

Studies on the Prevalence of Pebrine Disease in Magadi Taluk of Mysore Seed Area

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ABSTRACT

Preliminary survey on the current status of pebrine disease in Magadi taluk of Mysore seed area was conducted from November 2022 to April 2023. The results revealed that the disease incidence in pupae and mother moths samples collected from two Government Model Grainages (Magadi and Kudur) was found to be on par with 6.66 per cent. However, the DFLs collected from both the grainages had no incidence of pebrine. The overall per cent mean pebrine disease incidence in two Government Model Grainages (Magadi and Kudur) in Magadi taluk of Mysore seed area was 4.44. On microscopic examination of each of the thirty samples collected from Mathikere and Koramangala CRCs no pebrine spores were detected, indicating no pebrine incidence in Mattikere and Koramangala CRCs of Magadi taluk in Mysore seed area. The pebrine disease incidence in late age silkworms collected from Soluru, Kudur and Kalya hoblies in farmer's field was 12.00, 8.00 and 8.00 per cent, respectively. The presence of pebrine in silkworm excreta collected from Soluru, Kudur and Kalya hoblies from farmer's field was 8.00, 4.00 and 4.00 per cent, respectively. The overall average disease incidence covering the three different hoblies including 15 villages surveyed in Magadi taluk of Mysore seed area was 9.33 per cent in late age silkworms and 5.33 per cent in silkworm excreta.

Keywords : Pebrine disease, Grainages, Chawki rearing centers, Farmer's field

SILKWORM, *Bombyx mori* L. a lepidopteran insect has marvellous ability to produce silk is considered as the most elegant textile. Owing to its unparalleled grandeur, natural sheen, high absorbance, light weight, soft touch and high durability. World wide silk is known as 'Queen of Textiles' (Vishaka and Narayanaswamy, 2018).

Mulberry silkworm, *B. mori* is the primary producer of commercial silk and domesticated for many centuries. Continuous indoor rearing of silkworms has rendered to lose its natural ability to withstand adverse climatic conditions resulting in infections causing crop loss or low yield leading to low silk productivity. Mulberry silkworm *Bombyx mori* L. is susceptible to number of diseases like flacherie,

grasserie, pebrine and muscardine (Mankani *et al.*, 2017) Silkworm diseases are highly contagious and the causative agents get easily dispersed leading to outbreak of diseases (Selvakumar *et al.*, 2005). Among all the diseases pebrine is the deadliest disease caused by microsporidian parasite, *Nosema bombycis* Nageli. The name pebrine was coined by De Quadrefagues (1860) because of pepper like spots on the diseased larvae in advanced stage of infection (Bhat and Nataraju, 2005).

The microsporidiosis of silkworm, commonly known as pebrine is the earliest known menace to the silk industry. Several historical evidences in various countries of the world showed that the outbreak of pebrine disease had greatly influenced the decline of

the sericulture industry in the past (Kamili and Massodi, 2000). Pebrine disease holds significant importance in sericulture, particularly in seed producing areas because it is the only disease of silkworm which can be transmitted from mother to egg (Transovarial transmission) and by contaminated egg surface (Transovum transmission) and majorly by the ingestion of contaminated mulberry leaf by silkworms (Horizontal transmission) (Bhat, 2007).

Even though the fight against pebrine has continued for more than a century, the loss due to the disease has not been completely eliminated (Chakrabarty *et al.*, 2012). During the year 2020-21 pebrine disease resurfaced in the grainages, CRCs and farmer's field which was noticed by the state Department of Sericulture, GoK, Magadi taluk of Mysore seed area, Karnataka. In order to find out the current status of the disease in this area, the research problem entitled 'Studies on the prevalence of pebrine disease in Magadi taluk of Mysore seed area' was formulated.

MATERIAL AND METHODS

The preliminary survey was conducted from November 2022 to April 2023 regarding current status of pebrine disease in Magadi taluk of Pure Mysore seed area. Simple random sampling procedure was adopted for the collection of samples from the selected areas. A single sample contains 10 specimens. Samples of disease free layings (DFLs), pupae, larvae, mother moths and silkworm excreta were collected to detect the extent of pebrine incidence in grainages, CRCs and farmers' field in Magadi taluk of Mysore seed area.

