Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2016; 4(3): 01-13

Received: 01-03-2016
Accepted: 02-04-2016

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Unprecedented first record of infestation level *Acarapis woodi* (Rennie) and overwintering ability in Savojbolagh regions of Alborz province in Iran

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Abstract
The aim of the present study was to demonstrate additional observations on the honeybee tracheal mite of colonies harboring highest and lowest infestations level of *A. woodi* and we evaluated various lines of Iranian honey bees for different characteristics such as mite intensity, bee population, colony weight change and tracheal mite infestations as well as their winter survival. We evaluated the overwintering ability of candidate breeder lines of Iranian honey bees, most of which are resistant to both *Varroa destructor* and *Acarapis woodi* (Rennie), during 2013-2015. Our results indicate that Iranian honey bee colonies (headed by original and supersedure queens) can successfully overwinter in the northwest provinces, thus, chemical treatment to control mites and feeding of colonies during winter must be used to increase winter survival of these Turkey-hybrid bees. It is necessary that researches in order to prevent the transmission and spread of the parasite in our country be performed.

Keywords: First record, Infestation level, *Acarapis woodi*, Overwintering ability, Alborz

1. Introduction
The present study was aimed to evaluate the infestation level and prevalence of bees colonies with *Acarapis woodi* (Rennie) in three sites in Savojbolagh county in the Alborz province and overwintering ability of Iranian honey bee was compared with Turkey honeybee colonies commercially available in the Iran and comparing the colony mortality between colonies headed by OI (Original Iranian) and SI (Supersedes Iranian) queens, and also tracheal mite dissection under laboratory. The tracheal mite infestation of honey bees is an unresolved problem for the beekeeping industry [74]. The mite *Acarapis woodi* is considered as the factor of *Acarin*e disease in the honey bee. This parasite was discovered in 1921 on the honey bee in the island create Britain for the first time [3]. In addition, infected swarms, drifting bees, and the distribution of *A. mellifera* around the world have contributed to the spread of this mite. Although its current range is not well known, honeybee tracheal mite (H.B.T.M) has successfully invaded most countries, including Europe, Asia, parts of Africa, North and South America, but is not known to occur in Australia, New Zealand or Scandinavia [72]. Tracheal mite is now well established in all countries with a significant beekeeping industry, with the exception of New Zealand, Australia, Norway and Sweden [58]. *Acarapisosis* has been found in North and South America, Europe and the Middle East (Figure 1) [58]. The identification and detection of the mite led to a law from the US Department of Agriculture restricting all live honey bee imports into the USA in 1922 [64]. Despite this restriction, honeybee tracheal mite (H.B.T.M) was first seen in the USA by beekeepers in Texas in 1984 and the prairie provinces of Canada in 1985. Thereafter, *A. woodi* spread throughout the USA and most Canadian provinces, facilitated by commercial beekeepers transporting bees for pollination, and the sale of mite- infested package bees. In addition, infested swarms, drifting bees, and the worldwide distribution of *A. mellifera* have contributed to the spread of this mite [73]. Although its current range is not fully known, the honeybee tracheal mite (H.B.T.M) has successfully been established in many countries in most continents, including Europe, Asia, parts of Africa, and North and South America [73]. Recent work reported *A. woodi* was found on Asian honey bees, *A. cerana japonica*, in Japan [40].

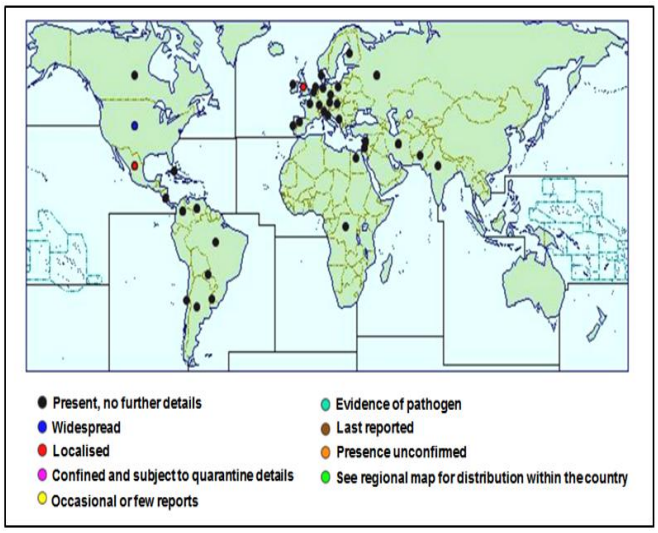

Fig 1: Distribution of tracheal mite in the world. Photograph by Claire Beverley, CABI, Nosworthy Way, Wallingford, OX10 8BU, UK (Source: http://www.cabi.org).

Parasitic mites such as *Acarapis woodi* and the more recently discovered *Varroa destructor* mite are examples of such threats that can cause a great deal of damage to honey bee populations. *Varroa* is currently viewed as the most economically significant parasite associated with honey bees. [38, 43, 72]. The present study was conducted with the aim of mite distribution level study in Savojbolagh regions of Alborz province in Iran; also the distribution level between three sites in Savojbolagh County in the Alborz province, the apiaries inside every city and the hives inside every apiary was studied. Results of this study were used to reinforce hives and fight to this pest. *Acarapis woodi* (Rennie), known as the honeybee tracheal mite (H.B.T.M.) reduces the resistance of bees to other parasites and diseases, shortens the life cycle and in heavy infestations can block the tracheae of bees. Known to western beekeepers as *Acarine* or *Acarine* disease, but better described as the honeybee tracheal mite or simply the tracheal mite, it was first discovered by John Rennie (1865-1928) and his coworker Bruce White. The mite was then thought to be the cause of this disease but that is now believed to be caused by a bee virus and not by the mite. Tracheal mites spread by transferring from bee to bee. The mites often go undetected because they are not visible to the naked eye and infested bees do not show visible symptoms. A beekeeper with dead honey bee colonies in the spring may attribute the mortality to normal winter mortality. The *Varroa* mite is spread locally by being carried on the bodies of adult bees. Illegal importations and the movement of hives have helped to increase its spread from country to country. The *Varroa* mite was originally a parasite of the Asian honey bee (A. *cerana*), but it has recently undergone a host shift to parasitize the European honey bee as well [4, 57]. Of the *Varroa* species complex that includes (*Varroa jacobsoni* Oud.) and (*V. underwoodi*), [23] only *V. destructor* is capable of reproduction within *A. mellifera* colonies [4]. The mite causes relatively little injury to its native host, because it generally reproduces only within the cells of developing males (drone brood), leaving the colonies' workers unharmed [8, 9, 16]. Moreover, Asian honey bee adults readily recognize the presence of phoretic mites and remove them with their mandibles, a grooming behavior that is elicited by a dance to recruit neighboring workers [10]. *A. cerana* nurse bees are more adept than European honey bees at recognizing and removing parasitized and diseased brood before it emerges, thus preventing mite spread [9]. These factors all help to stabilize the host-parasite relationship between *Varroa* and *A. cerana*. Similar behaviors are lacking in *A. mellifera*, leaving European honey bees vulnerable to parasite induced morbidity and mortality [22, 41]. A study by Buchler *et al*. (1992) have revealed that, in comparison to *Apis mellifera*, the Asian honey bee is much quicker to respond to phoretic mites, and it is more successful in removing them. They found that workers of Asian honey bees begin autogrooming much more quickly and with a higher frequency than European bees when a mite was experimentally placed on the thorax of an adult bee. *A. cerana* workers also performed allogrooming, which is almost never observed in *A. mellifera* in response a *Varroa* mite. The behavior is initiated by a parasitized bee when it performs a shaking dance, after which neighboring worker bees come to the parasitized bee and comb its hairs with their mandibles, paying special attention to the petiolus region usually presented to them by the parasitized bee [10]. There are at least three species of *Acarapis* mites [23]. They are clearly distinct from other honeybee mites, but closely resemble each other [23]. The tracheal mite was first described to *Acarapis woodi* [38, 65]. Two other external species, *Apis. Dorsalis* and *Apis. Externus* were described [54]. *Acarapis* mites are known to be present on every inhabited continent except Australia. They have not been found in Scandinavia, China, Japan, and the Caribbean [11, 23, 50]. Also there are no documents on the first infestation of honeybee, *A. mellifera*, with *A. woodi*, there is a report of its occurrence in Iran [31]. Furthermore *A. woodi* was not found during a three year survey in the Iran country [33]. The object of this survey was to investigate and confirm the presence or absence of *A. woodi* as well as other species of *Acarapis* mites in Iran. From what we currently know, *A. mellifera* is the original host of honeybee tracheal mite (H.B.T.M), as this mite has only been recorded on other *Apis* species following the introduction of *A. mellifera* to Asia. The exact causes of the loss of colonies infested with honeybee tracheal mite (H.B.T.M) are still unknown. This problem is exacerbated by the lack of unique symptoms associated with tracheal mite.

