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Virulence of *Varroa destructor* in Colonies of Honey Bee *Apis mellifera*

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Abstract

Although *Varroa* mite infestation of honeybee is widespread throughout the world, data about the level of infestation in the bees of our area are inadequate. *Varroa* mite infestation was first detected in Iraq in the mid-1980s. A large investigation was carried out to determine *Varroa* infestation level in the apiaries of Duhok Province, Northern Iraq. Otherwise, this study aimed to clarify the parasitic effect of the mite *Varroa destructor* on the mandibular and hypopharyngeal glands of *Apis mellifera*. A total of 1000 samples of adult workers of different ages from more than 20 separated apiaries were collected from August to the end of October 2013. Capped brood, drone and worker brood, from five apiaries were examined. A total of 450 newly emerged workers from three separated apiaries of the same area were collected during the late summer 2013. Effects of *Varroa* infestation on mandibular glands and hypopharyngeal glands of newly emerged workers of honeybees were investigated. High level of the infestation was found in all apiaries of Dohuk region and may act as a risk factor to the bee health. Results showed significant differences in the size of hypopharyngeal gland acini of newly emerged workers infested with one to three mites compared to noninfested newly emerged workers, while only newly emerged workers infested with three mites showed significant differences in the size of mandibular glands as compared to noninfested 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. Considerable numbers of *Varroa* mites can be controlled inside bee colonies without chemicals by removal of drone pupae or sometimes if necessary removing generations of worker pupae before emergence as adults. Using movable screened bottom boards in the opposite side of the hive entrance for the observation and counting naturally felled down *Varroa* mites were very beneficial in this area during hot summer periods.

Keywords: *Varroa* mite, parasite, honeybees, mandibular glands, hypopharyngeal glands

1. Introduction

Honeybees are proving to be excellent indicators of the state of an ecosystem, in addition to their products that contribute significant economic values to humans. The loss of natural pollinators as a result of habitat loss and pesticide application

has forced farmers to depend on domesticated honeybees for pollination. While natural habitats can provide full pollinator services, conventional agriculture clears these lands and adds pesticide amendments for crop production, greatly reducing habitat availability for pollinators and killing beneficial insects [1].

Honeybees are the most efficient pollinators of 80% of the crops worldwide, and farmers prefer their services because they greatly improve crop yields and can be transported when pesticides are applied. The decline of pollinators in recent decades is threatening the structure and function of natural and agricultural ecosystems. Pollinators provide essential ecosystem services by aiding plant and tree reproduction that require pollination assistance. Large-scale production of food crops in agricultural systems is, in many cases, only possible with the assistance of pollinators, primarily honeybees [2].

Recent declines in many of these pollinators have been blamed on land-use changes, diseases, chemicals, and climate change [3]. A typical established honeybee colony is made up of one mated queen, 0–300 male drones, and 20–200,000 small sterile female workers. The drones' primary function is mating with virgin queens. On the other hand, workers clean and rebuild the hive, cap/uncap, and tend larvae cells, gather nectar, pollen, and propolis, make honey, and defend the colony. The queen can lay around 1500–2000 eggs/day and can last up to 2 years. The queen communicates with colony members through pheromone secretions that, among other functions, signal attacks on the colony or promote swarming. Through their egg laying patterns, queens can indicate colony strength and therefore adequate honey production and pollination services [2, 3].

The life cycle of honeybees is different for each caste. The metamorphic process of worker bees takes 21 days and begins with fertilized eggs deposited at the bottom of a cell. After 3 days, the egg hatches into a first-instar larva and is fed by nurse bees. The first-instar larva grows through successive molting stages. The brood is then sealed by the nurse bees to allow pupa growth. The adult worker bee chews its way out its cell after spending 1 day as an unemerged adult. Complete metamorphosis in drones is similar to workers; however, it takes 3 days longer to fully develop due to the larger size of the cell. Queens take only 16 days to develop from egg to adult as a result of the nutritious royal jelly feeding [4].