Survey in Grainages

A survey was conducted in two Government Model Grainages in Magadi taluk of Mysore seed area located at Magadi and Kudur. During the survey, collection of 15 samples each of pupae, mother moths and DFLs from both grainages. In total, 45 samples were collected from each of the grainages, resulting in a total of ninety samples (Pupae, mother moths and DFLs) collected from two grainages (Table 1). The collected samples were utilized for examination of pebrine spore. During the survey, pupae, moths and

TABLE 1
Details of samples collected from Government Model Grainages of Magadi and Kuduru in Magadi taluk of Mysore seed area

Grainage	Stage	No. of sample
Government Model Grainage, Magadi	Pupae	15
	Mother moths	15
	DFLs	15
Total		45
Government Model Grainage, Kudur	Pupae	15
	Mother moths	15
	DFLs	15
Total		45

DFLs in the grainages were observed for external symptoms of pebrine disease as given by Jolly (1986). Pupal symptoms *viz.*, soft abdomen and irregular black spots on the body wall and highly infected pupae fail to metamorphose into adult. Moths symptoms *viz.*, irregular moth emergence, clubbed wings and distorted antennae, improper mating and low fecundity, easily coming off scales from wings and abdomen. Symptoms of silkworm eggs *viz.*, lack of uniformity in egg shape, reduction in egg size, eggs laid in clumps and poor egg attachment to egg sheet, eggs with less gluey substance resulting in their detachment from the egg sheets.

Survey in Chawki Rearing Centres (CRCs)

There are two CRCs in Magadi taluk of Mysore seed area located at Mathikere and Koramangala. Samples of chawki silkworms were collected randomly from both the CRCs. A total of sixty samples were collected from two CRCs, including thirty samples from each CRC (Table 2). The collected samples were utilized for examination of pebrine spores.

Survey in Farmer's Field

The survey was conducted in farmer's field including three different hoblies *viz.*, Soluru, Kudur and Kalya. In each of these hoblies, five sericultural villages were randomly selected from each hoblies *viz.*, Soluru (Kempapura, Kannasandra, Chikkamudegere,

TABLE 2
Details of samples collected from the CRCs in
Magadi taluk of Mysore seed area

CRC	Samples	No. of sample
Mathikere	Chawki silkworms	30
Koramangala	Chawki silkworms	30
Total		60

M. R. Palya and S. L Palya.), Kudur (Sugganahalli, Kumbarpalya, Honnapura, Biskur and Gangonahalli) and Kalya (Halasinganahalli, Basavanapalya, Shivanasandra, Upparthi and Singrigowdanapalya). Subsequently, five silkworm rearers were chosen at random from each of these villages. From each selected silkworm rearer, one sample of late age silkworms was collected, along with a sample of silkworm excreta. In total fifty samples were collected from each hoblies (Table 3).

During the survey, larvae in the farmers' field were observed for external symptoms of pebrine disease as given by Jolly (1986). Larval symptoms *viz.*, unequal sized larvae, sluggish, irregular moulting, appearance of black spots on the integument of larvae, pale colour and translucent with wrinkled skin shrunken in size and becomes flaccid.

A total of three hundred samples were collected from Magadi taluk of Mysore seed area including ninety samples from grainages, sixty samples from CRCs and one hundred and fifty samples from farmers' field. The collected samples were utilized for examination of pebrine spores.

