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Cross Infectivity of *Glyphodes pyloalis* Walker Infested Mulberry Leaves on the Rearing Performance of Silkworm, (*Bombyx mori* L.)

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A B S T R A C T

In the present study, susceptibility of the silkworm, *Bombyx mori* to the pathogens of mulberry pest *Glyphodes pyloalis* was ascertained. Silkworm, *Bombyx mori* inoculated with the pathogens isolated from *G. pyloalis* (Microsporidian and Nuclear Polyhedral Virus) showed high mortality at larval and pupal stages. 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 survival rate and pupation rate besides the other economical characters were also affected in the inoculated batches of silkworm. The *v* larvae inoculated with the pathogens of silkworm showed mortality of 90.64% and 92.00% due to Microsporidian and Nuclear Polyhedral Virus inoculation respectively. The study has 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.

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. The production on scientific lines is essential for organizing sericulture on sound economic lines. From the economic point of view, moriculture coupled with silkworm rearing remains a highly labour intensive activity providing vast scope for employment. 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 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 (Sharma and Tara, 1985; Khan *et al.,* 2004). Over 300 insect and non-insect species of pests are known to attack mulberry (Narayanaswamy *et al.,* 1996; Reddy and Kotikal, 1988). From Karnataka alone more than 100 while from Maharashtra 20 insect pests have been reported so far from mulberry (Sathe and Mulla, 1999). A number of parasitoids and predators have been recorded on this pest from most parts of Jammu and Kashmir and elsewhere in Asia (Mathur, 1980). 53 natural enemies comprising of 37 parasitoids, 6 predators and 10 pathogens are known to attack mulberry leaf Webber (Manjunathgowda *et al.,* 2005) The presence of various silkworm pathogenic microbes have been reported in Bihar hairy caterpillar (*Diacrisia oblique*) which is a major pest of mulberry (Sharma *et al.,* 1989).

Silkworms are susceptible to a number of diseases caused by different infectious agents such as protozoan, viral fungal and bacterial diseases. It is the main factor seriously affecting the cocoon production. During the silkworm rearing, the silkworm comes into contact with pathogenic agents (viz., Microsporidian, virus, fungi and bacteria) which accounts for considerable loss to cocoon production (Samson 1995). Mulberry (*Morus* sp.) is the only host plant of silkworm, *Bombyx mori* L. Different mulberry pests and lepidopterans were known to harbor microsporidian (Sharma *et al.,* 1989; Srikanta, 1987 and Chandra, 1987). Ishihara &Iwano, (1991) reported that the perpetual incidence of microsporidian infection in silkworm may be due to various sources of secondary contamination or cross infection from the alternate hosts.

The periodic occurrence of Pebrine disease in the rearing field indicates the possibility of cross infection of Pebrine spore from the other alternate host, (Bashir and Sharma, 2008). *Nosema* spp, *Pleistophora* spp and *Leptomonas* spp. infect silkworm *Bombyx mori* and causes microsporodiosis (Jolly, 1986 and Abe, 1978). There are other microsporidia besides *Nosema bombycis* that infect silkworm, *Bombyx mori*, which is generally thought to be the result of cross infection of microsporidia in silkworm and other insects (Kishore *et al.,* 1994; Bhat *et al.,* 2009).

Bombyx mori Nucleopolyhedrovirus (B*m*NPV) is the most harmful virus causing severe economic loss to the sericulture industry (Ponnuvel *et al.,* 2003). This disease is commonly termed as grasserie/ jaundice/ milky disease/fatty degeneration or hanging disease. The *Glyphodes pyloalis* Walker is the habitual host of non-occluded viruses pathogenic to the silkworm *Bombyx mori* L. (Watanabe *et al.,* 1988).

Materials and Methods

Collection of mulberry pest *G.pyloalis* Walker

A survey was conducted of the mulberry farms of College of Temperate Sericulture (CTS), Mirgund and Mulberry Farms of Sericulture Development Department, J&K located at Mirgund and Tulsibagh, Srinagar respectively during Spring 2017. Survey during the incidence of *Glyphodes pyloalis* Walker was conducted by adopting fixed plot method. In each mulberry garden five micro plots were considered (4 at corners and 1 at the middle of mulberry farm). 15 randomly selected plants in each micro plot were observed for pest infestation (75 plants/ mulberry farm). The per cent pest infestation was calculated by using the formula:

$$PI = \frac{\text{No. of infested leaves}}{\text{Total no. of leaves observed}} \times 100$$

The healthy samples collected during survey

were brought to the laboratory of CTS, Mirgund which were reared and utilized for further experiments. However the diseased and dead samples were taken to isolated place and processed/homogenized and smear prepared thereof was centrifuged for 5 minutes at 3000 rpm and then examined under microscope (600x) to observe presence of pathogens. The morphological details of the pathogens were recorded for their identification.

