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Infestation levels of the mite (*Varroa destructor*) between two honeybee races *Apis mellifera meda* (Indigenous) and *Apis mellifera carnica* (Imported) in Savojbolagh regions of Alborz province in Iran

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Abstract
The present study was aimed to evaluate the infestation level of two honey bee races *A. mellifera meda* and *A. mellifera carnica* with *Varroa destructor* and comparing the colony mortality in Savojbolagh regions of Alborz province in Iran in 2016. Estimation of different mites populations was based on daily mites downfall and the mites on adults and brood itself. The results of the present study was based on adult bees and brooded showed high mites development in *A. mellifera meda* bees where the population increased to 109.05, 48.5, 124.3 and 91.85 folds where as in carnica colonies the increase was very low 25.59, 24.89, 51.84 and 33.36 during their respective months of inspection. Based on daily mites downfall the population estimates revealed 9.4, 12.1, 68.4 and 42.35 fold increase in Indigenous colonies where as in carnica colonies (Imported) the mites population was increased to 5.24, 7.3, 38.14 and 26.5 fold folds.

Keywords: Infestation level, V. destructor, *A. mellifera meda*, *A. mellifera carnica*, Iran

1. Introduction
Presently, the beekeepers rear both the indigenous bee (*A. mellifera meda*) and Carniolan bee (*A. mellifera carnica*) [74]. One of the major problems, facing beekeeping industry in Iran is the infestation of honeybee colonies with parasitic mite *Varroa destructor* [74]. The initial observations have indicated that indigenous bee race (*A. mellifera meda*) is less susceptible to Varroa mites as compared to the imported bee (*A. mellifera carnica*) and this quality may be attributed to its more efficient defensive behavior [74]. Unfortunately, few literatures have reported the presence of this dreaded honey bee parasites such as *Varroa destructor* in Savojbolagh regions of Alborz province in Iran [75]. In Sub-district in the Alborz province, beekeeping activities have long been plagued with many problems such as honeybee diseases (Varroa mites), Fluctuations in the price of inputs and lack of effective drug, low honey yield, frequent bee swarming and honeybee mortality due to herbicides and spraying, extreme temperatures and drought [76]. In the past few years in Iran, the use of chemical pesticides to control varroa mite has led to parasite's resistance and contamination of hive products [43]. The frequency of damaged *Varroa destructor* Anderson and Trueman (Mesostigmata: Varroidae) found on the bottom board of hives of the honey bee, *A. mellifera L.* (Hymenoptera: Apidae) has been used as an indicator of the degree of tolerance or resistance of honey bee colonies against mites [1]. Relatively harmless on its natural host, the Eastern honeybee *Apis cerana*, the varroa mite has recently crossed onto the Western honeybee *Apis mellifera* and spread from its Asian origins throughout most of the world [8]. The spread of *Varroa destructor* from infested to uninfested colonies is accomplished by migration of female mites on foragers to another colony [33] and robbing among colonies [63]. Swarming, or colony reproduction, is also a possibility for the mite to spread. The mite migrates on bees in a swarm that will establish the new colony [25, 44]. The mite later switched host to the western honeybee *Apis mellifera* and now has become a serious pest of that bee wordwide [2, 50, 64]. There are several factors that contribute to population growth rate of *Varroa destructor* such as duration of brood rearing season, presence of drone brood, host specific effects of the bee [12, 39, 53], geographic and climatic factors [17, 52] and possibly varroa genotypes [20, 32]

