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Study on impact of parasite (*Nosema* species) on characters of tropical tasar silkworm *Anthereae mylitta drury*

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Abstract

Infection of the pebrine disease has been found to be highly virulent and harm the cocoon yield as well as characters of silkworm *Anthereae mylitta*. Therefore, an attempt was made to evaluate the impact of parasite *Nosema species* on the ecorace (*Sukinda)* of *A.mylitta* in respect of transovarial transmitted (T1), secondary infection (T2) and healthy silkworm (T3). In comparison to T3, the number of larval mortality was 16 and 11 in T1 and T2 respectively; whereas as number of pupal mortality was 6 and 5 in T1 and T2 respectively.The larval weight, number of moths emerged, number of eggs laid and percent hatchability were reduced in T1 and T2 in comparison to T3.The infected layings were high in T1(51%) and T2(42%) as against T3(0%). Similarly, the infected moths were 34% in T1 and 15% in T2 as against 0 percent in T3.All the characteristics parameters of cocoon were reduced in T1 and T2 against T3. The study explains that there was no significant variation between T1 and T2 on different parameters of larva, pupa and cocoon characters.

Key words

Nosema impact, Ecorace, Pebrine disease, Infection, *Anthereae mylitta*

Introduction

Antheraea mylitta drury a sericigenous endemic species of India is often infected with an intracellular parasite of the genus *Nosema* which causes pebrine disease (Rath *et al.,* 2003). Pebrine can be acquired from the mother moth (primary infection) or from the environment through food (secondary infection) (Bansal *et al*.,1997). Infected larvae show black pepper like spots on the integument are the infected hypodermal cells which become enlarged, vacuolated and get blackened due to the formation of melanin (Ganga, 2003). *A.mylitta* larvae infected with *Nosema* sp. show extended development period, reduced size and larval weight in comparison to uninfected ones (Rath *et al*., 2003). The infected larvae of *Bombyx mori* show significant changes in the cocoon weight, shell weight, denier, reelability etc., (Bhat and Nataraju, 2005). Chakrabarti and Manna (2006) identified three *Nosema spp*. from three non-mulberry silkworms as *Nosema mylitta* from A*ntherae*a *mylitta*, *N.ricini* from *Philosamia ricini* and *N.assamensis* from *A. assamensi*s. Some of the microsporidia show transovarial transmission and some are not (Fujiware, 1980; Fujiware, 1984; Ananthalakshmi et al., 1994; Nageshwara Rao *et al*., 2004). Most of the species are highly virulent and mortality caused by them also varies. No silkworm race reported to be completely immune to *pebrin*e. Spores of *Nosema sp*. can be detected at any stage of life cycle in Tasar silkworm (Sharan *et al*., 1992) and are different in size, shape and pathogenecity(Bhat *et al*.,2009).

The present investigation was carried out to evaluate the level of impact of parasite *Nosema* on various characters of *Anthereae mylitta drury(Sukinda)* attained through transovarial and secondary modes of infection.

Materials and Methods

The present work was done at Basic Seed Multiplication and Training Centre, Central Silk Board,

Rampachodavaram, East Godavari district, Andhra Pradesh, India by collecting the *Anthereae mylitta* cocoons (*Sukinda)* during the month of June 2010. The cocoons were preserved in the cages made up of wire mesh of size 2ftx2ftx2ft under temperature of 28-30ºC and humidity 69-71%. The emerged moths were tested for *pebrine* disease by a method derived from that used in sericulture **(**Pasteur, 1870). In this method, the abdomen of an adult was severed with scissors, placed in a small mortar, mixed with water and crushed with pestle. A drop of the smear was placed on a clean slide and examined under a microscope of 600X magnification for *Nosema sp.*, spores. The eggs laid by healthy and infected moths were collected and incubated for further research.

Isolation of *Nosema* spores: *Nosema* spores were isolated from diseased larvae of Sukinda homogenized in 0.6% K_2CO_3, filtered and the filtrate was centrifuged at 3000 rpm for 15min. The supernatant was discarded and sediment suspended in 1 ml distilled water, mixed with percoll (poly vinyl silica particles**)** and centrifuged at 5000 rpm for 15 min **(**Sato and Watanabe, 1980). The spores were collected from the sediment and washed in distilled water thrice and stored as stock at 4°C in 0.85% NaCl until use. The spores were suspended in distilled water and spore count was enumerated by Neubaeur haemocytometer. The stock solution was diluted to obtain an inoculum dosage of $1x10^7$ spores ml^{-1}. Healthy third instar larvae were starved for 3-4 hrs to induce hunger and than fed on the *Terminalia arjuna* leaves smeared with inoculum dosage.

