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The Incidence of *Acarapis woodi* and *Varroa destructor* in Kurdistan Apiaries, Iran

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Abstract: Parasitic mites such as *Acarapis woodi* and *Varroa destructor* mite are examples of such threats that can cause a great deal of damage to honey bee populations. The present study was aimed to evaluate the infestation level and prevalence of apiaries and bees colonies with *Acarapis woodi* and *Varroa destructor* in Kurdistan province. A total of 100 apiaries were sampled from April to September 2014 and April to September 2015. Samplings were performed and samples were examined to detect the presence of *Varroa destructor* and *Acarapis woodi*. The prevalence of *Acarapis woodi* infestation in apiaries of this region 1% was calculated. In addition, *Acarapis woodi* infestation rate of hives was 0.26%. Among 100 apiaries, 61% (95% CI, 45.9-76.1) apiculture was infected by varroa mite and 39% not infected. The average value of *Varroa destructor* in adult bees colonies of Kurdistan apiaries was 7.5% (95% CI, 5.9-9.1). Regarding high rate of infestation and incidence of mites in the apiaries of Kurdistan province, it seems necessary to have a regular control program in order to decrease the rate of infestation in this region.

Keywords: *Acarapis woodi*, *Varroa destructor*, Prevalence, Kurdistan, Iran

1. Introduction

There are approximated 165000 colonies of honey bees in the Kurdistan province, kept by about 44000 beekeepers. Bees make an essential portion of agriculture and the environment through pollination. Here, the honey bee (Apis mellifera L.) plays a presiding role through the pollination of both wild plants and commercial crops. Honey bees are currently the major managed pollinator available within the Kurdistan. In addition to this, the bees also produce honey and wax. Honey bees, as well being affected by contaminants and pesticides, can be affected by a range of diseases, pests, and parasites [1] that are of significance for the health of colonies and also from the point of view of regulation and the movement of bees in trade around the world. Pests and diseases can cause high levels of colony losses, creating a reduction in available pollinators for important crops [2]. Acarapis mites are parasites of bees which can reason intense damage to the bee industry by subtractive honey production, eradicating colonies in both managed and feral honey bees and decreasing pollination [3]. In addition, honey bee tracheal mites (HBTM) affect the overwintering susceptibility of bee colonies and have been related with paralyzed bees displaying disjointed wings (called 'K-wing') and crawling on the ground near hives. A heavy HBTM load causes diminished brood area, smaller bee populations, looser winter clusters, increased honey consumption, lower honey yields and, ultimately, colony demise. In temperate regions, mite populations increase during the stress of cold winter temperatures, when bees are confined to the hive; this stress and the inability of bees to keep the winter cluster warm may be the cause of colony loss [4]. Currently, there are 3 species of Acarapis mites: A. woodi, A. externus, and A. dorsalis [5]. These mites are known to parasitize specific locations on bees. A. externus is mainly found on the external

surface of the head and thorax of bees. A. dorsalis, on the other hand, is mostly found on the thorax especially in a groove between the mesoscutum and mesoscutellum of bees. Unlike these 2 species, A. woodi is an internal parasite that lives in the abdominal and thoracic air sacs of bees [6]. All 3 species are prevalent all over many countries including UK, USA, Iran, Turkey, China, and Japan [7]. In addition, infected swarms, drifting bees, and the distribution of A. mellifera around the world have donated to the spread of this mite. However, its current range is not well known [4].

