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STUDIES ON MULBERRY PEST (*GLYPHODES PYLOALIS* WALKER) AS AN ALTERNATE HOST TO THE PATHOGENS OF SILKWORM (*BOMBYX MORI* L.) DISEASES

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ABSTRACT

In the present study, susceptibility of the mulberry pest *G. pyloalis* to the pathogens of silkworm, *Bombyx mori* was ascertained. The survey was carried out at the three locations viz. Mulberry farms of College of Temperate Sericulture, Mirgund and two mulberry farms of Sericulture Development Department, J&K located at Mirgund and Tulsibagh, Srinagar. 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). Under temperate conditions, *G. pyloalis* (Walker) causes severe damage to mulberry crop and is considered as a major pest of mulberry in Jammu and Kashmir (Fotadar *et al.*, 1998). Zeya *et al.* (2003) reported 20-25 percent damage to mulberry foliage in Kashmir is due to *G. Pyloalis* during July to October. The survey study showed high infestation of *G. Pyloalis* during the month of October, 2017 and 2018. The maximum infestation of 73.03% was observed at SDD Tulsi Bagh followed by 45.20% at SDD Mirgund and 32.99% at CoTS Farm. During the study various stages of *G. Pyloalis* were found infected with the Microsporidian and Nuclear Polyhedral Virus 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% percent respectively during October, 2017. In the present study, susceptibility of the mulberry pest *G. Pyloalis* to the pathogens of silkworm, *Bombyx mori* was ascertained so that management strategies are taken accordingly.

Keywords **:** *Bombyx mori* L., *Glyphodes pyloalis* , Host , Mulberry, Silkworm, Pathogen.

Introduction

Sericulture is an art of rearing silkworms for the production of cocoons which is the raw material for silk production. Asia is the major producer of silk in the world and produces over 98.5 percent of global output, although there are more than 40 countries in the world silk map, but the bulk of silk comes from China and India, followed by Brazil, Uzbekistan, Thailand Vietnam, Korea and Japan (Bhat, 2014). Sericulture is grouped under village and small enterprises sector that plays major role for the creation of sustainable employment and income generation (Ishtiaque *et al.,* 2013). The productivity of mulberry silk cocoons and superior quality of silk is dependent on rearing of healthy rearing of silkworm, *Bombyx mori* L., which in turn is closely related to quality feed (mulberry leaf). The importance of quality of mulberry leaves on the growth, development and silk production in silkworm is well documented (Dasgupta, 1961; Yokoyama, 1963; Das and Sikdar, 1970; Radha *et al.,* 1978).

The mulberry varieties like *Morus alba, Morus indica* are attacked by a number of pests like *P. comstocki, A. ipsilon, M. hirsutus, D. pulverulentalis* and *G. 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, *G. pyloalis* (Walker) causes severe damage to mulberry crop and is considered as a major pest of mulberry in Jammu and Kashmir (Fotadar *et al.*, 1998). In recent years, mulberry pyralid, *G. pyloalis* Walker (Lepidoptera: Pyrilidae) has assumed a serious status as it not only causes loss in leaf yield but also transmit diseases (Watanabe *et al.*, 1988). In Jammu & Kashmir, the maximum damage (71.26%) due to *G. pyloalis* Walker was recorded during October and minimum (2.23%) during July (Anonymous, 1996).

Silkworms are susceptible to a number of diseases caused by different infectious agents such as protozoa, virus, fungi and bacteria and cause considerable crop loss. Microsporidians are the most important protozoan pathogens of insects and fishes and over seven hundred species of them were recorded from these hosts (Canning, 1977; Sprague, 1977). 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). The *G. pyloalis* Walker is the habitual host of non-occluded viruses pathogenic to the silkworm *Bombyx mori* L. (Watanabe *et al.,* 1988). Pyralid pests are regulated by several natural enemies during its egg, larval and pupal stages (Ramakrishna Ayyar and Margabandu, 1934; Vishakantaiah and Jagadeesh Babu, 1980; Gautam, 1986).

Materials and Methods

Silkworm rearing 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. Survey during the incidence of *G. 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 *G. pyloalis* to the pathogens of silkworm *B. mori*

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

Results

Infestation of *G. pyloalis* at various locations

The observations recorded during the survey conducted at various locations *viz*., CTS Mirgund, SDD Mirgund and SDD Tulsibagh, during the year 2017 are presented in Table 1. The infestation showed significant difference in various months of the year as well as in different stations. *G. Pyloalis* infestation initiated in the month of June and percent infestation increased in the subsequent months. Mean infestation of 9.13%, 12.36%, 16.79%, 40.56% and 49.05% was recorded during the months June, July, August, September and October respectively. So for as stations are concerned, mean infestation ranged from 14.05% to 39.67% with maximum mean infestation at SDD, Tulsibagh (39.67%) followed by SDD, Mirgund (23.00%) and the minimum infestation was recorded at CTS, Mirgund (14.05%).