In temperate climates the average increase in mites population is about 10-fold per year [28, 37, 61] while it increase up to 100-fold within one summer [27]. In tropical climate the parasites seems to be less virulent [61] whereas in sub-tropical climates the infestation rate is lower than in temperate climates [52]. In Yemen, in *A. mellifera yemenitica* found different rate of varroa infestation from one place to another. Thus the increasing mites population can wipe out the colonies in three to four years if not controlled [60]. Lange and Natskii 1990 [40], determined the varroa infestation level by using method of counting the mites fall down on the hive floor during winter and flying season and reported maximum mites' mortality in spring and autumn. By using the same method [26] investigated that the number of brood cells decreased in population with the end of spring flowering season when the average number of fallen mites increased from 0.4 to about 15 mites/day in two weeks. The number of fallen mites in late July and in August was 132.7 and 50.3 mites/day respectively. Romaniuk (1983) [63], found that the number of live *Varroa destructor* females per 100 bees was low in the period from April to July (3.2: 7.7) respectively. This number increased to 15.9 and 23 in August and September respectively. The number of female per 100 worker larvae was 7.5 in May, 8.1 in Jun, 14.9 in July, 29.7 in August and 134.7 in September. Rademacher (1985) [56], found the natural death rate of mites varies with season; it increases slowly from May to July, reaches at peak in September and then falls in October. He also added that there are considerable variations between different years and locations. A large proportion of non-reproduction females were observed in autumn. Such variations in reproduction levels could produce differential growth rhythms in mite population during different seasons. Detection of mites' attack in a colony at initial stage is imperative for effective control of *Varroa destructor* [17, 60]. For low infestation levels (below ten mites) the use of acaricide may be the only effective method for detecting the mites with acceptable level of precision in broodless colonies [60]. If mites population is between 10 and 100, then the examination of hive debris should allow for detection [61]. A study by Fries *et al*. 1991 [27], compared different diagnostic methods for detection of varroa mite at allow infestation levels. They concluded that debris was more effective than examining the brood itself for low infestation rates. Liebig *et al.* [42], reported a close correlation between mites collected in hive debris and the size of the varroa mite population. Most migration of varroa into colonies occurs in the late summer and fall [23, 66]. This was determined by measuring mite drop on sticky boards in colonies established with few or no mites [22, 23, 38, 66].

The life cycle of V. destructor is spent inside sealed brood cells, feeding on the developing bee's larvae, with a preference for drone pupae at the edge of the brood nest before they come out to be transferred to another host as the honeybees emerge [65]. *V. destructor* has spread at remarkable speed throughout most of the world by now. Other factors might contribute to the growth of varroa populations including mite migration into colonies on foragers from other hives. The V. destructor, **(**Anderson and Trueman) is a native parasite of the Asian honeybee and was originally found only in Asia [19, 16, 24, 57, 11]. The mite, *V. destructor,* is one of the most destructive pests of the honeybee, *A. mellifera mellifera* in the United States, first reported in North America in 1987 [11, 19] in United Kingdom (UK) in 1992. The first incidence of the presence of *Varroa destructor* was reported in Iran in 1984 [46].

The objective of the present study was to compare between two different Honey bee races in the degree of infestation which may indicate the bee race genetic influence on the colony infestation and comparing the colony mortality in Savojbolagh regions in Alborz province.

2. Materials and Methods

We used method of sampling is the examination of hive debris for mites on the bottom boards Tewarson *et al*. 1992 [72]. A sticky adhesive is applied to the cardboard to prevent living mites from leaving the debris and reconnecting to a passing bee. These "sticky boards" method can be enhanced by adding acaricides to the colonies to increase the number of mites that fall down from the bees Devlin (2001) [16]. Dropped mites were recorded daily as a method of predicting the mite population. Naturally felled down varroa mites were recorded for 6 Months using IPM sticky board with 200 colonies in three sites in Savojbolagh county in the Alborz province (Central district, Chaharbagh district and Chendar district) respectively (Fig.4). The number of dead/ fallen mites on the thick white paper sheet at bottom of the hives was used to determine the mite mortality.