Recent huge losses of honeybee colonies are causing overall population declines in the large parts of our planet. This occurrence is threatening the apicultural industry, while causing economic and ecological pressures on agricultural crop production and ecosystem services. Recent reports point to the spread of honeybee diseases and parasites as an explanation for these colony losses [5]. The ectoparasitic mite, *Varroa destructor* [6], is at the core of colony losses worldwide and has been responsible for the nearly complete eradication of wild and feral honeybee populations in Europe and North America since it was introduced to this new honeybee host species [7].

The apicultural industry depends heavily on chemical *Varroa* control treatments to keep managed colonies alive. These chemical controls can leave residues in hive products, have negative impacts on honeybee health, and remove selective pressures that would be required for host or parasite adaptations [4]. *Varroa destructor*, as a major pest of *Apis mellifera*, under different agricultural settings, in organic and conventional farms, has caused massive economic losses and expense for beekeepers. Different treatments were applied in colonies to determine impacts on mite loss that may influence colony health, life span, and individual health that affect honey yield and other products. *Varroa* mites remain the number one management problem for beekeepers and scientists alike. The onset of resistance to the treatments available, and the potential impact of secondary infections, will make controlling

the mite more difficult in the future. Knowledge of bee biology and both the biology and the pathology of *Varroa* mites are essential for understanding possible tolerance mechanisms in the honeybee host. Other factors that encourage spread of many bee infections in this area including *Varroa* mites are using traditional style of bee hives, depending mainly on natural swarming to obtain new colonies, poor knowledge of the beekeepers on using pesticides, and illegal entrance of bee colonies from the neighboring countries. The discrepancies in the rates of *Varroa* mite infestation levels in the different countries or even in the same region of the country could be attributed to many factors such as temperature, humidity, availability of pollen, numbers of apiaries, and density of honeybee colonies.

In spite of widespread of *Varroa* mite in the bees of the Northern Iraq but the data are poor about the level of infestation in the apiaries of the region, the aim of the present study is to determine the level of *Varroa* mite infestation in the apiaries of Duhok Province, Northern Iraq. Otherwise, several research studies worldwide have demonstrated distinct levels of virulence of the mite and the 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. Therefore, this study aimed to clarify the parasitic effect of the mite *Varroa destructor* on the mandibular and hypopharyngeal glands of *Apis mellifera*.

2. *Varroa* mite infestation in honeybee colonies

The mite *V. destructor* Anderson is the species of ectoparasitic mites (Acari: Varroidae) that causes serious disease (Varroosis) of larvae, pupae, and adults of honeybee *Apis mellifera* L. The mite is also known to transfer pathogenic viruses into the bee [8] and is suspected to be one of the agents causing colony collapse disorder [9]. Currently, the disease represents one of the most important problems of the world beekeeping and is attributed by the International Epizootic Bureau to the list "B" of quarantine diseases of bees along with American foulbrood and Acariosis. Therefore, Varroosis must be regularly controlled to predict colony loss [10]. The hemophagous honeybee mite *V. destructor* is still the greatest threat for apiculture. No other pathogen has had a comparable impact on both beekeeping and honeybee research during the long history of apiculture. The mites have negatively affected the apiculture industry in every country that it has been introduced. Accurate estimates of the effect of *V.* mites on the apiculture industry are hard to find, but it is safe to assume that the mites have killed thousands of colonies worldwide; *V.* mites also have affected the feral (wild) population of bees in many areas. Since feral colonies were not managed for *V.* mites and the colonies were left unprotected, the loss of feral colonies quickly resulted as *V.* mites continued to spread. This external parasite feeds on the hemolymph of adult bees, larvae, and pupae. Heavy parasitism results in heavy bee mortality and subsequent weakening of the colony and can lead to colony death [11]. High levels of *Varroa* mite infestation was found in apiaries of the Dohuk region, northern Iraq [12].

The mite *V. destructor* is an ectoparasite that feeds on the hemolymph of adult bees and their brood in the postcapping stage [13]. 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 [14, 15]. The mite acts as a vector for viruses that may cause problems, such as bees growing with defective wings and high bee mortality rate. In addition, adult bees originating from parasitized pupae will have lower body weight, orientation problems, and lower life spans [13, 15, 16].

