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Studies on the impact of *microsporidiosis* on tropical tasar silkworm *Anthereae mylitta Drury*

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ABSTRACT
Objective: Microsporidiosis (Pebrine) is one of the dreadful disease seen in *Anthereae mylitta Drury (Andhra local ecorace*), caused by *Nosema* species. Infections of the disease are highly virulent and harm the cocoon yield as well as cocoon characters. Therefore an attempt has been made to evaluate the impact of *Microsporidiosis* on *Andhra local* in respect to transovarial transmission (observed as T1), secondary infection (observed as T2) and Healthy silkworm (observed as T3).
Results: The larval and pupa mortality was 28%, 8% in T1, 20%, 6% in T2 and in case of T3 there was no mortality. In comparison with the control, there was a significant impact ($p \leq 0.05$) of infection on larval weight, Number of moths emerged, fecundity, hatching percentage cocoon weight, shell weight, filament length, reelability and weight of the silk reeled. Statistical analysis explains that there is no significant ($p \leq 0.05$) variation between T1 and T2 groups.
Conclusion and application: A control over the secondary infection will reduce the transovarial infection and also yield could be increased quantitatively and qualitatively.
Key words: *microsporidiosis, nosema,* transovarial, secondary infection, Andhra *local.*

INTRODUCTION
The tasar silkworm, *Antheraea mylitta Drury*, *Andhra local* ecorace is an exclusive race of Andhra Pradesh. In view of its superior cocoon characters such as compact and hard cocoons, high reelability, high shell ratio and low denier this ecorace deserves to be developed further (Thangavelu, 1992). However since it suffers from climatic hazards, prolonged larval period, heavy larval mortality, indefinite period of diapause, erratic moth emergence and poor egg laying behavior (Sen and Jolly., 1967) the ecorace has been thoroughly neglected leading to the extinction of this ecorace
Andhra local is often infected with an intracellular parasite of the genus *Nosema*. *Pebrine* can be acquired from the mother moth (primary infection) or from the environment through food (secondary infection). Infected larvae show black pepper like spots on the integument. These infected hypodermal cells become enlarged and vacuolated and blackened due to the formation of melanin (Ganga, 2003). 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, and reelability (Shabir Ahmad Bhat and Nataraju 2005). Several strains and species of microsporidia have since been isolated from silkworms and other insects (Kishore *et al*., 1994; Bhat and Nataraju 2004). Chakrabarti & Manna (2006) identified three *Nosema sp*. from

three non-mulberry silkworms as *Nosema mylitta* from *Antheraea mylitta*, *N.ricini* from *Philosamia ricini (Eri silkworm)* and *N.assamensis* from *A. assamensis* (Muga silkworm). 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 is reported to be completely immune to *pebrine*. 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 pathogenicity (Shabir Ahmad Bhat *et al*.,, 2009). The present experiment is an attempt to protect the ecorace.

MATERIALS AND METHODS:

Andhra local cocoons were collected as per the standard norms such as weight, colour, size of cocoons and length of the peduncle from the forest patches of Jakaram, Warangal District, Andhra Pradesh, India. The cocoons were preserved in the wire mesh cages of size 2 ft x2 ft x2 ft under temperature of 29±1 ºC and humidity 70 % ± 1 %. 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 is severed with scissors, placed in a small mortar, mixed with water and crushed with pestle. A drop of the smear is placed on a clean slide and examined under a microscope of 600x magnification for *Nosema sp.*, spores. Eggs are collected from both healthy and pebrine infected moths and kept in incubation for further research.

Isolation of microsporidian spores: Microsporidian spores were isolated from diseased larvae of Andhra local ecorace homogenized in 0.6% Potassium Carbonate (K_2CO_3), filtered and the filtrate was centrifuged at 3000 rpm for 15 minutes to sediment the spores. The supernatant was discarded. The sediment was suspended in 1 ml distilled water and 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^{o}C in 0.85% Sodium Chloride (Na Cl) until use. The spores were suspended in distilled water and spore count was enumerated by a Neubaeur haemocytometer. The stock solution was diluted with water to obtain an inoculum dosage of 1x10^{7} spores/ml. Healthy third instar larvae were starved for 3-4hours to induce hunger and than fed on the *Terminalia arjuna (Arjun)* leaves smeared with inoculums dosage.

