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IMPACT OF *VARROA* MITE INFESTATION ON THE MANDIBULAR AND HYPOPHARYNGEAL GLANDS OF HONEY BEE WORKERS

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ABSTRACT: *Varroa* mite infestation was first detected in Iraq in the mid 1980s (Food and Agriculture Organization). High level of the infestation was found in all apiaries of Dohuk region and may act as a risk factor to the bee health. The mite *V. destructor* feeds on the haemolymph of the developing and adult bees. The structures that may be directly affected by *Varroa* mite infestation are the bee glands. Therefore this study aimed to investigate the effect of the parasitic mite *V.destructor* on the mandibular and hypopharyngeal glands of *A. mellifera* in the late summer 2013. Our results show significant differences in the size of hypopharyngeal gland acini in newly emerged workers infested with 1–3 mites compared to non-infested newly emerged workers, while only newly emerged workers infested with 3 mites showed significant differences in the size of mandibular glands as compared to non-infested newly emerged workers.
Management strategies of the mid and late summer treatment are necessary to keep the mite population at low levels before and during the period when the winter bees emerge.

KEY WORDS: *Varroa* mite, Honey bees, mandibular gland, hypopharyngeal gland, parasite

INTRODUCTION

The mite *V. destructor* is an ectoparasite that feeds on the haemolymph of adult bees and their brood in the post-capping stage (Bailey and Ball 1991).

Its reproductive potential and virulence are multifactorial and might vary according to the region of occurrence and bee race; *V. destructor* damages may lead to its complete death of a colony (Rosenkranz et al. 1999; Rosenkranz et al. 2010). The mite acts as a vector for viruses that may cause problems, such as bees growing with defective wings and high bee mortality rate (Rosenkranz et al. 2011). In addition, adult bees originating from parasitized pupae will have lower body weight, orientation problems and lower life spans (Bailey and Ball 1991; Chen and Siede 2007; Rosenkranz et al. 2010).

Varroa mites have two distinct life stages: a phoretic phase spent on the adult bees traveling within or between colonies; and a reproductive phase that occurs in the capped brood cells during honey bee pupal development. Generally, mites are significantly more often found in brood cells than on adult bees, with up to 90 % of the colony's mites found within the brood (Boot et al. 1993; Rosenkranz and Renz 2003). During the phoretic phase the mite can be found between the abdominal segments of the adult bee where they can reach the intersegmental membrane for feeding.

Feeding behavior of *V. destructor* is poorly understood, for feeding process, the individual parasite pierces the body wall of its host, and then extracts the haemolymph. All *V. destructors* resident within the brood cell can repeatedly revisit this feeding site because it remains open for several days (Kanbar and Engels 2003, 2005).

This unique ability of *V. destructor* to repeatedly feed on its bee host, suggests that they probably secrete anti-wound healing factors from their salivary glands (Barbara Locke 2012).

One of the structures that may be directly affected by *Varroa* mite infestation is the hypopharyngeal gland (Schneider and Drescher 1987), which is located in the head and produces a protein-based substance that is used to feed larvae, the queen and the drones (Feng et al. 2009). Another structure that can be affected by *Varroa* mite infestation is the mandibular gland (Teixeira et al. 2008). The mandibular glands of *A. mellifera* are exocrine glands responsible for the production of pheromones, which play a direct role in communication among members of the colony (Cruz-Landim and Mello 1967).

The honey bee infestation by *Varroa* mite was first recorded in Iraq in the mid 1980s (FAO). Beekeepers lost most of their colonies particularly those with traditional hives. *Varroa* mite was recorded in all Arab countries in 1990 (Haddad 2011). Despite of extensive using of acaricides by beekeepers, the parasite remains threat to the bee hives of the area including feral colonies. The apiculture sector was destroyed after the gulf war, at that time only feral colonies existed in the moun-

