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

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Authors' contributions

This work was carried out in collaboration among all authors. Author PS designed the study and wrote the first draft of the manuscript. Author SC performed the statistical analysis. Author AS wrote the protocol. Author KA managed the analyses of the study and author SA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Silkworm *Bombyx mor*i L. is one of the most important domesticated insects, which produces luxuriant silk thread in the form of cocoon by consuming mulberry leaves during larval period. The growth and development of silkworm is greatly influenced by environmental conditions. The seasonal differences in the environmental components considerably affect the genotypic

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expression in the form of phenotypic output of silkworm crop. *Bombyx mori* nucleopolyhedrovirus (*Bm*NPV) is a pathogen that causes great economic losses in sericulture. In the present investigation, twelve bivoltine silkworm breeds (WM, ND_5, NB_4D_2, U-4, PO_1, ND_3, U-6, CSR_2, SH_6, SPO, U-3 and NSP) were studied for temperature and *Bm*NPV stress. After third and fourth moult silkworm larvae were treated with *Bm*NPV inoculum at 25±1ºC and 30±1ºC temperature respectively. Increased mortality was observed at 30±1ºC when compared to 25±1ºC. However, with respect to breeds the highest total mortality was recorded in breed PO_1 and least in breed U-3. Silkworm breed U-3 may have the ability to tolerate *Bm*NPV 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 METHODS

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 surface sterilized by putting eggs in 2% formaldehyde solution for 2-3 minutes. After sterilization, the eggs were washed in running water till smell of formalin goes off and then dried the eggs in shade. The eggs were then incubated for twelve days at optimum temperature (25±1ºC) and 80±5 per cent relative humidity, followed by black boxing. On Thirteen day, the silkworm larvae were hatched out and the newly hatched worms were then shifted to rearing beds and reared as per rearing method suggested by Krishnaswami [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. Each batch having two treatments and each treatment consists of three replications with 50 larvae in each replication. In the first treatment- silkworm breeds after third moult were kept at 25±1ºC temperature and then inoculated, in second treatment - silkworm breeds after third moult were kept at 30±1ºC temperature and inoculated, in third treatment - silkworm breeds after fourth moult were kept at 25±1ºC temperature followed by inoculation and in fourth treatment - silkworm breeds after fourth moult were kept at 30±1ºC temperature and then inoculated. The silkworm larvae of fourth and fifth instar were selected because the silk gland is well developed during these instars and occurrence of disease is maximum at these stages. The silkworm larvae were starved during the temperature treatment period for six hours. The infection was carried out per orally by feeding silkworm with virus suspension smeared leaves. The leaf bits (10×12 cm size) were prepared, washed in running water and shade dried. These mulberry leaves were smeared evenly with the virus suspension at 1×10^3 POB per ml by using non-absorbent cotton. After shade dried for five minutes, the leaf fed to the silkworm larvae. For subsequent feeding inoculum free leaves were used for treated and untreated batches. The observation on mortality due to BmNPV was recorded up to pupal stage.

3. RESULTS AND DISCUSSION

3.1 Temperature Variation and *Bm*NPV Inoculum after Third Moult

Exposure of silkworm breeds to room temperature (25±1ºC) and high temperature (30±1ºC) for six hours followed by *Bm*NPV (1×10^3 POB/ml) inoculation on first day of fourth instar recorded significant variation in total mortality. At 25±1ºC temperature, the significant variations were observed in total mortality and the maximum total mortality was recorded in breed PO_1 (40.33±1.45) followed by breeds SPO (35.67±1.73) and NSP (33.67±1.15). The lowest total mortality was observed in U-3 (17.33±1.20) (Table 1). At 30±1ºC temperature, the total mortality varied significantly and the highest total mortality was recorded in breed PO_1 (52.00±1.00) followed by breed SPO (48.67±0.66) and NSP (47.67±0.66), whereas least total mortality was recorded in breed U-3 (21.00±1.20) (Table 1). With respect to control batch the maximum total mortality was recorded in breed PO_1 (5.33±0.33) followed by breed NSP (5.00±0.57) and NB_4D_2 (4.66±0.88), whereas least total mortality was recorded in breed U-3 (2.00±0.57) (Table 1).