Varroa destructor, *Acarapis woodi*, and Tropilaelaps spp. are the main parasitic honey bee mites. Among them, V. destructor is more prevalent throughout the world [8]. The mite V. destructor (formerly Varroa jacobsoni) is an ectoparasite of adult bees and their brood. It penetrates the intersegmental skin between the abdominal sclera of adult bees to ingest hemolymph. It can sometimes be found between the head and thorax. The number of parasites steadily increases with augmenting brood activity and the growth of the bee population, mainly late in the season when clinical signs of infestation can first acknowledge [9]. The varroa mites are parasites of adult bees and their brood. Essentially, the brood is damaged by the parasitic mites. Bees and their offspring that have been infected during the brooding phase by only one parasitic mite show various ill effects, such as a shortened life span, changes in behavior and an increased disease aptitude [10]. The parasitism is censorious if more than one mite enters the brood cell for reproduction. Only in the lethal stage immediately before the collapse of the colonies do clinical signs, such as shrunken wings and shortened abdomen, appear. This is due to an increased susceptibility to deformed wing and acute paralysis virus, as well as to the infection of wounds and loss of hemolymph [11]. Four species have been recorded: Varroa jacobsoni, V. destructor, V. underwoodi and V. rinderi. Until recently varroa mites that affect A. mellifera worldwide were assumed to be V. jacobsoni. However, it has been shown that these mites are V. destructor [12]. Despite the presence of beekeeping industry in Kurdistan province West of Iran, there is not any published information about parasitic bee mites in this region. In addition, in 2012-2014 there were some complaints about hive decline and low productivity in honeybee colonies of this area. The aim of the present study was to determine the situation of parasitic honey bee mite infestation in the apiaries of this region of Iran.

2. Materials and Methods

Sampling

2.1. Acarapis woodi

A number of bees to be examined: In general, about 30-50 bees are examined per colony [13]. There are different ways of determining the sample size needed to accurately detect tracheal mite infestation of a colony. The following equation developed by Cochran [14] is a way of finding the number of bees needed to be sampled for each colony (equation 1),

$$n_0 = \frac{Z^2 pq}{e^2}$$

and another equation is a way of finding the number of the colony for sampling in the province (equation 2).

$$n = \frac{\frac{z^2 pq}{d^2}}{1 + \frac{1}{N}\left(\frac{z^2 pq}{d^2} - 1\right)}$$

Infestation is estimated to be 5% [15], equation 1 shown about 73 bees should be examined per colony. Equation 2 shown 383 colonies should be sampled.

The technique of cutting thoracic disks and clearing them with lactic acid or KOH permits sampling large numbers of bees in a short time [16]. However, the time saved initially is lost later when the disks must be mounted on slides for examination (Figure 1-5).

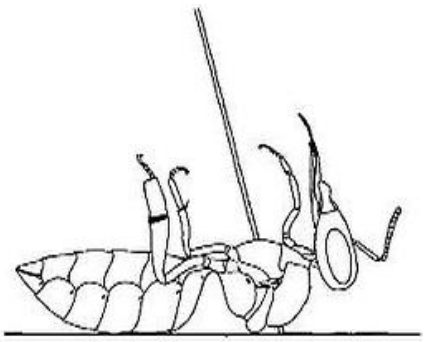

Figure 1. *An insect pin is inserted vertically through the thorax.*

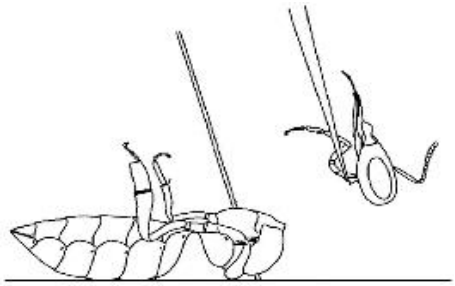

Figure 2. *Head and forelegs are removed by grasping the coxae with forceps and pulled anteriorly to expose the first pair of thoracic tracheae.*

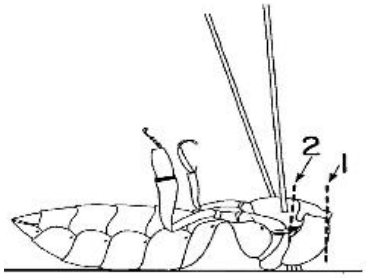

Figure 3. *Insertion of a second insect pin stabilizes the body position during dissection. Locations are shown for the initial cut (1) anteriorly and the second cut (2) in the middle of the thorax. Note that the second cut terminates halfway through the body.*

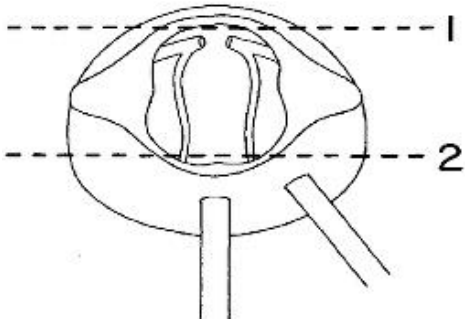

Figure 4. *Anterior/ventral angular view (as seen through the microscope) showing the exposed tracheae and the locations of the two cuts to be made.*