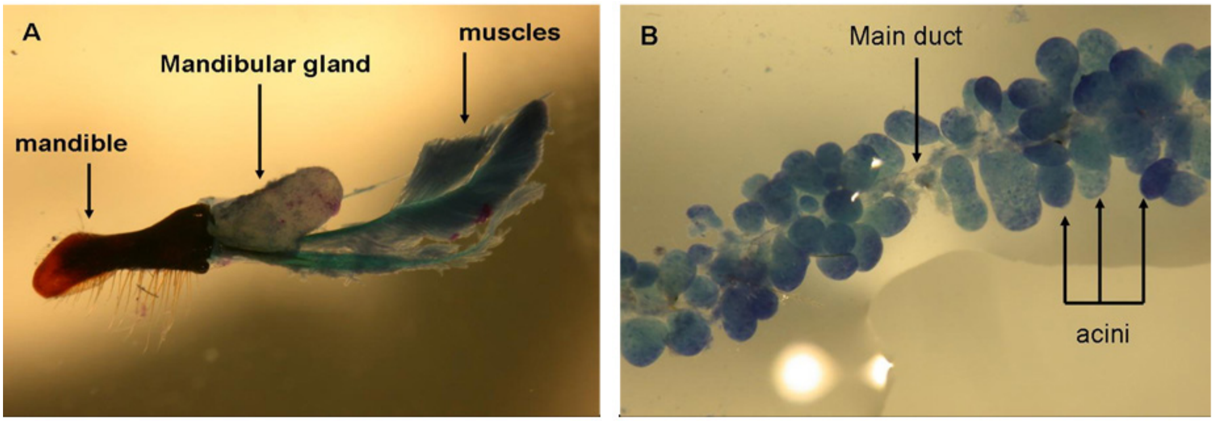

Fig. 1. A — the mandibular gland of a honeybee worker; B — part of the hypopharyngeal gland of a honeybee worker.

tains. Beekeeping process started again after 1991 and infested honey bee colonies were illegally entered the area from neighboring countries. *Varroa* mite infestation widely spreaded in apiaries of the Dohuk region (Ayoub 2014).

The mite *Varroa destructor* (Anderson and Truemann 2000) (Acari: Varroidae) is one of the most studied parasites of *Apis mellifera*, as it causes many losses in apicultural business worldwide. Several research studies have demonstrated distinct levels of virulence of the mite and increased colony mortality rates due to its infestation; however only a few studies report the mite's effects on specific tissues, glands or other organs in bees. This study was conducted with an aim of studying the parasitic effect of the mite *V. destructor* on the mandibular and hypopharyngeal glands of *A. mellifera*.

MATERIALS AND METHODS

Adult worker collection

A total of 450 samples (newly emerged workers) from three separated apiaries of the Duhok Province were collected during the late summer, 2013.

Effects of *Varroa* mite infestation on mandibular glands and hypopharyngeal glands of newly emerged workers of honey bees were investigated using three colonies of *Apis mellifera* from each apiary. The tested colonies headed with young active queens. The frames containing sealed brood with emerging workers were transferred into suitable room at 32 to 34ºC and fifty newly emerged bees were collected from each colony.

Collected bee samples were individually kept in eppendorf tubes containing 30 % alcohol and carefully examined under a dissecting microscope, and then the number of mites on the individual workers was counted. The samples of each apiary were separately grouped into non-infested newly emerged workers and infested with one (1M), two (2M) and three mites (3M). Collected bees were stored in a freezer for dissection.

Dissecting

Frozen samples were thawed at room temperature and immediately dissected to prevent tissue deterioration. The bees were dissected under a stereomicroscope at ×40 magnification. The size of mandibular glands and size of acini in hypopharyngeal glands of all workers from three groups (apiaries) was recorded.

Mandibular glands

In each dissected worker, mandibular glands from both sides of the head were dissected (the mandible with the gland separated from the head), placed on the surface of a clean glass slide, stained by diluted Giemsa stain and after few minutes washed by physiological saline. The longest and shortest dimensions of each gland were measured (Fig. 1A). The length and width of each gland were used to calculate the product for each gland (length × width). The average size of two glands for each dissected worker was calculated.

