EVALUATION OF SUSCEPTIBILITY TO MICROSPORIDIASIS IN ECORACES OF TROPICAL TASAR SILKWORM AND ITS IMPACT ON PHENOTYPIC AND COCOON CHARACTERS

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ABSTRACT

Microsporidiasis caused by Nosema found to be highly virulent and harm the phenotypic and cocoon characters. Therefore an attempt has been made to compare the pathogenicity rate on various characters of Sukinda and Daba T.V ecoraces in respect to T1 batch (Infected Sukinda), T2 batch (Infected Daba T.V), T3 batch (Healthy Sukinda) and T4 batch (Healthy Daba T.V.). In comparison with the T1 and T2 batches with T3 and T4 batches it was noticed that there is a significant impact of infection on various characters like larval weight, larvae and pupa mortality, number of moths emerged, percentage of infected moths, fecundity, hatching%, cocoon weight, shell weight, SR%, filament length, denier, reelability and weight of the silk reeled of both infected ecoraces. Statistical analysis explains that there is a significant variation between T1 and T2 groups for all the above characters with least variation between T2 and T4 rather than T1 and T3. A drastic increase of haemocytes were recorded in T1 in comparison with all the other three batches. The present results show that Sukinda ecorace is more susceptible to microsporidiasis than Daba T.V. ecorace. A control over the infection will increase the yield quantitatively and qualitatively.

Keywords: Daba T.V., Haemocytes, Microsporidiosis, Nosema, Sukinda

I INTRODUCTION

The tasar silk is produced by *Antheraea mylitta Drury* (Lepidoptera: Saturnidae), a wild polyphagous tropical sericigenous insect distributed over central India. The species has wide distribution over diverse ecological niche as forty four ecoraces but only a few are semi-domesticated and applied commercially for seed (egg) and silk production [1].

Microsporidiasis caused by Nosema 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 are the infected hypodermal cells which become enlarged and vacuolated get blackened due to the formation of...
Larvae infected with Nosema sp. show extended development period, reduced size and larval weight in comparison to uninfected ones [3]. The infected larvae of Bombyxmori show significant changes in the cocoon weight, shell weight, denier, reelability etc., [4]. Several strains and species of microsporidia have since been isolated from silkworms and other insects [5,6]. Three Nosema sp. from three non-nulberry silkworms as Nosemamyllitta from Antheraeamylittta, N.ricini from Philosamia ricini and N. assamensis from A. assamensis were identified [7]. Most of the species are highly virulent and mortality caused by them also varies. No silkworm race reported to be completely immune to pebrine. Spores of Nosema sp. can be detected at any stage of life cycle in Tasar silkworm [8] and are different in size, shape and pathogenicity[9]. The present experiment is an attempt to compare the pathogenecityrate on various phenotypic and cocoon characters of Sukinda and Daba T.V ecocoraces.

Haemocytes are the important component of the insect immune system. Cellular responses are direct interactions between hemocytes and non-self-materials. The interactions results in responses like nodulation, phagocytosis and encapsulation [10]. In insects several types of hemocytes are observed in the haemolymph [11]. Various functions like mechanization and immobilization of invading organism by encapsulation and phagocytosis, wound repair, coagulation have been attributed to haemocytes [12]. The studies on the susceptibility of three ecocoraces of Anthereamylittta against Am CPV reported that ecocoraces showing reduced number of haemocytes are tolerant to pathogen [13].

II MATERIALS AND METHODS

The present work was performed by collecting Daba T.V and Sukinda cocoons of 200 each during the month of April from the forest patches as per the standard norms like cocoon colour, cocoon shape, cocoon weight and peduncle length. The cocoons were accommodated separately in wire mesh cages of size 2ftX2ftX2ft. Cages were disinfected with 2% Formaldehyde [14]. From April to May 42 ±2% relative humidity and 30±2°C room temperature were maintained. In the month of June temperature has been reduced to 29±1°C and relative humidity increased to 70 ± 5% to get uniform moth emergence. The emerged moths were tested for microsporidiosis by a method derived from that used in sericulture [15]. 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. The eggs from healthy and infected moths of both the ecocoraces were prepared and incubated separately. The hatched larvae were reared in separate fields. To study the effect of nosemia infection on phenotypic characters and cocoon characters in both the ecocoraces the first instar larvae hatched from the eggs laid by the infected, healthy moths were kept as T1 batch (Infected Sukinda), T2 batch (Infected Daba T.V), T3 batch (Healthy Sukinda) and T4 (Healthy Daba T.V.). Each batch had five replications of 50 larvae and was reared till cocooning following standard procedure. Larvae that were died because of microsporidiosis were examined for the presence of spores under light microscope everyday till spinning and included for data analysis. Larvae that were died due to other reasons were excluded from the statistical analysis.
III STATISTICAL ANALYSIS

To estimate the impact of microsporidiosis one way ANOVA was used for the four batches T1, T2, T3 and T4. Critical differences (CD5%) was analysed by Tukeys post hoc procedure. All the data presented were the average values of five replications.

