysis and concluded that temperature and pathogenic virulence are the most important physical agent for induction of diseases in silkworms. Temperature acts as an important external physical factor for both larval susceptibility and multiplication of viruses in the host. Silkworms are adapted to rearing at 25^0 C and temperature higher and lower than this limit increases the susceptibility of silkworm to the virus and this susceptibility decreases with the larval stages. Steinhaus [12] reported that environmental factor such as temperature activates the latent virus and once the occult virus reaches the infective stage, the virus multiplication proceeds, in the same manner as in case of natural infections.

4. CONCLUSION

Observations from the current study, such as larval mortality and pupal mortality of twelve silkworm breeds after temperature variation and inoculation with BmNPV at fourth and fifth instar showed that silkworm breed U-3 recorded the least larval and pupal mortality at both 25±1^0 C and 30±1^0 C. Thus, silkworm breed U-3 may have the ability to tolerate BmNPV incidence and can be exploit for evolving disease-tolerant silkworm hybrids.

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