Tracheal mites, *Acarapis woodi* (Rennie), remain a serious problem for the Iran. *Acaricidal* resistance, especially pyrethroid resistance in some countries, is a serious problem and there is an associated problem of chemical residues in bee products. In the Pacific Northwest, bee colony losses of 31% were observed during winter 1988 and early spring 1989 because of high levels of tracheal mites [13]. Similar results were observed in Pennsylvania in 1989 [30]. A higher winter mortality of 25-85% was observed in the northeastern USA during the 1995-1996 seasons [29]. The authors claimed that this increase in colony losses was caused by *Varroa* and tracheal mites and also by secondary infections as a consequence of feeding by the parasitic mites.

Honey bee colonies in northeastern Mexico have relatively high populations of (*Acarapis 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 [27]. More recently, high levels of infestation have been found in the area [26, 42]. The prevalence of *A. woodi* both among and within colonies in northeastern Mexico currently appears similar to those reported in England about 60 years ago [14, 52, 64]. Although economic thresholds have not been firmly established, several studies have reported adverse effects when mite populations exceeded a stated level. Otis

and Scott-Dupree (1992) have revealed that, when the infestation level was 50% or greater, colony value was doubtful. Similar views, stating that a 50% infestation in the autumn was likely to result in colony death during winter [53]. 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. [18, 35]. 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 [75]. 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, death of the colony [75]. Surprisingly, few productivity studies have been reported [27]. Infested colonies produced less honey, but data were not included [38, 41]. *A. woodi* shortens bee longevity by 20%, and then a colony that was 100% infested should be 20% less productive [12]. This may, however, be an underestimation, because if bees live about 35 days in the summer, then a 20% reduction in longevity represents about a 50% reduction in foraging lifetime. Colonies with infestations lower than 35% produced more honey than those whose infestations were higher [34]. 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. However, A study by De Guzman *et al*. (2001) have stated that susceptible stocks can suffer harmful effects of tracheal mites in the mild winter conditions of southern Louisiana. The researchers observed serious losses (≥50%) of overwintering Italian honey bee colonies. Surviving Italian colonies were too small to be able to build up in spring to sizes that could be divided [22]. In contrast, tracheal mite-resistant Iranian honey bee colonies that were maintained in the same locations suffered only minor mortality and developed into sufficiently large colonies in spring that could be divided. Importantly, their study underlines the importance of genotype in affecting the abundance of tracheal mites in honey bee colonies. Environment does play a role in determining the growth of tracheal mite populations in honey bee colonies. Previous studies showed that colonies of Italian stock (from the same source and bought at the same time), which suffered from a strong growth of tracheal mite populations in Iowa and Louisiana during the summer were not subjected to any growth of tracheal mite populations in Mississippi [24]. Synergistic interaction between *Varroa* and tracheal mites had been reported [25]. These observations suggest that environmental as well as genetic factors affect the abundance of tracheal mites in honey bee colonies.

Tracheal mites, *Acarapis woodi* (Rennie), remain a serious problem for the Savojbolagh region of Alborz province in Iran in the winters of (2013-2015). From what we currently know, *A. mellifera* is the original host of honeybee tracheal mite (H.B.T.M), as this mite has only been recorded on other *Apis* species following the introduction of *A. mellifera* to Asia [73]. The exact causes of the loss of colonies infested with honeybee tracheal mite (H.B.T.M) are still unknown. This problem is exacerbated by the lack of unique symptoms associated with tracheal mite [73]. Complete control is unrealistic but beekeepers practice control methods in order to keep the mite at a low level in colonies. Integrated pest management is recommended, using a variety of methods and good husbandry is the key. *Acaricidal* resistance, especially pyrethroid resistance in some countries, is a serious problem and there is an associated problem of chemical residues in bee products [73]. Breeding more tolerant bee strains and effective methods of biological control could be the way forward. Safe and efficacious control measures would offer relief to infested areas and provide assurance to uninfested regions that this parasite is manageable. Recently the systemic *Acaricide* cymiazole has been found efficacious in controlling the parasitic honey bee mite, *Varroa jacobsoni* Oudemans [31, 32, 67, 71]. *Acarapis woodi* (Rennie), known as the honeybee tracheal mite (H.B.T.M.) reduces the resistance of bees to other parasites and diseases, shortens the life cycle and in heavy infestations can block the tracheae of bees. *Acaricidal* resistance, especially pyrethroid resistance in some countries, is a serious problem and there is an associated problem of chemical residues in bee products.

1.1 Charting the Distribution of Bee Mites

Several papers have been published on the world distribution of honeybee parasites and diseases. The first world maps appeared in 1981 followed by updates [62, 63], which included *Tropilaelaps* [11]. A study by Eischen *et al*. (1988) have charted and updated earlier records tracheal mite (*Acarapis woodi*) [52, 54]. In 1997 the same author produced country records for honeybee diseases, parasites and pests [65]. Records for honeybee mites, especially for species such as *T. clareae*, need continual updating for accuracy, changes in the distribution of the mites as well as for new species and country records [68, 74].

1.2 Some Recent Work in Iran

Workers in Iran have been researching potential control methods for *Varroa* using chemicals, natural substances and the behavioral features of bees. These include studies on different *Acaricides* and their effect in controlling the parasite, including formic acid and Apistan and comparisons between their efficacies [6, 7,]. Surveys of bee colonies in Iran for *A. woodi* demonstrated that the mite was found in 19 of the 139 apiaries sampled [46]. The two external species, *A. externus* and *A. dorsalis* were found in eight provinces of Iran. A study by Ahmadi *et al*. (2014) have revealed that, 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, Isfehan, Lorestan, Kerman and Yazd.

1.3 Honeybee Tracheal Mite (*Acarapis Woodi*)

In addition to *A. woodi*, at least two other species of *Acarapis* (Acarus = mite and Apis = bee) are found on the external surface of bees. These are referred to as *A. dorsalis* Morgenthaler and *A. externus* Morgenthaler but are thought to be harmless [55, 56]. The tracheal mite lives, as the name implies, in the thoracic tracheae of adult bees drones, workers and queens being infected. Smaller numbers occur in the abdominal air sacs. Heavy infestations block the tracheae and thus prevent air reaching the body tissues of the bee. In older bees the mite population declines. The mite can it is found by careful dissection of the thoracic tracheae using a binocular dissecting microscope (Figure 2).

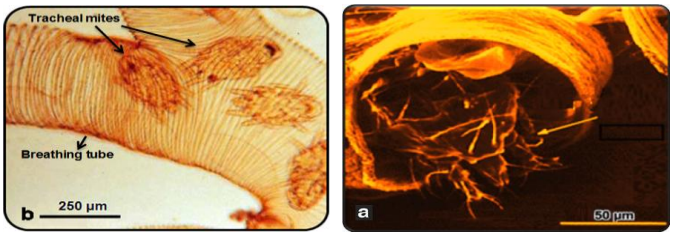

Fig 2: (a). Scanning electron photomicrograph and Immunofluorescence of the thoracic tracheae (*Apis mellifera* meda) and binocular dissecting microscope in the laboratory and (ponceau arrowhead) mark the tracheal mite inside honey bee trachea. (b). Mites are also occasionally found in air sacs in the thorax and abdomen. Within the trachea, the mite reproduces and feeds. Mites penetrate the breathing tube walls with their mouthparts and feed on blood. Scale bars (a) 50 µm, (b) 250 µm.