HYPOPHARYNGEAL GLANDS

A longitudinal incision was made in the top of the head. Then the hypopharyngeal glands were dissected on the surface of a clean glass slide, stained by diluted Giemsa stain and washed by physiological saline. The longest diameter of fifteen acini from each side (thirty acini from each worker) of the head was measured (Fig. 1B). The average size of acini for each dissected worker was calculated.

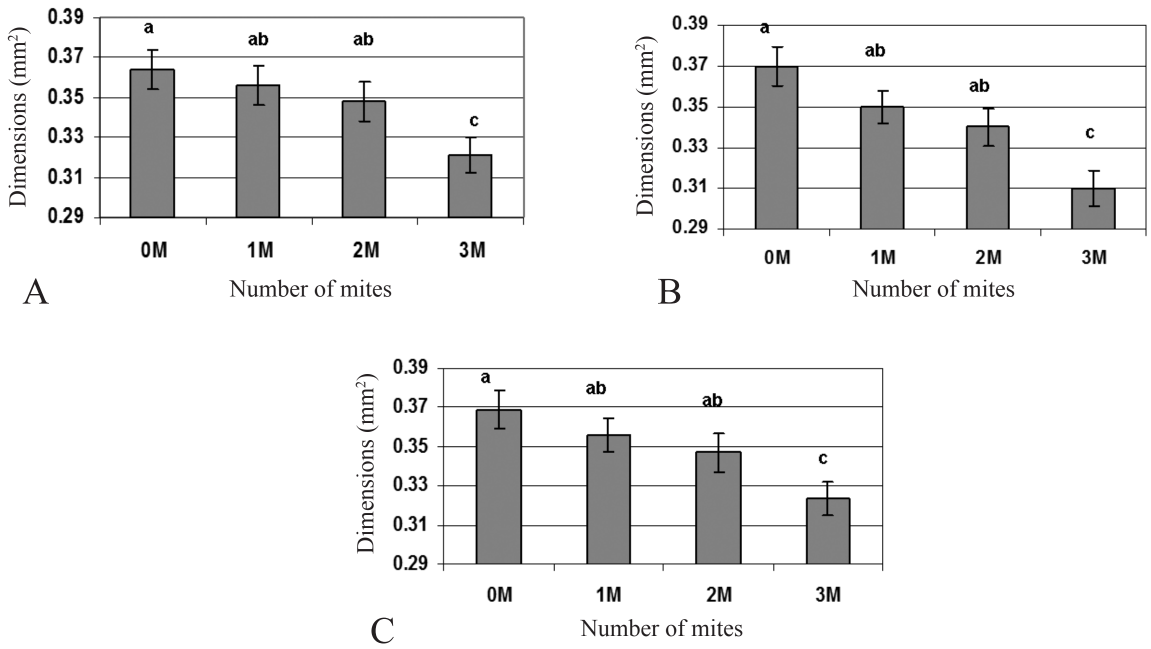

Fig. 2. Dimensions of mandibular glands (mean ± SE) of non-infested newly emerged workers compared to infested groups with one mite (1M), two mites (2M) and three mites (3M) in three apiaries: A1, A2 and A3.

STATISTICAL ANALYSIS

Two-way ANOVA was used to compare non-infested newly emerged workers with infested groups (1M, 2M, and 3M) of newly emerged workers collected from three colonies of the same apiary. Duncan's multiple comparison tests used to detect significant differences among groups of workers. The data of each apiary were separately analyzed.

Means with the same letter are not significantly different; 1M, 2M, 3M = newly emerged workers with one mite, two mites and three mites respectively. A1, A2 and A3= apiary one, apiary two and apiary three.

RESULTS

Non-infested newly emerged workers in the three apiaries were significantly ($P = 0.0002$) different in the average size of mandibular glands only from bees infested with three mites, while the size of this gland of non-infested bees was not significantly different ($P = 0.052$) from those infested with one mite or with two mites.

