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Studies on the susceptibility and cross infectivity of mulberry pest *Glyphodes pyloalis* Walker to silkworm, *Bombyx mori* L

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Abstract

In the present study, susceptibility of the mulberry pest *G. pyloalis* Walker to the pathogens of silkworm, *B. mori* L. and susceptibility of the silkworm, *B. mori* to the pathogens of mulberry pest *G. pyloalis* was ascertained. During the study various stages of *G. pyloalis* were found infected with the Microsporidian (Pebrine) and Nuclear Polyhedral Virus (*BmNPV*) whereas, fungal and bacterial pathogens were not observed during the present study. The mean incidence of microsporidian and NPV was observed as 4.22% and 5.99%, respectively. Silkworm, *Bombyx mori* inoculated with the pathogens isolated from *G. pyloalis* (Microsporidian and Nuclear Polyhedral Virus) showed high mortality at larval and pupal stages. However, silkworms inoculated with Microsporidian showed mortality of 53.66% at larval stage whereas silkworms inoculated with Nuclear Polyhedral Virus showed mortality of 61.00% at larval stage. The study revealed that *G. pyloalis* besides being a major pest of mulberry causing severe damage to the leaves also acts as alternate host for the pathogens causing diseases of silkworm. As such, this pest needs to be managed to prevent loss to the mulberry plantations as well as spread of silkworm disease causing pathogens.

Keywords: *Bombyx mori*, cross infectivity, *Glyphodes pyloalis*, mulberry, pest, susceptibility

1. Introduction

Sericulture is an art of rearing silkworms for the production of cocoons which is the raw material for silk production. India has a unique distinction of being the only country producing all the four known commercial silks namely Mulberry, Tasar, Eri and Muga. Mulberry (*Morus* sp.) is the only host plant of silkworm, *Bombyx mori* L. Sericulture is grouped under village and small enterprises sector that plays major role for the creation of sustainable employment and income generation [1]. There are several factors that hinder the productivity as well as quality of mulberry leaves, among them incidence of pests and diseases acts as major one. The importance of quality of mulberry leaves on the growth, development and silk production in silkworm is well documented [2].

The plantation is ravaged by different pests and diseases and as many as 11 major and 10 minor insect pests have been reported from Jammu and Kashmir [3]. The mulberry varieties like *Morus alba*, *Morus indica* are attacked by a number of pests like *Pseudococcus comstocki*, *Agrotis ipsilon*, *Macanalicoccus hirsutus*, *Diaphania pulverulentalis* and *Glyphodes pyloalis* Walker causing heavy damage to the mulberry foliage by defoliation and skeletonization. *G. pyloalis* mostly prevalent in summer and autumn season is a threat to the second commercial crop. Under temperate conditions, Susceptibility (Walker) causes severe damage to mulberry crop and is considered as a major pest of mulberry in Jammu and Kashmir [4].

The perpetual incidence of microsporidian infection in silkworm may be due to various sources of secondary contamination or cross infection from the alternate hosts [5]. The periodic occurrence of Pebrine disease in the rearing field indicates the possibility of cross infection of Pebrine spore from the other alternate host, [6]. The *G. pyloalis* Walker is the habitual host of non-occluded viruses pathogenic to the silkworm *Bombyx mori* L. [7].

2. Materials and Methods

Healthy *G. pyloalis* larvae collected from the mulberry farms of College of Temperate sericulture, Mirgund were screened against the pathogens of silkworm, *B. mori* viz.

Microsporidia and Nuclear Polyhedrosis virus. The pathogens isolated from the silkworm, *B.mori* L. were tested against leaf roller, *G. pyloalis* for their pathogenicity. The suspension of the pathogen was prepared in distilled water and diluted to obtain an inoculum of 1×10^6 units/ml.