Studies for the susceptibility of silkworm, *Bombyx mori* to the pathogens of *G. pyloalis*

Under this experiment the pathogens collected/isolated from *G. pyloalis* were tested for their pathogenicity to the silkworm, *B. mori.* Disease free layings 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 *Glyphodes 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 (Cantwell, 1974). One ml of inoculums was smeared 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 h to ensure complete consumption of the contaminated leaves. After 12h, 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.

Results and Discussion

Incidence of Pathogens in *G. pyloalis* under natural conditions

Survey conducted during the year 2017 at various locations viz., CTS Mirgund, SDD Mirgund and SDD Tulsibagh, to ascertain the incidence of pathogens in *Glyphodes pyloalis* showed presence of Microsporodia (*Nosema bombycis*) and Nuclear Polyhedrosis virus (NPV). The percent incidence of these pathogens in *G.pyloalis* larvae is presented in Table 1which showed mean incidence of *Nosema bombycis* in *G.pyloalis* as 1.11%, 2.22%, 1.99%, 3.33% and 4.22% during the months of June, July, August, September and October respectively. So for as stations are concerned, the maximum mean incidence was recorded in CTS Mirgund (3.59%) followed by SDD Mirgund (3.19%) and minimum mean incidence was recorded in SDD Tulsibagh (0.92%). The mean incidence of NPV in *G.pyloalis*is presented in table 2 as 1.10, 2.44%, 4.21%, 5.10% and 5.99% during the months June, July, August, September and October respectively. Among the stations, the maximum incidence was recorded in SDD Mirgund (4.66%) followed by CTS Mirgund (4.39%) and the minimum incidence were recorded in SDD Tulsibagh (2.26%). The low incidence of the disease at SDD, Tulsibagh is mainly due to less silkworm rearing in the vicinity of the area.

Effect of pathogens isolated from *G. pyloalis* on Survival rate and economic parameters of silkworm, *Bombyx mori* L.

The effect of pathogens Microsporodia (*Nosema bombycis*) and Nuclear Polyhedrosis virus (NPV) isolated from *Glyphodes pyloalis* on the Survival rate and economic parameters

is presented in tables 2, 3, 4 and 5 observations recorded are described as under.

Total larval duration

No significant difference with regards to the total larval duration was observed. However, minimum larval duration was recorded in T2 (648.33h) followed by T3 (652.16 h) and T1 (654.66 h).

Average larval weight

There was significant difference in average larval weight and the highest larval weight was recorded in T3 (32.51 g) followed by T2 (31.68 g) and the lowest was recorded in T1 (27.21 g).

Larval mortality

In case of larval mortality it shows 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%).

Cocoon yield by number

The cocoon yield per 10,000 larvae by number was recorded in various treatments. 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 were 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).

Cocoon yield by weight

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).

Survival percentage (%)

In Survival percentage, significant difference was 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%).

Pupation rate (%)

In case of pupation rate, the lowest was recorded in T2 (63.31%), T1 (77.42%) and T3 (96.86%). Significant differences were observed in pupation rate where lowest was recorded in T2 (63.31%) and highest was recorded in T3 (96.86%).

Cocoon parameters

There was no significant difference between the treatments so for as single cocoon weight is concerned and the data ranged from 1.60 g to 1.85 g. 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%).

No significant difference was recorded in filament length as well as in raw silk percentage; however the filament length ranged from 816.03 to 887.70 and raw silk percentage ranged from 12.32% to 12.77%.

In case of Denier 2.20, 2.26 and 2.68 were recorded T1, T2 and T3 respectively. Significant difference was recorded in denier being lowest in T1 and highest in T3.

