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Hemocyte Changes During the Progressive Infection of *Beauveria bassiana* in Different Breeds of Silkworm (*Bombyx mori L.*)

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Abstract : The immune system in insects involves in clearing the haemolymph of foreign pathogens by haemocytes and humoral factor. They can accomplish this in a couple of different ways. One is a cellular mediated response by physically clearing the haemolymph by phagocytosis, nodulation and encapsulation and the other is by humoral secretion of proteins. Pathogenic infection in silkworm, *Bombyx mori* L. is common and there is a possibility of differential response by haemocytes in different breeds to microbial infection. Hence, the differential response in the form of difference in Total haemocyte count (THC) and Differential haemocyte count (DHC) in susceptible and tolerant breeds were investigated under normal and *Beauveria bassiana* invasion conditions. Under normal condition, there was a gradual increase in THC as the age of the silkworm increases and high THC was recorded in Nistari, PM and NB4D2 (tolerant breeds) compared to susceptible breeds (NB7, NB18 and KA). There was a gradual increase in THC, gradual decrease in prohaemocyte and oenocyte counts and increase in plasmatocyte and granulocyte counts during the progressive infection by *Beauveria bassiana*.

Key words : Bombyx mori L., Silkworm breeds, Beauveria bassiana, Total haemocyte count, Differential haemocyte count)

Introduction

Infection in insect stimulates a complex of cell mediated and humoral responses. It involves the recognition of non-self and the effector mechanism. The effector mechanism includes cellular defense reactions and rapid synthesis of anti microbial polypeptides by the fat bodies and midgut. However, the first response is cellular immunity involving the haemocytes. Haemocytes are the complex of several types of circulatory cells in hemolymph of insects. There are three well-defined types of haemocytes *viz.*, prohaemocytes, plasmatocytes and granulocytes in most of the insects and one or more of other types such as coagulocytes, spherulocytes, adipocytes and oenocytes. The white muscardine disease of silkworm caused by *Beauveria bassiana*, has been known for long to be highly pathogenic to the silkworm. Kawakami (1965) reported that the haemocytes of silkworm are capable of phagocytizing the hyphal bodies of low pathogenic muscardine fungi, *Isaria fumosorosea* and *Harziella entomophila*, but not highly pathogenic *Beauvaria bassiana.* It is possible that the virulent pathogens are able to over come the phagocytic activity and destroy phagocytes (Hou and Cheng, 1985). Although a successful defense of insects may not take place, at least initial or temporary cellular response of insect hemocytes to foreign bodies does occur. The interaction of haemocytes is immediate and includes phagocytosis, nodulation and encapsulation (Gupta, 1986). This response differs and could be manifested in the form of total haemocyte count and differential haemocyte count. Indirectly the total haemocy count and differential haemocyte count may indicate the susceptibility status of the insect. In the present study the relationship of THC and DHC with regard to

Table 1. Total haemocyte counts in different silkworm breeds during the progressive infection of *Beauveria bassiana*

Breed	Treatment	Total haemocyte counts (No. of cells/mm³ of haemolymph)				
		1 day	2 days	3 days	4 days	5 days
NB7	Control	5875±52	6383±61	6667±98	7083±113	7333±129
	Treatment	6192±213 +5.40% **	8042±143 +26.00% **	6842±67 +2.53% *	4025±262 -43.17% **	0 -100% **
NB18	Control	5950±145	6408±407	7142±132	7742±136	8283±151
	Treatment	6292±80 +5.75% **	8558±116 +30.16% **	7325±52 +2.56% *	4033±279 +47.91% **	0 -100% **
NB4D2	Control	6375±108	6833±157	7458±139	8000±95	8858±97
	Treatment	6750±63 +5.87% **	8967±41 +31.23% **	7667±61 +2.80% *	5117±125 -36.04% **	0 -100% **
KA	Control	5392±74	5850±219	6308±107	6742±59	7108±72
	Treatment	5667±52 +5.10% **	7383±271 +26.21% **	6483±137 +2.08% *	3417±82 -49.32% **	0 -100% **
PM	Control	6342±132	6975±52	7542±67	8033±61	8933±103
	Treatment	6908±180 +8.93% **	9167±151 +31.43% **	8158±153 +8.17% *	5733±169 -28.63% **	3058±116 -65.77% **
Nistari	Control	6417±82	7275±52	7825±69	8242±250	9225±144
	Treatment	7092±124 +10.52% **	9783±52 +34.47% **	8567±103 +9.48% *	6242±250 -25.48% **	3383±144 -63.33% **

** = Significant at 1% level * = Significant at 5% level

susceptibility status of silkworm to *Beauveria bassiana* infection was investigated and results are presented.