2.1 Origin and distribution of *Varroa* mites

V. jacobsoni Oudemans was first described as a natural ectoparasitic mite of the Eastern honeybee *A. cerana* in Java [17] and has a wide distribution on this bee throughout Asia [18]. The ectoparasitic honeybee mite *Varroa destructor* was originally confined to the Eastern honeybee *Apis cerana*. After a shift to the new host *Apis mellifera* during the first half of the last century, the parasite dispersed worldwide and is currently considered the major threat for apiculture. The mite that is responsible for the clinical symptoms of “Varroosis” in *A. mellifera* belongs to the species *Varroa destructor*, which was assumed to be *Varroa jacobsoni* until the year 2000 [6].

Prior to recent studies, *Varroa jacobsoni* a species of mite that parasitizes *Apis cerana* (Asian honeybees), was considered homogeneous. The more damaging *Varroa destructor* was previously included under the name *Varroa jacobsoni*, but the two species can be separated on the basis of the mitochondrial DNA (mtDNA) sequence.

Although *Varroa* mites from different populations are physically alike, their virulence toward *A. mellifera* is not uniform. The greatest variation is associated with *V. jacobsoni* of Javanese origin, these mites completely lack the ability to reproduce on *A. mellifera* [19, 20] and their mitochondrial DNA (mtDNA) cytochrome oxidase I (CO-I) gene sequences differ from those of phenotypically similar mites that reproduce on *A. mellifera* in Europe [21].

The *Varroa* mite did not receive much attention by scientists until a host shift occurred and it became a pest on *A. mellifera* in Europe. The mite was first found in Europe in 1977 and in North and South America in 1977 and 1971, respectively [22]. Since then, it has spread throughout the world with the help of honeybee importations [23, 24]. Today, only Australia [6, 15], Northern Scandinavia [25], and some extremely isolated island populations [26] remain free of *Varroa*. *Varroa destructor* [6] is the only identified *Varroa* species parasitizing European honeybees; it is an exotic and relatively recent invasive species to parasitize the European honeybee (*Apis mellifera*).

Varroa mites are widely considered the biggest honeybee health problem worldwide. Until recently, *Varroa jacobsoni* has been found to live and reproduce only in Asian honeybee (*Apis cerana*) colonies, while *V. destructor* successfully reproduces in both *Apis cerana* and *Apis mellifera* colonies [27]. The mite affecting honeybee *Apis mellifera* now has been officially renamed to *Varroa destructor*.

The only mite of economic importance is *Varroa destructor*, after which successfully shifted from the original host, *Apis cerana* to the western honeybee, *A. mellifera*. The new host lacks features that obviously established a stable host-parasite relationship in *Apis cerana* during a long period of coevolution [28].

The details of the host shift are unclear. Most likely, this shift occurred when *Apis mellifera* colonies were transported to Eastern Russia or the Far East in the first half of the past century, which led to a sympatric distribution of both honeybee species [23], and might have allowed the parasite to infest the new host. *Varroa* mites are widely considered the biggest honeybee health problem worldwide. Until recently, *Varroa jacobsoni* has been found to live and reproduce only in Asian honeybee (*Apis cerana*) colonies, while *Varroa destructor* successfully reproduces in both *A. cerana* and *A. mellifera* colonies [27].

2.2 Morphology of the mite

Varroa mites show a distinct sexual dimorphism [29], with many morphological adaptations to their host. A common feature of both sexes is the division of the body into two well-defined parts, the idiosoma and the gnathosoma. The idiosoma

comprises the larger part and one dorsal shield and different ventral shields. The female mites have a flattened, ellipsoidal idiosoma with greater width than length. The legs of the female are short and strong and show specialized structures, the apoteles, for adherence to the host. The dorsal and ventral shields are highly sclerotized and show a reddish-brown coloration. Thin and flexible membranes between the shields enable the mite to dilate during feeding and egg formation. The male body is pear shaped and shows only weak sclerotization, which is mainly present in the legs and the dorsal shield. Males are clearly smaller than females in all developmental stages. The legs of the males are longer in relation to the body size than the legs of females [30].