Table.1 Incidence of *Nosema bombycis* in *G. pyloalis* under natural conditions (2017)

Stations / Months	S1 CTS, Mirgund	S2 SDD, Mirgund	S3 SDD, Tulsibagh	Mean
M1(June)	1.33 (1.43)	1.99 (1.64)	0.00 (1.00)	1.11 (1.36)
M2(July)	3.33 (2.00)	2.66 (1.78)	0.66 (1.21)	2.22 (1.66)
M3(Aug)	2.66 (1.78)	2.66 (1.78)	0.66 (1.21)	1.99 (1.59)
M4(Sep)	4.66 (2.35)	3.99 (2.13)	1.33 (1.43)	3.33 (1.97)
M5(Oct)	5.99 (2.60)	4.66 (2.35)	1.99 (1.56)	4.22 (2.17)
Mean	3.59 (2.20)	3.19 (1.93)	0.92 (1.28)	

Table.2 Incidence of NPV in *G. pyloalis* under natural conditions (2017)

Stations / Months	S1 CTS, Mirgund	S2 SDD, Mirgund	S3 SDD, Tulsibagh	Mean
M1(June)	1.33 (1.43)	1.33 (1.43)	0.66 (1.21)	1.10 (1.35)
M2(July)	2.66 (1.78)	3.33 (2.00)	1.33 (1.43)	2.44 (1.73)
M3(Aug)	5.99 (2.60)	4.66 (2.24)	1.99 (1.64)	4.21 (2.16)
M4(Sep)	5.32 (2.49)	6.66 (2.74)	3.33 (2.00)	5.10 (2.41)
M5(Oct)	6.66 (2.74)	7.33 (2.84)	3.99 (2.13)	5.99 (2.57)
Mean	4.39 (2.20)	4.66 (2.25)	2.26 (1.68)	

*Values in parenthesis are Arc sine transformed
C.D (P≤0.05)
Months (M): 1.44
Stations(S): 1.11
Month× Stations (M×S): NS

Table.3 Effect of pathogens isolated from *G.pyloalis* on various economic characters of Silkworm, *Bombyx mori* L.

Pathogen Inoculated	Total larval Duration (hrs)	Weight of 10 mature larvae (g)	Larval mortality (%)	Cocoon yield /10000 larvae by number (No.)	Cocoon yield/1000 0 larvae by weight (kg)	Survival Percentage (%)	Pupation rate (%)
***Nosema bombycis* (T1)**	654.66	27.21	53.66 (7.38)	4,633	6.58	36.33 (36.89)	77.42 (8.83)
NPV (T2)	648.33	31.68	61.00 (7.86)	3,800	5.58	38.00 (6.23)	63.31 (7.99)
Control (T3) (Without inoculation)	652.16	32.51	4.00 (2.20)	9,600	17.33	94.00 (9.75)	96.86 (9.99)
C.D.	**NS**	**1.110**	**0.536**	**624.484**	**1.156**	**5.695**	**0.765**

Table.4 Effect of pathogens isolated from *G.pyloalis* on various economic characters of silkworm, *Bombyx mori* L.

Pathogen Inoculated	Single Cocoon weight (g)	Single shell Weight (g)	Shell ratio (%)	Filament Length (m)	Raw Silk (%)	Denier
***Nosema bombycis* (T1)**	1.60	0.24	14.88	848.87	12.32	2.20
NPV (T2)	1.58	0.31	19.57	816.03	12.50	2.26
Control (T3) (Without inoculation)	1.85	0.36	21.67	887.70	12.77	2.68
C.D.	**NS**	**0.445**	**2.578**	**NS**	**NS**	**0.197**

The pathogens observed in the present study clearly establish that *G.pyloalis* harbor microbes pathogenic to silkworm where Microsporidian (*N.bombycis)* and Nuclear Polyhedrosis virus (NPV) causing Pebrine and Grasserie diseases in silkworm *Bombyx mori* respectively. The present findings are therefore, in conformity with the results reported earlier. In the present study the pathogen incidence of *Nosema bombycis* varied in different months as well as in different stations.

The maximum mean pathogen incidence was recorded during October (4.22%) and minimum mean incidence was recorded during June (1.11%). So for as stations are concerned the maximum mean incidence was recorded in CTS Mirgund (3.59%) followed by SDD Mirgund (3.19%) and the least incidence was recorded in SDD Tulsibagh (0.92).

With regards to the Nuclear Polyhedrosis virus (NPV) observation in the *Glyphodes pyloalis* during the present study causing maximum incidence of 5.99 % during October and minimum mean incidence was recorded during June (1.10%). So for as stations are concerned the maximum mean incidence was recorded in SDD Mirgund (4.66%) followed by CTS Mirgund (4.39%) and the minimum mean incidence was recorded in SDD Tulsibagh (2.26%).