The second instar larvae of *G. pyloalis* were inoculated with the pathogens by smearing the inoculums on the mulberry leaf @ 1 ml/10 sq.cm leaf area, allowing it to shade dry and fed to the larvae in the sterilized glass jars which were covered with the muslin cloth and ensuring that larva consume leaf for at least 12 hours. After which fresh leaf was provided to the larvae till feeding period is over. Three replications of 25 larvae were maintained for each inoculating pathogen. The mortality due to infection by specific pathogen was recorded and the dead/diseased larvae were regularly examined for the presence of pathogen to determine the pathogenicity.

Disease free laying's of silkworm race (CSR4) obtained from Division of Sericulture Crop improvement of CTS, Mirgund were reared in Silkworm rearing laboratory CTS, SKUAST-K Mirgund during Autumn (September-October 2017) upto 3rd instar. Immediately after 3rd moult larvae were inoculated with the pathogens isolated from the *G. pyloalis* Walker. For each inoculating pathogen six replications were maintained with 150 worms in each replication. Required concentration was prepared (1×10^6 spores/ml) from stock solution and quantified to estimate the spore concentration following standard haemocytometer count [8]. One ml of inoculums was

smear on mulberry leaves, the leaves were allowed to shade dry and then fed to the third instar silkworm larvae of CSR4 breed immediately after 3rd moult. The larvae were allowed to feed on the treated leaves for 12 hrs. to ensure complete consumption of the contaminated leaves. After 12th hour, the larvae were fed with normal mulberry leaves and reared till cocooning. A control batch of healthy larvae was also maintained separately under the same laboratory conditions. Any larval mortality observed during the rearing process was recorded.

3. Results

3.1 Susceptibility of *G. pyloalis* larvae to the pathogens isolated from silkworm, *B. mori* L.

The observations recorded on the susceptibility of *G. pyloalis* larvae inoculated with the pathogens of microsporidian (*Nosema bombycis*) and Nuclear Polyhedrosis virus (NPV) isolated from the silkworm *B. mori* showed high incidence of both the pathogens in the *G. pyloalis* with high mortality at larval and pupal stages (Table 1). Total mortality due to *N. bombycis* inoculation was recorded as 90.64% where as the mortality rate due to Nuclear Polyhedrosis virus (NPV) was recorded as 92.00%. This high mortality of larval and pupal stages of *G. pyloalis* clearly showed that *G. pyloalis* serves as alternate host to the pathogens of the silkworm *B. mori*, thereby acting as potential source for the spread of the silkworm diseases.

Table 1: Susceptibility of *G. pyloalis* to the pathogens of silkworm *B. mori* L

Silkworm Pathogen Inoculated	Mortality at Larval and Pupal stages of <i>G. pyloalis</i>		Total mortality (%)
	Larval Stage	Pupal Stage	
<i>Nosema bombycis</i>	15	7.66	90.64
Polyhedral virus	21	2	92.00
Control (No inoculation)	1	0	4.00

3.2 Effect of pathogens isolated from *G. pyloalis* on survival rate and economic parameters of silkworm, *B. mori* L.

The results with respect to effect of pathogens isolated from *G. pyloalis* on survival rate and economic parameters of silkworm, *B. mori* L. are presented in Table 2. The study revealed that no significant differences with regard to the total larval duration were observed. However, minimum larval duration was recorded in T2 (648.33h) followed by T3 (652.16 h) and T1 (654.66 h). Larval mortality recorded significant difference between the treatments. The highest larval mortality was recorded in T2 (61.00%) followed by T1 53.66% and the lowest larval mortality was recorded in normal batch (4.00%). The cocoon yield per 10,000 larvae by number was recorded in various treatments (Table 2). The lowest cocoon yield of 3800 was recorded in T2, followed by 4633 in T1 and the highest cocoon yield of 9600 in T3 (normal batch). Significant decrease was observed in cocoon yield by number was recorded in T2 (3,800), followed by T1 (4,633) and highest was recorded in normal batch (9,600).