Tracheae are colourless or pale in uninfected bees. In low infestations patchy discolouration of the tracheae occurs and in heavy infestations brown blotches or even a black appearance of the tracheae may be found. Obstruction of the tracheae by mites and eggs occurs in heavy infestations. Mating takes place in the tracheae. Females produce several large eggs in batches of 5 or more. Eggs hatch (in 3 to 4 days) into larval mites which feed and development to the adult stage takes place, via a non-feeding nymphal stage (referred to as a resting stage), in about 10-15 days depending on the ambient temperature. Young females climb out of the tracheae attaching to the hairs of bees and via this route transfer to other hosts (Figure 3).

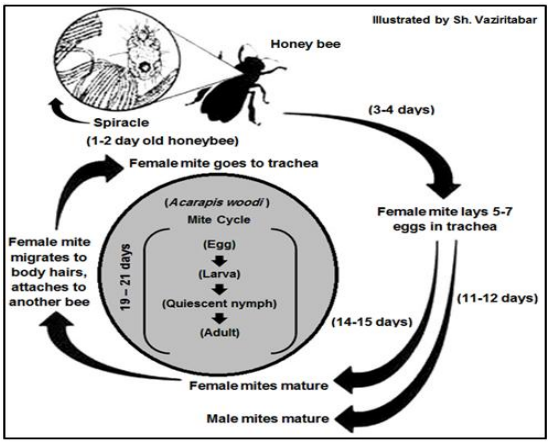

Fig 3: Honeybee tracheal mite (*Acarapis woodi*) life cycle. (Photograph by Jane Wells).

Honeybee tracheal mite (HBTM) can cause diminished brood area, smaller bee populations, looser winter clusters, increased honey consumption, lower honey yields and frequently, colony demise [73]. In temperate regions, mite populations increase during the winter, when bees are confined to the hive in the winter cluster [74]. Heavy mite infestation affects bee metabolism and the ability of colonies to regulate the cluster temperature and chilling may be a significant cause of colony death [74]. *Acariosis* is caused by *Acarapis woodi* (an internal mite). The mite lives in tracheal tubes (mainly in the first thoracic pair). Involvement is only in adult bees and cannot be found in broods and Most of mortality happens in winter and spring [1]. The presence of this mite in large numbers, combined with other diseases and unfavourable conditions, could contribute to a reduction in activity, a shorter life cycle and the early death of a bee colony. *Acarapis woodi* Rennie, known as the honeybee tracheal mite (H.B.T.M.) reduces the resistance of bees to other parasites and diseases, shortens the life cycle and in heavy infestations can block the tracheae of bees (Figure 2, 5). The present study aimed to evaluation of the infestation level and prevalence of bees colonies with *Acarapis woodi* (Rennie) in three sites in Savojbolagh county in the Alborz province and overwintering ability of Iranian honey bee was compared with Turkey honeybee colonies commercially available in the Iran and comparing the colony mortality between colonies headed by OI and SI queens, and also tracheal mite dissection under laboratory. The aim of the present study was to demonstrate additional observations on the honeybee tracheal mite of colonies harboring highest and lowest infestations of *A. woodi* and we evaluated various lines of Iranian honey bees for different characteristics such as mite intensity, bee population, colony weight change and tracheal mite infestations as well as their winter survival. Additionally, we have found it effective in controlling *A. woodi* in laboratory. The discovery and identification of the honeybee tracheal mite (H.B.T.M.) in Europe prompted the USA Congress in 1922 to ban the importation of all bees and intensive sampling occurred. Despite these precautions it was first detected in Texas in the USA in 1984, having probably migrated from South America. In Northwest of Iran the tracheal mite has been regarded with somewhat greater importance and the picture appears rather different to that in Europe, although it is not so serious a pest as was previously thought. The literature states that the mite shortens the life of adult bees (but only by a few days) and heavy infestations can affect their flight. A reduction in bee activity has also been suggested which could lead to reduced honey production. It is possible those Iranian bees are somewhat less resistant to the mite and/or that there are different strains of bees involved.

1.4 Tracheal Mite Life Cycle

Acarine mites (*Acarapis woodi*) live and reproduce in bee's trachea, disrupting respiratory functions and introducing viruses. The tracheal mite lives, as the name implies, in the thoracic tracheae of adult bee drones, workers and queens being infected. Smaller numbers occur in the abdominal air sacs. Heavy infestations block the tracheae and thus prevent air reaching the body tissues of the bee and mating takes place in the tracheae. Females produce several large eggs in batches of 5 or more. Eggs hatch (in 3 to 4 days) into larval mites which feed and development to the adult stage takes place, via a non-feeding nymphal stage (referred to as a resting stage), in about 10-15 days depending on the ambient temperature. Young females climb out of the tracheae attaching to the hairs of bees and via this route transfer to other hosts and briefly very small parasitic mite *Acarapis woodi* attaches inside trachea and feeds on hemolymph of adult bees causes damaged/obstructed trachea and flight muscle atrophy lowers flight efficiency and reduces thermoregulatory ability (Figure 2).

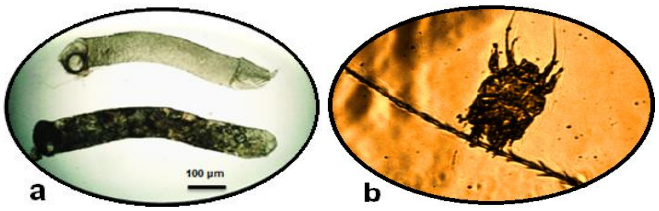

Fig 2: (a). Small parasitic honeybee mite *Acarapis woodi*, attaches inside trachea and feeds on hemolymph of adult bees. Image of the larval mites from (*Acarapis woodi*) view by scanning electron microscopy (SEM) under laboratory condition in Iran. (b).Tracheal mites are microscopic in size (as long as 1.5 times the diameter of a human hair) and spend their entire life cycle within the tracheae (breathing tubes) of adult honey bees. Scale bars (a) 100 µm, (b) 20 µm.

Female mite crawls into spiracle of adult bee < 4 days old female feeds and lays a few eggs in spiracles offspring hatch develop, mate, migrate out of spiracle onto hairs female mites crawl into spiracles of other young bees and mature female *Acarine* mites leave the bee's airway and climb out on a hair of the bee, where they wait until they can transfer to a young bee. Once on the new bee, they will move into the airways and begin laying eggs. (Figure 3, 4). The tracheal mite population may vary seasonally. During the period of maximum bee population, the number of bees with mites is reduced.

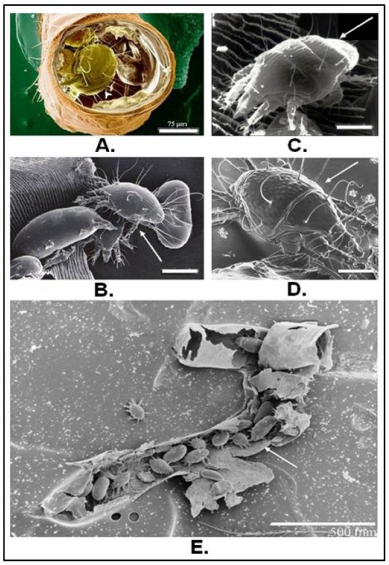

Fig 5: Illustrations are show with low-temperature scanning electron micrograph (LT-SEM) of thoracic tracheae of Iranian honey bee (*Apis mellifera* meda). A. Tracheal mite live in the tracheal tubes in the thorax of adult honey bees (breathing airways) and Adult mites penetrate the tracheal wall with their piercing mouthparts and feed on bee blood. B, C, D images (adult and egg) mites exit the bee trachea and climb to the tip of a body hair. E. White arrowhead marks show (*Acarapis woodi*) locations in the tracheal tubes and honeybee airsac under a microscope. The Obstruction of the tracheae by mites and eggs occurs in heavy infestations and numerous mites in varying stages of development and mite debris inside the tracheae are thought to reduce capacity for airflow. Scale bars A. 75 µm, B. 100 µm, C. 60 µm, D. 65 µm, E. 500 µm. [LT-SEM photos A, B, E of: (Ochoa *et al*. 2005)].