The aim of present study was to investigate the population growth of *Varroa destructor* in *A. mellifera meda* (Indigenous) and *A. mellifera carnica* (Imported) under Savojbolagh regions condition in Alborz province in Iran and comparing the colony mortality between colonies Iranian honey bees (*A. mellifera meda*) and Western honey bee (*A. mellifera carnica*) for *Varroa destructor* (Acari: Varroidae) population estimation in Savojbolagh apiaries in the Alborz province. This study was conducted on 20 apiaries and 10 hive from each apiary. Apiaries were chosen from 3 different Savojbolagh regions of Alborz province. 200 honeybee colonies, 100 *A. mellifera meda* (Indigenous) and 100 *A. mellifera carnica* (hybrid bee race) were used in the research program which was carried out in the Department of Entomology Research and Department of Animal Science, Islamic Azad University Varamin, Pishva Branch, Tehran, Iran. Assessment of parasitic mites *Varroa destructor* (Acari: Varroidae) infesting the colonies of Iranian honeybees (*A. mellifera meda*) and Western honey bee (*A. mellifera carnica*) was counducted in 3 districts of Savojbolagh regions condition in Alborz province in Iran from 15th June, 2016 - 15th September, 2016 and Field work was started in the end of February 2016 at Savojbolagh apiaries. The objective of the present study was to compare between two different Honey bee races in the degree of infestation which may indicate the bee race genetic influence on the colony infestation.

The Carnica race honey bee (hybrid bee race) was imported from Germany the past few years whereas the *A. mellifera meda* is indigenous honey bee race in Iran. In the end of February the indigenous bees were transferred from log hives to modern hives where the frames were provided with one-inch foundation strip to give them the chance to build the comb with small sells. After transfer, the bees were treated with perizin drug to make sure that the colonies were free of the mites. Then the bees were left till 15-April to get adaptation and were given intensive feeding during that period. The mites were removed from adult bees (host) by shaking the bees' combs in a polyethylene bag and after filling the bag with varroatester mite Co_2 spender and then bees were shacked to separate the mites from the bees after anesthetize them with Co_2 gas after 10-20 seconds shake the varroa counter for 10 seconds and they were shaken several times until mites were separated. They were then removed from the

cylinders and placed on a piece of white paper (Fig. 1). The varroa mites are also affected by the anesthetic and will fall off the bees. So they are easy to count and its easy to evaluate the number of mites in hives. Then the mites were collected in small tubes and thereafter, 20 mites were introduced directly upon the bees in each colony on 15-April, 2015. The sticky boards were examined from each hive after 2 hours and 24-hours after inoculation to record the number of mites falls down. After 2-hours in Carnica colonies an average of 8 mites fall down and 10 mites in Indigenous colonies. After 24-hours the average number of mites fall down was recorded 12 mites in Carnica and 10 mites in Indigenous colonies. Then after 24-hours more mites were added to make mites population upto 20. In first two weeks after inoculation an average of 10 mites fall down on sticky board so we considered 15 mites instead of 20 mites.

Fig 1: Varroatester mite Co_2 spender used for separate the mites from the bees after anesthetize bees with Co_2 gas after 10-20 seconds and Co_2 dosing arrangement with 2×16 gram co_2 bottles (enough for up to 12 tests and 200 bees from the brood frame).

The groups were separated in different locations and robbing screens were installed on the colonies to prevent robbing and drifting [35]. Colonies were provided with sugar and pollen during the study period. The mite population was estimated using bee and brood samples and hive debris. Between 400 and 500 live adult bees were taken from brood combs and stored in a deep freezer until examination. The mites were separated from the bees by vigorously shaking the bees in 70% ethanol for 5 to 6 minutes and this was repeated 3-times. The mites were washed from the bees using a hand shower over a double wire screen. Number of bees and mites in a sample were counted to determine the level of infestation on adult bees. Data were adjusted to number of mites/200 colonies.