2.3 Mite biology and behavior

Varroa destructor is closely linked to its honeybee host and lacks a free living stage. There are two distinct phases in the life cycle of *V. destructor* females: a phoretic phase (transport phase) as a parasite on adult bees and a reproductive phase within the sealed drone and worker brood cells. Males and nymphal stages of the mite are short lived and can only be found within the sealed brood cells. On the adult bees, the *Varroa* females are transported to brood cells for their reproduction or spread by foraging and swarming bees [31]. On the adult bees, the *Varroa* mite female usually is hidden under the sternites of the bee [32]. The mites suck substantial amounts of hemolymph from both the adult bees and the preimaginal host stages within the sealed brood cells. 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 [33, 34]. The mites can feed and survive on both adult bees and their brood. The mites feed on their host's hemolymph (blood) through punctures made in the body wall with their sharp mouthparts.

The host finding and reproductive behavior of *Varroa destructor* is essential for understanding the population dynamics of the parasite, but it is also of particular significance for the beekeeping practice. Certain cues for the orientation of the mites could be used for development of biological control methods such as traps, repellents, or mating disruption by certain pheromones. The control of reproduction of a parasite is a crucial point for the stability of a host-parasite relationship [35]. The mites feed on both adult and brood of bees, weakening them and spreading harmful pathogens such as bee viruses. Infested colonies eventually die out unless control measures are regularly applied. *Varroa* mites cannot be completely eradicated, but beekeepers can successfully keep productive bees despite the presence of the mite. *Varroa* can be controlled by monitoring the infestation in their colonies and the use of appropriate control methods to keep mite numbers below levels that are harmful. The mites reproduce inside the sealed brood cells; to breed, a gravid (egg-carrying) female mite enters occupied brood cells just before the cell is capped over, where she remains in the brood food under the larva until the cell is sealed. She breathes through a respiratory organ, common in mites. Five hours after cell capping, the bee larva consumes the rest of the food. Mites prefer to breed in drone brood but will also breed in worker brood. About 4 hours after capping, the mite starts feeding on the immature bee and establishes a feeding site on this host that her offspring can feed from as they develop.

The life expectancy of *Varroa* mites depends on the presence of brood and will vary from 27 days to about 5 months. During the summer, *Varroa* mites live for about 2–3 months during which time, providing brood is available, they can complete three to four breeding cycles. In winter, when brood rearing is restricted, mites overwinter solely on the bodies of the adult bees within the cluster, until brood rearing commences the following spring [4]. *Varroa* mites are mobile and can readily move between bees and within the hive, to travel between colonies they

depend upon adult bees for transport—through the natural processes of drifting, robbing, and swarming. *Varroa* can spread slowly over long distances in this way.

Both wild and managed colonies may exchange mites via another mechanism that has received remarkably little attention or study, floral transmission. Recent study tested the ability of mites to infest foragers at feeders or flowers. They found that *Varroa destructor* mites are highly capable of phoretically infesting foraging honeybees; this details the mechanisms and maneuvers and describes mite behaviors postinfestation [36]. However, the movement of infested colonies by beekeepers as well as the natural swarming of feral colonies is the principal means of spread over long distances.

3. Recent developments for *Varroa* management

Many beekeepers and farmers are familiar with the *Varroa destructor*—the parasitic mite that essentially sucks the life out of honeybees, transmits diseases, and is considered a major contributor to colony collapse around the world. Several potential solutions have been in the works in the last few years, and last year, Bayer Bee Care registered a new tool that may give beekeepers in Europe a simple way to prevent the *Varroa* mite from spreading using an already-existing active ingredient; this new tool, called the “*Varroa* gate.” Development of methods for administering existing varroacides, particularly organic acids, to allow safer and more effective controls with fewer adverse effects is most likely to be based on active ingredients of existing veterinary medicines and pesticides reformulated for *Varroa* control. Novel varroacides based on other (often naturally occurring) active ingredients are also under development. The development of synthetic pheromones (natural chemical messages) to provide control over *V. mites*, for instance, by inhibiting their feeding or reproduction.

