STUDIES ON EFFICIENT CONCENTRATION OF BENZIMIDAZOLE INCONTROLLING MICROSPORIDIASIS AND IMPROVING COCOON CHARACTERS IN AN THEREAEAE MYLITTA DRURY (DABA TV)

Lakshmi Marepally¹, G. Benarjee²

¹Post Doctoral Fellow (UGC), ²Professor, Department of Zoology, Kakatiya University, Warangal, Telangana, (India)

ABSTRACT

Microsporidiosis (Pebrine) is one of the dreadful disease seen in tropical tasar silkworm Antheraea mylitta. Drury, caused by Nosema species. Infections of the disease found to be highly virulent and harm the cocoon characters. Therefore an attempt has been made to evaluate the efficient dosage concentration of Benzimidazole in controlling the disease through studies on cocoon characters and hemocyte count in the fifth instar larvae. The results reveal a significant increase in shell weight, cocoon weight, filament length, reelability, reeled silk weight and denier in B1 batch cocoons (0.005% Benzimidazole Treatment) than B2 (0.04% Benzimidazole Treatment), B3 (0.02% Benzimidazole Treatment) and B4 (0.01% Benzimidazole Treatment) batches respectively in comparison with infected control. In comparison with the healthy control, the hemocyte count in B1 batch larvae (14802±143.56) were almost same but, found to be high in B2 (14903±148.16), B3 (14928±153.18) and B4 batches (14932±168.21) respectively. Based on the results obtained from present study 0.05% Benzimidazole is found efficient in controlling microsporidiasis in Antheraeaemylittadrury (Daba TV).

Keywords: microsporidiosis, nosema, Benzimidazole, Antheraeaemylittadrury.

I. INTRODUCTION

Microsporidiosis (pebrine) is one of the dreadful disease seen in Antheraea mylitta. Drury (Daba TV) caused by intracellular parasite Nosema species. Pebrine can be acquired from the mother moth (primary infection) or from the environment through food (secondary infection). The pathogen is causing considerable yield loss upto 40% in combination with other pathogens [1]. Black pepper like spots on the integument of infected larvae are the infected hypodermal cells which become enlarged and vacuolated get blackened due to the formation of melanin [2]. The infected larvae of Bombyxmori and Antheraeaemylittadrury show significant changes in the cocoon weight, shell weight, denier, reelability etc. [3,4]. Three Nosemasp. were identified from three non-mulberry silkworms as Nosemamylittra from Antheraeaemylittadrury, Nosema. ricini from Philosamiaricini and
Nosema assamensis from Antheraea assamensis. No silkworm race reported to be completely immune to pebrine [5]

Hemocytes 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 [6]. In insects several types of hemocytes are observed in the haemolymph[7]. Various functions like mechanization and immobilization of invading organism by encapsulation and phagocytosis, wound repair, coagulation have been attributed to haemocytes[8]. The studies on the susceptibility of three ecoraces of Anthereaemylittadrury against Am CPV reported that eco races showing reduced number of haemocytes are tolerant to pathogen [9]. Recently, some work has been carried out on the haemocytes and protein changes in tasar silkworm[10,11].

Effectiveness of fungicides to control the disease has been investigated by several workers [12,13]. Carbendazim(fungicide) and Chloroquine(antimalarial drug) were found effective against microsporidias[14]. 

The present work was carried out to understand the efficient concentration of a systemic fungicide, Benzimidazole in controlling microsporidiasis by studying the cocoon characters and hemocytes in Anthereaemylittadrury.(Daba TV)

II. MATERIALS AND METHODS

Daba TV 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, Telangana, India. The cocoons were preserved in the cages made up of wire mesh of size 2ft x 2ft x 2ft under temperature of 29±1ºC and humidity 70±1%. The emerged moths were tested for microsporidiasis by a method derived from that used in sericulture [15]. In this method, the abdomen of moth 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 laid by healthy and infected moths were collected and incubated for further research. To evaluate the efficient concentration of Benzimidazole against microsporidiasis first instar larvae of Andhra localecorace, were divided as Healthy control, Infected control and Benzimidazole treated. Larvae hatched from the eggs laid by the healthy Andhra local moth were kept as healthy control batch. Larvae hatched from the eggs laid by the infected moths were kept as infected control batch. Second and third instar larvae hatched from the eggs laid by the infected moths and fed with four different concentrations of Benzimidazole like 0.005%, 0.01%, 0.02%, 0.04% respectively were kept under Benzimidazole treated as B1, B2, B3 and B4 batches. The infected control and Benzimidazole treated batches were brushed and reared separately on Terminaliaarjunaplantation in the same field and healthy control batch larvae in the other field to avoid secondary contamination. Chemicals were administered by using foliar sprayer. Each batch had five replications of 100 larvae and was reared till cocooning. Cocoon weight, Shell weight following standard procedure.