Materials and Methods

Four bivoltine silkworm breeds viz., NB7, NB18, NB4D2 and KA and two multivoltine silkworm breeds viz., PM and Nistari were received from the Germplasm Bank, Central Sericultural Research and Training Institute, Mysore and reared following the standard method (Datta, 1992) up to III moult.

Immediately after III moult, larvae were divided into two sets. In first set the fourth instar silkworms of each breed (NB7, NB18, NB4D2, KA, PM and Nistari) were bled individually from the first abdominal leg every day up to 5 days. The total and differential haemocyte counts were estimated using haemocytometer following standard procedure (Cantwell, 1973). Total haenocyte counts (THC) were determined per ml of haemolvmph and THC per mm³ of haemolymph was estimated according to the formula suggested by Jones (1962). The

Table 2. Percentage of Differential Haemocyte Counts in different silkworm breeds under normal and *Beauveria bassiana* inoculated condition

Breed	Treatment	1 day					2 day					3 day					4 day					5 day				
		PR	PL	GR	SP	OE	PR	PL	GR	SP	OE	PR	PL	GR	SP	OE	PR	PL	GR	SP	OE	PR	PL	GR	SP	OE
NB7	Control	25	25	31	14	05	23	23	33	16	05	22	21	37	15	06	21	19	39	15	05	21	19	39	15	
	Treatment	21	27	33	15	04	16	27	37	16	04	12	31	40	13	04	06	38	46	07	03	0	0	0	0	
NB18	Control	23	25	34	13	05	21	22	37	15	05	21	21	38	16	04	20	19	40	16	05	20	19	40	16	
	Treatment	19	27	37	13	04	15	26	39	16	04	12	31	40	13	04	09	38	45	05	03	0	0	0	0	
NB4D2	Control	21	23	38	12	06	19	22	40	13	06	18	20	43	15	04	18	19	44	14	05	18	19	44	14	
	Treatment	13	26	43	13	05	11	28	42	15	04	09	32	45	10	04	05	35	50	07	03	0	0	0	0	
KA	Control	23	22	32	17	06	21	21	35	17	06	20	20	38	17	05	19	18	40	18	05	19	18	40	18	
	Treatment	19	24	34	18	05	18	25	36	16	05	13	30	40	13	04	10	30	45	12	03	0	0	0	0	
PM	Control	15	22	45	11	07	14	20	47	12	07	12	19	50	16	06	11	18	53	12	06	11	20	54	10	
	Treatment	14	25	53	13	05	10	26	55	05	04	06	31	56	04	03	05	33	57	03	02	03	35	58	02	
Nistari	Control	14	22	49	09	06	12	22	50	09	06	12	21	51	10	06	12	19	53	11	OS	10	22	55	08	
	Treatment	08	25	55	07	05	06	28	56	06	04	04	32	58	03	03	OS	32	58	03	02	02	35	59	02	

PR = Prohaemocyte; PL = Plasmatocyte; GR = Granulocyte; SP = Spherulocyte; OE = Oenocyte

differential haemocyte count (DHC) was estimated by counting different haemocytes from a haemocyte population of 200. Different haemocytes *viz.*, prohaemocytes, plasmatocytes, granulocytes, spherulocytes and oenocytes were identified based on the morphological features as described by Nittono (1960).

In another set, larvae of NB7, NB 18, NB4D2, KA, PM and Nistari were inoculated with *Beauveria bassiana* conidia (1 X 10^7 conidia/ml) immediately after third moult and the haemolymph was collected for estimation of total and differential haemoctye counts and results were compared with that of same breeds of larvae reared under normal rearing condition.

