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## Research Article

### STUDIES ON TRANSMISSION RATE OF PEBRINE (*NOSEMA MYLITTA*) TO HEALTHY TROPICAL TASAR SILKWORM (*ANTHRAEA MYLITTA* D.) FROM SECONDARY SOURCES OF CONTAMINATION IN SILKWORM REARING PLOTS

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#### ABSTRACT

Tropical tasar silkworm rearing is mainly being practiced by tribal peoples of Middle and north eastern states of India. The productivity to tasar is being affected by the different pathogens which are causing considerable yield loss to silkworm farmers. Among the different diseases pebrine which is caused by protozoa, *Nosema mylitta* is most important pathogen causing majority of damage due to its transovarial mode of transmission. To overcome the disease spread, the strict mother moth examination is being used to prepare disease free layings for the silkworm rearers. Although the disease free layings are being supplied and used for the rearing of tasar farmers, the pebrine disease was noticed in farmers fields. The present study was carried out to understand the mode of transmission of pebrine disease by secondary sources such as, leaf, bark, soil and excreta present in the tasar silkworm rearing plots. The results reveals that, all the secondary sources studied in the present study showed the transmission of pebrine disease to healthy tasar silkworm larva in the rearing plots. Among the different secondary sources, the rate of transmission of pebrine disease is more in soil contaminated plots when compared to other secondary sources of infection.

**Key words:** Tasar, Pebrine, *Nosema*, Secondary sources, Bark, Leaf, Soil & excreta.

#### INTRODUCTION

The Tasar silkworm, *Antheraea mylitta* Drury is prone to infection of various organisms. Microsporidiosis of the silkworm caused by highly virulent parasitic microsporidia *i.e.*, *Nosema mylitta*, is one of the most serious malady which determines the success or failure of sericulture industry in any country<sup>1</sup>.

Pebrine is a dreaded disease of Tasar silkworm caused by *Nosema mylitta* (Protozoa: Microsporidia). It is a unique pathogen transmitted through egg (transovarial transmission), by the ingestion of the contaminated leaf (horizontal transmission) and by contaminated egg surface (transovum transmission).

Pebrine, a chronic and disastrous disease of the silkworm is responsible for the sudden collapse of industry in France and Italy in 1965. Even though the fight against this disease in all the Sericulture practicing countries is going on since more than 100 years, the disease is under check by following the techniques of strict mother moth examination for the disease free silkworm eggs, in addition to disinfection and hygienic rearing. Though the disease is under reasonable control, it appears sporadically due to infected seed and persisting secondary contamination in the rearing plot<sup>2</sup>.

The silkworm larvae infected during early stages of 1<sup>st</sup> and 2<sup>nd</sup> instar die up to 5<sup>th</sup> instar, but if infection occurs during 4<sup>th</sup> and 5<sup>th</sup> instar, larvae manages to survive and form cocoons but the silk from the cocoon of infected larvae is usually much inferior.

The earlier report reveals that, the rate of transovum transmission of *Nosema mylittans* in Tasar silkworm ranges from 52.68% to 92.24%<sup>3</sup>.

#### MATERIALS AND METHODS

##### Selection of Plots & Collection of samples

Selection of plots of this experiment was done in plots where 2<sup>nd</sup> crop rearing was done (previous year) during July, 2015 in rearing plots of PPC, Kharsawan. Samples of soil, leaves, bark and excreta were collected from different plots. Five grams of soil samples were collected from top, middle (1 feet deep) and lower (3 feet deep) portion of the plot from 5 different places.

Leaves (5 numbers) were collected from 5 different trees from one plot. Bark samples from 5 trees were collected from the plot. Samples were collected from all the plots and labeled accordingly. The collection of soil samples were mainly done around the trees where 2<sup>nd</sup> crop silkworm rearing was conducted. The moisture content in the soil was further removed by drying the samples in the shade.

The excreta samples were collected from the rearing plots where rearing is being conducted (2<sup>nd</sup> Crop). The excreta (50 grams) was collected randomly with three replicates from the vicinity of trees where rearing is being conducted. The faecal matter was air dried before conducting experiments. Microscopic examination of each sample was done at 600x presence of pebrine spores and concentration was recorded.