The first instar larvae were divided into three treatments, Transovarian infection-T1,Secondary infection-T2 and Healthy worms (Control)-T3**.** Larvae hatched from the eggs laid by the infected moths were kept as T1. Larvae fed on the leaves treated with the *Nosema* spores of $1x10^7$ spores ml^{-1} were kept as T2. Larvae hatched from the eggs laid by the healthy moth were kept as T3. The three treatments were brushed and reared separately on freshly cut *Terminalia arjuna* leaves in the laboratory. Each treatment had five replications of 50 larvae and reared till cocooning following standard procedure.

Larval weight, larval mortality, pupa mortality, moth emergence, fecundity, hatchability% and cocoon characters for treatments T1, T2, T3 were recorded. Larvae that died because of pebrine disease were examined for the presence of Nosema spores under light microscope everyday till spinning were included and those died otherwise excluded for data analysis.

Statistical analysis : One-way analysis of variance (ANOVA) was used for comparison between T1, T2 and T3 groups. Critical differences (CD5%) was analysed by Tukeys post-hoc procedure (John Wilder Tukey, 1984).

Results and Discussion

Present studies shows that the microsporidian (Nosema) isolate from the *Sukinda* can cause secondary infection in the healthy larvae and the infection can also pass from infected moths to the progeny through transovarial mode. The findings of Remadevia *et al*. (2010) working on pathological effects of microsporidian isolate from teak defoliator observed 88.7% of transovarial transmission. In case of T1 and T2, the larval weight was found to be less by 28.8 and 22.7% (9.02, 7.1gm) respectively in comparison to control. The decrease in food consumption, digestion, relative consumption rate, efficiency of conversion of ingested food in fifth instar of *A.mylitta* infected with *Nosema sp*. reduced the relative growth rate of the larvae (Rath *et al*., 2003).

Table 1 : Impact of pebrine on the larval weight, survival, fecundity and hatching of *Anthereae mylitta. D* (*Sukinda*, First crop)

Treatment	Larval weight (gm)	Larval mortality (number)	Pupa mortality (number)	No.of moths emerged	% of infected moth	No. of eggs laid	% of infected layings	Hatchability (%)
T1	22.26±1.02	16±1	6±1	28±1	34.1±1.22	112±2	50.96±1.03	46±1
T2	24.18±1.25	11±1	5±1	34±1	15.4±1.34	131±3	41.88±0.93	52±1.5
T3	31.28±1.52	0	0	49	0	167±2	0	79±1
CD5%	-	0.17	0.12	0.17	0.13	11.3	-	-
Statistical difference of								
T1 T2	-	5.0±1	1±1	6±1	18.7±1	19±2	-	-
T3 T1	-	16±1	6±1	21±1	34.1±1	55±2	-	-
T3 T2	-	11±1	5±1	15±1	15.4±1	36±2	-	-

CD: Critical difference. Each value represents the mean of five replications ± SE. The values presented in parentheses indicate the increase (+) or decrease (-)

Table 2 : Impact of pebrine on the cocoon characters of *Anthereae mylitta.D* (*Sukinda*,First crop)

Treatment	Single cocoon weight (gm)	Single shell weight (gm)	SR%	Single cocoon filament length (m)	Denier (%)	Reelability	Weight of silk reeled from single cocoon (gm)
T1	7.08±0.32	0.89±0.08	12.56±0.78	148.15±2.13	16.41±1.05	38.13±1.56	0.27±0.05
T2	7.52±0.58	0.94±0.23	12.36±0.13	195.74±3.86	16.55±0.85	47.87±1.68	0.36±0.04
T3	9.56±0.53	1.12±0.32	11.88±0.23	273.21±3.25	14.49±0.58	50.21±1.13	0.48±0.06
CD5%	0.35	0.04	-	96.23	0.47	1.31	0.03
Statisticaldifference of							
T1 T2	0.44±0.26	0.05±0.15	-	47.59±1.73	0.14±0.2	9.74±0.12	0.09±0.01
T3 T1	2.48±0.21	0.23±0.24	-	125.06±1.12	1.92±0.47	12.08±0.43	0.21±0.01
T3 T2	2.04±0.05	0.18±0.09	-	77.47±0.61	2.06±0.27	2.34±0.55	0.12±0.02