Results and Discussion

The total haemocyte count estimated for different untreated breeds of silkworm from fourth instar onwards showed significant differences among the breeds. Among them, the multivoltine breeds *viz.*, Nistari and Pure Mysore and bivoltine breed NB4D2 which are reported to be tolerant had high THC (Table 1). As the age of larvae increased the THC also increased in all the breeds from I[st] day to 5[th] day (5875 - 7333 cells/mm^3 of haemolymph in NB7; 6376 - 8858 cells/mm^3 of haemolymph in NB18; 6376 - 8858 cells/mm^3 of haemolymph in NB4D2; 5392 - 7108 cells/mm^3 of haemolymph in KA; 6342 - 8993 cells/mm^3 of haemolymph in PM and 6417 - 9225 cells/mm^3 of haemolymph in Nistari breeds). The THC was peak at 5[th] day (IV moult) in all breeds. The THC after inoculation with *Beauveria bassiana* increased during first 2 day after infection and then there was a decrease. The increase on 1[st] and 2[nd] day was 5.40 and 25.99% for NB7; 5.75 and 30.16% for NB18; 5.87 and 31.23% for

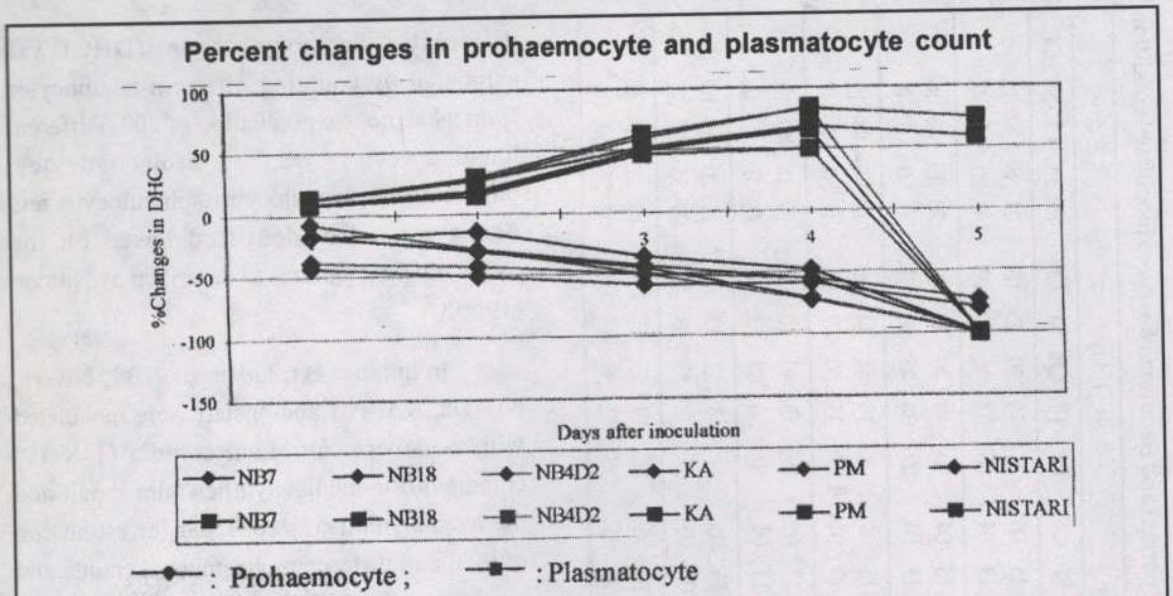

Fig. 1. Prohaemocyte and Plasmatocyte response during progressive B. bassiana infection in different silkworm breeds.

NB4D2; 5.10 and 26.21% for KA; 8.93 and 31.43% for PM and 10.52 and 34.47 % for Nistari respectively. From 3rd day onwards THC started decreasing as it was drastic by the 5th day of progressive infection and the decrease was 100%. The decrease in THC was 100% by 5th day of progressive infection in NB7, NB18, NB4D2 and KA. In case of PM and Nistari only 65.77 and 63.33% decrease was recorded by 5th day and 100% by 6th day.

The differential haemocyte count (DHC) in different silkworm breeds both in control and *Beauveria bassiana* treated batches are presented in Table 2. Under normal conditions (control), the DHC indicates higher number of granulocytes followed by plasmatocytes and prohaemocytes in tolerant breeds *viz.*, NB4D2, PM and Nistari. As the larval age increases during IV instar (1-5 days), the granulocyte count increased (31-39% in NB7, 34-40% in NB18, 38-44% in NB4D2, 32-40% in KA, 45-54 in PM and 49-55% in Nistari breeds) and the prohaemocyte count decreased (25-21% in NB7, 23-20 % in NB18, 22-19% in NB4D2, 23-19% in KA, 15-11% in PM and 14-10% in Nistari breeds) in all the breeds from 1st to 5th day.