Samples of 400-sealed worker brood cells were examined as we could not find the drone brood because a few cells were scattered in the colony while others were opened so we only used worker brood cells, with a month interval. The cells were opened and the number of adult females in the cells and on the bees was counted. The total amount of brood in the colonies was estimated in each colony using the double-sampling technique described by Rogers *et al*. 1993[63]. The adult bee population was estimated as described by Burgett and Burkikam 1985[13]. The collection of the samples was started 60-day after inoculation to give a chance to mite population for infestation levels of the *Varroa destructor* mite. To reproduce, the parent varroa enter the brood cell of the honey bee right before the cell is capped (Fig. 2). Once the cell is capped, the parent varroa lays eggs and pierces the developing bee brood, leaving a wound open in it.

Fig 2: Varroa infested brood cell of the honey bee before the cell is capped.

2.1 Different methods for evaluation of *Varroa destructor* in honeybee colonies

A sticky adhesive was applied to the cardboard to prevent living mites from leaving the debris and reconnecting to a passing bee. These "sticky boards" can be enhanced by adding acaricides to the colonies to increase the number of mites that fall down from the bees [16]. Most migration of varroa into colonies occurs in the late summer and fall [23, 67]. This was determined by measuring mite drop on sticky boards in colonies established with few or no mites [22, 23, 38, 67]. The mites on the sticky boards were assumed to have entered the hive on foragers from other colonies. We have chosen "Sticky board" method because this technique is very friendly for bees and the while the other techniques is recommended by OIE (Organization for Animal Health). Biotechnical methods involve beekeeping management techniques specifically designed to reduce mite levels in a colony. The "Sticky board" method was found better than adult bees and brood samples for the detection of mite population at low infestation level and also recommends "Sticky board" method for bee sample examination.

2.2 "Sticky Board" test

Probably the best sampling method involves inserting a sticky board at the bottom of the hive (Fig. 3). Mites are constantly falling off and climbing back onto bees, and this method will sample those mites. The method is fairly easy because it can be done without even opening the hive. But it involves two trips to the apiary, and you first need to make a sticky board. "Sticky board" into the bottom of the hive and leave it for 24-48 hours to allow mites to fall through the mesh onto the contact paper. Then, remove the whole thing and count the number of mites that fell off the bees onto the sticky contact paper (brown hive in plate A).

The hive debris was collected weekly from April until September on sticky board placed on the bottom of the hive to estimate the natural mortality. A wire screen prevented bees from gaining access to debris or mites. The adult female was counted directly on the paper (Fig. 3).

Fig 3: (Plate A). Sticky board placed on the bottom of the Iranian hive to estimate the natural mortality and check the population of varroa mites in a colony, (Plate B). Varroa floor with insert covered in hive debris and wire screen prevented bees from gaining access to debris or mites, (Plate C). Natural mites downfall on sticky boards and hive floor debris containing mites.

2.3 Study area

The present study was aimed to evaluate the infestation rate and prevalence of two honey bee races with *Varroa destructor* in three sites in Savojbolagh county in the Alborz province and comparing the colony mortality between colonies Iranian honey bees (*A. mellifera meda*) and Hybrid western honey bee (*A. mellifera carnica*) for *Varroa destructor* (Acari: Varroidae) population estimation in Savojbolagh apiaries in the Alborz province (Central district, Chaharbagh district and Chendar district) respectively (Fig.4). 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.