The samples collected from both Magadi and Kudur grainages were examined for presence of pebrine spores using Fujiwara method at silkworm pathology laboratory, P2 grainage, Magadi and P1 grainage, Kuduru, respectively and samples from CRCs and farmers' field were examined for presence of pebrine spore at silkworm pathology laboratory, office of the Assistant Director of Sericulture, Magadi. The incidence of the pebrine disease was calculated using the formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of samples infected}}{\text{Total number of samples collected}} \times 100$$

Examination of DFLs for Pebrine Spores as given by Rahul *et al.* (2021)

- The eggs from each sample were examined for the presence of pebrine spores at 600X under light microscope

TABLE 3
Details of samples collected from farmers' field in Magadi taluk of Mysore seed area

Hobli	Soluru	Kudur	Kalya
Village	Kempapura	Sugganahalli	Halasinganahalli
	Kannasandra	Kumbarpalya	Basavanapalya
	Chikkamudgere	Honnapura	Shivanasandra
	M. R Palya	Biskuru	Upparthi
	S. L Palya	Gangonahalli	Singrigowdanapalya
Samples collected from each hobli	50	50	50

- Pebrine spores were identified based on characteristics such as luster, shape, size and Brownian movement

Examination of Larvae for Pebrine Spores Rahul *et al.* (2021)

- The collected larvae were crushed in a domestic mixer for 2 minutes by adding 4ml of 0.6 per cent of K_2CO_3 solution in the ratio of 1:4 (larvae:ml).
- The homogenate was transferred into a beaker and allowed to settle for 3-5 minutes.
- The liquid was filtered through double layered fine mesh plastic strainer.
- The filtered liquid was centrifuged at 3000 rpm for 3 minutes.
- Later the supernatant was discarded and sediment was dispersed in a few drops of 2 per cent KOH solution over a cyclomixer
- Two smears from each sample were examined for pebrine spores using light microscope (600X)

- Pebrine spores were detected and identified by observing luster, shape, size and Brownian movement.

Same procedure was followed for examination of mother moths and pupae for pebrine spores but for pupae 0.6 per cent K_2CO_3 was added in the ratio of 1:8 (pupae: ml).

Examination of Silkworm Excreta for Pebrine Spores Rahul *et al.* (2021)

- A handful of silkworm excreta was gathered from various locations from the rearing bed. Subsequently, the collected excreta was thoroughly mixed and one gram was weighed for the purpose of examining pebrine spores.
- One gram of silkworm excreta was soaked in 8 ml of 0.6 per cent solution K_2CO_3 solution.
- The soaked mixture was crushed using mortar and pestle.
- The crushed sample was filtered through double layered fine mesh plastic strainer.
- Two smears from each sample were examined using light microscope (600X) for pebrine spores.



Plate 1 : Sample collection at grainages



Plate 2 : Sample collection at CRCs



Plate 3 : Sample collection at farmer's field

- Pebrine spores were detected and identified by observing luster, shape, size and Brownian movement.

RESULTS AND DISCUSSION

Incidence of Pebrine Disease in Government Model Grainages

The survey was conducted in two Government Model Grainages in Magadi taluk of Mysore seed area located at Magadi and Kudur.

During the survey, pupae, moths and DFLs in the grainages were observed for external symptoms of pebrine disease. However, no external symptoms of pebrine disease were observed. Further, random samples of pupae, moths and DFLs were collected and examined under microscope for the presence of pebrine spores.

As far as external symptoms of pebrine disease are concerned Bhat (2007) reported that pebrine infection rates are typically low in natural populations and often do not exhibit external symptoms. This necessitates the examination of a large number of individuals to detect and estimate the incidence of infection. Also Bhat and Nataraju (2005) reported that microsporidian strain (Lbms) could invade all the tissues but failed to cause any larval or pupal mortality prior to the adult eclosion due to low rate of proliferation and low infectivity. They also reported that sometimes pebrine spores can occur in numbers that appear to entirely fill the silkworm without any symptoms of infection and this could be attributed to low virulence in this particular strain.