During the progressive infection of *B. bassiana,* there was a gradual decrease in the prohaemocyte and gradual increase in plasmatocyte counts in all the breeds from 1st day of infection to 5th day. 100% decrease in count of these two haemocytes was observed on 5th day of progressive infection in bivoltine breeds (NB7, NB18, NB4D2 and KA) and 72.73-80.00% decrease in prohaemocyte and 75.00 and 59.10% increase in plasmatocyte in PM and Nistari breeds respectively (Fig. 1). There was a gradual increase in granulocyte count in all the breeds up to 4th day. There was drastic decrease in granulocyte counts to an extent of 100% in the bivoltine breeds on 5th day and gradual decrease in PM and Nistari on 5th day (Fig. 2) and 100% decrease on 6th day. There was

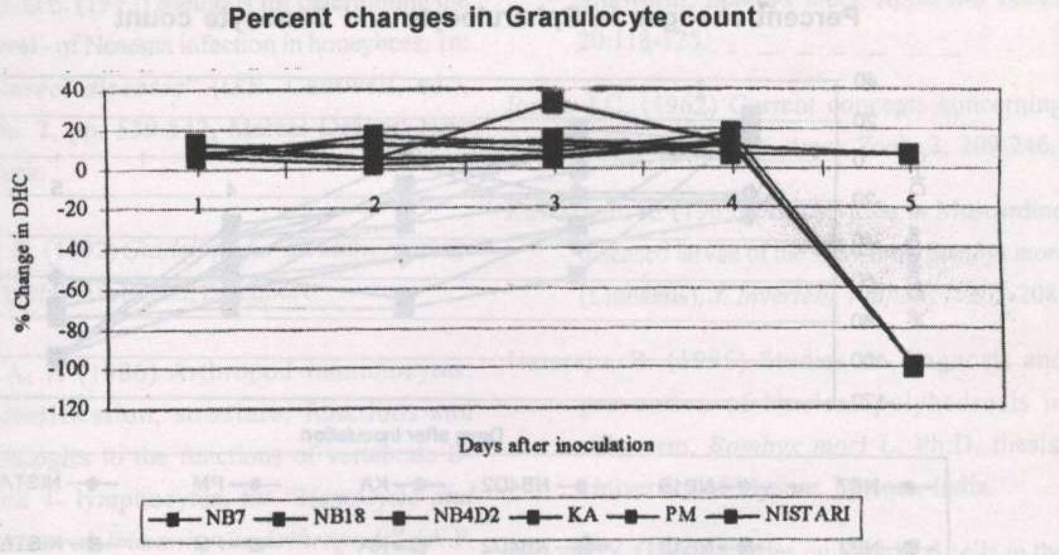

Fig. 2. Granulocyte response during progressive B. hassiana infection in different silkworm breeds.

no change in the spherulocyte count with regard to NB7 and NB18 breeds up to 2 days of infection and then there was a gradual decrease. 100% decrease was recorded on 5th day of infection. In NB4D2 the increase was up to 2 days of post infection and then count decreased up to 100% by 5th day of infection. In KA, PM and Nistari breeds the increase in spherulocyte count was observed only on 1st day of infection and then decreased. 100% decrease was recorded on 5th day of infection in KA and 80.00 and 75.00% decrease in PM and Nistari respectively on 5th day of infection. In the case of Oenocyte count, during the progressive infection, there was a gradual decrease and 100% decrease was recorded in bivoltine breeds (NB7, NB18, NB4D2 and KA) and 60.00% decrease in multivoltine breeds (PM and Nistari) on 5th day of infection (Fig. 3) and 100% decrease on 6th day of infection.

The results clearly indicated that all the bivoltine and multivoltine breeds tested were found to be susceptible to *Beauveria bassiana* infection, but there was a difference in tolerance level. The observations of high THC in the tolerant silkworm breeds and the response of insect host in terms of THC during progressive infection point to the possibility of a correlation between the tolerance of the breed to infection and THC. The multivoltine breeds *viz.*, PM and Nistari which are reported to be comparatively more tolerant to BmNPV and other pathogen infections (Nataraju, 1995 and Baig, 1994) recorded the maximum increase in THC (31.43 - 34.47%) on 2nd day of infection and 100% of decrease in haemocyte resulted only on 6th day of progressive infection while in bivoltine breeds, 100% decrease in THC was reached by 5th day. Similar observations of 100 % loss in haemocyte on 7-8th day of progressive infection of BmNPV in PM and Nistari breeds were reported and in susceptible breeds (KA, NB7 and NB18), it was on 5th or 6th day (Balavenkatasubbaiah *et al.*, 2001).