Further, there was reduction in cocoon yield by weight also. The lowest was recorded in T2 (5.58kg) followed by T1 (6.58kg) and highest cocoon yield by weight was recorded in T3 (normal batch) (17.33kg). With respect to survival percentage, significant differences were recorded between the treatments and the lowest was recorded in T1 (36.33%) followed by T1 (38.00%) and the highest was recorded in T3 normal batch (94.00%). There were no significant difference between the treatments so far as single cocoon weight is concerned and the data ranged from 1.60 g to 1.85 g (Table 2). The lowest single shell weight was recorded in T1 (0.24 g) followed by T2 (0.31 g) and T3 (0.36 g). There were significant difference in single shell weight and T3 was recorded statistically superior over other treatments. The lowest shell ratio percentage was recorded in T1 (14.88%) followed by T2 (19.57%) and the highest was recorded in T3 (21.67%). However, no significant differences were recorded in raw silk percentage as raw silk percentage ranged from 12.32% to 12.77% (Table 2).

Table 2: Effect of pathogens isolated from *G. pyloalis* on various economic characters of silkworm, *B. mori* L.

Pathogen Inoculated	Total larval Duration (hrs.)	Larval mortality (%)	Cocoon yield /10000 larvae by number (No.)	Cocoon yield/ 10000 larvae by weight (kg)	Survival Percentage (%)	Single Cocoon weight (g)	Single shell Weight (g)	Shell ratio (%)	Raw Silk (%)
<i>Nosema bombycis</i> (T1)	654.66	53.66 (7.38)	4,633	6.58	36.33 (36.89)	1.60	0.24	14.88	12.32
NPV (T2)	648.33	61.00 (7.86)	3,800	5.58	38.00 (6.23)	1.58	0.31	19.57	12.50
Control (T3) (Without inoculation)	652.16	4.00 (2.20)	9,600	17.33	94.00 (9.75)	1.85	0.36	21.67	12.77
C.D.	NS	0.536	624.484	1.156	5.695	NS	0.445	2.578	NS

4. Discussion

In the present study with regards to the susceptibility of *G. pyloalis* of silkworm *B. mori* L. Total mortality rate of 90.64% and 92.00% when *G. pyloalis* was inoculated with *N. bombycis* and Nuclear Polyhedrosis virus (NPV) respectively. Studying the cross infectivity between pathogens of silkworm, *Bombyx mori* L. and mulberry leaf roller, *D. pulverulentalis* (Hampson) observed that out of 1,000 larvae screened, 85 larvae were infected with microbes ^[9]. The pathogenic microbes isolated from *D. pulverulentalis* i.e. Microsporidian, *B. bassiana* and *bacteria* caused mortality of 66-80%, 100% and 12-28% respectively in silkworm. The cross infectivity of *B. mori* L. Nuclear Polyhedrosis virus (NPV) and *B. mori* Kenchu virus to the larvae of *D. pulverulentalis* infesting mulberry ^[10]. It has been reported that 60% infection in silkworm by a microsporidian isolated from a butterfly, *peris* sp ^[11].

The effect of pathogens isolated from *G. pyloalis* on the survival rate and economic parameters of silkworm *Bombyx mori* showed no significant difference for total larval duration. The longest total larval duration was recorded in T1 (654.66 h), followed by T3 (652.16 h) and T2 (648.33 h). The highest larval mortality (61.00%) was found in T2 (NPV) which was followed by T1 (*N. bombycis*) 53.66% and lowest was (4.00%) recorded in T3 (normal) which may be due to the inoculums given to the silkworms. Similar findings were observed while studying the impact of new microsporidian infection on larval and cocoon parameters of the silkworm *Bombyx mori* L. ^[12]. The microsporidian has resulted in low larval and pupal mortality but remarkably high infection percentage in moth stage at 1×10^5 and 1×10^6 spore/ml inoculation doses. It has been reported the cross infectivity of Pebrine disease from muga to eri silkworm ^[13]. While studying the cross infectivity between pathogens of silkworm, *Bombyx mori* L. and mulberry leaf roller, *D. pulverulentalis* (Hampson) it has been observed that out of 1,000 larvae screened, 85 larvae were infected with microbes ^[9]. The pathogenic microbes isolated from *D. pulverulentalis* i.e. microsporidian, *B. bassiana* and *bacteria* caused mortality of 66-80%, 100% and 12-28% respectively in silkworm.