Data pertaining to incidence of pebrine disease in Government Model Grainages in Magadi taluk of Mysore seed area is presented in Table 4, which shows

TABLE 4

Incidence of pebrine disease in Government model grainages in Magadi taluk of Mysore seed area

Grainage	Sample	No. of sample collected	No. of sample infected	Disease incidence (%)
Government Model Grainage, Magadi	Pupae	15	01	6.66
	Mother moths	15	01	6.66
	DFLs	15	00	0.00
	Mean pebrine incidence (%)			4.44
Government Model Grainage, Kudur	Pupae	15	01	6.66
	Mother moths	15	01	6.66
	DFLs	15	00	0.00
	Mean pebrine incidence (%)			4.44
Per cent mean pebrine incidence in grainages				4.44

that, among fifteen samples of pupae collected, only one sample exhibited the presence of pebrine spores on microscopic examination, revealing pebrine disease incidence of 6.66 per cent. Among fifteen mother moth samples collected, only one sample showed the presence of pebrine spores on microscopic examination, indicating pebrine disease incidence of 6.66 per cent. However, none of the fifteen samples of DFLs collected showed any pebrine spores on microscopic examination, indicating no incidence of pebrine in DFLs in Magadi grainage (Table 4, Fig. 1).

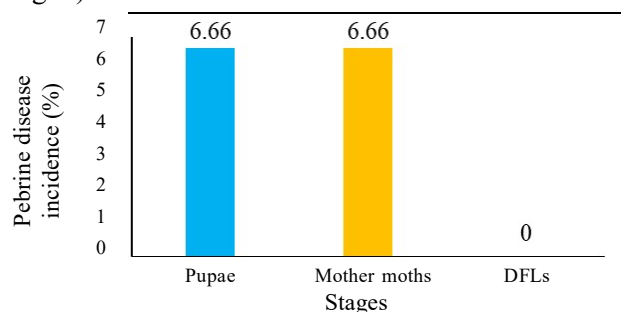


Fig 1 : Incidence of pebrine disease in Government Model Grainage, Magadi in Magadi taluk of Mysore seed area

The same trend was seen in Kudur grainage were in among fifteen samples of pupae collected, only one sample exhibited the presence of pebrine spores on microscopic examination, revealing pebrine disease incidence of 6.66 per cent. Among fifteen mother moth samples collected, only one sample showed the presence of pebrine spores on microscopic examination, indicating pebrine disease incidence of 6.66 per cent. However, none of the fifteen samples of DFLs collected showed any pebrine spores on microscopic examination, indicating no incidence of pebrine in DFLs (Table 4, Fig 2).

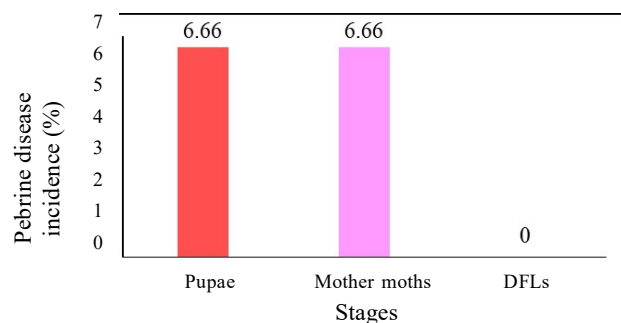


Fig 2 : Incidence of pebrine disease in Government Model Grainage, Kudur in Magadi taluk of Mysore seed area

The overall per cent mean pebrine disease incidence in the two grainages (Magadi and Kudur) in Magadi taluk of Mysore seed area was 4.44 (Table 4).

Though pebrine spores were detected in samples of pupae and moths in both the grainages, but not in DFLs, because mother moth examination is conducted strictly and in case pebrine spores are detected the egg lots are burnt immediately.

As per available literature regarding pebrine incidence the surveys conducted are of general nature and not specific to different stages including pupae, mother moths and DFLs.

Ping and Jie (2011) studied the pattern and prevalence of *N. bombycis* in Guangdong silkworm egg production centers and reported that pebrine incidence has shown seasonal variations. The incidence rate in autumn was high while it was low in spring and summer and increased again in winter. Weather conditions, geographical location and agronomic activity influenced pebrine prevalence.

Vijayakumari *et al.* (2019) conducted a survey in Basic Seed Farms of Karnataka and reported that incidence of pebrine was 4.2 per cent in P4 Basic Seed Farm, Hassan and 0.34 per cent in P3 BSF, Mysore.