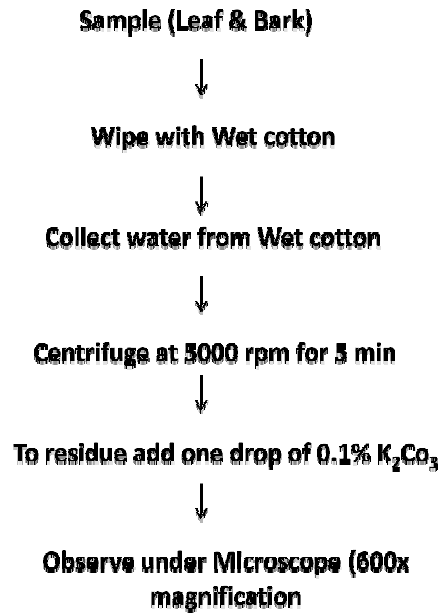


Figure 1: Detection of pebrine spores in bark and leaf

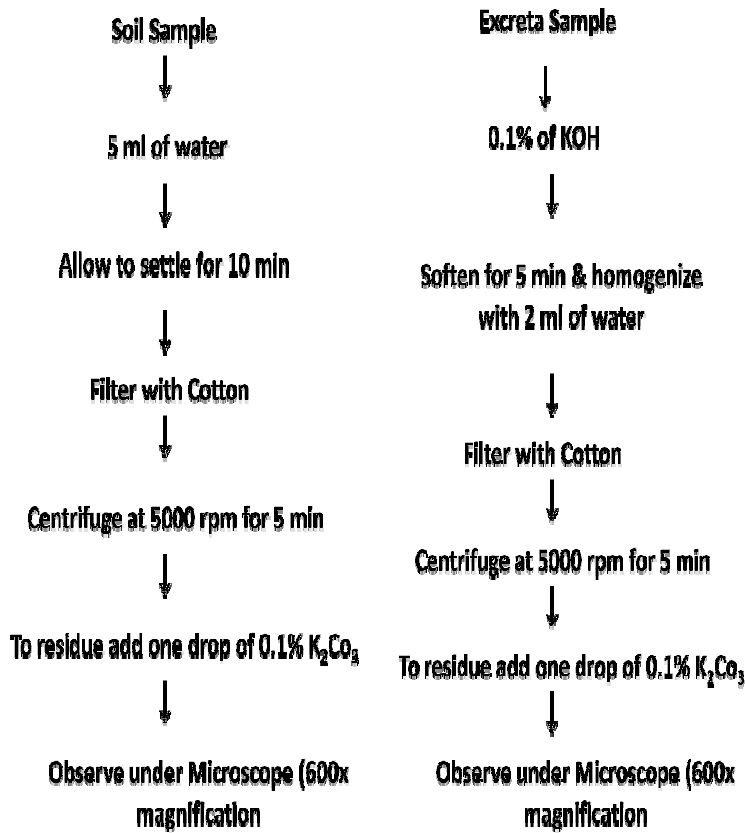


Figure 2: Detection of pebrine spores from soil and Excreta

**Table 1: Examination of different layers of soil for presence of pebrine spores**

Sl. No.	Upper soil				Middle soil (1 feet depth)				Lower soil (3 feet depth)			
	Field1	Field2	Field3	Avg	Field1	Field2	Field3	Avg	Field1	Field2	Field3	Avg
1	++	+++	++	++	++	+	+	++				-
2	+++	++++	+++	+++	+	+++	++	++			+	+
3	++	+++	+	++	++	+	++	++	+			+
4		++	+	+	++	+	+	+			+	+
5	++			+	+			+				-

NOTE: 0-3 +, 3-5 ++, 5-7 +++, 7-9 +++++, - No pebrine spores were found in soil supernatant sample.

**Table 2: Examination of Bark, Leaf and Excreta for the presence of pebrine spores**

Sl.No	Bark				Leaves				Excreta			
	Field1	Field2	Field3	Avg	Field1	Field2	Field3	Avg	Field1	Field2	Field3	Avg
1	+	++	+	+	+	+		+	++	+	++	++
2	+++	+++	+++	+++				-	+++	++	+++	+++
3	+	++	+	+				-	++	++	++++	+++
4				-	+			+	++	+++	++	++
5	+	++	+	+				-	++	++	+	++

**Table 3: Microscopic observation of larval sample for presence of pebrine spores**

Days after brushing	Bark	Leaves	Soil	Excreta
1	No Spores	No Spores	No Spores	No Spores
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16	+		+	
17	+		+	
18	+		+	
19	+		+	
20	+		+	
21	+		+	
22	+		+	
23	+		+	
24	++		+	
25	++		+	
26	++		++	+
27	++	+	++	+
28	++	+	++	+
29	++	+	++	+
30	++	+	++	++
31	++	+	++	++
32	+++	+	++	+++



**Figure 1: The typical symptoms of pebrine disease noticed in healthy tasar silkworm reared on the secondary source of infection**