2. Materials and Methods

2.1 Study area

Field and laboratory studies were conducted overwintering honey bee colonies (*A. mellifera* mada) with *Acarapis woodi* (Rennie) infested in Savojbolagh county, Alborz province. A survey of honey bee colonies infested was conducted from winter 2013 to winter 2015 for presence, prevalence and detection of *Acarine* mites; *Acarapis woodi* (Rennie) in Savojbolagh apiaries in the Alborz province (Central district, Chaharbagh district and Chendar district) respectively (Figure 6). Savojbolagh is a county in Alborz province in Iran. The county is subdivided into three districts: the Central district, Chaharbagh district and Chendar district. Fieldwork was conducted in the following three districts in Alborz (Northwest province), located between (35°.50'8″ North latitude and 50°.40'0″ East longitude) in Iran. Iranian bee colonies were obtained from Savojbolagh (Northwest province) for 2 years (starting over the winter of 2013-2015). The average monthly temperatures in Savojbolagh county between 2013 and 2015 was ranged from (-20 to 25 °C) and annual rainfall varied from (1.4 to 86.9 mm).

Fig 6: Savojbolagh apiary in the Alborz province during the winter

2.2 Apiary trial

The overwintering ability of Iranian honey bees was compared with Turkish honeybee colonies commercially available in the Iran. The colonies were divided between three sites in Savojbolagh county in the Alborz province (Central district, Chaharbagh district and Chendar district) respectively (Figure 6). There were 30 Iranian and 27 Turkey colonies in site 1 and 40 Iranian and 25 Turkey colonies in site 2 and 35 Iranian and 20 Turkey colonies in site 3. Of these, 105 Iranian colonies (thirty-five colonies for each site) and 72 Turkey (25 for site 1 and thirty five for site 2 and twelve for site 3) were placed individually onto weighing scales (load cells) to monitor colony weight change from November 2013 to April 2015. For each site, individual scales were connected to a centralized switchboard with 24 channels. Colonies were equalized for the amount of honey available for each colony. In September 2013, all colonies were fed with (equal 1 gallon. of 95%) high sugar syrup. Thereafter, no additional food was provided. In November 2013, each colony was packed for winter by using cardboard winter wrap cartons with Styrofoam on top of the inner cover. An upper entrance hole was provided for each colony. All colonies were treated with antibiotics Fumidil-B and one strip of Apistan to control *Varroa* mites in November 2013. Colony weight change was estimated by subtracting the final weight of each colony recorded on 20 April 2014 from its initial weight on 12 November 2013. Adult bee populations were estimated as described by which visually estimated the percentage of adult bees occupying a comb [17]. In winter (2014–2015), A total of 267 queen-right colonies from the 1500 multistate evaluation of 3 different Iranian queen lines, [2]. In this study, samples were collected from savojbolagh apiaries of three different regions and were used in this study (only some of these lines were retained in the Iranian honey bee breeding program. Others were discarded because they displayed some tracheal mite susceptibility in this and other tests.) In chendar districts, 105 colonies were divided between two sites, two sites were used for 96 colonies in Central districts, and two sites were used for 66 colonies in Chaharbagh districts. Test colonies were either headed by Original Iranian (OI) or Supersedure Iranian (SI) queens. Colonies in Chaharbagh districts and Central districts received no chemical treatment. In Chendar districts, check mite besides (Bayer Corporation) treatment was used in August 2013 because of high *Varroa* mite infestations. These colonies started as highly infested divisions in April 2013 for *Varroa* mite research [42]. Chendar districts colonies were fed with diluted high sugar syrup and then packed using black cardboard boxes as described above. Colonies in Chaharbagh districts and Central districts were not packed for the winter. In Chendar districts, 43 colonies were positioned individually onto load cells to monitor colony weights.

However, colony weight measurements were halted because of apiary inaccessibility caused by heavy snowfall and foul weather. Initial adult bee population was estimated as described which visually estimated the percentage of adult bee population in honeybee colonies [38]. The amount of brood was estimated using the method with TDM (Thoracic disc method) of [21, 68], which visually estimated the percentage of the total comb area covered by capped brood and TDM is a technique developed for detailed assessment of mite infestations. Because of cold weather, cluster size was estimated by counting the number of frames covered by adult bees in March 2015. The numbers of frames with brood and covered by adult bees were recorded in May 2015. At the end of the experiment, all broodless colonies with two frames or less of adult bees with or without queens were considered dead.

2.3. Tracheal mite dissection

A. woodi prevalence (proportion of adult workers infested) and mite intensity (number of mites per infested bee) were determined by dissecting 30 bees per colony subsampled from 400 to 600 bees. Examination of bees was done using thoracic dissection [49]. Infested tracheae were pulled and placed on a glass slide with double-sided tape. The tracheae were then dissected and individual tracheal mites were counted. For winter (2013-2014), tracheal mite infestations were recorded from September to November 2013 before the colonies were packed for the winter and from March to April 2014 when temperature permitted the sampling of colonies. For (2014-2015), sampling was done in August and November 2014 and in March and May 2015. All surviving colonies from Central districts and Chaharbagh districts were sampled at the end of the experiment. In Chendar district, a total of 55 colonies were randomly sampled (8-10 colonies per site) at the end of the experiment.

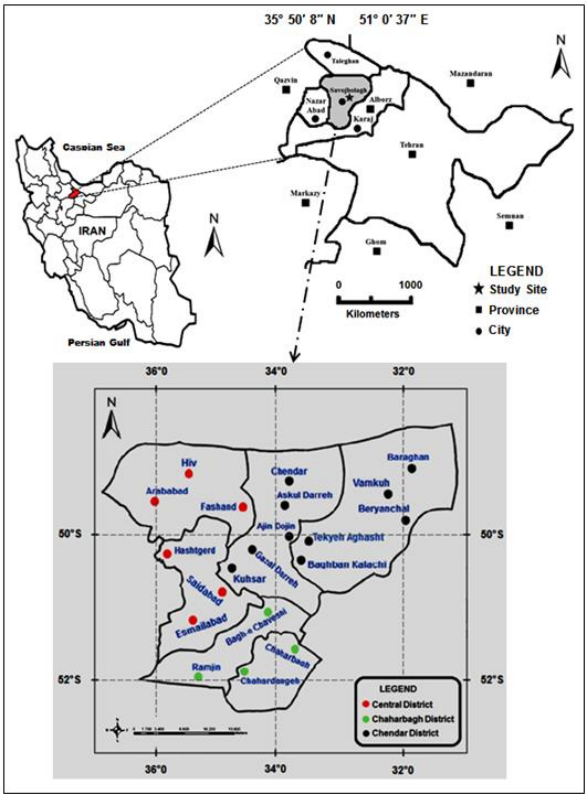

Fig 6: Map of Iran and the study area of Savojbolagh regions in Alborz province showing the sampling sites and distribution areas of *Acarapis* mite into three districts: the Central district, Chaharbagh district and Chendar district.

2.4. Weather data

Weather information was obtained from the weather underground web site (http:// weatherstationalborz.com); the weather station Alborz province for three sites in Savojbolagh county in the Alborz province (Central district, Chaharbagh district and Chendar district) respectively. The average monthly temperatures in Savojbolagh county between 2013 and 2015 was ranged from (-20 to 25 °C) and annual rainfall varied from (1.4 to 86.9 mm).