To study the influence of Benzimidazole on the total hemocyte counts (THC), fifth instar larvae were selected from all the batches separately. The hemolymph was drawn into a Thoma white blood cell pipette up to 0.5
mark and diluted up to the 11 mark with tauber–yeager fluid [16]. The pipette was then shaken for several minutes and the first three drops were discarded. A double line with improved Neubauer ruling Hemocytometer was filled with diluted hemolymph and the hemocytes counted in its four corner and one central (1mm²) squares. The number of circulating hemocytes per cubic millimeter was calculated using the following formula [17].

\[
\text{Hemocytes in five } 1\text{mm}^2 \times \text{Dilution} \times \text{Depth factor of chamber} / \text{No. of squares counted}
\]

Where dilution = 20 times, Depth factor of the chamber = 10 (constant) and No. of squares counted = 5.

**Statistical analysis**

Each assay was replicated 3 times. Values were expressed as mean ± SE of replication and Student’s *t*-test was applied to locate significant (*P* < 0.05) differences between treated and untreated larvae. Critical differences (CD5%) was analysed by Tukey’s post hoc procedure.

**III.RESULTS AND DISCUSSION**

Table 1 indicate the rearing performance of Benzimidazole in controlling microsporidiasis and the influence on cocoon characters. In comparison with the healthy control and infected control, cocoon weight of B1, B2, B3 and B4 batches found decrease by 6.3, 12.8, 16 and 12.5% respectively and increase by 13.4, 6.8, 3.3 and 7.3% respectively. It is observed that high shell weight were recorded in B1 batch cocoons compared to B2, B3 and B4 which may be due to the resistance attained against Nosema infection. [3] working on microsporidiasis in *Daba TVecorace, Anthereaemylittadrury* have recorded low cocoon weight and shell weight in the cocoons of both trasovarial, secondarily infected larvae rather than the healthy control. *A. mylitta* larvae infected with *Nosema* species have shown decrease in shell weight [18]. 0.005% carbendazim treatment of larval stages during rearing has a definite effect in suppressing the development of *Nosemasp*. in *A. mylitta* and increases the cocoon weight and shell weight [14]. The administration of certain neurohumoral factors, vertebrate hormones, chemicals like prostaglandins increases the larval life cycle, cocoon weight, shell weight, reproductive potential in silkworm [19,20, 21].

It is evident from the results that the SR% of B1, B2 and B3 were not deviated much from healthy control but shown an increase of 11, 7.59, 7.39, 1% respectively over infected control.[12] have reported that there is no significant variation in the shell ratio of Nosema infected cocoons and healthy control cocoons. It is evident from the results that filament length of B1, B2, B3 and B4 batch cocoons have decreased by 9.69, 18.21, 22.43 and 23.78% respectively when compared to that of healthy control. In comparison with the infected control the filament length of healthy control and B1,B2,B3 and B4 batch cocoons found to be more by 60, 55.52, 50.56, 47.87 and 46.95% respectively. There is no much variation in the filament length values for healthy control and B1 batch cocoons. Microsporidiasis in *Daba TVecorace* will seriously affect the filament length [3]. It is found that the B1, B2, B3 and B4 batch cocoons have decreased in reelability by 1.17, 3.11, 2.33 and 3.11% respectively than that of healthy cocoons. Thereelability of healthy, B1,B2,B3 and B4 batch cocoons was found to be increase significantly by 16.28, 15.3, 13.6, 14.29 and 13.6% respectively over the infected control. Microsporidiasis will significantly affect the reelability[3]. When compared with the healthy control the weight of the silk reeled of B1 batch cocoon was low by 8%, whereas in B2, B3 and B4 batch cocoons it was low by
16, 28 and 16% respectively. It is evident from the results that the reeled silk weight of healthy, B1, B2, B3 and B4 batch cocoons it was 47%, 36%, 23.5%, 20.5% and 23.5% more from infected control. The weight of the silk gland will reduce in A. mylitta larvae infected with Nosema sp. which finally reduces the silk production[18]. It is observed that, the denier of B1, B2, B3 and B4 batch cocoons was more than healthy cocoons by 1.89, 2.68, 5.67 and 10.17% respectively. Lowest denier value can be attributed to healthy cocoons next comes the B1 treated batch of cocoons. A significant increase in the denier values of B2, B3 and B4 batches were noticed which can be attributed to the serious impact of microsporidian infection and reduction in silk quality.[3] working on microsporidiasis in Daba TVecorace, A. mylittadrury have reported a significant variation between the denier values of nosemainfected cocoons and healthy control. Silk produced from the cocoons ofpebrineinfected larvae is usually much inferior[22].