Among samples collected from all tested apiaries, mandibular glands were bigger in non-infested newly emerged workers (A1 = 0.364 mm^2 ; A2 = 0.370 mm^2; A3 = 0.369 mm^2) than those of bees infested with one mite (A1 = 0.356 mm^2; A2 = 0.358 mm^2; A3 = 0.356 mm^2) followed by bees infested with two mites (A1 = 0.348 mm^2; A2 = 0.349 mm^2; A3 = 0.347 mm^2),while the smallest mandibular glands were found in newly emerged bees infested with three mites (A1= 0.321 mm^2; A2= 0.318 mm^2;A3= 0.323 mm^2) (Fig. 2).

Infested newly emerged workers were significantly ($P = 0.0001$) differed in the average size of the acini of hypopharyngeal glands from non-infested bees in the three apiaries. The average dimension of the hypopharyngeal gland acini of non-infested newly emerged workers was the highest (A1 = 116 μm; A2 = 121 μm; A3 = 115 μm) followed by those of bees infested with one mite (A1= 100 μm; A2 = 106 μm; A3 = 98 μm) then bees infested with two mites (A1= 96 μm; A2= 93 μm; A3 = 92 μm), while the lowest dimension was found in newly emerged bees infested with three mites (A1 = 80 μm; A2= 79 μm; A3 = 77 μm) (Fig. 3).

DISCUSSION

Varroa mite infestation strongly affects colony health in two ways, directly, when the mites feed on the haemolymph of the developing and adult bees, affecting indirectly the population growth of the colony. This leads to shortage of pollen and nectar gathering by foragers as well as insufficient quantities of royal jelly secreted by nurse bees to provide the developing bees. Both larval and adult nutrition have important effects on the honey bee body growth.

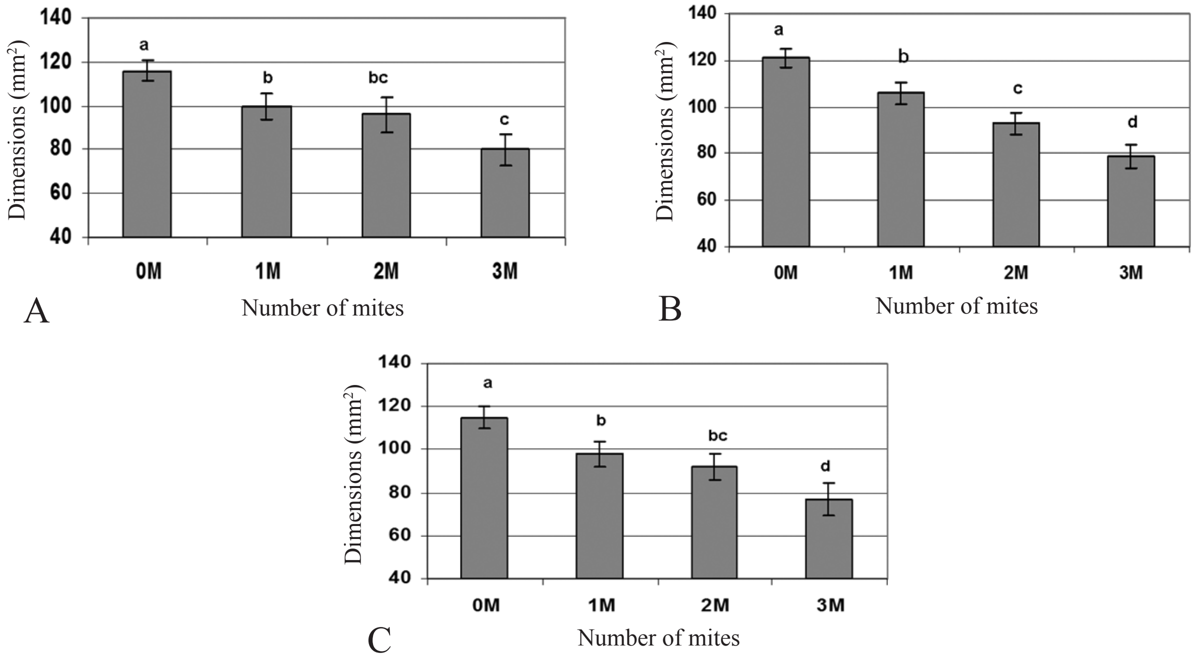

Fig. 3. Dimensions of hypopharyngeal gland's acini (mean ± SE) of non-infested newly emerged workers compared to infested groups with one mite (1M), two mites (2M) and three mites (3M) in three apiaries: A1, A2 and A3.