Rahamathulla *et al.* (2012) studied the incidence of microsporidian disease in silkworm for a period of 3 years (2008-10) in sericulture basic seed farm, Mysore, Karnataka, India. The results indicated that the disease incidence was higher during the beginning of winter (October-January) followed by rainy (July-September) and low or zero level of disease infection in summer season (March-May).

Incidence of Pebrine Disease in CRCs

Pebrine disease symptoms were not encountered in young age silkworms in CRCs during the current survey. Further, random samples were collected and observed for pebrine spores. On microscopic examination of each of the thirty samples collected from Mathikere and Koramangala CRCs no pebrine spores were detected, indicating no pebrine incidence in Mattikere and Koramangala CRCs of Magadi taluk in Mysore seed area (Table 5).

TABLE 5
Incidence of pebrine disease in CRCs in Magadi taluk of Mysore seed area

CRCs	Samples	No. of samples collected	No. of samples infected	Disease incidence (%)
Mathikere	Chawki silkworms	30	00	0.00
Koramangala	Chawki silkworms	30	00	0.00
Per cent mean disease incidence				0.00

Surveys related to pebrine incidence specific to CRCs has not been conducted so far as per available literature. Ganie (2008) conducted a study on the incidence of pebrine disease in Jammu & Kashmir. He reported that lower incidence of the disease was found in state departmental rearing centres and in the areas which were directly under the influence of state sericulture department compared to private farmers.

Incidence of Pebrine Disease in Farmer's Field

During the current field survey interaction with farmer's revealed that the farmers were totally unaware of pebrine disease and any symptoms related to pebrine disease in late age silkworms. On careful observation for symptoms including unequal sized worms, sluggish, irregular moulting, pale colour, wrinkled skin, shrunken body size, appearance of black spots if any on the integument, it was observed that the larvae were symptom free except for some uneven sized silkworms, here and there.

Incidence of Pebrine Disease in Late Age Silkworms

Five random samples were collected from each village *viz.*, Kempapura, Kannasandra, Chikkamudegere, M. R. palya and S. L. palya from Soluru hobli of Magadi taluk in Mysore seed area of which only one sample each from Kempapura, Chikkamudegere and M. R. palya revealed the presence of pebrine spores on microscopic examination, whereas the samples collected from Kannasandra and S. L. palya showed no incidence. The pebrine disease incidence in

late age silkworms collected from Soluru hobli was 12.00 per cent. In Kudur hobli only one sample each from Kumbarpalya and Biskuru villages showed the presence of pebrine spores on microscopic examination, whereas the samples collected from Sugganahalli, Honnapuar and Gangonahalli exhibited no incidence. The pebrine disease incidence in late age silkworms collected from Kudur hobli was 8.00 per cent. In Kalya hobli of Magadi taluk in Mysore seed area only one sample each from Halasinganahalli and Upparathi revealed the presence of pebrine spores on microscopic examination, whereas the samples collected from Basavanapalya, Shivanasandra and Singrigowdanapalya showed no incidence. The pebrine disease incidence in late age silkworms collected from Kalya hobli was 8.00 per cent. The overall average pebrine disease incidence in late age silkworms covering the three different regions including 15 villages surveyed in Magadi taluk of Mysore seed area was 9.33 per cent (Table 6, Fig 3).

So far the surveys conducted regarding pebrine disease incidence do not specify a particular stage of silkworm instead most of the surveys done are season wise.

Devaiah and Krishnaswamy (1975) reported that pebrine infection was highest during September - October (38.97%) followed by February - March (32.58%) and lowest in April - May (4.66%) under Mysore climatic conditions. Further, Balavenkatasubbaiah *et al.* (1992) investigated on status of pebrine disease in the seed areas of Attibele, Kunigal and commercial areas including Ramanagara and Chamarajnar. The highest infection (25.71%) of pebrine disease was reported during winter followed by rainy (15.72%) and least (5.75%) was observed during summer season.