Table 2 indicate the haemocyte count in healthy, infected control and the effect of selected dosage of Benzimidazole on hemocytecount. The infected control have shown more number of hemocytes in the fifth instar larvae in comparison with the healthy control. The infected larvae treated with 0.05% Benzimidazole have shown less number of hemocytes in comparison with other three treatments and almost equal number with healthy control. This shows the efficiency of the concentration.[1] have reported a variation in hemocyte counts in different breeds of silkworm, Bombyx mori L and also during progressive infection of BmNPV. Theecoraces showing reduced number of hemocytes are tolerant to pathogen [9]. Thus the earlier reports supports the present work by showing less number of hemocytes.

Thus in conclusion 0.05% Benzimidazole is efficient in controlling microsporidiasis and the treatment improves the cocoon characters of infected Daba TVecorace.

IV. ACKNOWLEDGEMENTS

Dr. Lakshmi Marepally would like to thank UGC- New Delhi for providing financial assistance in the form of Post doctoral fellowship to carry out this research work.

REFERENCES


Table 1: Rearing performance of Benzimidazole on cocoon characters of *Antheraeaamyllita* D (Daba TV) by controlling microsporidiasis.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cocoon Weight (g)</th>
<th>Shell Weight (g)</th>
<th>SR%</th>
<th>Filament Length (m)</th>
<th>Reelability (%)</th>
<th>Weight Of raw Silk (g)</th>
<th>Denier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control</td>
<td>9.72± 1.15</td>
<td>0.98± 0.22</td>
<td>10.8± 0.06</td>
<td>362.14± 2.85</td>
<td>5.19± 0.26</td>
<td>0.52± 0.1</td>
<td>12.65± 0.14</td>
</tr>
<tr>
<td>Infected control</td>
<td>7.75± 0.05</td>
<td>0.65± 0.04</td>
<td>8.54± 0.06</td>
<td>142.32± 1.54</td>
<td>4.25± 0.02</td>
<td>0.36± 0.08</td>
<td>22.32± 0.28</td>
</tr>
<tr>
<td>CD 5%</td>
<td>0.04± 0.04</td>
<td>0.04± 0.04</td>
<td>0.32± 0.04</td>
<td>0.48± 0.02</td>
<td>0.2± 0.04</td>
<td>0.04± 0.04</td>
<td>0.56± 0.04</td>
</tr>
</tbody>
</table>

CD: Critical difference. All the values are the mean values of five replications.
Table 2: Total haemocyte count (THC) in fifth instar larvae of healthy, infected control, Benzimidazole treated *Antherea mylitta* D (Daba TV)

<table>
<thead>
<tr>
<th>Type of the larvae</th>
<th>No. of hemocytes in the fifth instar larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control</td>
<td>14753±152.53</td>
</tr>
<tr>
<td>0.05% Benzimidazole treated</td>
<td>14872±133.56</td>
</tr>
<tr>
<td>0.04% Benzimidazole treated</td>
<td>14913±138.16</td>
</tr>
<tr>
<td>0.02% Benzimidazole treated</td>
<td>14935±143.18</td>
</tr>
<tr>
<td>0.01% Benzimidazole treated</td>
<td>14965±178.21</td>
</tr>
<tr>
<td>Infected control</td>
<td>14982±152.43</td>
</tr>
</tbody>
</table>

All the values are the mean values of five replications.