Results indicated that double application of oxalic acid (OA) is worthwhile to beekeepers in *Varroa* management. It is not harmful to colonies and it reduces *Varroa* populations to such an extent that seven to eight doublings, which would take more than 1 year, are needed to build back to the original level [37].

The acaricide toxicity to the *Varroa* mites was consistent in both the caged adult honeybees and workers in the queen-right colonies; two types of acaricides, coumaphos at the highest doses and hop acids, were comparatively more toxic to the worker bees. Results of the same study show that various acaricides are variably effective for *Varroa* suppression when the mite populations are rising and brood is present [38]. Research continues to identify naturally occurring fungi and bacteria that could kill *Varroa* mites within the bee colony and to develop these into practical control methods.

A large number of essential oils and plant extracts were successfully used to minimize mite populations.

Biotechnical applications to minimize *V. mite* populations as a part of colony management (drone brood trapping) were used against *Varroa destructor*. Development of more sophisticated and effective biotechnical controls is likely to become part of routine colony management. Some beekeepers in many developed countries can easily apply methods for removing considerable numbers of *Varroa* mites from their colonies without chemicals by removal of drone pupae or sometimes if necessary they can remove generations of worker pupae before emergence as adults. These techniques can be performed after queen confinement on empty combs inside especially plastic bags made from queen excluder, although this technique will cause worker loss but at the same time will minimize mite populations

inside colonies; therefore, this method is effective especially during the beginning of the brood rearing period [39].

Recent study estimated *Varroa* sensitive hygiene (VSH) by calculating the removal rates of parasitized brood, which is sometimes found to correlate with the removal of dead brood, either freeze-killed or pin-killed brood. They used an artificial mite introduction method; they introduced female adult mites in recently capped brood cells and assessed the removal rates. They could not conclude that *Varroa* sensitive hygiene only or preferentially targets reproducing mites, leaving nonreproducing mites undisturbed; they concluded that more than one mechanism of resistance may evolve in response to the selection pressure by *Varroa* mites [40].

4. Materials and methods

Sample collection and investigating the virulence of *Varroa* mites in the area were carried out in Duhok province, Northern Iraq, during August, September, and October 2013.

4.1 Adult worker collection

Adult worker samples included a large number of newly emerged workers before feeding, in addition to other group of adult workers those presented in brood chamber.

4.1.1 Adult worker samples of different ages

In order to obtain the level of infestation of *Varroa* mites in apiaries of this area, a total of 1000 samples (adult workers of different ages) from 20 separated apiaries in Duhok province, Northern Iraq, were collected from August to the end of October 2013. Adult bees (samples) were taken from both sides of three uncapped brood combs of five colonies in each apiary. Collected bee samples were kept individually in eppendorf tubes containing 30% ethanol, and then the number of mites on the individual worker was counted.

4.1.2 Samples of newly emerged adult workers

A total of 450 samples (newly emerged workers) from 30 colonies in three separated apiaries of the Duhok Province; Northern Iraq, were collected during the late summer 2013. Note that the egg laying ends by middle of October in this area. Effects of *V. mite* infestation on mandibular glands and hypopharyngeal glands of newly emerged workers of honeybees were investigated using three colonies of *Apis mellifera* from each apiary. The tested colonies headed with young active queens. The combs containing sealed brood with emerging workers were transferred into suitable room at 32–34°C; and 50 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 newly emerged workers was counted. The samples of each apiary were separately grouped into noninfested newly emerged workers and infested with one mite (1M), two mites (2M), and three mites (3M). Collected bees were stored in a freezer until dissection.

4.2 Direct examination of brood (brood samples)

4.2.1 Worker brood

A total of 300 worker pupae from 30 colonies in three separated apiaries of the Duhok Province during August, September, and October were collected. In each apiary, parts of the comb containing sealed worker brood in the center of brood chamber were transferred to the laboratory. Cells of pupae recently capped were scratched, and then all stages of *Varroa* female mites in each cell were counted, and the walls of the cells and the removed caps were also examined as the mite frequently hides there.

