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ESTIMATION OF EFFICIENT CONCENTERATION OF BAVISTIN IN CONTROLLING MICROSPORIDIASIS AND IMPROVING COCOON CHARACTERS IN *ANTHEREAE MYLITTA DRURY* (ANDHRA LOCAL ECORACE)

Lakshmi Velide[1]* and M.V.K. Bhagavanulu[2]

[1]*Department of biotechnology, Gokaraju Rangaraju Institute of Engineering and Technology, Bachupally, Kukaypally,Hyderabad, Andhra Pradesh, India.

[2]Basic Seed Multiplication and Training Center, Central Silk Board, Chennor, Adilabad, Andhra Pradesh, India.

Tel: +91- 9866950998 Email: lakshmi.velide@gmail.com

ABSTRACT: *Microsporidiosis(Pebrine)* is one of the dreadful disease seen in tropical tasar silkworm *Antheraea mylitta.Drury (Andhra local ecorace*), 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 concenteration of Bavistin 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%Bavistin Treatment) than B2(0.04% Bavistin Treatment), B3(0.02% Bavistin Treatment) and B4(0.01% Bavistin 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% Bavistin is found efficient in controlling microsporidiasis in *Andhra local ecorace*.

Key words*:* Microsporidiosis, Nosema, Bavistin, Andhra local.

INTRODUCTION

The tasar silkworm, *Antheraea mylitta Drury*, *Andhra local* ecorace is an exclusive race of Andhra Pradesh. In view of its superior commercial characters such as compact and hard cocoons, high reelability, high shell ratio and low denier this ecorace deserves to be developed further [25]. 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 the ecorace has been thoroughly neglected leading to the extinction of this ecorace [17].

*Microsporidiosis(pebrine)*is one of the dreadful disease seen in *Antherea.mylitta.Drury (Andhra local ecorace*), 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 [15]. 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 [6].The infected larvae of *Bombyx mori and Anthereae mylitta drury* show significant changes in the cocoon weight, shell weight, denier, reelability etc.,[10,19]. [4] Identified three *Nosema sp*. from three non-mulberry silkworms as *Nosema mylitta* from *Antherae*a *mylitta*, *Nosema.ricini* from *Philosamia ricini* and *Nosema assamensis* from *A*ntheraea *assamensi*s. No silkworm race reported to be completely immune to *pebrin*e.

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 [22]. In insects several types of hemocytes are observed in the haemolymph [16].Various functions like mechanization and immobilization of invading orgnism by encapsulation and phagocytosis, wound repair, coagulation have been attributed to haemocytes[12].The studies on the susceptibility of three ecoraces of Anthereae mylitta drury against Am CPV reported that ecoraces showing reduced number of haemocytes are tolerant to pathogen [21].Recently, some work has been carried out on the haemocytes and protein changes in tasar silkworm[9,23].

Effectiveness of fungicides to control the disease has been investigated by several workers [3,7].Carbendazim(fungicide) and Chloroquine (antimalarial drug) were found effective against microsporidias[13].

The present work was carried out to understand the efficient concenteration of a systemic fungicide, Bavistin in controlling microsporidiasis by studying the cocoon characters and hemocytes in *Andhra local ecorace* with which the ecorace can be protectedfromextinction.

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 Chennor, Adilabad District,Andhra Pradesh, 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 [11]. 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 efficienct concenteration of Bavistin against microsporidiasis first instar larvae of *Andhra local ecorace,*were divided as Healthy control, Infected control and Bavistin 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 concenterations of Bavistin like 0.005%, 0.01%, 0.02%, 0.04% respectively were kept under bavistin treated as B1, B2, B3 and B4 batches. The infected control and bavistin treated batches were brushed and reared separately on *Terminalia arjuna* plantation 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 bavistin 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 [24]. 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 ($1mm^2$) squares. The number of circulating hemocytes per cubic millimeter was calculated using the following formula [8].

Hemocytes in five $1mm^2$ x Dilution × Depth factor of chamber / 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 Tukeys post hoc procedure.

ESULTS AND DISCUSSION

Table 1 indicate the rearing performance of Bavistin 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 to 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. [10] working on microsporidiasis in *Andhra local ecorace,Anthereae mylitta drury* 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 [14]. 0.005% carbendazim treatment of larval stages during rearing has a definite effect in suppressing the development of *Nosema* sp. in *A. mylitta* and increases the cocoon weight and shell weight [13].The administration of certain neuro humoral factors, vertebrate harmones, chemicals like prostaglandins increases the larval life cycle, cocoon weight, shell weight, reproductive potential in silkworm [2,5,26].

Table 1: Rearing performance of Bavistin on cocoon characters of *Anthereae mylitta .D (Andhra local)* by controlling microsporidiasis.

Treatment	Cocoon Weight (g)	Shell Weight(g)	SR%	Filament Length(m)	Reelability (%)	Weight of raw Silk(g)	Denier
0.05%	9.07±1.02	0.88±0.03	9.71±0.05	320.45±2.07	5.1±0.13	0.46±0.05	12.92±0.25
0.04%	8.44±0.23	0.79±0.02	9.36±0.04	290.22±1.56	5±0.05	0.42±0.03	13.02±0.34
0.02%	8.13±0.05	0.76±0.03	9.34±0.03	275.26±1.35	5.04±0.06	0.41±0.04	13.40±0.48
0.01%	8.47±0.06	0.74±0.02	8.73±0.05	270.45±2.12	5±0.05	0.42±0.03	13.97±0.56
Healthy control	9.68±1.12	0.97±0.21	10±0.08	354.82±2.87	5.16±0.25	0.5±0.08	12.68±0.15
Infected control	7.86±0.06	0.68±0.02	8.65±0.05	143.5±1.65	4.32±0.04	0.34±0.05	21.32±0.25
CD 5%	0.06	0.03	0.21	0.54	0.3	0.03	0.48

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

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.[10] 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 Andhra local ecorace will seriously affect the filament length [10].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.The reelability 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 [10]. 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[14]. 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.[10] working on microsporidiasis in *Andhra local ecorace, A. mylitta drury* have reported a significant variation between the denier values of nosema infected cocoons and healthy control. Silk produced from the cocoons of pebrine infected larvae is usually much inferior [20].

Table 2 indicate the haemocyte count in healthy,infected control and the effect of selected dosage of bavistin on hemocyte count.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% bavistin 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 concenteration. [1] have reported a variation in hemocyte counts in different breeds of silkworm, Bombyx mori L and also during progressive infection of BmNPV.The ecoraces showing reduced number of hemocytes are tolerant to pathogen [21].Thus the earlier reports supports the present work by showing less number of hemocytes.

Table:2 Total haemocyte count(THC) in fifith instar larvae of healthy, infected control, bavistin trated *Anthereae mylitta .D (Andhra local)*

Type of the larvae	No. of hemocytes in the fifth instar larvae
Healthy control	14753±172.53
0.05% Bavistin treated	14802±143.56
0.04% Bavistin treated	14903±148.16
0.02% Bavistin treated	14928±153.18
0.01% Bavistin treated	14932±168.21
Infected control	14976±162.43

All the values are the mean values of five replications

Thus in conclusion 0.05% bavistin is efficient in controlling microsporidiasis and the treatment improves the cocoon characters of infected Andhra local ecorace.

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