2.5. Sampling colonies

2.5.1. When to sample

When trying to detect tracheal mites, sampling time is crucial. Infestation by tracheal mites varies through 2013-2015. For detection of honeybee tracheal mite (H.B.T.M) in colonies, bees should be collected in winter or early spring when (H.B.T.M) populations are highest because of the reduced bee brood production. During this time, a high proportion of older, overwintering bees is present in the colonies, and the mites have had a long time to reproduce. The large number of actively feeding mites can cause the tracheae to turn black.

2.5.2. Collecting bee samples

Because honeybee tracheal mite (H.B.T.M) infestations are influenced by the age of bees, the location within the hive from which bees are sampled should be considered. Since queens can be found on honey frames, it is recommended to examine frames of the entire colony to find the queen before taking any samples; this will ensure that the queen will not end up in the sample jar. Collect adult drones for sampling as well, as they tend to have higher mite abundance than worker bees [22, 68].

2.6. Laboratory trial

According recommended method by OIE (Office International Epizooties), a thin disk removed in front of middle pair of the legs and using of potassium hydroxide). A surveillance programme also aids in early detection of honey bee pests and diseases including any new introductions. One pest is the honeybee tracheal mite (H.B.T.M) *Acarapis woodi*, an obligate endoparasite of honey bees [58]. Samples examined in the Department of Entomology Research (honeybee laboratory) and Department of Animal Science Research Varamin - Pishva University. Positive diagnosis can be made only by microscopic examination of honey bee tracheae. In sampling for this mite, one should try to collect either bees that may be crawling near the hive entrance or bees at the entrance as they are leaving or returning to the hive (60 bees per colony sampled in each apiary) and selected colonies from different sites in each Savojbolagh county (Central district, Chaharbagh district and Chendar district). Total samples were randomly selected in different apiaries from each site (Central district, Chaharbagh district and Chendar district) for inclusion in this survey. For the internal mite, *Acarapis woodi*, samples of sixty adult bees/hive were taken from the hive entrance and when available 60 dead bees from the ground in front of the same hive. Sample was stored in separate containers in 75% ethanol. Some samples were also taken from feral colonies of *A. mellifera* meda in mountainous areas. The presence of tracheal mite was determined using the standard thoracic disc assay. For external mites, samples of 60 adults bees/hive were collected in a polyethylene bag and transported in an ice box to our laboratory, where they were refrigerated for further studies. The bees were examined singly under a binocular microscope. Each group of bees was

shaken and washed in 75% ethanol after microscopic examination, and the alcohol was examined for the presence of mite. The alcohol from the other bee samples was also examined for the presence of external mites.

2.7. Laboratory detection: microscopic detection of *Acarapis woodi*

The morphological technique involves examining the prothoracic tracheae under a microscope. Detection of low level infestation by *A. woodi* requires careful microscopic examination of tracheae, whereas when the infestation is heavy, the trachea will turn opaque and discolored and can be noticed without the aid of a microscope. Beekeepers often use unreliable bee stress symptoms, such as dwindling populations, abandoned overwintered hives full of honey, or weak bees crawling on the ground as symptoms of honeybee tracheal mite (H.B.T.M). One method is to pull off the head and collar of a bee and examine the trachea, [71, 73]. Conversely in low infestation levels, tracheae from an individual bee need to be examined. Bees may anesthetize or kill by freezing before examination. Developing a technique to locate the internal mites on individual bees (Figure 8 and 2 (b) for details) [52, 71, 73].

2.7.1. Diagnosis of tracheal mites in the laboratory

Acarapisosis can only be reliably diagnosed by carrying out dissection and microscopic examination of honey bees' primary trachea. The following tools are required for dissection and microscopic examination:

2.7.1.1. The bee is placed under a dissecting microscope, held prone with forceps (across abdomen) and the head and the first pair of legs scraped off by using a scalpel or razor blade.

2.7.1.2. The ring of prothoracic sclerite (collar) removed by using a fine forceps as well.

2.7.1.3. The exposed tracheae of both sides removed after carefully detaching them from the thoracic wall.

2.7.1.4. The tracheae removed and placed on a glass slide and examined under a microscope for mites; this technique is very time-consuming and also has the possibility to lose mites while separating tracheae from the thoracic wall and transferring them to the slide.

3. Data analyses

Analysis of variance ANOVA for repeated measures using evaluated the effects of honey bee type and sampling month on the prevalence and intensity of tracheal mites. A two-way factorial ANOVA was used regarding data on colony weight change (final-initial weight), and bee population with bee type and site modeled as fixed effects and colony within type by site as random effects. Before analyses, data for mite prevalence were transformed using arcsine transformation, and square-root transformation was used to transform data on mite intensity, colony weight change, and numbers of brood and adult frames. Degrees of freedom were estimated using the Kenward-Roger method. Analysis of Variance test (ANOVA) one way for the completely randomized design was used to compare mean of tracheal mite individuals collected from beehive's bottom and those living on worker bees during 12 months in twenty apiaries of three sites in Savojbolagh Region in Alborz province in Iran. In all cases, means were compared using Student-Newman-Keuls Test (LSD) ($p = 0.005$) [16].

4. Results

4.1. Tracheal mite infestation (prevalence)

The proportion of bees infested was monitored from September 2013 to April 2014. No significant interaction between bee type and sampling month ($F = 1.12$, df = 4, 52.6, $P = 0.346$) was detected (Table 1). However, tracheal mite infestations were significantly ($F = 5.88$, df = 4, 38.9, $P = 0.0005$) different among the sampling months. The highest infestation was observed in April 2014, and the lowest infestations were recorded from September to November 2013. The Iranian bees had significantly ($F = 6.3$, df = 1, 58.6 $P = 0.014$) lower infestation than the Turkey colonies. Overall, an increase in infestation **[**(final infestation - initial infestation) / initial infestation**]** was observed in both stocks. However, no differences ($F = 0.01$, df = 1, 48, $P = 0.940$) were found, with the Iranian bees having a 2.6 ± 1.43 fold increase compared with 3.14 ± 1.5 fold increase in the Turkey colonies.

4.2. Mite intensity

No significant interaction between bee type and sampling month ($F = 0.57$, df = 4, 54.8, $P = 0.682$) was detected (Table 1). However, significant ($F = 4.88$, df = 4, 49.7, $P = 0.002$) differences among sampling months were observed with the highest mite intensity recorded in October 2013. The lowest mite load was observed in September 2013. Both stocks had similar ($F = 1.81$, df = 1, 65.6, $P = 0.185$) numbers of mites per infested bee. Likewise, an increase in mite intensity was noted. However, both stocks supported comparable ($F = 0.01$, df = 1.45, $P = 0.819$) fold-increase in mite load with a mean increase of 2.86 ± 1.12 and 3.26 ± 1.20 for the Turkey and Iranian bees, respectively.

4.3. Infestation levels of *Acarapis woodi* (Rennie) living in bee hives

Collecting *Acarapis woodi* (Rennie) individuals during the whole period of experiment 2013-2015 showed that apiaries in Bagheban kalachi significantly recorded the highest level of infestation by an average of 184.7 mite hive. The infestation levels significantly decreased in two sites (Kuhsar and Hiv) followed by Gazal darreh, Saidabad, Chendar, Beryanchal, Fashand, Baraghan, Arababad, Askull darreh, Ajin Dojin, Hashtgerd by an average of 164.6, 164.6, 85.22, 18.3, 15.4, 13.2, 13.2, 11.5, 4, 3, 3 and 2.1 mite hive, respectively. On the other hand, from the earlier results, it was noticed that *Acarapis woodi* (Rennie) infestation level in Bagheban kalachi apiary presented about 14%, while the infestation levels declined in apiaries of Kuhsar, Hiv, Gazal darreh, Saidabad, Chendar, Beryanchal, Fashand, Baraghan, Arababad, Askull darreh, Ajin Dojin, Hashtgerd by 12, 12, 9, 8, 7 and 3% of the total infestation in 2013-2015 (LSD = 0.949, Figure 7).