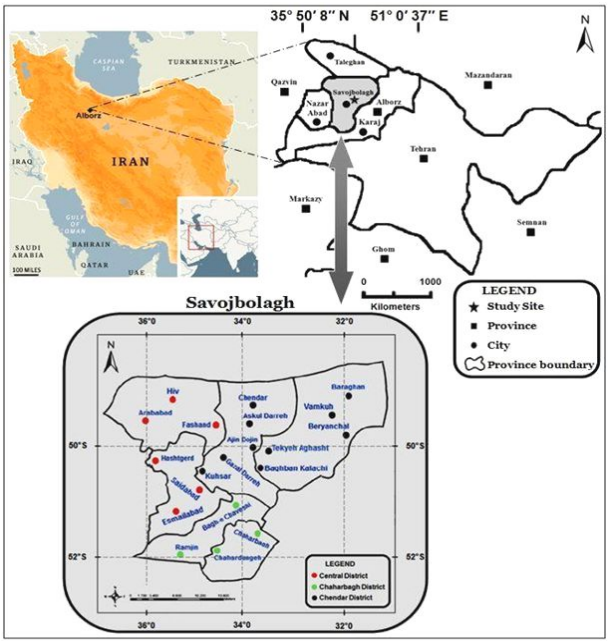

Fig 4: Map of Iran and the study area of Savojbolagh regions in Alborz province showing the sampling sites and distribution areas of *Varroa destructor* (Acari: Varroidae) mite into three districts: the Central district, Chaharbagh district and Chendar district.

3. Statistical analysis

The total number of mites in each colony with brood was estimated using the following two methods: first method is Average daily down fall × 120 [42], and second method is mites on bees and brood (the infestation rate of sampled bees × number of bees + infestation rate of sampled brood × number of brood cells [29]. Data collected were stored in Microsoft Office Excel 2007 and analyzed by SPSS software version 20 for presentation of the results.

4. Results

4.1 Estimating varroa population density in colonies

The study was started with 200 colonies, 100 colonies of *A. mellifera meda* (Indigenous) and 100 colonies of *A. mellifera carnica* (Hybrid bee race imported). Each colony was inoculated with 10-mites and the colonies were maintained by feeding them on sugar and pollen. The data was recorded with 1 month interval starting 15th June, 2016- 15th September, 2016 to study and comparing the colony mortality between

colonies Iranian honey bees (*A. mellifera meda*) and hybrid western honey bee race (*A. mellifera carnica*) for *Varroa destructor* (Acari: Varroidae) population estimation in Savojbolagh apiaries in the Alborz province. The mites population estimate based on average daily downfall in (hybrid bee race) *A. mellifera carnica* on inspection days were recorded 102.85, 192, 700.8 and 582 (5.24, 7.3, 38.14 and 26.5 folds increase, respectively) on every 15th of June, July, August and September respectively. While the mites estimate in *A. mellifera meda* (Indigenous bee colonies) were higher in number as 193.2, 308.4, 1692 and 1260 (9.4, 12.1, 68.4 and 42.35 fold increase) on their respective dates of inspection (Table 1; Fig. 5). The Fig. 5 showed increased in mites population from June to August and then decreased in September. In both hybrid honeybee race A. mellifera carnia and Iranian honey bee race (*A. mellifera meda*) the highest peaks in mites population were observed in August but the increase was more drastic in Indigenous bee colonies (*A. mellifera meda*).

Table 1: Estimation of the number of varroa mites in imported race () and Iranian honeybee colonies (Indigenous), calculation based on average daily mites downfall × 120.

	Honey bee races					
	A.mellifera carnica (Imported)			A.mellifera meda (Indigenous)		
Data	Number of mite fall/week	Average No. of mites fall/day	Estimated No. of mites	Number of mite fall/week	Average No. of mites fall/day	Estimated No. of mites
15/6/2016	4.2	0.85	102.85	8.1	1.61	193.2
15/7/2016	7.1	1.6	192	12.3	2.57	308.4
15/8/2016	43	5.84	700.8	101	14.10	1692
15/9/2016	24.5	4.85	582	47.9	10.50	1260

