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Changes in protein profile and RNA content of *Apis mellifera* worker pupa on parasitization with *Tropilaelaps clareae*

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Abstract: *Tropilaelaps* infestation of *Apis mellifera* pupa caused reduction in total protein concentration of the infested pupa. This may be due to protein feeding by mite. The additional protein fractions observed by SDS-PAGE and the difference in the nature of free amino acids suggested synthesis of newer proteins perhaps related to the stress response of the host. This opinion is strengthened by the increase in concentration of RNA (184.580±36.987 in non-infested pupa and 293.402±50.329 in infested pups) observed in the present study which was responsible for increased transcription of genes encoding antiparasite peptides as reported by other workers.

Keywords: *Apis mellifera*, Peptides, *Topilaelaps clareae*

INTRODUCTION

Tropilaelaps mites (Acari–Laelapidae) are ectoparasites of honeybees native to Asia (Delfinado and Baker, 1961; Laigo and Morse, 1968).The primary host of mite *Tropilaelaps clareae* is *Apis dorsata* (Laigo and Morse, 1968) but the mites also infested the western honey bee when the latter was introduced in India (Delfinado and Baker, 1961; Anderson and Morgan, 2007). Today mites (Acari) parasitizing honey bees have become a global problem. They are threatening the survival of managed and feral honey bees and have become the major problem for beekeeping industry (Kumar *et al*., 1993 ; and Kumar and Kumar, 1995).
According to Rinderer *et al.*, (1994), if population of mite increases rapidly, it can cause death of colony. Risk of mortality is high in case of *A. mellifera* which is a new host for the mite and has no inherent defense to fight attack. The correlation between influence of *T. clareae* and pathway leading to death of colony is not clear. It is suggested that *Tropilaelaps* associated pathogenesis may have roots in more basic pathophysiological mechanisms. It is to develop an understanding into these mechanisms that the present study was undertaken.

MATERIALS AND METHODS

The studies were carried out on *Tropilaelaps* infested and non-infested worker brood of the honey bee *A. mellifera* L. The parameters considered for the experiment included quantitative changes in protein, free amino acid and RNA content of the infested pupa.
Study area and study material: The work was carried out on European honey bee *A. mellifera* L. collected from the colonies maintained in a private apiary in village Tirra (Chandigarh). The late pupa (16-20 days old) was sampled. This is the most infestive stage in the life cycle of the host as reported by the earlier workers (Kumar *et al*., 1993). Both non-infested and infested pupae were collected.
Sample preparation: Each pupa was taken in 1ml of saline and was homogenized electrically. The homogenate was used for analysis of protein estimation by Lowry's Method (Lowry *et al*., 1951), for sodium dodecyl sulphate polyacrylamide Gel Electrophoresis (SDS–Page) by the method of Laemmli (1970), paper chromatography for the qualitative analysis of free amino acids (Swarup *et al*., 1981) and for the quantitative estimation of RNA was done by the method of Schneider (1945).

RESULTS AND DISCUSSION

Hive of honey bee provides good habitat for several parasites, bacteria, viruses and attracts various predators. The most serious bee parasites are ectoparasites *Tropilaelaps* and *Varroa* mites. Originally these species were pests of *A. dorsata* and *A. cerana* respectively i.e honey bee species with which the mites co-existed in the countries of their origin. The practice of migrating bees and exotic introductions have today led to the spread of these mites over a wide spectrum of honey bee species ranging from *A. mellifera*, *A. cerana, A. florea and A. dorsata.*
The present study was carried out to investigate the influence of the *Tropilaelaps* mites on protein profile, free amino acids, RNA content of *A. mellifera* L. worker pupa. The results have been compared with

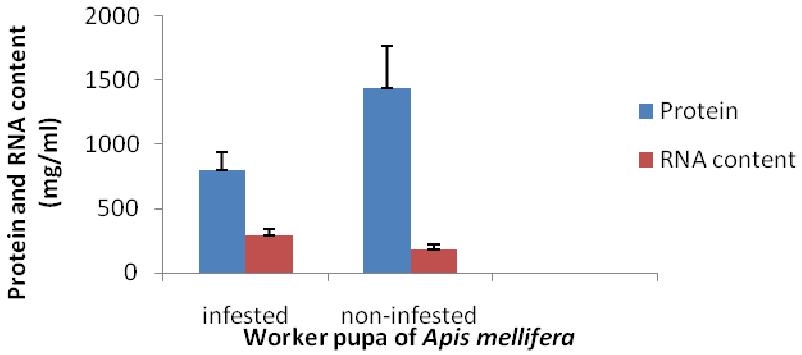

Fig.1. *Histogram showing concentration of protein and RNA in non-infested and infested worker pupa of A. mellifera.*

