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Studies on the Pathogenicity of Pebrine Spores Isolated from Ichneumon Fly (*Xanthopimpla Pedator)* Infesting Tropical Tasar Silkworm on Healthy Silkworm Larvae

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ABSTRACT

Tasar culture is the livelihood of the majority of poor tribal peoples of central and north eastern India. The tasar culture is being practiced in the outdoor condition it is amenable to different pests and predators which also harbors the tasar silkworm infecting diseases causing pathogens such as pebrine disease. The present study was aimed at isolating and purifying the pebrine spores from Ichneumon fly infesting tasar silkworm. The purified spores were artificially inoculated to healthy tasar silkworm larvae per orally. The observation of pebrine spores in inoculated larvae showed the presence of pebrine spores after 16 days post inoculation (dpi). The inoculated larvae showed typical symptoms of pebrine in tropical tasar silkworm *i.e.,* appearance of black pepper spots on the larval integument which were distributed sparsely. The result confirms that, pebrine disease can be transmitted by the Ichneumon fly carrying the pebrine spores. This also confirms that, Ichneumon fly can be secondary source of pebrine contamination in the tasar rearing fields.

Keywords: Ichneumon Fly, *Xanthopimpla Pedator,* Pebrine, Artificial Inoculation, Secondary Contamination.

I. INTRODUCTION

The tasar culture is a forestry based industry which is providing livelihood to poor tribal people residing in different parts of India. Tropical tasar silkworm (*Antheraea mylitta* D.) is a sericigenous, polyphagous insect which feeds on different plants in different states of India (Mahapatra, 2009). Since tasar culture is carried out in outdoor condition, the silkworm is easy target for more number of pests of both host plants and silkworm which can results in 70-80% crop loss (Mathur and Shkla, 1998).

Numerous insects thrive on tasar silkworm, among them *Xanthopimpla* (hymenoptera), *Blepharipa* (Diptera) are pupal and larval parasites, *Sycanus*, *Cantherona*, (hemiptera), *Hierodulla bipapilla* (dictyoptera), *Polistes* and *Oecophylla* (hymenoptera) are predators of different age groups of tasar silkworms. The cumulative effect of these results in 30 - 40% tasar crop loss (Shivakumar and Shamita, 2013).

The Ichneumon fly, *Xanthopimpla pedator* Fabricius (Hymenoptera: Icheumonidae) are the major endo-parasitoids of tasar silkworm. It is widely distributed in tropical tasar region of India and other parts of world. The ichneumon fly belongs to the order Hymenoptera, family ichneumonidae. Adult fly is bright yellow in colour with a number of black bands and there is a black spot on each sternum located dorso-ventrally. Length of the adult is about 2cm with 1cm long ovipositor in female with long stylets (two in number) (Shivakumar and Shamita, 2013).

The female fly lays eggs inside the pre-pupal body by inserting its ovipositor through freshly formed / flimsy cocoon shell. Only one egg is deposited in each host. The maggot after hatching consumes the entire pupal

content except the skin and pupates. The adult fly emerges from the cocoon by piercing the cocoon which renders the cocoon unfit for reeling (Aruna *et al.*, 2014).

Pebrine is a dreaded disease of Tasar silkworm caused by *Nosema mylitta* (Protozoa: Microsporidia) which is causing devastating effect with yield loss up to 25-40% (Sahay, 2000). Pebrine disease is transmitted through transovarial transmission viz., mother moth to offspring along with secondary source of infections (transovum and per oral) (Kumar *et al.*, 2013). No much information is available on the transmission of pebrine spores from pests infesting tasar silkworm. With this background, the present work has been carried out to purify the pebrine spores from the Ichneumon fly and study the pathogenicity of purified spores on the healthy tasar silkworm larvae.

II. METHODS AND MATERIAL

Collection and Examination of Yellow Fly:

During pre-grainage operations, the emerged Ichneumon flies were collected from different grainages in PPC, Kharsawan. The individual Ichneumon fly moths were subjected to microscopic examination to confirm the presence of pebrine spores. The spores bearing Ichneumon fly samples were further subjected to purification of spores.

Purification of Pebrine Spores from Yellow Fly:

The pebrine spore bearing Ichneumon fly samples were pooled and subjected to centrifugation at 4,000 rpm for 10 minutes. The supernatant was discarded and the concentrated sugar solution was added to sediment and centrifugation was carried out as described earlier. The white sediment in the tube was observed for the purity of pebrine spores.

Inoculation of Purified Pebrine Spores to Healthy Tasar Silkworm Larvae:

Newly hatched disease free larvae were fed with leaf sprayed with pebrine spore purified from Ichneumon fly in the plastic box. After complete consumption of leaf, the larvae were shifted to plant free from pebrine contamination. The larval samples were collected regularly and examined for the presence of pebrine spores and results were recorded.

III. RESULT AND DISCUSSION

Results

The results of the inoculation of pebrine spores purified from the pebrinized Ichneumon flies collected from grainage house to healthy tasar silkworm produced typical pebrine symptoms (retarded growth, irregular and inactive feeding along with black peppery spots on the integuments) (Figure 1). The distribution of black spots was very scanty and sparse.

Figure. 1. The pebrine challenge inoculated tasar silkworm larvae showing black peppery spots on the integument (4th instar larvae)

Regular microscopic examination of pebrine inoculated larva showed presence of pebrine spores from 17 days post inoculation (dpi). The intensity of pebrine spores was very less (1-3 spores) in 17-21 dpi. The frequent increase in concentration of pebrine spores (4-5 spores) was noticed from 22-24 dpi. The more increased concentration of pebrine spores (more than 5 spores) was noticed from 25 dpi till the harvest of the crop. The majority of the cocoons produced from pebrine inoculated larvae were of flimsy nature (Table 1).

Table 1. Observation for the presence of pebrine spores in yellow experiment:

Days post inoculation (DPI)	Observation
1	**No Pebrine spores noticed (Upto 16 DPI)**
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	+++

Discussion

The pebrine spores were noticed in the extract of Ichneumon fly which were collected from grainage house. The pathogenicity studies of isolated and purified pebrine spores from pebrinized Ichneumon fly revealed that, the pebrine spores present in the Ichneumon fly can also infect the healthy tropical tasar silkworm artificially (Figure 1). It is also understood that further studies on the incidence of Pebrine on Ichneumon fly and other insects may help provide better explanation for the high rates of horizontal transmission primarily in the tasar silkworm rearing fields (Table 1). The control over the secondary infection will reduce the damage caused and also increases the yield qualitatively and quantitatively (Shivakumar and Shamitha, 2013).

IV. CONCLUSION

The Ichneumon flies collected from the grainage showed the presence of pebrine spores. The pathogenicity test of purified from pebrine bearing Ichneumon flies showed the typical symptoms of pebrine in healthy tropical tasar silkworm. The mode of transmission of pebrine from Ichneumon fly to tasar silkworm larvae needs to be understood.

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