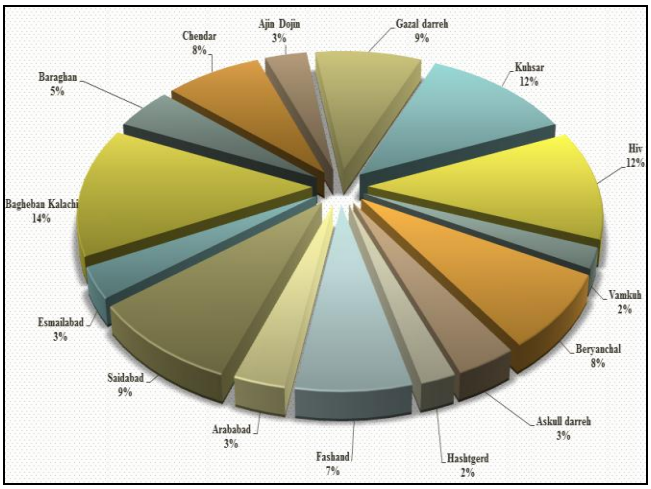

Fig 7: Infestation percentage of *Acarapis woodi* (Rennie) found in bee hives three different sites (Central district, Chaharbagh district and Chendar district) during 2013-2015

4.4. Colony weight change

No significant interaction between bee type and site was observed (F = 0.00, df = 1.36, P = 0.980). Colonies in both sites also showed no significant (F = 2.35,df = 1.36, P = 0.144) differences in weight change, with an average loss of 5.01 ± 0.34 and 5.68 ± 0.33 kg for colonies located in sites 1 and 2, respectively. However, significant (F = 38.18, df = 1.36, P < 0.0001) differences between stocks were detected. After twenty one week, the Turkey colonies lost equal 6.82 ± 0.34 kg, whereas the Iranian colonies lost only an average of 3.89 ± 0.33 kg.

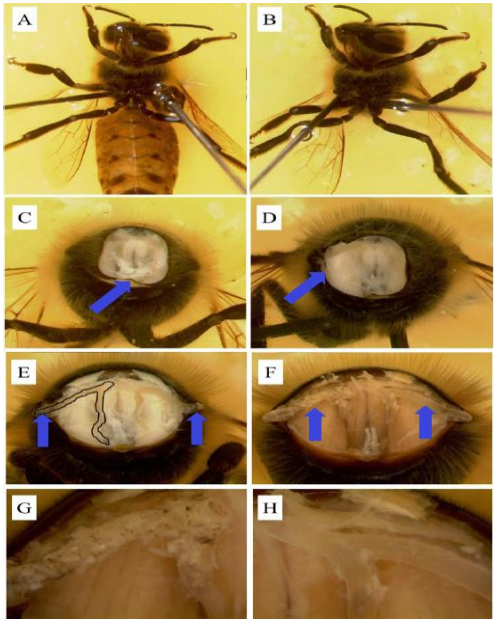

Fig 8: Dissecting a bee to determine tracheal mite infestation. A. Pin a worker bee through the thorax using two insect pins (the bee's body will pivot in the dish if pinned with only 1 pin). The bee in this figure is pinned into a petri dish of hardened beeswax. The bee is covered in 70% ethanol to facilitate dissection. B. Remove the abdomen. This is a helpful technique to limit contents from the bee's digestive systems from emptying into the field of view. C. Remove the head and front pair of legs. The "collar" junction is arrowed. D. The beginning of collar removal. The area where the collar has been removed is arrowed. E. The collar has been removed, including the part that covers the spiracles (Blue arrowhead). The bee's right trachea (the one on the left in the figure) is outlined to show shape and position. F. Trachea infested with mites (on left, blue arrowhead) and not infested with mites (on right, arrowed). G. Close-up of infested trachea and H. close-up of uninfested trachea. (Photos of: Lyle Buss and Tricia Toth, University of Florida, USA).

Table 1: Mean infestation levels (mean ± SE) of tracheal mites in Turkey and Iranian honey bee colonies during winter (2013-2014) in Chendar districts

Date	Honey bee type		Mean
	Domestic	Iranian	
Prevalence			
September, 2013	25.37 ± 3.54	8.68 ± 4.90	15.44 ± 2.79^{c}
October, 2013	24.05 ± 3.35	9.40 ± 3.56	15.44 ± 2.52^{c}
November, 2013	23.38 ± 3.67	10.4 ± 4.21	18.10 ± 2.48^{c}
March, 2014	27.88 ± 4.44	12.34 ± 4.98	20.62 ± 3.53^{b}
April, 2014	36.55 ± 5.36	15.07 ± 6.14	26.44 ± 3.61^{a}
Mean	27.63 ± 5.0^{a}	11.31 ± 4.45^{b}	-
Intensity			
September, 2013	11.33 ± 1.35	7.33 ± 1.53	9.71 ± 1.04^{c}
October, 2013	17.49 ± 2.93	16.31 ± 2.43	16.76 ± 1.79^{a}
November, 2013	16.65 ± 2.23	10.76 ± 2.33	13.49 ± 1.50^{b}
March, 2014	11.75 ± 1.66	10.21 ± 1.37	11.41 ± 1.76^{b}
April, 2014	12.99 ± 1.56	10.90 ± 1.62	12.41 ± 1.76^{b}
Mean ^{a}	14.12 ± 1.29	10.85 ± 1.52	-

Numbers within a column and row followed by the same letters are not significantly different (P > 0.05). ^{a} Not significant (P > 0.05).

4.5. Bee population dynamics

Analysis on the number of adult bees in September 2013, showed no significant interaction between stock and site (F = 0.53, df = 1, 33, P = 0.458). No site effect (F = 0.60, df = 1.33, P = 0.430) was detected, with a mean of 14,363 ± 1,246 and 15,389 ± 944 bees for sites 1 and 2, respectively. However, the Turkey bee colonies (18,105 ± 747 bees) were significantly (F = 13.64, df = 1.33, P = 0.0008) bigger than the Iranian bee colonies (11,088 ± 673 bees) at the beginning of the experiment. In April 2014, no interaction between stock and site (F = 0.01, df = 1.30, P = 0.945) was observed regarding the number of adult bees in the colonies. Likewise, colonies in site 1 (6,453 ± 510 bees) had similar (F = 0.00, df = 1.30, P = 0.965) numbers of bees as those colonies located in site 2 (6,258 ± 456 bees). Both stocks had comparable (F = 0.24, df = 1.30, P = 0.628) numbers of adult bees with the Turkey bees having a mean of 6,946 ± 476 bees and the Iranian colonies an average of 5,405 ± 465 bees. Despite having equal colony strength at the end of the experiment, the Turkey colonies significantly (t-test, df = 32, P < 0.0001) lost equal 66% of their initial population compared with 45% in the Iranian colonies.

4.6. Winter mortality in area

Colony mortality was very similar (Fisher's exact test, n = 66, P = 0.363) between the two stocks. Only five colonies (two Iranian [8%] and six Turkey [18%] colonies) died between March and April 2014. Tracheal mite infestations of these colonies before their death were very high (one Iranian, 43%; four Turkey, 60, 88, 88, and 100%). Approximately 50% (14 of 29) of the surviving Turkey colonies had > 25% (20-63%) tracheal mite infestations, whereas only 19% (five of 27) of the Iranian colonies had high (27-60%) levels of infestation.

4.6.1. Winter (2014–2015) in the study areas

Winter was unusually cold and long during (2014-2015). Heavy snow started falling early (November 2014) and frequent snowstorms occurred for equal 4 month in chendar districts (Figure 9).