Impact of microsporidiosis on the larval weight, survival and the cocoon characters: To study the effect of the microsporidian parasite infection on the larval weight, survival and commercial characters of *Andhra local* first instar larvae were divided into the following 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 microsporidian spores of 1x10^{7} spores/ ml were kept as T2. Larvae hatched from the eggs laid by the healthy *Andhra local* 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 was reared till cocooning. Larval weight, larval mortality, Pupa mortality, Moth emergence, Hatchability percentage were recorded for all the three treatment. Cocoon characters for treatments T1, T2, T3 were also recorded. Larvae that died because of *microsporidiosis* were examined for the presence of spores under light microscope everyday till spinning and included for data analysis. Many spores of oval shape were identified. Larvae that died due to other reasons were excluded from the statistical analysis.

Statistical analysis: To estimate the impact of *microsporidiosis* one way ANOVA was used for the three groups T1, T2 and T3. One concerned approach is whether there would be equal performance in the three groups. Critical differences (CD5%) were analyzed by Tukeys post hoc procedure. All the three groups were compared with each other to find the resultant difference between the groups which explains whether there is a significant variation between the groups on correlation with CD5%. All the data presented were the average values of five replications.

RESULTS:

Table 1 show that the larval weight in case of transovarial infection was 20.17gms while that of T2 and T3 batch was 21.14 and 27.23gms respectively.

Mortality rate of the larvae and pupa found be 28%, 8% in T1 batch due to transovarial transmission and 20%, 6% in T2 batch due to secondary infection.

Table 1: Impact of microsporidiosis on the larval weight, survival, fecundity and hatching of *Anthereae mylitta .D (Andhra local)*

Treatment	Larval Weight (gms)	Larval Mortality (number)	Pupa Mortality (number)	No. of Moths emerged	% of Infected moths	Fecundity	% of Infected layings	Hatching (%)
T1	20.17	14.0	4.0	32	75.87	88	80	26
T2	21.14	10.0	3.0	37	60.88	107	65	48
T3	27.23	0.0	0.0	47	0.00	174	0.0	71
CD at 5%	-	0.13	0.09	-	0.175	1.816	-	-
Difference of means of								
T1 and T2	-	0.08	0.02	-	0.14	0.94	-	--
T1 and T3		0.28	0.08		0.76	1.92		
T2 and T3		0.2	0.06		0.62	1.91		

CD: Critical difference. All the values are the average values of five replications.

In T1 and T2 batches 64% and 74% larvae survived to form cocoons while incase of T3 batch 94% larvae survived to form cocoons. There was 75.87% infection found in moths emerged from transovarial infection and in case of secondarily infection 60.88% of the moths emerged found to be infected. Fecundity found to be 174 in case of the moths emerged from T3 batch and it was 88 in case of moths emerged from T1 batch. The infected layings were found to be 80% in T1 batch and 65% in T2 batch. Hatchability was found to be at the top in case of eggs laid by healthy moths (71%).When compared with the T3 batch hatchability found to be very less in T1 batch (26%) and in T2 batch it was 48%.

Table 2: Impact of microsporidiosis on the commercial characters of *Anthereae mylitta.D (Andhra local)*

Treatment	Single Cocoon Weight (gms)	Single Shell weight (gms)	SR%	Single Cocoon Filament Length (mtrs)	Denier	Reelability (%)	Weight Of Silk Reeled From single Cocoon(gms)
T1	5.95	0.82	13.78	109.12	17.76	36.4	0.21
T2	6.91	0.86	12.44	145.23	16.92	39.5	0.27
T3	8.21	1.11	13.52	266.27	13.47	48.4	0.39
CD at 5%	0.05	0.008	-	-	0.39	0.158	0.011
Difference of means of				-			
T1 and T2	0.952	0.037	-		0.836	0.3	0.056
T1 and T3	2.254	0.292		-	4.288	1.2	0.18
T2 and T3	1.302	0.255			3.451	0.9	0.12

CD: Critical difference. All the values are the average values of five replications.

