Influence of *Varroa jacobsoni* Oudemans Parasatization on the Protein profile and RNA content of *Apis mellifera* L. worker brood

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Abstract

Protein profile and RNA content of *Varroa jacobsoni* Oudemans infested *Apis mellifera* L. worker brood was studied and compared with non- infested brood. It was observed that total protein concentration in whole body extract was higher in non-infested pupa. The number of protein fractions obtained on SDS-PAGE were however more in the pupa infested with mite. The concentration of RNA was higher in healthy pupa as compared to infested one suggesting reduced transcription of genes encoding peptides and proteins.

Keywords: *Apis mellifera, Varroa jacobsoni, Protein profile, Worker brood.*

Introduction

The ectoparasitic mite *Varroa jacobsoni* Oudemans is today regarded as the most serious malady of honey bee colonies. *V. jacobsoni* was first detected by Dutch acarologist Jacobson on the Eastern honey bee, *Apis cerana* (Oudemans ,1904). *A. cerana* has been recognized as the mite's native host. Delfinado (1963) collected specimens of *V. jacobsoni* from *A. mellifera* brood in Hong Kong in 1962. This was the first report of the utilization of *A.mellifera* as an alternative host by *V.jacobsoni*. *Varroa* feeds on the haemolymph of adult bee, larvae and pupae. Haemolymph is probably lost at a variable rate in each bee, depending upon the time of feeding by parent mites and their progeny in relation to the bees development.

Cacho *et al.* (1996) studied the effect of *Varroa* parasitization on the glycoprotein expression of *A. mellifera* spermatozoa. They (Cacho *et al.*, 1996) compared the lectin binding patterns of the spermatozoa of non- parasitized and parasitized bees and observed that presence of *Varroa* altered the expression of glycoprotein on the spermatozoa.Yang and Cox-Foster (2005) reported that infestation by *Varroa* led to reduction in the transcription of genes encoding antimicrobial peptides and immunity- related enzymes causing immunosuppression in the infested bees. The present investigation were undertaken to study influence of parasitization on the protein profile of the infested worker pupa and to compare the RNA content of the infested and healthy pupae

in order to understand the pathophysiological changes exhibited by the infested bees.

Materials and Methods

Samples of *A.mellifera* worker brood were drawn from the colonies maintained by Department of Zoology, Panjab University Chandigarh. A random sample of 10 infested and 10 non- infested worker pupae (brown eye stage) was taken for each test after brushing off the bees from the comb.Each pupa was taken in 1ml of PBS and electrically homogenized. Estimation of total protein in the infested and non-infested sample was done following Lowry's standard procedure (Lowry *et al.*,1951). The protein types and protein fractions were determined by standard SDS-PAGE technique (Laemmli,1970). For the estimation of RNA the procedure of Schneider (1945) was utilized.

Results

Protein concentration was found to be higher (0.260 $\pm$ 0.0030mg/ml) in whole body extract of healthy pupa as compared to 0.176 $\pm$ 0.002mg/ml in the pupa infested with mite (Results are mean + SD. Values are significantly different from control at $p<0.0001$). A total of ten bands corresponding to different protein fractions were observed in worker brood not infested with mites. The molecular weights of these proteins ranged between 38.0 to 97.6kDa while the distance traveled was in the range of 0.8 and 5.6 cm. In case of infested sample on the other hand, twelve bands were observed (Table1).

Table-1: Protein types in non- infested and infested worker pupa of *A. mellifera* as observed by SDS-PAGE.

S.No.	Standards		*Apis mellifera* late pupa Non-infested		*Apis mellifera* late pupa Infested	
	Molecular weights (kDa)	Distance travelled (Cm)	Molecular weights (kDa)	Distance travelled (Cm)	Molecular weights (kDa)	Distance travelled (Cm)
1.	97.4	0.8	97.6	0.7	97.6	0.7
2.	66.0	2.5	90.2	1.1	90.2	1.1
3.	43.0	4.5	88.1	1.3	88.1	1.3
4.	29.0	5.6	79.4	1.5	79.4	1.5
5.	-	-	70.7	1.8	70.7	1.8
6.	-	-	67.5	2.2	67.5	2.2
7.	-	-	-	-	65.2	2.7
8.	-	-	59.3	2.8	59.3	2.8
9.	-	-	53.6	3.3	53.6	3.3
10.	-	-	48.1	3.8	48.1	3.8
11.	-	-	-	-	40.7	4.6
12.	-	-	38.0	4.8	38.0	4.8

Comparison of electropherogram of infested and non-infested sample revealed that the protein types with molecular weights of 65.2 and 40.7kDa were absent in case of non-infested brood of *A. mellifera*. The proteins fraction of 97.6, 90.2, 88.1, 79.4, 70.7,67.5, 59.3, 48.1 and 38.0kDa were common in both cases.

The RNA concentration in whole body extract of non-infested late worker pupa (brown eye) was found to be 0.017 ± 0.002mg/ml as compared to 0.008 ± 0.003mg/ml in late worker pupa infested with mite (Result are mean ± SD .Values are significantly different from control at $p<0.05$) .

Discussion

Physiological interference due to mite infestation was reported by Ball (1997) who observed depletion in host hemolymph as a consequence of feeding by the mite.The reduction in total proteins (estimated by Lowry's method) of the infested pupal extract observed during the present study could be a consequence of hemolymph depletion .However the protein types established by SDS-PAGE were two more in the infested than in the non-infested brood. The additional proteins are perhaps contributed by mite feeding or could be produced by the host in response to the presence of the parasite. The later however seems unlikely because the RNA content of the parasitized pupa was observed to fall suggesting reduced transcription of genes encoding for polypeptides. Bees parasitized by *V.jacobsoni* have been reported to the immunosupressed due to reduction in the synthesis of immunity related enzymes as reported by Yang and Cox-Foster (2005).

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