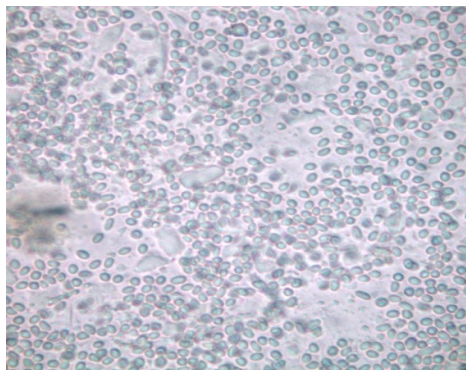


Figure 2: The pebrine spores observed under phase contrast microscope (600x magnification)

## RESULTS

During the selection of plants for experimentation, the different soil samples were subjected to microscopic examination to confirm the presence of pebrine spores. During the examination, varying concentration of pebrine spores were noticed in different soil strata. The upper soil layer has more pebrine spore in comparison with middle and lower soil layer. The plants having more spores around the vicinity of plants were selected for brushing of healthy larvae to understand the transmission of pebrine from soil (Table 1).

Similarly collected Bark, Leaves and Excreta samples were subjected to microscopic examination for the presence of pebrine spores. More concentration of pebrine spores was noticed in excreta when compared to leaf and bark samples. Leaf samples of plants showed very scanty presence of spores. Only two samples among five samples showed the presence of spores in very less concentration (1-3 spores) during the examination. The plants having more spores in the surrounding areas of plants were selected for brushing of healthy larvae to understand the transmission of pebrine from other sources (Table 2).

The testing of larvae which were reared on the plants having different secondary source of contamination showed the different pattern of pebrine disease expression (presence of pebrine spores during microscopic examination). The examination of larva in the soil contaminated plants showed early pebrine symptoms (black peppery spots and stunted growth) when compared to other secondary sources (Figure 3). The spores showed the typical characteristics of pebrine such as oval shape, refractile nature and Brownian movement (Figure 4). The larva during examination showed presence of pebrine spores from thirteen days after brushing in soil, sixteen days in bark, twenty six days in excreta and twenty seven days in leaf as pebrine transmission source (Table 3).

## DISCUSSION

Transmission of pathogen is a key factor in pathogen-host interactions that can influence the population dynamics of the host<sup>4</sup>. There are several potential pathways by which pathogens are transmitted within a host population – the most common are vertical transmission, i.e. the direct transfer of infection from parent to progeny<sup>5</sup>, and horizontal transmission, i.e. the transmission of the pathogens from one individual to another of

the same generation. The results of our study also confirm that, the horizontal spread of disease is occurring due to secondary contamination at various levels during rearing. Therefore, it is essential to keep continuous surveillance during rearing of silkworm and take preventive measures.

With the introduction of Mother-moth examination, Pebrine free eggs are obtained and used for all rearing purposes. As the rate of horizontal transmission of *Nosema mylittans* in Tasar silkworm ranges from 52.68% to 92.24% when 2%, 4%, 6%, 8% and 10% of carriers were introduced in the healthy population<sup>3</sup>, as such, a major problem for its control arises outside the lab where all environmental factors are at play. This is where majority of its control is required and the control would determine the outcome of the crop. Presently monitoring of silkworm crops is done by random sampling of unequal worm homogenizing the entire worm and microscopic examination of homogenate to detect the spores. This is rather a cumbersome process and the homogenate contains tissues debris, fat globules and undigested food particles which are likely to mark or interfere with the appearance of spores, especially, when initial infection is low, in addition to this, the scope for larval examination in the field has limitations of restricted sampling where in majority of farmers are reluctant to waste worms, if the crop looks healthy.

Infection also results from diseased and dead larvae, faeces of larvae, moths, diseased egg shells, larval and pupal exuviae etc. In the rearing bed major source of infection is the faeces of diseased larvae, contaminated tray, seat paper and dust from infected rearing and leaf storage rooms. Sometimes infection takes place through contaminated mulberry leaf from field. The excreta and dead larvae of pebrine infected wild insects may also form a source of infection. Our results also confirm that, faecal matter (excreta) is also source of pebrine infection to tasar silkworm larvae during rearing period. Examination of incidence of Pebrine in leaves, barks, soil and silkworm litter via the use of microscopes and the severity of each incidence has been noted. The results of the present study revealed that, pebrine disease is transmitted from different secondary sources. The time taken for the expression of the disease varies from source to source and concentration of spores present in the source of infection. Based on the results of the present study, methodologies used in our studies can be used for the effective identification of pebrine spores in different contamination source which can be used to assess the health status of the larvae during rearing (Table 1-3).

## CONCLUSION

Based on the results of the present study, pebrine disease is transmitted from secondary sources mainly soil, leaf, bark and excreta. The rate of transmission of pebrine disease is more in soil contaminated plots when compared to other secondary sources of infection. The time taken for expression of typical pebrine symptoms was less in larvae reared in plots having soil contamination in comparison with other secondary sources of infection.

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