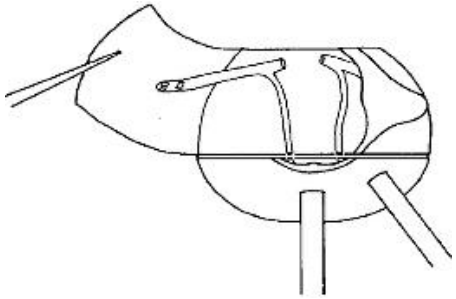

Figure 5. *A flap of the exoskeleton is shown pulled to one side with forceps to reveal the large thoracic trachea that is visible along its entire length.*

Conversely, in low infestation levels, tracheae from an individual bee need to be examined. Bees may anesthetize or kill by freezing before the examination. Developing a technique to locate the internal mites on individual bees.

2.2. Varroa destructor

Due to the protocol of the Iranian Veterinary Organization [17], varroa sampling was done and 5% of hives in each beekeeping center were investigated. Approximately 100 nurse bees are removed from combs. Samples should be taken from both sides of at least three uncapped brood combs. To determine later the average infestation rate based on these individual results. Test procedure: a) kill the bees in a special container by submersion in alcohol. b) Stir the container for 10 minutes, the solution (bees + alcohol) was then agitated so that the mites would be released from the bees' bodies. c) Separate the bees from the mites by means of a sieve with a mesh size of approximately 2–3 mm, finally, the actual number of bees and mites were counted and recorded [9]. V. destructor prevalence per beehive was estimated in the phoretic state following the protocol; the V. destructor prevalence per hive was calculated as% V. destructor= (Number of phoretic mites/number of adult bees ×100 [18]. Infestation is estimated to be 31.5% [19]. Equation 2 shown 322 colonies should be sampled.

Samplings were performed and samples were examined to detect the presence of V. destructor and A. woodi.

3. Results

A total of 100 apiaries were sampled from April to September 2014 and April to September 2015.

Acarapis woodi: 73 adult honeybees were taken from each colony and 5% of hives in each beekeeping center were investigated. During the study, a total of 383 colonies were sampled and studied. A. woodi was found in 1 of the 100 beekeeping center sampled and prevalence of A. woodi infestation in apiaries of this region 1% was calculated. In addition, A. woodi infestation rate of hives was 0.26%.

Varroa destructor: During the study, a total of 322 colonies were sampled and studied. Among 100 apiaries, 61% apiculture was infected by varroa mite and 39% not infected. The average value of V. destructor in adult bees colonies of Kurdistan apiaries was 7.5%.

4. Discussion

One pest is HBTM A. woodi, an obligate endoparasite of honey bees. This mite reduces the resistance of bees to other parasites and diseases, shortens the life cycle and in heavy infestations can block the tracheae of bees. Tracheal mites spread by transferring from bee to bee. There are no documents on the first infestation of the honey bee, A. mellifera, with A. woodi, there is a report of its occurrence in Iran [20]. Reported that, the average prevalence rate of Acariosis in total population 0.8, highest prevalence in Ardebil province (2.3%), the lowest prevalence in Fars province (0.3%) and also negative total samples from West Azarbayejan, Isfahan, Lorestan, Kerman, and Yazd [21]. The infestation level and prevalence of bees colonies with A. woodi in Savojbolagh county in the Alborz province was 13% [22]. Distribution of mite A. woody in two Mazandaran and Kurdistan provinces was 5.5 and 4.4%, respectively [15]. Honey bee colonies in northeastern Mexico have relatively high populations of A. woodi, found that 46% of apiaries in Nuevo Leon and 17.6% in Tamaulipas were infested. These surveys indicate that during the winter of 1985, 61% of colonies were infested and that 42% had infestations in which 30% or more of the bees were parasitized [23]. The environment does play a role in determining the growth of tracheal mite populations in honey bee colonies and parasitism by tracheal mites has been thought to have minimal consequences to honey bee colonies in regions where winter is mild [24]. Tracheal mites are most damaging to honey bees during the winter and Tracheal mite effect is weather dependent; serious damage can be encountered in cooler areas whilst warmer zones do not experience major impact and Colonies are seldom seen with symptoms in summer or autumn [25]. Tracheal mite could also be responsible for lower honey yield and increased honey consumption, lower pollen collection, dysentery, smaller brood area, looser winter cluster, excessive swarming, higher bacterial and viral level and, eventually, the death of the colony [25]. Colonies with infestations lower than 35% produced more honey than those whose infestations were higher [26]. Contrarily, the presence of A. woodi has not always been associated with decreased honey production. Tracheal mites can harm honey bees in mild climates. For years, parasitism by tracheal mites has been thought to have