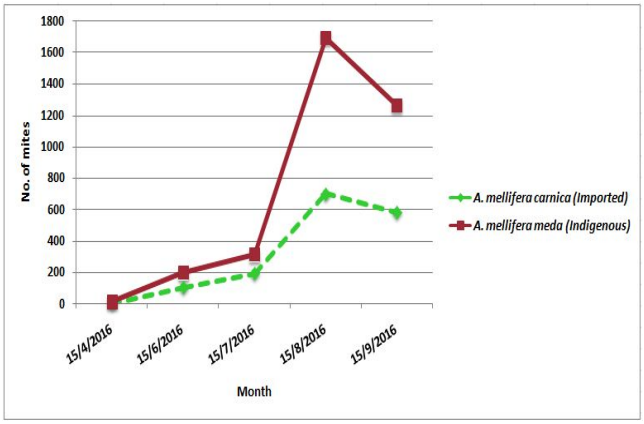

Fig 5: Estimation of the number of varroa mites in Carnica (dashed, green line) and Indigenous honeybee colonies (solid, red line), from June 2016 to September 2016 calculation based on average daily mite downfall × 120.

The average mites population calculated based on infestation rate of live bees × number of bees + infestation rate of brood × number of brood cells in Indigenous colonies (*A. mellifera meda*) presented higher mite population 2039, 2685, 3804 and 1876 as compared to Carnica bee colonies (Imported race) as 435.9, 271.2, 974.9 and 657.2 calculated during their respective dates of inspection (Fig. 6; Table 2). The graph depicted high mite population in the where the increase in honey bee colonies *A. mellifera meda* mite population reached to 109.05, 48.5, 124.3, and 91.85 folds in June, July and August respectively and then it declined toward September where the population remained 91.85 folds. The mite population in Carnica honeybee colonies increased 25.59, 24.89, 51.84 and 33.36 folds in June, July, August and September.

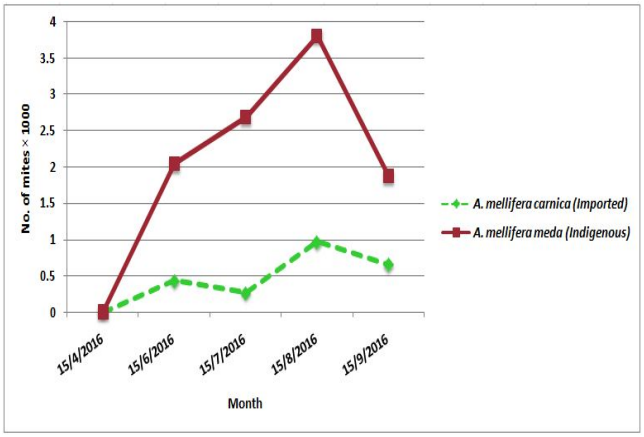

Fig 6: Estimation of the number of varroa mites in Carnica (dashed, green line) and Indigenous honeybee races (solid, red line), calculation based on infestation rate of live bee × number of bees + infestation rate of brood × number of brood cells.

Table 2: Estimation of the number of varroa mites in Carnica honeybee colonies (imported) and Indigenous race (*A. mellifera meda*), calculation based on number of mites per live bee × number of bees + number of mites per brood cell × number of brood cells.

Data	Honey bee colonies	
	A. mellifera carnia (Imported)	***A. mellifera meda* (Indigenous)**
15/6/2016	435.9	2039
15/7/2016	271.2	2685
15/8/2016	974.9	3804
15/9/2016	657.2	1876

The mites estimate from the adult bee population alone was also higher in Iranian colonies (Indigenous bee) 532.7, 1220.3, 1752 and 774 as compared to Carnica colonies (Imported honey bee race) 435.9, 271.2, 974.9 and 657.2 calculated during their respective dates of inspection. The graph showed an increase in mites population from the month of June in both imported hybrid bee race (*A. mellifera carnica*) and Indigenous honeybee race (*A. mellifera meda*) where the mites population reached to its maximum in the month of August and then declined toward September. The mites population recorded from broods of indigenous colonies (*A. mellifera meda*) was 1482.3, 1387.5, 1800 and 850.6 in June, July, August and September respectively in comparison with Carnica bee colonies (imported race) where the mites population remained nil during their respective dates of inspection (Fig. 7).