4.6.1.1. Chendar region Situation

Hundered-five colonies 57 Original Iranian (OI) and 48 Supersedure Iranian (SI) were evaluated throughout the winter. No significant interaction between site and stock was detected for both the number of adult bees (F = 0.00, df = 1. 50, P = 0.966) and amount of brood (F = 1.02, df = 1.50, P = 0.336) in August 2014. Initially, OI and SI colonies had similar (F = 0.00, df = 1.50, P = 0.998) adult bee population with a mean of 33,641 ± 1,450 and 26,407 ± 2,541 bees, respectively. The amount of brood for OI (45,570 ± 2,451 cells) and SI (39,414 ± 3,971 cells) bees was also similar (F = 3.22, df = 1.50, P = 0.083) during this month. Likewise, no significant differences between the two sites were detected for both the number of bees (F = 0.00, df = 1.50, P = 0.9995) and the amount of brood (F = 0.08, df = 1.50, P = 0.768) in August 2014. Colonies in site 1 had an average of 29,053 ± 1,595 bees and 42,637 ± 3174 brood cells, whereas site 2 had 31,083 ± 2,287 bees and 42,252 ± 3,444 brood cells. In March 2015, the analysis on cluster size also showed no interaction between site and stock (F = 2.35, df = 1.57, P = 0.125). No stock differences (F = 0.18, df = 1.57, P = 0.666) were detected, with an average of 4.3 ± 0.26 and 4.6 ± 0.39 frames for OI and SI, respectively. However, there were significant (F = 41.61, df = 1.57, P < 0.0001) differences between the two sites, with site 2 colonies (5.99 ± 0.33 frames) having bigger cluster sizes than those colonies located in site 1 (2.94 ± 0.53 frames). At the end of the experiment in May 2015, no interaction between site and stock (F = 1.36, df = 1.42, P = 0.25) and no site effect (F = 0.73, df = 1.42, P = 0.389) were detected regarding the number of brood frames. However, SI colonies had significantly (F = 4.52, df = 1.42, P = 0.04) more frames of brood (5.52 ± 0.46 frames) than OI (4.24 ± 0.37 frames) colonies. For the number of frames covered by adult bees, no site by stock interaction (F = 0.24, df = 1.42, P = 0.625) was detected as well as no site (F = 1.51, df = 1.42, P = 0.229) and stock (F = 1.90, df = 1.42, P = 0.172) effects. OI and SI bees had similar number of frames covered by adult bees with an average of 6.64 ± 0.56 and 8.06 ± 0.49 frames, respectively. For the mite prevalence, our results showed no interaction between honey bee type and sampling month (F = 0.07, df = 3.52.9, P = 0.974) (Table 2). However, significant (F = 6.20, df = 3, 52.9, P = 0.001) differences among the sampling months were detected, with a peak infestation observed in March 2015. The lowest infestations were observed in August and November 2014 and May 2015. OI and SI colonies showed similar (F = 0.01, df = 1.56.7, P = 0.919) infestations of tracheal mites. A similar trend was observed with the number of tracheal mites per infested bee (mite intensity) (Table 2). There was no stock by sampling month interaction (F = 0.28, df = 3, 51.6, P = 0.844) and no stock (F = 0.23, df = 1, 58.1, P = 0.637) effect was observed. However, significant (F = 6.24, df = 3, 54.5, P = 0.001) differences among sampling months were recorded. The highest mite intensity was observed in August 2014 and May 2015, whereas the lowest mite load was observed in November 2014, but it was comparable with the mite load observed in March 2015. There were no differences (Fisher's exact test, n = 105, P = 1.0) in colony mortality between colonies headed by OI and SI queens. A total of 48 colonies (36 OI and 12 SI) died between November 2014 and May 2015 (Table 3). Among the 48 dead colonies, 20 colonies were mite-free, 14 colonies had 6-20% tracheal mite infestations, and 14 colonies had infestations >45% when the colonies were packed in November 2014. By March 2015, all of the 73 OI colonies were still alive, although 28 colonies had infestations ranging from 35 to 83%. Three of these highly infested OI colonies died between March and April with infestations of 65, 88, and 90%, although they had adequate food stores.

Table 2: Mean infestation levels (mean ± SE) of tracheal mites in colonies of Iranian honey bees located in Chendar districts and Central districts during winter (2014-2015)

Date	Honey bee type		Mean
	Original Iranian	Supersedure Iranian	
Prevalence			
August, 2014	6.73 ± 2.15	7.94 ± 2.67	7.23 ± 1.08[b]
November, 2014	6.86 ± 2.44	7.81 ± 3.50	8.30 ± 1.78[b]
March, 2015	15.45 ± 3.44	11.67 ± 5.35	12.58 ± 2.73[a]
May, 2015	4.65 ± 1.41	6.19 ± 2.14	5.87 ± 1.27[b]
Mean [a]	8.60 ± 2.3	8.52 ± 2.94	-
Intensity			
August, 2014	7.59 ± 1.79	12.53 ± 2.68	8.87 ± 1.16[c]
November, 2014	3.60 ± 0.85	4.96 ± 1.35	13.27 ± 1.63[b]
March, 2015	7.01 ± 1.21	6.54 ± 1.66	11.08 ± 1.26[b]
May, 2015	10.48 ± 2.38	9.50 ± 3.48	12.37 ± 1.24[b]
Mean [a]	7.10 ± 0.86	8.20 ± 1.43	-

Numbers within a column and row followed by the same letters are not significantly different (P > 0.05). [a] Not significant (P > 0.05).

Thirty two SI colonies also were alive in March; ten had 28-48% infestation and 18 colonies had 0-27%. fourteen of the thirteen SI colonies that died between November 2014 and May 2015 had low infestations (0-10%), and one colony had 43% infestation. At the end of the experiment, 93% (28 of 30) of the surviving OI and 82% (10 of 15) of the SI colonies had 0- 10% mite infestations. Only seven OI colonies (27 and 30%) and three SI (30 and 34%) had infestations above the economic thresholds. Between the two sites, there were significantly (Fisher's exact test, n = 105, P = 0.025) more (61 versus 18% colonies) dead colonies in site 1 than in site 2. Sixty-five colonies survived the winter. In May 2015, equal 68 divisions were made from all the surviving colonies, including eight colonies of unknown origin (queens were not seen either at the beginning or at the end of the experiment but known to be of Iranian origin).

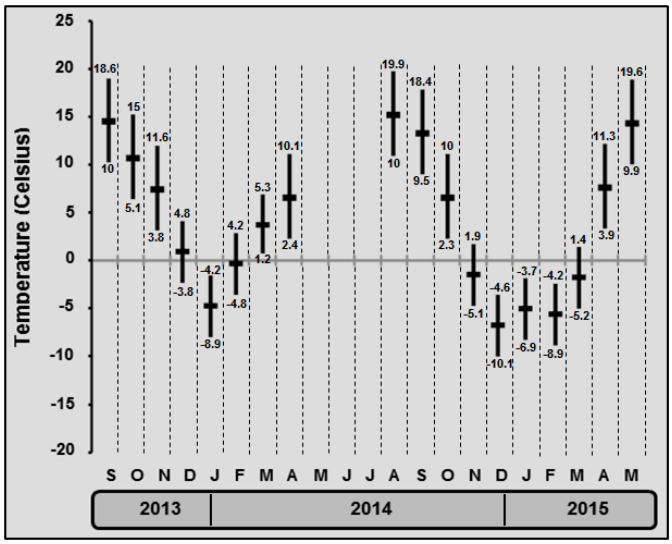

Fig 9: Monthly maximum, mean, and minimum temperatures (°C) for chendar districts in the Alborz province in Iran from (September 2013 to May 2015).

4.6.1.2. Central region situation

Among the 96 colonies at the beginning of winter, 88 were headed by OI and eight by SI queens (Table 3). Initially, tracheal mite infestations were very low (equal 1% on average). Only four of 25 colonies (three OI and one SI) sampled were infested with 10-13% at the end of the experiment. All colonies were alive in February 2015. However, in late March, one OI colony was queen less. This was the only colony death. Approximately 143 divisions (three deep brood frames, three frames of honey, enough adult bees to cover at least five frames and four empty frames) were made in March. Some of the divisions/packages also were made from 14 colonies with queens (of Iranian origin), which were not seen either at the beginning or at the end of the experiment. The lowest temperature of -3.8°C was recorded in December 2014.