Infestation of *G. pyloalis* at various locations during the year 2018:

The observations recorded during the survey conducted at various locations viz., CTS Mirgund, SDD Mirgund and SDD Tulsibagh, during the year 2018 are presented in Table 2. The infestation showed significant difference in various months of the year as well as in different stations. *G. Pyloalis* infestation initiated in the month of June and percent infestation increased in the subsequent months. Mean infestation of 9.00%, 14.89%, 22.05%, 47.98% and 51.77% during the months June, July, August, September and October respectively. Among the stations, mean infestation ranged from 20.06% to 42.29% with the maximum mean infestation at SDD Tulsibagh (42.29%) followed by SDD Mirgund (25.06%) and the minimum mean infestation was recorded at CTS, Mirgund (20.06%).

Table 1: Month and year-wise infestation of *G. pyloalis* at three locations during 2017

Stations / Months	S1 CTS, Mirgund	S2 SDD, Mirgund	S3 SDD, Tulsibagh	Mean
M1(June)	4.51 (2.24)	7.24 (2.73)	15.65 (3.92)	9.13 (2.97)
M2(July)	7.03 (2.70)	10.09 (3.30)	19.94 (4.54)	12.36 (3.52)
M3(Aug)	10.22 (3.32)	15.92 (4.06)	24.24 (4.99)	16.79 (4.13)
M4(Sep)	17.56 (4.17)	37.55 (6.20)	66.58 (8.20)	40.56 (6.19)
M5(Oct)	30.95 (5.64)	44.24 (6.71)	71.96 (8.53)	49.05 (6.97)
Mean	14.05 (3.62)	23.00 (4.60)	39.67 (6.04)	

Values in parenthesis are square root transformed

C.D ($P \leq 0.05$) Month (M): 4.484 Station (S): 3.473 Month × Station (M×S): 7.766

Table 2: Month and year-wise infestation of *G. pyloalis* at three locations during 2018

Stations / Months	S1 CTS, Mirgund	S2 SDD, Mirgund	S3 SDD, Tulsibagh	Mean
M1(June)	5.19 (2.35)	5.28 (2.49)	16.54 (4.15)	9.00 (12.99)
M2(July)	10.10 (3.30)	13.84 (3.70)	20.72 (4.63)	14.89 (3.88)
M3(Aug)	16.48 (4.13)	18.92 (4.41)	30.76 (5.63)	22.05 (4.72)
M4(Sep)	33.50 (5.87)	41.08 (6.48)	69.36 (8.38)	47.98 (6.91)
M5(Oct)	35.03 (5.99)	46.16 (6.86)	74.10 (8.66)	51.77 (7.17)
Mean	20.06 (4.33)	25.06 (4.79)	42.29 (6.29)	

*values in parenthesis are square root transformed

C.D (P ≤0.05) Month (M): 3.722 Station (S): 2.883 Month × Station (M×S): 6.447

Pooled data of infestation of *G. pyloalis* at various locations during the years 2017 and 2018:

Pooled data of infestation of *G. pyloalis* walker at various locations during 2017 & 2018 presented in Table 3 and Fig.1 and Fig. 2 respectively. The observations recorded during the survey conducted at various locations viz., CTS Mirgund, SDD Mirgund and SDD Tulsibagh. The infestation showed significant difference in various months of the year as well as in different stations. *G. Pyloalis* infestation initiated in the month of June and percent infestation increased in the subsequent months. Mean infestation of 9.07%, 13.62%, 19.42%, 44.27% and 50.40% during the month June, July, August, September and October respectively. Among the stations, mean infestation ranged from 17.06% to 40.99% with maximum infestation at SDD Tulsibagh (40.99%) followed by SDD Mirgund (24.03%) and minimum infestation was observed at CTS Mirgund (17.06%). The high infestation at SDD, Tulsibagh is attributed to the low maintenance of the mulberry plantation there, as poor ground sanitation and management results in the multiplication of the pests.

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

The observations recorded on the susceptibility of *G.pyloalis* larvae inoculated with the pathogens Microsporodia (*Nosema bombycis*) and Nuclear Polyhedrosis virus (NPV) isolated from the silkworm *Bombyx mori* are presented in Table 4 which showed high incidence of both the pathogens in the *G.pyloalis* with high mortality at larval and pupal stages. Total mortality due to *Nosema 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 *Bombyx mori*, thereby acting as potential source for the spread of the silkworm diseases.