The cocoon yield by number (No.) as well as weight (kg) was found lowest in T2 (3800 and 5.58 kg respectively) followed by T1 4633 and 6.58 kg as compared to the normal control (9600 and 17.33 kg) respectively. The highest survival percentage (94.00%) was recorded in T3 (normal) & lowest was (38.00%) was recorded in T2 (NPV). The cross infectivity of *B. mori* L. has been reported that Nuclear polyhedrosis virus (NPV) and *B. mori* Kenchu virus to the IV instar larvae of *D. pulverulentalis* infesting mulberry ^[10].

The cocoon characters viz., single cocoon weight, single shell weight and shell ratio are adversely affected. There was no significant difference between the treatments so far as single cocoon weight is concerned and the data ranged from 1.60 g

to 1.85 g. The highest single cocoon weight (1.85 g) was recorded in T3 (normal) & lowest was (1.58 g) was recorded in T2 (NPV). The highest single shell weight (0.36 g) was recorded in T3 (normal) and lowest (0.24 g) was recorded in T1 (*Nosema bombycis*) which may be due to inoculum given to the silkworms. In case of Shell ratio the highest was recorded in T3 (normal) 21.67% followed by T2 (NPV) 19.57% and the lowest was observed in T1 (*N. bombycis*) 14.88% which may be due to the inoculum given to the silkworms, as the diseases spread by the occluded bodies (OBS) in the blood cells of infected silkworm. These results are inconformity with the results of ^[14] who reported that the spread of Grasserie (*BmNPV*) disease of silkworm in India and concluded that the disease spreads by the occluded bodies (OBS) in the blood cell of infected silkworm as it effects the consumption of less food of the silkworm Silk from the cocoons of Pebrine infected larvae is inferior in strength and uniformity of thickness to that of healthy larvae ^[15]. It has been reported that Pebrine infected silkworms spin flimsy and poor quality cocoons ^[16].

The effect of pathogens on raw silk percentage was recorded and there was no significant difference recorded in raw silk percentage. In case of raw silk percentage the highest was recorded in T3 normal (12.77%) followed by T2 (NPV) 12.50% and lowest was recorded in T1 (*N. bombycis*) 12.32%.

5. Conclusion

The study was conducted to ascertain the susceptibility of the mulberry pest *G. pyloalis* Walker to the pathogens of silkworm, *B. mori* L. and susceptibility of the silkworm, *Bombyx mori* to the pathogens of mulberry pest *G. pyloalis*. The observations recorded on the susceptibility of *G. pyloalis* larvae inoculated with the pathogens Microsporidian (*N. bombycis*) and Nuclear Polyhedrosis virus (NPV) isolated from the silkworm *Bombyx mori* showed high incidence of both the pathogens in the *G. pyloalis* with high mortality at larval and pupal stages. The study undertaken to ascertain the susceptibility of silkworm, *B. mori* to the pathogens isolated from *G. pyloalis* by inoculation method showed mortality of 53.66% at larval stage due to Microsporidian (*Nosema bombycis*) and 61.00% when inoculated with Nuclear Polyhedrosis Virus. The overall survival rate and pupation rate was significantly lower as compared to the normal rearing (without inoculation). These observations clearly showed that silkworm *Bombyx mori* is highly susceptible to the pathogens isolated from *G. pyloalis* and call for devising propre management strategies against the pest.

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