minimal consequences to honey bee colonies in regions where winter is mild [24]. It is important to use apiaries that are protected from chilly winds when overwintering colonies, especially under northern weather conditions such as a cold snap, blizzard, and cold weather. These events left most sectors of West Iranian beekeeping in a difficult position. Safe and efficacious control measures would offer relief to infested areas and provide assurance to uninfested regions that this parasite is manageable. These observations suggest that greater loss of Iran bees may occur during harsh winter because of an increased death of infested adult bees and also from starvation. Thus, chemical treatment to control mites and feeding of colonies during winter must be used to increase winter survival of colonies bees. Colonies with high tracheal mite infestations generally are expected to die during winter. Treatments for HBTM include using vapors from menthol crystals, synthetic acaricides, and oil or grease patties made from vegetable shortening and sugar [13].

This mite was first reported as an ectoparasite from Iran in 1983 [27]. The new worldwide distributed and hematophagous mite V. destructor is considered as the main pest of honey bees A. mellifera. According to the results, this high prevalence of infestation shows the high spread of V. destructor in apiaries in Kurdistan Province. This result is similar to other sites of the world that had reported. The study on the prevalence of V. destructor infestation in apiaries of Golestan province in Iran showed that 92% of apiaries were infested [28]. The prevalence of varroa infestation in apiaries of North East of Iran was 31.5% [19]. The annual average of varroosis incidence in Eastern-Azerbaijan province was reported 23.39% [29]. In Fars province, varroa infestation during the year is less than 4% [30]. In Turkey, 25.61% of the hives were infested by varroosis [31]. In Poland 30% of the hives [32] and in Serbia 21.5% of the hives [33] were reported as infested. The studies conducted by De Jong et. al. (1984) demonstrated that prevalence of varroosis is usually more common in cold regions rather than warm climates [34]. The results from this study showed the high rate of varroosis incidence in this province comparing with the results of Eliami et al. (2005) in Fars province (warm climate). and also, confirms the results of De Jong et al. (1984). In the study conducted by Eliami et al. (2005), a significant association was witnessed between climate and the rate of varroosis incidence; and in the cold regions. It is proposed that varroa infestation in cold climates is more than that of warm climates and varroa rate of incidence in cold seasons is more than warm and hot seasons. The rapid spread of varroa mites among bee colonies is due to a number of factors such as drifting of diseased bees, movement of bee swarms, and robbing of weakened colonies [35]. In addition, migratory beekeeping practices and the importation of infested bees lead to a rapid distribution of varroa mites [6], in addition, environmental factors like temperature, humidity or the availability of pollen and nectar can indirectly influence parasite population. Under temperate climatic conditions, yearly treatments against the parasite are therefore indispensable to prevent damage of infested honey bee colonies. The effective control of V. destructor after the honey yield, but before the production of winter bees, is a crucial element of sustainable treatment concepts [36]. Regarding high rate of infestation and incidence of varroosis in the apiaries of Kurdistan province, it seems necessary to have a regular control program in order to decrease the rate of infestation in this region.

5. Conclusion

A. woodi and V. destructor remain a serious problem for the Iran. Acaricidal resistance in some countries is a serious problem and there is an associated problem of chemical residues in bee products. Thus, chemical treatment to control mites and feeding of colonies during winter must be used to increase winter survival of colonies bees. Colonies with high mite infestations generally are expected to die during winter. It is necessary that researchers in order to prevent the transmission and spread of the parasite in our country be performed.

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