A survey on the incidence of pebrine disease was carried out by Ganie (2003) who reported that pebrine disease incidence was lower in state departmental rearing centres and highest in farmer's rearing houses because of secondary contamination. In case of primary infection (transovarian transmission), the larvae do not grow and survive beyond the II-instar (Jolly, 1986). Further, (Hukuhara, 2017) reported that

TABLE 6
Incidence of pebrine disease in late age silkworms collected from Soluru, Kudur and Kalya hoblies of Magadi taluk in Mysore seed area

Hobli	Village	Late age silkworms	
		No. of sample collected	No. of sample infected
Soluru	Kempapura	05	01
	Kannasandra	05	00
	Chikkamudgere	05	01
	M. R Palya	05	01
	S. L Palya	05	00
	Pebrine (%)	12.00	
Kudur	Sugganahalli	05	00
	Kumbarpalya	05	01
	Honnapura	05	00
	Biskuru	05	01
	Gangonahalli	05	00
	Pebrine (%)	8.00	
Kalya	Halasinganahalli	06	01
	Basavanapalya	06	00
	Shivanasandra	06	00
	Upparathi	06	01
	Singrigowdanapalya	06	00
	Pebrine (%)	8.00	
Per cent mean pebrine incidence		9.33	

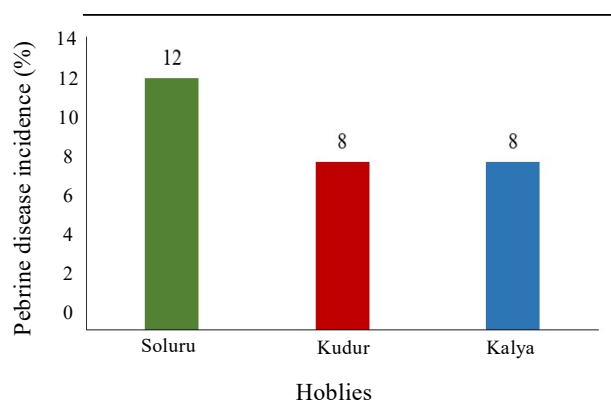


Fig. 3 : Incidence of pebrine disease in late age silkworms collected from Soluru, Kudur and Kalya hoblies of Magadi taluk in Mysore seed area

the silkworms infected during early stages of I and II instar die by V instar, but if infection occurs during IV or V instar, the larvae survive up to the moth stage to become the parents of congenitally infected progeny.

Presence of Pebrine in Silkworm Excreta

Among the five random samples of silkworm excreta collected from Kempapura, Kannasandra, Chikkamudgere, M. R. palya and S. L. palya of Soluru hobli, Magadi taluk of Mysore seed area, only one sample each from Kempapura and Chikkamudgere revealed the presence of pebrine spores on microscopic examination, whereas the samples collected from Kannasandra, M. R. palya and S. L. palya showed no pebrine spores. The presence of pebrine in silkworm excreta collected from Soluru hobli was 8.00 per cent. In Kudur region only one sample from Kumbarpalya village showed the presence of pebrine spores on microscopic examination, whereas the samples collected from Sugganahalli, Honnapuar, Biskuru and Gangonahalli exhibited zero presence of pebrine spores. The presence of pebrine in silkworm excreta collected from Kudur hobli was 4.00 per cent. In Kalya hobli of Magadi taluk in Mysore seed area only one sample from Upparathi revealed the presence of pebrine spores on microscopic examination, whereas the samples collected from Halasinganahalli, Basavanapalya, Shivanasandra and Singrigowdana palya villages showed zero presence of pebrine spores. The presence of pebrine in silkworm excreta collected from Kalya hobli was 4.00 per cent. The overall average presence of pebrine spores in silkworm excreta covering the three different hoblies including 15 villages surveyed in Magadi taluk of Mysore seed area was 5.33 per cent (Table 7, Fig. 4).

Available literature regarding survey on incidence of pebrine disease are only seasonal and now here is it specific to silkworm excreta.