4.6.1.3. Chaharbagh region situation

Among the 66 colonies that survived the winter, 38 were headed by OI and 28 by SI queens (Table 3). All colonies were nearly mite-free throughout the experiment. At the end of the experiment, only two OI colonies (queenless with laying workers) was infested with tracheal mites at 4%. No differences (Fisher's exact test, $n = 66$, $P = 0.204$) in colony mortality between Original Iranian (OI) and Supersedure Iranian (SI) were detected. Of the 66 colonies, nine colonies (13%) died; seven OI colonies and two queenless SI colony. Of the seven OI colonies, two colony had the lid blown off, and five colonies had either a virgin queen or were queenless and were considered dead because they were too weak to receive new queens. There were 100 divisions made, which consisted of three deep frames of brood, four frames of food, enough bees to cover five frames, and four empty frames.

Table 3: Numbers of colony survivors and divisions made from colonies overwintered in different locations in Savojbolagh regions during winter (2014-2015)

Location Districts		Original Iranian	Supersedure Iranian	Total	No. divisions [a]
Chendar	Initial	73	32	105	88
	Final	36	12	48	
	Dead [b]	36 (32%)	14 (32%)	50 (32%)	
Central	Initial	88	8	96	143
	Final	58	8	66	
	Dead	2 (4%)	1(3%)	2 (4%)	
Charbagh	Initial	38	28	66	100
	Final	25	25	50	
	Dead [b]	7 (15%)	2 (4%)	9 (13%)	

[a] Includes divisions from colonies headed by unidentified queens of Iranian origin. [b] Not significant ($P > 0.05$).

5. Infestation level of *Acarapis woodi* found during 12 months (in different apiaries)

Data presented in (Figure 10) showed that population of *Acarapis woodi* (Rennie) was high in winter time and gradually increased from March and May with an average of 18.32 and 22.2 mite bee (presented 13 and 7% of the total annual number of *Acarapis woodi* (Rennie) mite, respectively. In July, the average number of mites parasitizing reached its peak of an average of 23.5 mite hive which is almost 13% of the total infestation (Figure 9). Reduction in infestastion level was significant in later months (from August till October).

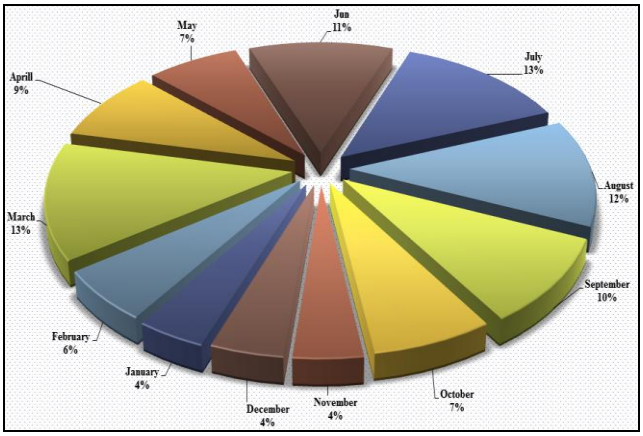

Fig 10: Infestation percentage of *Acarapis woodi* (Rennie) found during 12 months in three sites in Savojbolagh region 2013-2015

6. Discussion

Substantial differences in overwintering characteristics between Iranian and Turkey honey bees were observed in (2013-2015), despite that season's mild winter. One important difference was that Iranian colonies lost less weight. Colonies in both sites also showed no significant ($F = 2.35$,df = 1.36, $P = 0.144$) differences in weight change, with an average loss of (5.01 ± 0.34 and 5.68 ± 0.33) kg for colonies located in sites 1 and 2, respectively. However, significant ($F = 38.18$, df = 1.36, $P < 0.0001$) differences between stocks were detected. After twenty one week, the Turkey colonies lost equal 6.82 ± 0.34 kg, whereas the Iranian colonies lost only an average of (3.89 ± 0.33) kg. This observation corroborates observations of determination of prevalence rate of Iranian honey bees (*Acarapis woodi*) [1]. Additionally, we have found it effective in controlling *A. woodi* in laboratory. Another factor that contributes to successful transfer of parasites among colonies is the aggressive protection of stored food reserves in many social insects however, although differential food consumption contributed to the differential weight loss by the Iranian and the Turkey colonies, the differential loss of adult bees also played a role. The Turkey colonies started as significantly bigger (16,000 versus 10,000 bees) colonies, but the two stocks had similar strength at the end of the experiment. The Turkey colonies lost equal 56% of their adult bees, whereas Iranian colonies lost equal 48%. Hence, Turkey colonies lost more weight in part because they lost more adult bees. This worker bee loss was at least partially because of tracheal mite infestations. Turkey colonies had infestation rates that were above the economic threshold [34, 64] throughout the winter and rose as winter proceeded. Because infested bees die sooner than uninfested bees [9, 14, 15, 32, 49], it is clear that tracheal mite infestations led to a disproportional early death of worker bees in the Turkey colonies, even during unusually mild winter. These observations suggest that greater loss of Turkey 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 these Turkey bees. Colonies with high tracheal mite infestations generally are expected to die during

winter. The thirty colonies that die in the different sites (2013-2014) study were all highly (35-68%) infested. Fifty-two percent of the surviving Turkey colonies had infestations of >52%. These results are generally in consistent with previous studies where tracheal mite infestations in Iowa, Louisiana, and Mississippi were always higher in Italian colonies compared with Russian colonies [18, 19, 20]. The survival of these colonies can be attributed to the mildness of the winter because cold temperature stress accelerates colony deaths because of tracheal mites [51]. Various chemical and *Acaricides* have been used extensively against *Varroa* in colonies of the honey bee, *A. mellifera*, since September 1984, in Iran [47, 48]. Despite eight years of heavy usage of various *Acaricides* and other chemicals for *Varroa* control and the negative results by Ghasemi and Tirgari (1991) through three years of survey in the Iran, this work revealed the presence and infestation of honey bee colonies by *A. woodi* (R.) in Iran.

7. Conclusion

Tracheal mite infestation can be fatal to a honey bee colony preparing to overwinter in temperate climates, since overwintering requires brood production to cease and workers to extend their longevity. Colony survival was excellent in Central districts and Chaharbagh district. Some colony deaths in Chendar districts were clearly the result of tracheal mite infestations in lines that proved susceptible to tracheal mites in harsh conditions and also because of starvation (isolation of clusters from food supply). However, most lines displayed good tracheal mite resistance. Hence, in this study the same lines gave somewhat different results in the three districts in Savojbolagh County in the Alborz province. In Central districts, no colonies were troubled by tracheal mites although mites were present at very low levels in the colonies. In Chaharbagh districts, once again the majority of colonies had no tracheal mites as observed in previous studies [46]. Only colonies that were severely stressed for other reasons had low infestations. In Chendar districts, tracheal mite pressure was the greatest. Chendar conditions were necessary to provide a good evaluation of Iranian honey bee tracheal mite resistance. However, the mild winters of Central districts are sufficiently cold to have resulted in tracheal mites killing ≥50% of highly susceptible colonies [1]. Our results also showed higher mortality (10 versus 36% colonies) of colonies located in the more exposed apiary than the apiary located on top of a slope and surrounded by polystyrene in Chendar districts. Hence, it is important to use apiaries that are protected from chilly winds when overwintering colonies, especially under northern weather conditions such as cold snap, blizzard and cold weather. These events left most sectors of Northwest 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 Turkey 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 these Turkey bees. Colonies with high tracheal mite infestations generally are expected to die during winter. It is necessary that researches in order to prevent the transmission and spread of the parasite in our country be performed.

8. Acknowledgement

We thank all the beekeepers who contributed to this study and also for providing the hives infested with tracheal mites, *Acarapis woodi* (Rennie). I also thank the beekeepers who donated their bee colonies to be used for the experiments. I wish to thank S.M. Esmaeilzade, for assisted with data collection and endless help during field work and the three anonymous reviewers for the improvement of the manuscript. We would like to thank Department of Entomology Research (honeybee laboratory) and Department of Animal Science Research Varamin - Pishva University for their cooperation and excellent facilitation during study period and we are also grateful to two anonymous reviewers for their insightful comments.

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