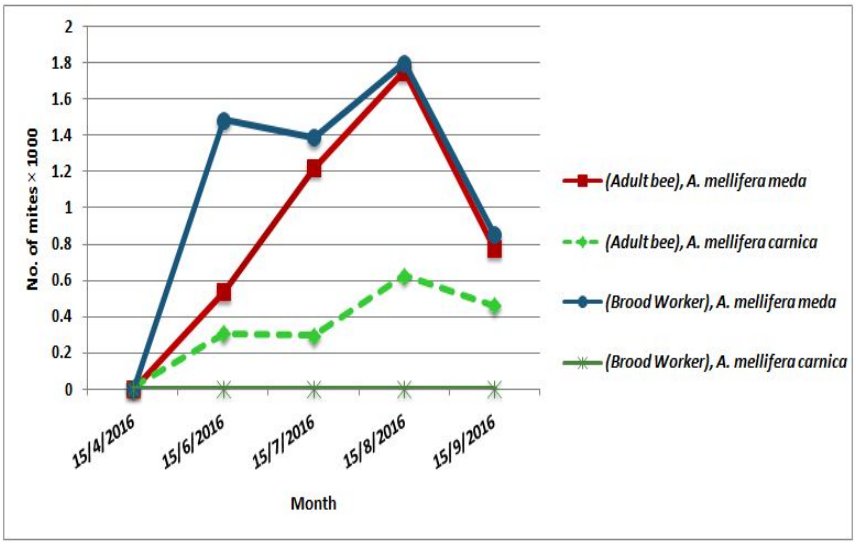

Fig 7: Estimation of mite populations based on mites in Cacnica (Imported hybrid honeybee race) and Iranian honeybee race (*A. mellifera meda*) in both adult and worker brood.

The rate of infestation was determined for adult bees which presented lower infestation rate in Carnica bees (imported race) 0.046, 0.042, 0.98 and 0.074 recorded on every 15th of June, July, August and September respectively as compared to indigenous bees (*A. mellifera meda*) 0.96, 0.184, 0.318 and 0.206 while the data regarding the infestation rate calculated for brood also showed higher degree of infestation on *A. mellifera meda* (indigenous colonies) 0.27, 0.48.9, 0.65.6 and 0.44.5 as compared to Carnica bee colonies where the infestation rate was found nil, estimated during their respective dates of inspection (Fig. 8).

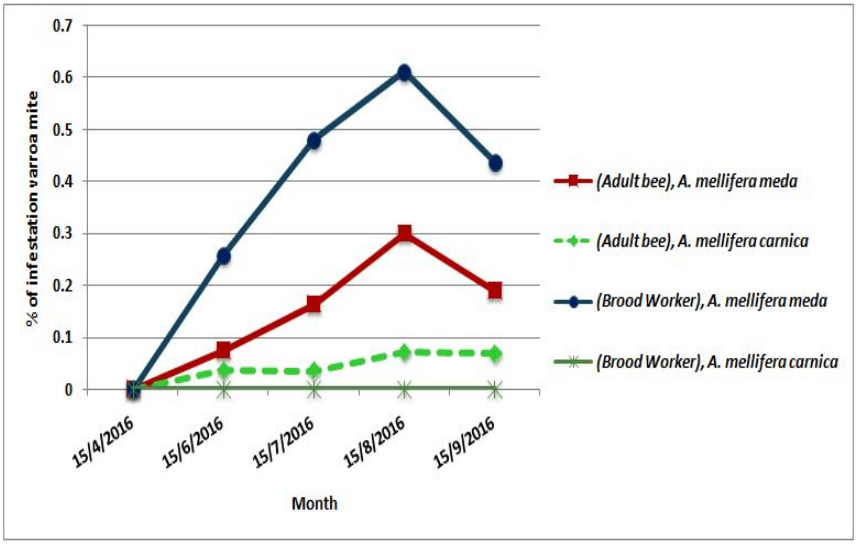

Fig 8: The percentage of mites infestation based on mites in both races (Exotic race) and *A. mellifera meda* (Indigenous race) in both adult and worker brood in honeybee colonies.