IV RESULTS AND DISCUSSION

Present studies shows that the microsporidian virulence was high and had much impact on various characters of the ecoraces. The pathological effects of microsporidian isolate from teak defoliator have observed to be 88.7% [16]. TABLE 1 show that the larval weight in case of T1 batch was 22.86% less than T2 batch. T1 batch and T2 batch larvae have shown 28.4% and 21.86% reduction in their weights in comparison with T3 and T4. 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 [17]. Mortality rate of the larvae and pupa found be 80% and 50% more in T1 batch than in T2 batch whereas in T3 and T4 batches it was zero. Bombyx.mori have shown maximum mortality of silkworm larvae during early stages than fourth and fifth instars infected by Nosema sp.[9]. In T1 and T2 batches 64% and 80% larvae survived to form cocoons but in case of T3 and T4 it was recorded as 100%. In comparison with all the four batches the % larvae survived to form cocoons was too low in T1 batch. In T1 batch the moths emerged were 66% less than T2 batch. In comparison with T3 and T4 batches the emerged moths in T1 and T2 batches were less by 56.9% and 77.6%.There was 72% infection found in moths emerged from T1 batch and in case of T2 batch 39.5% of the emerged moths found to be infected whereas in T3 and T4 batches the total emerged moths are free of infection.

Fecundity found to be 33% more in case of the moths emerged from T1 batch than T2 batch. Fecundity in T1 and T2 batches was less by 36.3% and 51.8% in relation to T3 and T4 batches. Hatchability was found to be more in case of eggs laid by T2 batch moths (56%) when compared with T1 batch (32%). Hatching% in T1 and T2 batch was recorded low by 39% and 26% in comparison with T3 and T4 batches. Among T1, T2 and T3 and T4 batchessignificant decrease in the hatchability was noticed in case of T1 batch There was a decline in ovary weight, fecundity, and fertility in A.mylitta larvae infected with Nosema sp.[3].The high spore concentration of Nosema in the gonads of A.mylitta, A.assamensis and B. mori will affect the reproductive potential and fertility [18].

TABLE 2 shows the results of cocoon characters. The single cocoon weight of T2 batch was 27% more than T1 batch cocoons. The cocoon weights of T1 and T2 batches was found to be less by 30% and 17% when compared with T3 and T4 batches. The shell weight of the cocoon in T2 batch was 13.3% more when compared with T2. But the shell weights of T1 and T2 were found to be less by 37.5% and 39.3% when compared with T3 and T4 batches. Fifth instar larvae of A.mylitta infected by Uzifly have got decreased (27-63.5%) in its cocoon weight and shell weight [17]. The shell weight will get reduced in the A.mylitta larvae infected with
Nosema sp.[3]. Low shell weight in Andhra local ecorace can be attributed to minimal disease resistance [19]. There was no significant variation between the shell ratio (SR%) of T1, T2 and T3 batches but it was recorded more in T4 batch.

It is evident from the results that single cocoon filament length was more by 16% in T1 batch than in T2 batch. The filament length of the T1 and T2 batch cocoon has decreased by 46% and 44% when compared with T3 and T4 batches. There was a significant difference in the filament lengths of all the four batch cocoons. Microsporidiasis seriously effects the filament length in Andhra local ecorace[19]. The silk obtained from the cocoons of infected larvae is usually much inferior with high denier[9]. The denier of T1 batch cocoon was found to be low by 8.5% than in T2 batch cocoons. A significant increase in the denier values of T1 (10.4%) and T2 (18%) batches were noticed in relation to T3 and T4 batches which can be attributed to the serious impact of microsporidian infection. The reelability of T1 batch cocoon was 18% more than T2 batch cocoons, whereas the T1 and T2 batch cocoons show a reduction in their reelability % by 45.4% and 44.3% in comparison with T3 and T4 batch cocoons.

The weight of the silk reeled of T1 batch cocoon was 13.7% more in relation with T2 batch. Whereas the T1 and T2 batch cocoons show a reduction in their reelved silk weight by 52% and 50% when compared with T3 and T4 batch cocoons. The reelved silk weight from single cocoon was reduced a lot in case of infection rather than the healthy cocoons. Microsporidiasis reduces the silk quality and silk yield in Andhra local ecorace[19]. The decrease in silk gland weight in A. mylitta larvae infected with Nosema sp. reduces the silk production [3].

TABLE 3 indicate the haemocyte count in healthy and infected Sukinda and Daba T.V. ecoraces. The infected Sukinda (T1) have shown more number of hemocytes in the fifth instar larvae in comparison with the healthy control Sukinda (T3) and also Infected Daba T.V. (T2) and Healthy Daba T.V. (T4). In comparison with the infected Daba T.V. the healthy control Daba T.V. had shown less number of hemocytes. This shows the Susceptibility of Sukinda ecorace to microsporidiasis. The progress of infection during BmNPV in different breeds of silkworm, BombyxmorilL. causes a drastic variation in haemocytes [20]. The ecoraces showing reduced number of haemocytes are tolerant to pathogen[13]. Thus the earlier reports supports the present work by showing less number of haemocytes.