Similar observations were recorded by Sharma *et al.,* (2003) who isolated Nuclear Polyhedrosis virus (NPV) from natural population of leaf roller, *D. pulverulentalis* and they found 10.58 per cent infection. Narayanaswamy and Priyadarshini (2009) isolated the NPV from the diseased caterpillars of *D. pulverulentalis* and inoculated per-orally into healthythird instar larvae for mass production of *Dp*NPV polyhedral bodies under *in-vitro* condition. The NPV infection was observed after fourth day of inoculation. The NPV infected caterpillars exhibited symptoms like sluggishness, gradual changes in colour of the body (pink), shining of integument with opaqueness and milkiness, expansion of entire larval body, hanging upside down, body becoming soft, fragile and cuticle ruptured by liquefaction of the larval carcass releasing polyhedral bodies.

The effect of pathogens isolated from *Glyphodes 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). Weight of 10 mature larvae was recorded highest in T3 (normal) i.e. 32.51 g and lowest (27.21g) were recorded in T1.The highest larval mortality (61.00%) was found in T2 (NPV) which was followed by T1 (*N.bomycis*) 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 by Sharma *et al.,* (2014) while studying the impact of new microsporidian infection on larval and cocoon parameters of the silkworm *Bombyx mori* L.

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. Das *et al.,* (2014) reported the cross infectivity of Pebrine disease from muga to eri silkworm. Sharma *et al.,* (2003) while studying the cross infectivity between pathogens of silkworm, *Bombyx mori* L. and mulberry leaf roller, *Diaphania pulverulentalis* (Hampson) observed that out of 1,000 larvae screened, 85 larvae were infected with microbes. 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 highest pupation rate (96.86%) was recorded in T3 (normal) and lowest (77.42%) was recorded in T1 (*Nosema bombycis*). Srinivasagowda *et al.,* (2000) reported the cross infectivity of *B. mori* L. Nuclear polyhedosis virus (NPV) and *B. mori* Kenchu virus to the IV instar larvae of *D.pulverulentalis* infesting mulberry.

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 for 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 occulation bodies (OBS) in the blood cells of infected silkworm. These results are inconformity with the results of Nataraju *et al.,* (2000) who reported that the spread of Grasserie (BmNPV) disease of silkworm in India and concluded that the disease spreads by the occulation 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 (Steinhaus, 1949). Jameson (1922) and Ghosh (1944) have also reported that pebrine infected silkworms spin flimsy and poor quality cocoons.

The effect of pathogens on filament length and Raw silk percentage was recorded and there was no significant difference recorded in filament length as well as in raw silk percentage; however the highest was recorded in T3 normal (887.70 m) followed by T1 (*N.bombycis*) 848.87 m and lowest was recorded in T2 (NPV) 816.03 m. 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%. The thinnest denier was recorded in T3 (normal) 2.68 followed by T2 (NPV) 2.26 and lowest was recorded in T1 (*N. bombycis*) 2.20.

The results of the current study revealed that *Glyphodes pyloalis* is susceptible to various pathogens and in the present study the incidence of pathogens in *Glyphodes pyloalis* was recorded at three locations viz., CTS Mirgund, SDD Mirgund and SDD Tulsibagh which showed presence of Microsporidian (*Nosema bombycis*) and Nuclear Polyhedrosis virus (NPV). Maximum mean incidence of *Nosema bombycis* in *G.pyloalis* was observed in the month of October (4.22%).

The observations recorded with respect to the incidence of Nuclear Polyhedrosis virus (NPV) in *G.pyloalis* showed maximum mean incidence during the month of October (5.99%). Among the stations, the maximum incidence was recorded at SDD Mirgund (4.66%) followed by CTS Mirgund (4.39%) and the minimum incidence was recorded in SDD Tulsibagh (2.26%). The low incidence of the disease at SDD, Tulsibagh is mainly due to less silkworm rearing in the vicinity of the area.

In conclusion, the study undertaken to ascertain the susceptibility of silkworm, *Bombyx 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). Besides the other economical parameters such as cocoon yield by number and weight (ERR), cocoon weight, shell weight, shell ratio, filament length and raw silk percentage were also adversely affected in the inoculated batches of silkworm as compared to the normal batches. These observations clearly showed that silkworm *Bombyx mori* is highly susceptible to the pathogens isolated from *G.pyloalis.*

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Competing interest

The authors declare no conflict of interest in the publication of this manuscript.

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