Chakrabarty *et al.* (2013) reported that pebrine spores present in dead silkworms and excreta can serve as a

TABLE 7
Presence of pebrine in silkworm excreta collected from Soluru, Kudur and Kalya hoblies of Magadi taluk in Mysore seed area

Hobli	Village	Silkworm excreta	
		No. of sample collected	No. of sample infected
Soluru	Kempapura	05	01
	Kannasandra	05	00
	Chikkamudegere	05	01
	M. R Palya	05	00
	S. L Palya	05	00
	Pebrine (%)	8.00	
Kudur	Sugganahalli	05	00
	Kumbarpalya	05	01
	Honnapura	05	00
	Biskuru	05	00
	Gangonahalli	05	00
	Pebrine (%)	4.00	
Kalya	Halasinganahalli	06	00
	Basavanapalya	06	00
	Shivanasandra	06	00
	Upparathi	06	01
	Singrigowdanapalya	06	00
	Pebrine (%)	4.00	
Per cent mean pebrine		5.33	

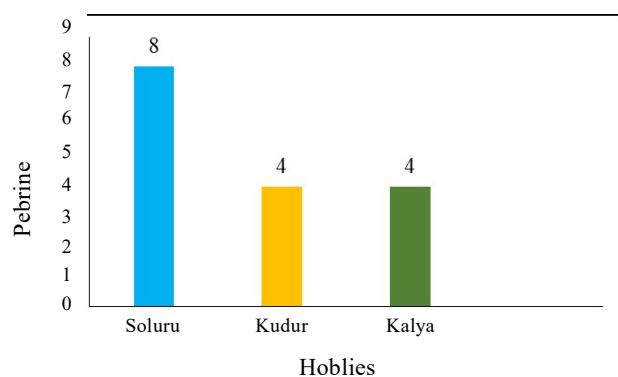


Fig. 4 : Presence of pebrine in silkworm excreta collected from Soluru, Kudur and Kalya hoblies of Magadi taluk in Mysore seed area

secondary means of spreading contamination. These spores survive in the external environment for many years unless they are dehydrated, heated or exposed to sunlight and act as a source of secondary contamination. Further, Vijayakumari *et al.* (2019) reported that pebrine incidence ranged from 1-3 per cent in Hindupur area, Andhra Pradesh. In Karnataka including multivoltine seed area of Kunigal / Magadi and the bivoltine seed area of Anekal / Attibele and in Tamil Nadu including Hosur bivoltine seed area and Denkanikottai multivoltine seed area regions were all found to be free from pebrine disease.

Samson *et al.* (1999) conducted survey on incidence of pebrine disease in Karnataka and reported 2.32 per cent of pebrine disease incidence. They also noted the seasonal variation in the incidence of pebrine disease. Rainy and winter seasons were reported as the most favourable for disease development and prevalence.

The study on the prevalence of pebrine disease in Magadi taluk of Mysore seed area was carried out during November 2022-April 2023 in Magadi taluk of Mysore seed area, Karnataka, India to assess the incidence of pebrine disease in graiages, CRCs and farmer's field.

The results revealed that the disease incidence in pupae and mother moth samples collected from two Government Model Grainages (Magadi and Kudur) was found to be on par 6.66 per cent. However, the DFLs collected from both the grainages had no incidence of pebrine. The overall per cent mean pebrine disease incidence in two Government Model Grainages (Magadi and Kudur) in Magadi taluk of Mysore seed area was 4.44. On microscopic examination of each of the thirty samples collected from Mathikere and Koramangala CRCs no pebrine spores were detected, indicating no pebrine incidence in Mattikere and Koramangala CRCs of Magadi taluk in Mysore seed area. The pebrine disease incidence in late age silkworms collected from Soluru, Kudur and Kalya hoblies in farmer's field was 12.00, 8.00 and 8.00 per cent, respectively. The presence of

pebrine in silkworm excreta collected from Soluru, Kudur and Kalya hoblies in farmer's field was 8.00, 4.00 and 4.00 per cent, respectively. The overall average disease incidence covering the three different hoblies including 15 villages surveyed in Magadi taluk of Mysore seed area was 9.33 per cent in late age silkworms and 5.33 per cent in silkworm excreta.

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