Our results show that significant differences in the size of both mandibular and hypopharyngeal glands exist among non-infested newly emerged workers with *Varroa* mites and infested groups. A significant difference in the size of hypopharyngeal gland acini was found in bees infested with 1, 2 and 3 mites compared to non-infested newly emerged workers, while only bees infested with 3 mites showed significant differences in the size of mandibular glands compared to non-infested bees. It seems that the hypopharyngeal glands are more affected by the mite infestation than mandibular glands. Deficiency of protein strongly affects hypharyngeal glands because the development of these glands requires sufficient amount of protein which severely reduced by *Varroa* infestation. Depletion of protein level in the body of infested pupae is due to the reduction of haemolymph which is consumed by direct feeding of the parasite as well as indirectly by improper feeding during larval stage which reared by previously infested nurse bees in the colony. The reduction in the size of hypopharyngeal glands has a potential adverse effect on the production and quality of royal jelly that causes abnormal development of the broods (Pinto et al. 2011). The results of this study are similar to that reported by Pinto et al. (2011) and Wegener et al. (2009) who found a significant decrease of hypopharyngeal gland acini diameter in bees parasitized with *Varroa* mites. Mandibular glands appeared less sensitive to the *Varroa* infestation compared to hypopharyngeal glands in which newly emerged workers resulted from infested pupae with 1 and 2 mites did not show significant differences in the size of mandibular glands compared to non infested broods. This may be attributed to the earlier development of the hypopharyngeal glands than mandibular glands in both larval and adult stages. Feng et al (2009) found that the worker bee can secrete royal jelly since it emerges.

Varroa mite acts as a vector for several viruses which have tropism to specific structure of the body. Teixeira et al. (2008) found that Deformed Wing Virus (DWV) has a tropism to the hypopharyngeal, mandibular and salivary glands, while Lanzi et al. (2006) found that Acute Bee Paralysis Virus (ABPV) affected hypopharyngeal gland development.

The open wound at the feeding site resulted by the *Varroa* bite in the body wall of the bee remains open and is used for repetitive feeding site for several days by all the mites living on the body of that bee (Kanbar and Engels 2003, 2005). After injuring the pupae's epicuticle, the mite feeds from its haemolymph (Bailey and Ball 1991). This process may compromise the bee development due to disturbances of natural hormonal regulatory mechanisms, considering that the pupal stage is critical to its later development (Schneider and Drescher 1987). Experimental evidence for ecdysteroid-induced suppression of general and specific protein synthesis has been demonstrated (Amdam et al. 2004). The majority of workers infested as pupae do not accumulate haemolymph proteins, including vitellogenin, to the same extent as in noninfested bees (Amdam et al. 2004). Vitellogenin acts as a storage protein that appears to be involved in various metabolic functions including the production of hypopharyngeal gland secretion (Amdam and Omholt 2002). This suggests that workers infested by *V. destructor* as pupae fail to develop key physiological characteristics of normally developed winter bees. These winter bees will be less likely to survive until spring or results in colonies containing a large number of workers with underdeveloped hypopharyngeal and mandibular glands. According to Deseyn and Billen (2005) hypopharyngeal gland secretions of winter bees are probably stored until spring, when reactivated workers use them to feed new cohorts of larvae. If a considerable fraction of the wintering bee population is infested during the pupal stage, it is natural to ask how these doomed bees may affect the spring population of bees and thereby the overall colony survival in the next season.

Beekeepers in temperate climates should therefore combine the late autumn management strategies with mid and the late summer treatment protocols to keep mite population at low levels before and during the period when the winter bees emerge.

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