Table 3: Pooled data of infestation of *G. pyloalis* at three locations during the years 2017 and 2018

Stations / Months	S1 CTS, Mirgund	S2 SDD, Mirgund	S3 SDD, Tulsibagh	Mean
M1(June)	4.85 (2.29)	6.262 (2.608)	16.093 (4.041)	9.079 (2.981)
M2(July)	8.566 (3.005)	11.970 (3.503)	20.330 (4.586)	13.622 (3.698)
M3(Aug)	13.354 (3.730)	17.420 (4.239)	27.499 (5.314)	19.424 (4.428)
M4(Sep)	25.532 (5.020)	39.316 (6.343)	67.971 (8.293)	44.273 (6.552)
M5(Oct)	32.992 (5.823)	45.203 (6.786)	73.032 (8.602)	50.409 (7.070)
Mean	17.069 (3.974)	24.034 (4.696)	40.995 (6.167)	

*Values in parenthesis are square root transformed

C.D (P ≤0.05) Months (M): 3.077 Stations(S): 2.384 Month× Stations (M×S): 5.331

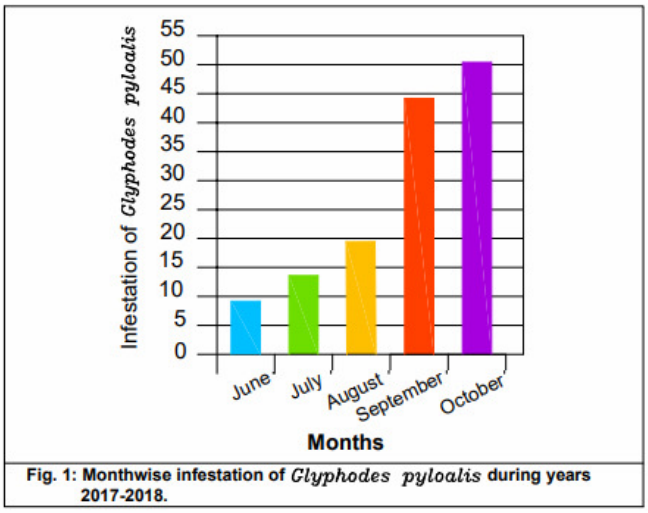

Fig. 1: Monthwise infestation of *Glyphodes pyloalis* during years 2017-2018.

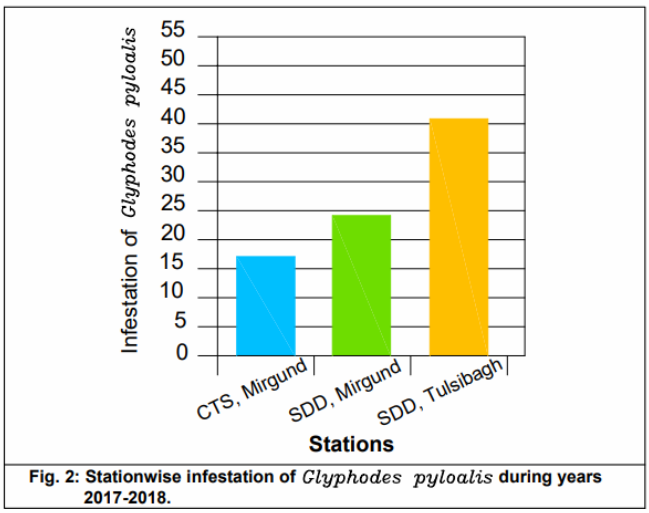

Fig. 2: Stationwise infestation of *Glyphodes pyloalis* during years 2017-2018.

Discussion

G. pyloalis causes severe damage to the mulberry plantation and is a major pest. The larvae of *G. pyloalis* web the tender leaves together, feed on the chlorophyll content of the leaves and skeletonize them. The early instar larvae of the pest inhibits the growth of the apical succulent portion of the shoot and this leads to its destruction, resulting in stunted growth of the plant, thereby leading to an appreciable decline in leaf yield. In case of severe infestation, the larvae of the leaf roller completely defoliate the mulberry garden. Grown up caterpillars feed voraciously on the tender leaves and their faecal matter can be seen on the leaves below the affected portion. Mathur (1980) also reported that the young larva feed on the tender epidermis of the under surface of leaf supported by a rough net, usually it eats the green tissue of the leaf from one side leaving the membranous epidermis on the other side.