All worker brood samples were individually kept in eppendorf tubes containing 30% alcohol and then carefully examined under binocular microscope. Empty brood cells (after brood removing) were observed using an appropriate source of light, and the numbers of mites were counted. It is worth to mention that honeybee brood production in our area starts from the beginning of April to the end of October.

4.2.2 Drone brood

A total of 300 drone pupae from 30 colonies in three separated apiaries of the Duhok Province in early summer were collected.

To obtain the level of infection, these drone brood were examined, the same was done as in worker brood collection; parts of the comb containing capped drone pupae were scratched, and then all stages of *Varroa* female mites in each cell were counted.

4.3 Examination of hive debris for mites on the bottom boards

Naturally felled down *Varroa* mites were also recorded for 6 weeks using sticky boards with 15 colonies in three separated apiaries. The distance among these apiaries was not less than 15 km. In each apiary, five untreated colonies were tested weekly for 6 weeks starting from the last week of September. A sticky board is placed below a screen mesh (3 mm × 3 mm) allowing for daily or weekly counting of *Varroa* mite. The number of dropped mites was recorded daily, using movable plastic sheets coated with vaseline placed on the bottom board of the tested hives; this board can be pulled from the opposite side of the hive entrance for the observation without irritating the foragers and guard bees in front of the hive. This method of the counting is very beneficial in this area because during hot summer days, it is impossible to open the hive for observations when temperature reaches 45°C. The total number of dropped mites during 1 week was considered the main indicator of the infestation level in each colony during the study.

4.4 Dissecting

The samples of each apiary those separately grouped into noninfested newly emerged workers and infested with one mite (1M), two mites (2M), and three mites (3M) were tested under binocular microscope to determine the effect of mite infestation on the mandibular glands and hypopharyngeal glands of these newly emerged workers.

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.

Figure 1.
 (A) The mandibular gland of a honeybee worker; (B) part of the hypopharyngeal gland of a honeybee worker.

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. The length and width of each gland were used to calculate the product for each gland (length $\times$ width). The average size of two glands for each dissected worker was calculated (**Figure 1A**).

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 15 acini from each side (30 acini from each worker) of the head was measured (**Figure 1B**).

5. Impact of *Varroa* mite infestation on immature and adult bees

The total number of colonies reared in traditional beehives around Duhok city was about 100,000–150,000, while the total number of bee colonies reared in modern beehives was about 400,000–450,000 in this area.

Wide variations in the infestation level were observed between apiaries even among colonies of the same apiary. These variations in the infestation could be attributed to several factors such as colony population, availability of nutritional resources, using of supplemental diets, and differences in the age of queens of tested colonies, in addition to the beekeeper experience in the apiary management.

Other factors that encourage spread of many bee infections including *Varroa* mites are using traditional style of beehives, depending mainly on natural swarming to obtain new colonies, poor knowledge of the beekeepers on using pesticides, and illegal entrance of bee colonies from the neighboring countries. The discrepancies in the rates of *Varroa* mite infestation levels in the different countries or even in the same region of the country could be attributed to many factors such as temperature, humidity, availability of pollen, numbers of apiaries, and density of honeybee colonies.

The levels of infestation were higher in drone pupae compared to worker pupae. Female mites prefer to enter cells and oviposit on drone pupae over worker pupae.

Significant differences were found in the size of both mandibular and hypopharyngeal glands among noninfested 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 one, two, and three mites compared to noninfested newly emerged workers (**Figure 2**), while only bees infested with three mites showed significant differences in the size of mandibular glands compared

Figure 2. Dimensions of hypopharyngeal gland's acini (mean $\pm$ SE) of noninfested newly emerged workers (0 Varroa) compared to infested groups with one Varroa (1), two Varroa (2), and three Varroa (3) in three apiaries: A1 (apiary 1), A2 (apiary 2), and A3 (apiary 3).

to noninfested bees (**Figure 3**). It seems that the hypopharyngeal glands are more affected by the mite infestation than mandibular glands. Deficiency of protein strongly affects hypopharyngeal glands because the development of these glands requires sufficient amount of protein, which is severely reduced by *Varroa* infestation. Depletion of protein level in the body of infested pupae is due to the reduction of hemolymph, 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.