CD: Critical difference. Each value represents the mean of five replications ± SE. The values presented in parentheses indicate the increase (+) or decrease (-)

The mortality rate of the larvae and pupa was high by 32, 12% (16,6) and 22 and 10% (11,5) in T1 and T2 than T3. Statistical analysis for larval mortality between the groups explains that T1 and T2 do not differ significantly where as, T1 and T3, T2 and T3 differ significantly because of pebrine. In case of pupal mortality, since the comparisons between T1 and T2, T1 and T3, T2 and T3 result in a mean difference less than CD5%, the three groups do not differ significantly. The previous reports of Bhat *et a*l. (2009) on *Bombyx mori* have shown maximum mortality of silkworm larvae during early stages than fourth and fifth instars infected by *Nosema sp*. In T1 and T2 batches 56% and 68% (28,34) larvae survived to form cocoons while incase of T3 batch 98% (49) larvae were survived to form cocoons.

The comparison between the groups for number of moths emerged explains that T1 and T2 do not differ significantly whereas T1 and T3, T2 and T3 differ significantly. There was 34.1% infection found in moths emerged from T1 and in case of T2, 15.4% of the moths emerged found to be infected. Since all three comparisons results in a difference greater than CD 5% the three groups vary significantly.Because of pebrine, eggs laid by the moths of T1, T2 batch was 33%, 21.6% less than T3 batch and the percentage of infected layings were also high. The comparison for fecundity between the groups explains a significant variation among all the three groups. The decline in ovary weight, fecundity, and fertility in *A.mylitta* larvae infected with *Nosema sp*. was reported by Rath *et al*. (2003). Bansal *et al*. (1997) have reported the high spore concenteration of *Nosema* in the gonads of *A.mylitta, A.assamensis* and *B. mor*i will effect the reproductive potential and fertility**.** Significant variation in the hatchability was also noticed among T1, T2 and T3 batches.

It is evident that the cocoon characters were poor in case of the cocoons obtained from T1 batch whereas the cocoons from T2 batch have shown medium results. Cocoon weight and shell weight in T1 and T2 batches were recorded less than the T3 batch and in T1batch it was less by 26% (2.48gm) and 20.5% (0.23gm). In case of shell weight, since all three comparisons result in a difference greater than CD5%, the three groups differ significantly. Rath *et al*. (2003) have reported the decrease in shell weight in *A.mylitta* larvae infected with *Nosema* sp. Rath and Sinha (2005) working on parasitization of fifth instar larvae of *A.mylitta* by *Uzifly* have reported the decrease (27-63.5%) in cocoon weight and shell weight in the infected larvae.

The filament length in T1 batch was recorded least and it was 45.7% (125.06m) less than the T3. The filament length of T2 batch was 28.4% (77.47m) less than T3 batch. The comparison for filament length between the groups explains that T1 and T3, T2 and T3 differ significantly because of pebrine. Highest denier value can be attributed to T1 batch and least denier value to T3 batch. Thus, the silk quality seems to be reduced due to the infection. According to Bhat *et al*. (2009) silk from the cocoons of infected larvae is usually much inferior.

The reelability also reduced in case of infection and it was 24% (38.13) less in T1 batch cocoons than T3 batch (50.21). The reeled silk weight was also reduced a lot in case of infection rather than the healthy cocoons. In case of denier and weight of the silk reeled, since all three comparisons result in a difference greater than CD5%, the three groups differed significantly.Rath *et al*. (2003) reported the decrease in silk gland weight in *A.mylitta* larvae infected with *Nosema sp*. which finally reduces the silk production.

Thus, in conclusion the impact of *Nosema* infection on various characters of Sukinda was high through transovarial

infection and secondarily infected. A control over the infection will reduce the damage caused and also increases the yield qualitatively and quantitatively.

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