V CONCLUSION

Thus in conclusion the impact of microsporidian parasite infection on Sukinda ecorace was high on characters like weight of larvae, mortality of larva and pupa, moth emergence, infected emerged moths, cocoon weight and shell weight whereas in case of Daba T.V the virulence had an impact on characters like fecundity, hatching%, SR%, filament length, denier, reelability and silkreeled. The infection also increased the haemocyte count in Sukinda ecorace. The present results shows that Sukinda ecorace is more susceptible to microsporidiasis than Daba T.V. ecorace. A control over the infection will reduce the damage caused and also increases the yield qualitatively and quantitatively.
ACKNOWLEDGEMENTS

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REFERENCES


**Table 1** Impact of microsporidiosis on the larval weight, survival, fecundity and hatching of *Sukinda* and *Daba T.V* ecorace

<table>
<thead>
<tr>
<th>Batch</th>
<th>Larval Weight (g)</th>
<th>Larval Mortality (number)</th>
<th>Pupa Mortality (number)</th>
<th>No. of Moths emerged</th>
<th>No. of Infected moths</th>
<th>Fecundity (Number)</th>
<th>Hatching (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>25.15±1.13</td>
<td>18</td>
<td>9</td>
<td>25</td>
<td>18</td>
<td>118</td>
<td>32</td>
</tr>
<tr>
<td>T2</td>
<td>32.18±1.15</td>
<td>10</td>
<td>6</td>
<td>38</td>
<td>15</td>
<td>89</td>
<td>56</td>
</tr>
<tr>
<td>T3</td>
<td>35.12±1.16</td>
<td>0</td>
<td>0</td>
<td>44</td>
<td>0</td>
<td>185</td>
<td>71</td>
</tr>
<tr>
<td>T4</td>
<td>41.18±1.12</td>
<td>0</td>
<td>0</td>
<td>49</td>
<td>0</td>
<td>172</td>
<td>82</td>
</tr>
<tr>
<td>CD 5%</td>
<td>-</td>
<td>0.16</td>
<td>0.14</td>
<td>0.13</td>
<td>0.12</td>
<td>1.24</td>
<td></td>
</tr>
</tbody>
</table>

CD: Critical difference. All the values are the averages five replications.
Table 2 Impact of microsporidiosis on the cocoon characters of Sukinda and Daba T.V. ecoraces

<table>
<thead>
<tr>
<th>Batch</th>
<th>Single Cocoon Weight (g)</th>
<th>Single Shell weight (g)</th>
<th>SR%</th>
<th>Single Cocoon Filament Length (m)</th>
<th>Denier</th>
<th>Reelability(%)</th>
<th>Weight Of Silk Reeled From single Cocoon (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>6.15±0.21</td>
<td>0.75±0.06</td>
<td>11.48±0.58</td>
<td>156.15±2.33</td>
<td>15.34±0.65</td>
<td>36.25±0.26</td>
<td>0.25±0.05</td>
</tr>
<tr>
<td>T2</td>
<td>7.82±0.48</td>
<td>0.85±0.27</td>
<td>11.42±0.13</td>
<td>134.74±2.76</td>
<td>16.78±0.75</td>
<td>30.72±0.34</td>
<td>0.22±0.04</td>
</tr>
<tr>
<td>T3</td>
<td>8.85±0.53</td>
<td>1.2±0.32</td>
<td>11.45±0.23</td>
<td>286.93±2.25</td>
<td>13.9±0.68</td>
<td>66.3±1.28</td>
<td>0.52±0.06</td>
</tr>
<tr>
<td>T4</td>
<td>9.48±0.87</td>
<td>1.4±0.41</td>
<td>12.46±0.42</td>
<td>242.34±1.23</td>
<td>14.23±0.54</td>
<td>55.15±1.42</td>
<td>0.44±0.05</td>
</tr>
<tr>
<td>CD 5%</td>
<td>0.25</td>
<td>0.05</td>
<td>-</td>
<td>87.38</td>
<td>0.25</td>
<td>2.14</td>
<td>0.02</td>
</tr>
</tbody>
</table>

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

Table 3 Total haemocyte count (THC) in fifth instar larvae of Infected and Healthy Sukinda and Daba T.V. ecoraces.

<table>
<thead>
<tr>
<th>Batch</th>
<th>No. of haemocytes in fifth instar larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1(Infected Sukinda)</td>
<td>15185±195</td>
</tr>
<tr>
<td>T2(Infected Daba T.V.)</td>
<td>14548±205</td>
</tr>
<tr>
<td>T3(Healthy Sukinda)</td>
<td>14685±178</td>
</tr>
<tr>
<td>T4(Healthy Daba T.V.)</td>
<td>13785±154</td>
</tr>
</tbody>
</table>

All the values are mean of five replications.