Figure 3. Dimensions of mandibular glands (mean $\pm$ SE) of noninfested newly emerged workers (0 Varroa) compared to infested groups with one Varroa (1), two Varroa (2), and three Varroa (3) in three apiaries: A1 (apiary 1), A2 (apiary 2), and A3 (apiary 3).

Mandibular glands appeared less sensitive to the *Varroa* infestation compared to hypopharyngeal glands in which newly emerged workers resulted from infested pupae with one and two mites did not show significant differences in the size of mandibular glands compared to noninfested brood. This may be attributed to the earlier development of the hypopharyngeal glands than mandibular glands in both larval and adult stages.

Without periodic treatment, most of the honeybee colonies in temperate climates would collapse within a 2–3 year period. The parasite pierces the body wall of its host and then extracts the hemolymph. All *Varroa destructor* resident within the brood cell can repeatedly revisit this feeding site because it remains open for several days [41]. This unique ability of *Varroa destructor* to repeatedly feed on its bee host suggests that they probably secrete antiwound healing factors from their salivary glands [4]. One of the structures that may be directly affected by *Varroa* mite infestation is the hypopharyngeal gland [42], which is located in the head and produces a protein-based substance that is used to feed larvae, the queen, and the drones [43]. Another structure that can be affected by *Varroa* mite infestation is the mandibular gland [44]. 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 [45]. The hypopharyngeal glands are more affected by the mite infestation than mandibular glands [46]. Several researches have demonstrated distinct levels of virulence of the mite and increased colony mortality rates due to its infestation; however, only a few studies reported the mite's effects on specific tissues, glands, or other organs in bees. Nevertheless, a little number of scientific papers had been published on the issue, glands, or morphological changes in other organs of the infected honeybee. After injuring the pupae's epicuticle, the mite feeds from its hemolymph [13]. 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. One of the structures that may be directly affected is the hypopharyngeal gland [28], which is located in the head and produces a protein-based substance that is used to feed larvae, the queen, and the drones [43].

6. Lack of biosecurity is a risk to honeybee health in developing countries

Honeybee biosecurity is a set of measures designed to protect a beekeeper's honeybees from the entry and spread of pests. Honeybee biosecurity is the responsibility of every beekeeper and every person visiting or working in an apiary. Implementing honeybee biosecurity is essential for a beekeeper's business. If an exotic or endemic pest establishes in an apiary, business costs will increase (for monitoring, cultural practices, additional chemical use, and labor), productivity will decrease (yield and/or colony performance), and markets may be lost. The health of the honeybee industry also ensures the continued success of many other plant industries that rely on honeybees for pollination. Early detection and immediate reporting increases the chance of an effective and efficient eradication.

The biosecurity measures of an individual beekeeper can be enhanced by collaborating with others in a particular region. Through this collaborative approach, biosecurity threats to all apiaries in a region can be minimized. Promotion of honeybee biosecurity at the regional level can be enhanced through the engagement of the community and by understanding the area's vulnerability, and the potential source and nature of threats. Neighboring apiaries (managed or abandoned), feral colonies, and/or unregistered hives are examples of potential biosecurity threats. Regional biosecurity efforts are strengthened by identifying what resources and

expertise are available and by having a commitment from stakeholders to implement biosecurity measures and surveillance programs. Implementation of honeybee biosecurity strategies underpins regional biosecurity, which in turn underpins national biosecurity.

7. Prospects for the development of new methods to deal with *V. mites*

Over the coming years, we can expect new developments that will change the way we control infestation with *V. mites*. Breeding programs in many countries are aiming to select and develop bees that are more tolerant of *Varroa*. These bees either may be able to naturally maintain better control over the mite population or may be more tolerant to the presence of the mites and their associated pathogens.