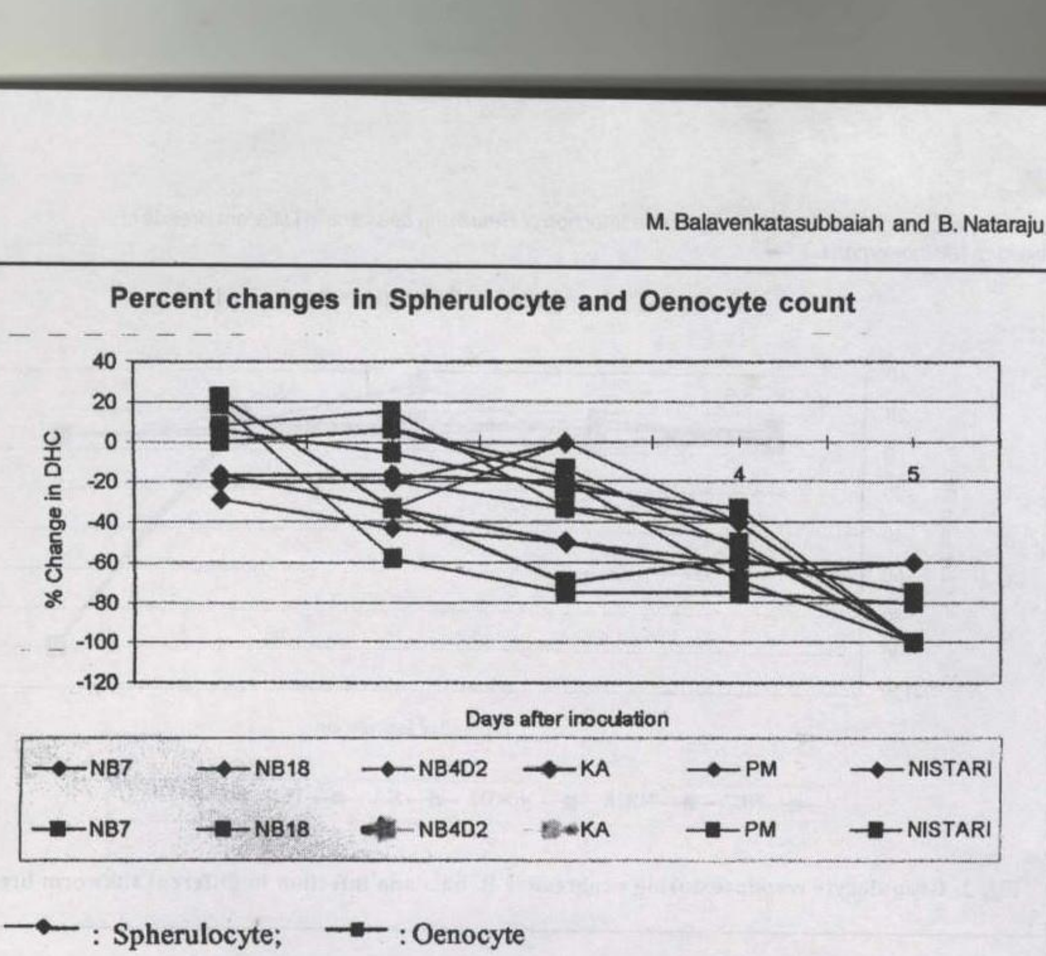

Fig. 3. Spherulocyte and Oenocyte response during progressive *B. bassiana* infection in different silkworm breeds

In case of DHC, the gradual decrease in prohaemocyte count may be due to the conversion of prohaemocytes to other types of haemocytes that is required for defensive during progressive infection of *B. bassiana*. The plasmatocytes and granulocytes, which are primarily involved in defensive showed gradual increase in all the breeds and finally 100% loss before death. This possibly indicates the primary role of plasmatocytes and granulocytes to fight against infection. Kawakami (1965) and Balavenkatasubbaiah *et al.* (2001) also made similar observations in silkworms against fungal and viral infections respectively. The drastic decrease in THC and DHC involves in defense on 5-6th day post infection may to due to high virulence of the pathogen involved. However, the increase in THC and DHC during first 4 days of post infection is clear indication of involvement of haemocytes in defense against infection. There is difference in level of increase in haemocytes in tolerant and susceptible breeds and it could form an index of resistance to *B. bassiana* infection in silkworm.

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