previous studies done on *Varroa* mite as very little is known regarding the pathophysiology of *Tropilaelaps.* Lowry's method used for the estimation of protein revealed that there was decrease in concentration of pupal protein from 1439.66 ± 324.28 mg/ml in the non-infested pupa to 802.70 ± 136.97 mg/ml in the case of infested pupa. This was expected in view of earlier studies where ectoparasitic brood mites *Varroa* and *Tropilaelaps* have been reported to feed on haemolymph thus depleting haemolymph proteins (Grobov, 1976; De Jong *et al*., 1982, Choi *and Woo* 1985 , Ball, 1997 and Badotra *et al.,* 2013). De Jong *et al*., (1982) further opined that *Varroa* infestation on honey bee decreased the haemolymph volume, total protein concentration, haemolymph protein concentration, adult weight at emergence and longitivity. Weight loss and shortening of lifespan of bee was reported to be due to reduction of the protein level in the haemolymph and Badotra *et al.,* (2013) and reduction of mass and protein content cause by Varroa mite infestation by Dooreememalen *et al.*,2013. Weinberg and Madel (1985) studied that the reduction in total protein in the haemolymph amounted to 27.0%, when the infestation rate was 1-3 mites/cell and 50.0%, when the rate was 4-6 mite/cell. Kolve and Shabanov, (1989) observed that protein depletion caused morphological damages in infested bees. In present study it was observed that the protein types established by SDS-PAGE were more in infested (16) than in the non-infested (8) brood. There were eight protein fractions which were similar in both the cases where as eight protein fractions with molecular weight 135.0, 124.0, 80.0, 64.0, 62.0, 42.0, 39.0 and 33.0 kDa were specific to the infested pupa. This is supported by the observation of Badotra *et al*. (2009) who also reported that protein fractions established by SDS – PAGE were 12 in infested pupa and 10 in non-infested pupa of *A. mellifera.*

This observation further gets support from the report of Glinski and Jarosz (1984) who described that the host increases production of certain proteins and immune polypeptides in response to parasitism. Thus while total protein concentration decreased, types of proteins synthesised increased. The new proteins were probably related to immune mechanism pathways.

In present study number of free amino acids was the same in both infested and non-infested samples, however the nature differed. In infested pupa L-Cystine, Histidine, L-Arginine, DL-Aspartic acid and L-Leucine were present while in case of non-infested pupa L-Cystine, Histidine, DL-Threonine, Glysine and DL-nor-Leucine were observed. L-Cystine and Histidine were present in both the cases where as L-Arginine, DL-Aspartic acid and L-Leucine were present only in infested pupa and DL-Therionine, Glysine and DL-nor-Leucine were found in non-infested pupa. Change in the composition of free amino acids was probably due to change in the nature of proteins/additional proteins synthesized in the body of host in response to the stimulus of mite infestation.

In the present study concentration of RNA in non-infested pupa was 184.580± 36.987 mg/ml and in infested pupa it was 293.402±50.329 mg/ml. The increased concentration of RNA (infested) observed during the present study explained the synthesis of newer polypeptides (observed by SDS-PAGE) under

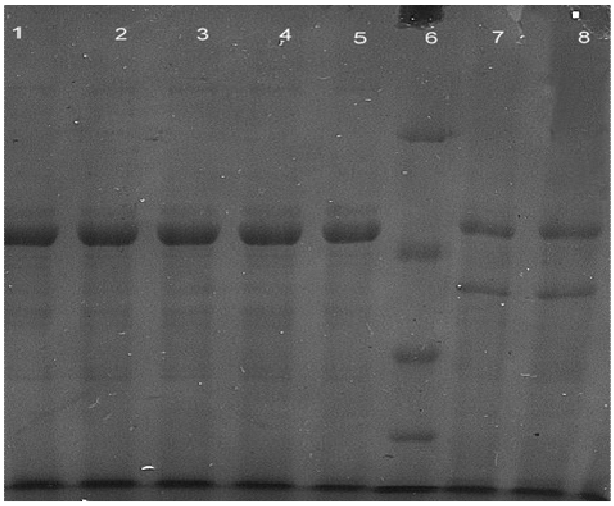

Fig. 2. *SDS PAGE of infested and non-infested worker pupa of A. mellifera, INFESTED: LANE 1, 2, 3, 4 and 5; NON-INFESTED: LANE 7 AND 8; STANDARD: LANE 6.*

the influence of mite parasitosis by increasing transcription of genes encoding antiparasitic proteins.

Conclusion

The following conclusions were drawn from the present study:

Tropilaelaps infestation of honey bee pupa caused reduction in total protein concentration of the infested pupa. This may be due to protein feeding by mite.

The additional protein fractions observed by SDS-PAGE and the difference in the nature of free amino acids suggested synthesis newer proteins perhaps related to the stress response of the host. This opinion is strengthened by the increase in concentration of RNA observed in the present study which was responsible for increased transcription of genes encoding antiparasite peptides as reported by other workers.

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