Table 2 shows the results of cocoon characters. The single cocoon weight of T1 batch was 5.95gms while that of T2 and T3 batch were 6.91, 8.21gms respectively. There was a significant decrease in the cocoon weight of T1 batch while some decrease in the T2 batch from the T3 batch. The shell weight of the cocoon in T1 batch was 0.82gms while that of T2 and T3 batch was 0.86 and 1.11gms respectively. It is observed that the shell weight of T1 batch cocoons has not varied much from that of T2 batch. It is observed

that SR% was high (13.79) in case of cocoons obtained from transovarian infection whereas lowest (12.44) was observed in the secondarily infected batch. The filament length of T1 batch cocoon was 109.12 meters, whereas T2 and T3 batch cocoons have shown 145.23 and 266.27 meters respectively. It is evident from the results that the filament length has seriously affected by the transovarian mode of infection. The filament length of the T2 batch cocoon has decreased by 54.54% when compared with T3. The denier of T1 batch cocoon was 17.76 while that of T2 and T3 batch cocoons were 16.92 and 13.47 respectively. The denier found to be less in T3 batch. A significant increase in the denier values of T1 and T2 batches were noticed which can be attributed to the serious impact of microsporidian infection. The reelability of T1 batch cocoon was 36.4%, while that in T2 and T3 batches were 39.5 and 48.4% respectively. There was a significant difference in the reelability of infected and healthy cocoons. The weight of the silk reeled of T1 batch cocoon was 0.21gms, whereas in T2 and T3 batch cocoons they were 0.27 and 0.39gms respectively. It is evident from the results that the reeled silk weight in T3 batch cocoons was more than 30.77% and 46.16% fromT2 and T1 batch cocoons.

DISCUSSION:

Present studies shows that the microsporidian isolated from the *Andhra local* can cause secondary infection in the healthy larvae and the infection can also pass from infected moths to the progeny through transovarial mode. The virulence was high and had much impact on various characters of the ecorace. 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 transovarian, secondary infection the larval weight found to be less by 26% and 22.4% when compared 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).

The mortality rate of the larvae and pupa in transovarial infection (T1) was high when compared to secondary infection (T2). The comparison for larval mortality between the groups explains that T1 and T2 do not differ significantly whereas T1 and T3, T2 and T3 differ significantly because of pebrine. In case of pupa mortality, since all three comparisons result in a difference less than CD5%, the three groups do not differ significantly. The previous reports of Shabir Ahmad Bhat *et al*.,(2009) on *Bombyx.mori* have shown maximum mortality of silkworm larvae during early stages than fourth and fifth instars infected by *Nosema sp*. In comparison with the control the percentage larvae survived to form cocoons was too low in transovarian infection and it was less by 21.2% in secondary infection. Only 88 eggs were laid by the moths of T1 batch whereas the number of eggs laid by the T2 batch moths was 38.5% less than the T3 batch and the percentage of infected layings were also high in T1 and T2 batch. The comparison for fecundity between the groups explains that T1 and T2 do not differ significantly whereas T1 and T3, T2 and T3 differ significantly because of pebrine. 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) reported that the high spore concentration of *Nosema* in the gonads of *A.mylitta, A.assamensis* and *B. mori* will affect the reproductive potential and fertility. Significant variation in the hatchability was noticed among T1, T2 and T3 *batches* and it was too low in case of T1 batch .

It is evident that the coccon characters were poor in case of the cocoons obtained from transovarian transmission (T1) whereas the cocoons from secondary infection (T2) 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 almost 27.6% less. Since all 3 comparisons for cocoon weight, shell weight result in a difference greater than CD5%, the three groups differ significantly. Rath *et al*.,(2003) 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 *Uzi fly* have reported the decrease (27-63.5%) in cocoon weight and shell weight in the infected larvae. There was no significant ($p \leq 0.05$) variation between the shell ratio of T1 and T3 batches and it was recorded less in T2 batch.

The filament length in T1 batch was recorded least and it was 59% less than the control. The filament length of T2 batch was 45.5% less from T3 batch. Highest denier can be attributed to T1 batch and least denier to T3 batch. Thus the silk quality seems to be reduced due to the infection. According to Shabir Ahmad 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 almost 25% less in T1 batch cocoons than T3 batch. The reeled silk weight from single cocoon was also reduced a lot in case of infection rather than the healthy cocoons. Since all 3 comparisons for reelability and reeled silk weight result in a difference greater than CD 5%, the three groups differ significantly. Rath *et al*., (2003) have 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 microsporidian parasite infection on *Andhra local ecorace* was high on commercial characters through transovarial infection rather than secondarily infected. A control over the secondary infection will reduce the damage caused and also increases the yield qualitatively and quantitatively

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