In the present study, it was observed that *G. pyloalis* infestation initiates during the month of June (9.07%) and considerable infestations in the following months with maximum increase in infestation during October (50.40%). So for as stations are concerned, the maximum infestation was recorded in SDD Tulsibagh (40.99%) followed by SDD Mirgund (24.03%) and the least was recorded in CTS Mirgund (17.06%). Similar observations were also reported by Farooz and Mir (2018) while studying *G.* infestation in mulberry and reported that *G. pyloalis* walker takes a heavy toll of mulberry especially from July to October under Kashmir climatic conditions. Similar findings were observed by Rajadurai *et al.,* 1999 and Narayanaswamy *et al*., 2003 in other Karnataka, Andhra Pradesh, and Tamil nadu. Zeya *et al.* (2003) also reported 20-25 percent damage to mulberry foliage in Kashmir is due to *G. pyloalis* during July to October. The infestation of *G. pyloalis* was reported to be 20-40 per cent at CTS, Mirgund (Anonymous, 1996-97). Fotadar *et al.*, 1998 also reported *G.pyloalis* as major pest of mulberry plantation in J&K causing severe damage to mulberry saplings.

The findings of the present study that the diseased samples in the population of *G. pyloalis* were infected with microsporidia suggest that the pest is a potential source of secondary contamination of microbes pathogenic to silkworm, *Bombyx mori* L. through mulberry leaf, which in turn can transmit the diseases. The natural population of leaf roller was found to be infected by fungus, *Beauveria bassiana* (Vuilleimin) (12%), Microsporidian (50%) and Nuclear Polyhedrosis virus (38 %) (Sharma*et al*., 2002; Narayanaswamy and Priyadarshini, 2009).Hayasaka and Yonemura, 1999 also reported that *Nosema bombycis* and *Nosema* sp. were infective to *G. pyloalis* in Japan and the infection ranged from 12.2 to 18.9 per cent. Manjunath, 2005 reported that *G. Pyloalis* causes extensive loss in leaf yield and also serves as an alternate host for the *Nosema bombycis* causing Pebrine in silkworm *B.mori*. As per Govindan *et al.* (1998), *D. pulverulentalis* is an alternative host forthe protozoa, *Nosema bombycis* Nageli.The cross infectivity of *Nosema bombycis* from mulberry silkworm *Bombyx mori* to lepidopteran and other insect pests has been reported by a number of authors. *Nosema bombycis* is known to infect several insects from different orders(Steinhaus, 1949). Cross infectivity of *Nosema bombycis* was studied on different insect pests like Turnip moth (*Agrotis segetum* Schift), heart and Dart moth (*A.exlamatiois* L.), Cotton Noctiuid moth (*Heliothis armigera*), Army worm (*Spodoptera exigua* Hb), Cabbage White moth (*P. brassicae*). In all the above species, the infected larvae died before adulthood except *H. armigera* (Kashkarova, 1981).

In the present study with regards to the susceptibility of *G. pyloalis* of silkworm *Bombyxmori* L. Total mortality rate of 90.64% and 92.00% when *G.pyloalis* was inoculated with *Nosema bombycis* and Nuclear Polyhedrosis virus(NPV) respectively. Similar findings were also observed by 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.Srinivasagowda *et al.* (2000) also reported the cross infectivity of *B. mori* L. Nuclear Polyhedrosis virus (NPV) and *B. mori* Kenchu virus to the larvae of *D.pulverulentalis* infesting mulberry. Samson *et al*. (1999) reported 60% infection in silkworm by a microsporidian isolated from a butterfly, *pieris* sp.

Conclusion

The survey conducted to ascertain the infestation of *G. pyloalis* walker at various locations viz., CTS Mirgund, SDD Mirgund and SDD Tulsibagh during the years 2017 & 2018 showed significant difference in various months as well as in

different stations. *G. pyloalis* infestation initiated in the month of June and per cent infestation increased in the subsequent months. The maximum infestation was recorded in the month of October (50.40%). Among the stations, the maximum infestation was observed at SDD Tulsibagh (40.99%) followed by SDD Mirgund (24.03%) and minimum infestation was observed at CTS Mirgund (17.06%). The high infestation at SDD, Tulsibagh is attributed to the low maintenance of the mulberry plantation there, as poor ground sanitation and management results in the multiplication of the pests.

The observations recorded on the susceptibility of *G. pyloalis* larvae inoculated with the pathogens Microsporidian (*Nosema 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. Total mortality due to *Nosema 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 *Bombyx mori*, thereby acting as potential source for the spread of the silkworm diseases.

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