Table 3: Mites populations based on different sampling method for the detection and estimating of *Varroa destructor* at different infestation levels.

Detection of mites from adult and worker brood			Sticky board method	
Data	**Carnica colonies**	**Iranian colonies**	**Carnica colonies**	**Iranian colonies**
5/6/2016	435.9	2039	102.85	193.2
5/7/2016	271.2	2685	192	308.4
5/8/2016	974.9	3804	700.8	1692
5/9/2016	657.2	1876	582	1260

One of the objectives of this study was also to compare the two methods used for the estimation of mites population between two hybrid bee race *A. mellifera carnica* and Indigenous race *A. mellifera meda* (Table 3). The sticky board method is better for the initial detection of mites population at low infestation levels. As in case of present study the mites were detected in all colonies whereas the adult and brood population detection method did not show mites in all colonies. Therefore, the sticky board method has have the advantage over the adult and brood method, sticky board detects the mites at initial infestation level and moreover, it is simple in use that can be used by the beekeepers.

5. Discussion

The results based on natural mites downfall on sticky boards revealed that *A. mellifera meda* (Indigenous) bees were more susceptible to *Varroa destructor* as compared to Carnica bees (Imported) and presented a continuous increase in mites population till August which is in agreement with [4, 26, 40]. The estimate based on average number of mites on adults and brood presented a similar trend where the population of mites was more abundant among the Iranian honeybee race (Indigenous) which showed there more susceptibility to *Varroa destructor* but yielded a higher population level of mites than those obtained from the natural mite fall on sticky board which was in agreement with [3, 4, 26, 27, 42]. The results also showed increase in mite population from June to August in both hybrid Carnica race (Imported) and indigenous bee colonies (*A. mellifera meda*) and then decrease in mites population toward September which was in line with those of [58, 64] who recorded the same trend in mites population growth rate. The mites population remained low in colonies (*A. mellifera carnica*) which could be attributed to its more efficient defensive behavior against parasitic mites population such as *Varroa destructor*.

A variation was observed in the rate of infestation both in adult and brood inside the colonies and between the honeybee colonies in the same treatment in both indigenous race (*A. mellifera meda* and hybrid bee race *A. mellifera carnica*), which were in agreement with [3, 29]. The infestation of the adult bees varies from one comb to another [55]. A study by Liebig 1996 [42], reported adult bee estimates are more likely to be affected by the part of the hive from which the sample was taken. Ellis and Baxendale 1994 [21], found that results of the sampling method. As in case of present study the mites were detected in all colonies with sticky board method whereas adult and brood population detection method did not show mites in all colonies which is in agreement with who compared different diagnostic methods for detection of varroa mite at allow infestation levels.

6. Conclusion

The sticky board is the most reliable method of detecting mite infestation when the population is low. The present study concluded that sticky board method was more effective than examining the adult and brood itself for low infestation rates. The mite population estimated directly from the live bees and broods was higher than mite estimates obtained from sticky board. The sticky board method was found better than adult bees and brood samples for the detection of mite population at low infestation level. Varroa mites in honey bee colonies are fluctuate during and between seasons and information about the number of colonies and rate of growth are required. Studying and comparing the results clearly indicate that the beekeeper awareness, and his way of management and fight against the parasites in the hives eventually has its effects on honey production. These results indicate success of future programming for breeding Iranian honeybees in terms of resistance characteristics against diseases and *Varroa destructor* mite. In addition, results of our research show that exotic honey bee race *A. mellifera carnica* (Imported) has good adaptation and potential of resistance against *Varroa destructor* mites.

7. Acknowledgement

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