3.2 Temperature Variation and *Bm*NPV Inoculum after Fourth Moult

Larvae of Silkworm breeds exposed to room temperature (25±1ºC) and high temperature (30±1ºC) for six hours followed by *Bm*NPV (1×10^3 POB/ml) inoculation on first day of fifth instar recorded significant variation in total mortality. At 25±1ºC temperature, the highest total mortality was recorded in breeds NSP (31.67±1.15) that was statistically at par with PO_1 (31.67±1.15). However, the lowest total mortality was found in breed U-3 (12.00±0.88). At 30±1ºC temperature, the total mortality varied significantly and the maximum larval mortality was observed in breed PO_1 (60.00±0.66) followed by breed SPO (55.67±1.15), while the least total mortality was observed in U-3 (27.00±0.88) (Table 2). With respect to control batch the maximum total mortality was recorded in breed PO_1 (5.33±0.33) followed by breed NSP (5.00±0.57) and NB_4D_2 (4.66±0.88), whereas least total mortality was recorded in breed U-3 (2.00±0.57) (Table 2).

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

Breeds	Total mortality		
	25±1ºC	**30±1ºC**	**Control**
WM	25.33±1.15	36.00±0.33	4.00±0.57
ND_5	20.67±1.45	27.33±0.88	3.66±0.88
NB_4D_2	26.67±1.20	39.33±0.88	4.66±0.88
U-4	18.00±1.15	25.00±0.57	3.00±0.57
PO_1	40.33±1.45	52.00±1.00	5.33±0.33
ND_3	32.33±0.57	40.33±1.20	4.66±0.33
U-6	23.00±1.15	30.67±0.57	4.00± 0.57
CSR_2	33.00±0.88	39.67±0.88	4.00±0.57
SH_6	32.00±1.45	41.00±1.20	4.33±0.33
SPO	35.67±1.73	48.67±0.66	4.00±0.57
U-3	17.33±1.20	21.00±1.20	2.00±0.57
NSP	33.67±1.15	47.67±0.66	5.00±0.57

Each value is a mean±standard error of three replications

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

Breeds	Total mortality		
	25±1˚C	**30±1˚C**	**Control**
WM	20.67±1.15	46.00±0.88	4.00±0.57
ND_5	16.67±0.66	34.33±0.57	3.66±0.88
NB_4D_2	21.67±1.45	45.33±0.88	4.66±0.88
U-4	15.00±0.57	31.33±0.57	3.00±0.57
PO_1	31.67±1.15	60.00±0.66	5.33±0.33
ND_3	26.67±0.88	45.33±0.57	4.66±0.33
U-6	18.67±1.15	36.00±0.33	4.00± 0.57
CSR_2	27.67±1.20	49.00±1.15	4.00±0.57
SH_6	25.33±1.45	50.00±0.88	4.33±0.33
SPO	31.00±0.57	55.67±1.15	4.00±0.57
U-3	12.00±0.88	27.00±0.88	2.00±0.57
NSP	31.67±1.15	49.33±1.20	5.00±0.57

Each value is a mean±standard error of three replications

Success of silkworm breeds depends largely on their adaptability to the environment in which it is destined to be reared. It is clear from the results, under BmNPV inoculated conditions the survival rate of silkworm breeds decline to that of control. Extensive work has been done on the induction of viral diseases. Among them the important physical agents which induce diseases are temperature and pathogenic virulence. Aruga et al. [8] found that temperature higher or lower than 25ºC act as a stress and tends to increase the larval susceptibility to viral infections. Sowmyshree et al. [9] studied the influence of temperature on resistance of the breed was much higher when the host was infected by the pathogen even at low dosage. Himeno et al. [10] concluded that virus multiplication at 25ºC 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 37ºC) 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 25ºC 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. Wojda [13] reviewed that heat-shock proteins are induced by infection as well as heat shock, and also play an important role in the immune response of insects. Such stress components function as immune modulators (Palmer [14]). Lakshmi et al. [15] studied the stability ten hybrids under fluctuating tropical environmental conditions with *Bm*NPV inoculation at room temperature (25ºC), low temperature (5ºC) and high temperature (32ºC). They recorded maximum mortality at 5ºC and minimum mortality at 32ºC. In this study, the larvae when exposed to 30±1ºC temperature, the least mortality was observed in U-3 breed that indicates its high level of tolerance to temperature and *Bm*NPV at fourth and fifth instar.

4. CONCLUSION

Observations from the current study, such as total larval mortality of twelve silkworm breeds (ND_5, NB_4D_2, U-4, PO_1, ND_3, U-6, CSR_2, SH_6, SPO, U-3 and NSP) after temperature variation and inoculation with BmNPV at fourth and fifth instar showed that silkworm breed U-3 recorded the least total larval mortality at both 25±1ºC and 30±1ºC. 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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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