Helping honeybees help themselves: breeding *Varroa*-resistant bee populations is a long-term solution for the mite problem. A number of honeybee colonies are showing the first signs of resistance—honeybees with a behavioral trait called *Varroa*-sensitive hygiene can detect the *Varroa* mites in the closed brood cell. These bees pull out the infested pupae and thus stop the *Varroa* mite from multiplying in the colony. This behavior was originally only known to occur among Asian honeybees. On the basis of these observations, researchers intend to strengthen this defensive capability by practicing selective breeding among European bee populations and thus create long-lasting protection against the parasites; but several more years of study are required. It is therefore still necessary that researchers and beekeepers continue to work on developing new methods to combat the *Varroa* mite.

It is especially important to know the enemy better through intensive monitoring of the *Varroa* mite. In long-term observations, researchers gain significant facts and figures about the mites' population and the efficacy of current countermeasures. These results help them optimize and complement *Varroa* treatments. So far, they were even able to identify sexual attractants called pheromones that can help to specifically develop new natural or synthetic varroacides in the future. Currently, experts are examining and testing the pheromones in the laboratory. Other treatments are used, for example, the sterilization of male and female mites or heat treatment, to protect the bee colonies from the *Varroa* mite.

8. Conclusions

Bee population in northern Iraq witnessed dramatic decreases at the late 1980s due to the wide spread of *Varroa* mite infestation according to the interviews made with local beekeepers. Moreover, war conditions led to the migration from the rural areas to the urban areas and economic sanction imposed by United Nation on Iraq; all these participated in the heavy loss of bee industry in the area.

Based on the results obtained throughout investigating a large number of colonies in Northern Iraq, high level of *Varroa* mite infestation found in all apiaries of the region and may act as a risk factor on the health of bees. Beekeepers lost most of their colonies, particularly those with traditional hives. This kind of hives are made of simple wood baskets covered with clay; they cannot be inspected and should be destroyed for honey harvesting; therefore, this type of hives should be neglected and it is necessary to encourage local beekeepers to use modern bee hives instead of these undeveloped hives.

Despite 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 in Iraq after the gulf war; at that time, only feral colonies existed in the mountains.

Beekeeping process started again after 1991, and infested honeybee colonies were illegally entered the area from neighboring countries; Therefore, *Varroa* mite infestation is widely spread in apiaries of the Dohuk region, northern Iraq.

The simple method of counting mites in hive debris is a useful parameter for monitoring the population development of *Varroa* in colonies with hatching brood. Using movable screened bottom boards placed in the opposite side of the hive entrance for the observation and counting naturally felled down *Varroa* mites were very beneficial in this area during hot summer periods.

Varroa mite infestation strongly affects colony health in two ways: directly, when the mites feed on the hemolymph of the developing and adult bees, and indirectly affecting 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, because both larval and adult nutrition have direct effects on the honeybee body growth.

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 brood. Mandibular glands appeared less sensitive to the *Varroa* infestation compared to hypopharyngeal glands; this may be attributed to the earlier development of the hypopharyngeal glands than mandibular glands in both larval and adult stages. *Varroa* mite can act as a vector for several viruses, which have tropism to specific structure of the body. Workers infested by *Varroa destructor* as pupae fail to develop key physiological characteristics of normally developed winter bees. The winter bees that develop as larvae in the presence of highly infested nurse bees in the late autumn will be less likely to survive until spring or result in colonies containing a large number of workers with underdeveloped hypopharyngeal and mandibular glands. If a considerable fraction of the wintering bee population is infested during the pupal stage, definitely these bees will affect the spring population of newly emerged workers and thereby the overall colony survival in the next season. Beekeepers in this area should therefore combine the late autumn management strategies with the mid- and late summer treatment protocols to keep mite population at low levels before and during the period when the winter bees emerge. This compilation of present-day knowledge on *Varroa* honeybee interactions emphasizes that we are still far from a solution for *Varroa* infestation and that, therefore, further research on mite biology, tolerance breeding, and *Varroa* treatment is urgently needed.

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