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VARROA CAUSES OXIDATIVE STRESS IN *APIS MELLIFERA* L

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Abstract

The present study was designed to study the influence of *Varroa* on honey bee *Apis mellifera*.The antioxidant defense system of the brown eye pupa- the infestive stage in the life cycle of the host- was evaluated. This was manifested as elevated activity of antioxidant defense enzymes, with Superoxide dismutase and Catalase activities being almost two times higher in infested versus non- infested pupae. Total protein content during the present observation was higher in case of uninfested pupae as compared to infested pupae.

Key words: *Varroa*, *Apis mellifera,* LPO, GST, GSH, Catalase.

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INTRODUCTION

The hive of honey bee is a suitable habitat for a variety of mites including non- parasitic, omnivorous, and pollen- feeding species, and parasites. The mites that parasitize honey bees have become a global problem. They are threatening the survival of managed and feral honey bees, the beekeeping industry and due to the role of bees in pollination, the future of many agricultural crops.

The *Varroa* mite, *Varroa destructor* [1] is an ectoparasite of cavity nesting honey bees. The mite is considered a serious threat to world beekeeping [2, 3] and the loss of complete colonies of *Apis mellifera* has been attributed to infestation with the parasite [4]. Adult female *Varroa* feeds on the haemolymph of adult bees by piercing the thin membranous areas between the thorax and abdominal sclerites. Feeding by the mite has been reported to lower the protein content of the haemolymph [5, 6, 7] especially the level of low molecular weight proteins. This is linked to depressed immunological functions observed in parasitized bees [8]. An enquiry into the biochemical changes in parasitized brood is essential to understand the pathophysiology of *Varroa* infestation.

MATERIAL AND METHODS

Study Area

The samples of *A.mellifera* worker brood were drawn from the colonies maintained by department of Zoology, Panjab University, Chandigarh.

Study Material

A. mellifera worker brood was taken for investigations with respect to influence of mite parasitosis.

Sample Collection

A random sample of 10 infested and 10 non- infested worker pupae (brown eye stage) was taken for each test after brushing off bees from the comb.

Sample preparation

The pupa was homogenized in 100 mM tris – Hcl (pH7.4). The homogenate was subjected to cold centrifuge at 10,000 x g for 30 minutes. The pellet was discarded and supernatant was used for various biochemical estimations.

Biochemical estimations

The total quantity of protein in the infested and uninfested sample of *A. mellifera* was determined by following the standard procedure of Lowry's method [9]. The activity of GST was estimated by method of Habig [10]. GSH content in pupal homogenate was estimated by the method of

[11]. Activity of SOD was determined by the procedure described by [12].Catalase was determined by the method of [13]. LPO was calculated by the method of [14].

RESULTS

Total protein content

In the pupal extract of uninfested sample, the total protein concentration (mg/ml) was higher (0.265+0.003), as compared to the infested pupa, (0.238+0.001).

Antioxidant defense enzymes

The mean activity of the four antioxidant enzymes tested was significantly higher in the infested pupa than in the uninfested pupa .However, in case of LPO this difference was non- significant. The data is presented in Table I.

Table I. Influence of *Varroa* parasitisation of worker pupae on enzyme activities in *A. mellifera.*

SAMPLE	SOD	CATALASE	GST	REDUCED GSH	LPO
Uninfested	8.130±1.990 Units / mg of protein	553.2 ± 3.508 U M H2O2 decomposed/ min/mg of protein	1.70±0.025 UM GSH adduct formed/min./mg of protein	0.0002±0.0001 nM MDA formed/ mg of protein	0.073±0.004* nM/ mg of protein
Infested	16.713±2.649 Units/ mg of protein	1015.5±4.551 UM H2O2decomposed/ min./ mg of protein	1.78±0.020UM GSH adduct formed/ min./ mg/ of protein	0.0003±0.0001 nm MDA formed/mg of protein	0.084±0.004 nM/ mg of protein

P value < 0.05, statically significant for all enzymes,
*Except LPO where P value = 0.56, not significant.

DISCUSSION

Biochemical indications of oxidative stress in *Varroa* infested worker pupae were manifested in the form of elevated activity of antioxidant defense enzymes, with SOD and Catalase activities almost two times higher in infested versus non- infested pupae. The present observations are congruent with other known pathologies associated with *Varroa*, namely (i) significant reduction in drone body weight upon emergence at infestation rates of one mite per cell [15] (ii) significant reduction in size of seminal vesicles and mucous glands[16] and (iii) reduced drone life expectancy at rates > 1 mite per cell [17].

Reduction of body weight [18] and aging [19] are typical somatic and physiological symptoms of oxidative stress in insects which, as a taxon, are naturally sensitive to disturbances of antioxidant defenses due to their high oxygen consumption rates and relatively high stress sensitivity. There are normally comparatively high levels of antioxidant enzymes (CAT, GST, SOD) in somatic and reproductive tissues of drones [20] and mature queens [21] that contribute to the protection of spermatozoa from oxidative stress and facilitate their long term survival.

Recently, Lipinski and Zoltowaska [22] reported significantly high activity of three anti- oxidant enzymes in *Varroa* infested drone pre-pupa and suggested that oxidative stress might be one of the pathogenic pathways of varroasis. They found the average activity of SOD, GPX and ceruloplasmin (CP), in infested pre- pupae was approximately 2-4 times higher than the levels in mite free pre-pupae.

It was of interest to determine the influence of mite pathogenesis on metabolically active enzymes/ pathways. Stress related increase in activity of acid phosphatase, glucose- 6- phosphatase and hexokinase on mite parasitisation in the late worker pupa(brown eye) has been reported from this laboratory [23].Total protein content during the present observation was higher in case of uninfested pupae as compared to infested pupae. It is known that the abdomen of bees parsitized by *Varroa* mites during their development contains not only less proteins but also lesser amount of sugars.

It can therefore, safely be concluded that parasitisation by *Varroa* on worker brood caused significant loss in total protein content and antioxidant enzymes as compared to uninfested worker brood.

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