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# Behavioral Defenses of Iranian Honey Bees (*Apis mellifera* meda) Against *Varroa destructor*

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## ABSTRACT

*Applying pesticides against Varroa destructor mite in most part of Iranian apiculture is inevitable. Therefore, we tried to investigate the resistance mechanisms of Iranian honeybees (Apis mellifera meda) against Varroa destructor. In this research, records of 50 colonies of the honeybees collected from five different regions of East Azarbayjan province. Recorded traits of each hive were number of adult bees, number of sealed brood, number of mites in phoretic phase, number of Varroa infestation brood, and number of Varroa mites falling on the hive bottom board (major variable). A multiple regression model studied effect of noise variables on the interested major variable. Then, Principal Component Analysis (PCA) was used to survey variables in different regions of apiculture in terms of number of Varroa fallen down on the bottom board of hives together with four above mentioned variables to examine all variables simultaneously. Results of variance analysis showed significance differences among different regions of apiculture in terms of number of Varroa mites down on the hives bottom board. In addition, results of correlation survey indicated that there is no significance correlation between number of the mites on the hive floor and population of adult bees of colonies and number of the sealed brood. However, there is a positive and significance correlation between number of Varroa fallen down on the bottom board of hives and number of mites in phoretic phase (r = 0.575); and number of Varroa infestation brood (r = 0.625). These results indicate the presence of a high variance of resistance behavior against Varroa destructor; and verify the possibility of breeding and producing bees of Iranian honeybee stocks which are resistant against Varroa.*

**Keywords**: Grooming, Hygienic behavior, Apis mellifera meda, resistance, Varroa destructor.

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## INTRODUCTION

The parasitic mite, *Varroa destructor* Anderson and Trueman, previously named *Varroa* jacobsoni Oud, is an external parasite of honey bees which was reported for first time by Oudemans in 1904 from *Apis cerana* colonies in Java and then from Singapore in 1957. For the

first time, in Hong Kong and Philipine in 1962-63, saw the *Varroa* mite in *Apis mellifera* L. colonies. Since then, the mite spread worldwide. In Asia, six *Varroa* haplotype that has selected *Apis cerana* as a host, named *Varroa* destructor [1]. Only two Japanese and Korean genotypes of *Varroa destructor* have been external parasites of *Apis mellifera* [2].

**Life cycle of *Varroa destructor:*** *Varroa destructor* can reproduce just in sealed brood cells of drone or worker bees. 15-20 hours before capping of the brood cells, the female *Varroa* leaves the adult bee body (which was its parasite) and transfer into brood, which are in their 5.instar period. While the young bee completes its metamorphosis period and leaves its cell, the female mite and its mature daughters leave the cell. These female mites transformed to other young bees easily. They live 4-13 days on mature bees before turning their parasite stage to 5.instar larvae of bees. This part of live of *Varroa* called as Phoretic phase [3]. Number of *Varroa* mite reproduction cycle in bee populations is 2-3 period [4].

**Resistance behaviors against *Varroa destructor*:** A worker bee can groom all the mites stuck to its body by its legs and jaws; otherwise, it does some special movements to attract other bees attention to make clean up the parasites from its body [5]. Frequency of grooming behavior in *Apis mellifera* L. is less than *Apis cerana.* Several researches show that parasites injured due to bee bites [6-7]. A negative and meaningful correlation between number of parasites injured by bees and amount of infestation of the whole colony with *Varroa destructor* had reported [8]. Therefore, grooming behavior is expressed on the basis of the injured parasites; and it is considered an important parameter in selecting colonies resistant against *Varroa*; however, frequency of this behavior in Apis mellifera L. is not as severe as that in Apis cerana [9]. Grooming behavior is one of important defensive mechanisms of honeybees against *Varroa* [10]. Regular and small pits at the back of *Varroa* mites related to growth period and parasite evolution; and should not be mistake with probable hurts by bees or other insect hunters [11]. In some race of honey bees, worker bees detect fertile and egg-laying parasites, and try to take the infested brood out of the hives, while they do not care those honey bee pupa that have been attacked by sterile mites. At first, this behavior of bees was called “Suppressed Mite Reproduction (SMR)”, but later or and due to result of several researches it was substituted by “*Varroa* Sensitive Hygienic (VSH)” [12]. *Varroa* sensitive hygienic behavior is a congenital trait and controls the considered parasite population [13].

## MATERIALS AND METHODS

This research started by purchasing 50 colonies of honeybees from five apiaries of East Azarbayjan province from April to November 2010. Then, these experimental colonies transported to apiary of Agriculture Education and Research Station. Some points in purchasing and selecting the experimental colonies were as follow: 1) no immigration of the hives by the beekeepers, 2) Breeding the queen by the beekeeper and from his own colonies, 3) Far distance of the apiary from other migrating beekeepers and other apiaries.

In this research, criteria of measuring hygienic behavior of bees against *Varroa* mites was number of the mites fallen down on the hives sticky bottom board and studying factors that put an effect on it. Therefore result of resistance against *Varroa* in honey bee colonies has direct relationship with number of the fallen mites on hives bottom board, the experimental colonies were divided into 5 groups of 10 sets and numbered according to where they have been purchased. Since the floors of the hives were set up with a 3 mm wire mesh in a wooden frame with a tray under it acting as a removable drawer. Before counting the number of the fallen down mites on the hive floor (NFV), it is necessary to estimate *Varroa* population inside each hive. To

do so, number of the mites in phoretic phase (NVF), number of the mites that infest the brood (NVB), adult bee population (NAB), and number of the bee brood (NBB) counted in each colony. In each experimental colony to estimate the percent of the mites in phoretic phase about 150-200 adult bees were taken directly from the combs of the hives into a jar with Choloroform. In the laboratory, after the bee samples and the mite stuck to their bodies anaesthetized with Choloroform, the separated mites and bees finally were count. Then, percent of the mites in their phoretic phase were calculated.

The adult bee populations, was estimated visually by counting number of combs where both sides are covered with bees. As 1500 worker bees are enough to cover one side of a comb, it is enough to count number of combs whose both sides are covered with bees and then to multiply them in 3000 to calculate adult bee population in each colony. These figures calculated according to length and width of an adult bee's body and dimensions of a standard frame [14].

The infestation rate (the sum of multiply and singly infested cells) of brood with *Varroa* mites in each experimental colony, was found by taken a comb of capped brood and sampling 100 capped brood cells in straight line transects on each side (50 cells per comb side) of comb [12].

Normally, each comb of honeybee has 7000 cells (3500 cells on each side of comb). In order for estimating number of capped brood cells, first the inner surface of one empty frame divided into ten equal parts by a thin metal wire. As each side of a comb include 3500- capped brood cells, each part of the wired empty frame includes 350 cells of waxy comb. Putting the wired frame on each comb of the experimental colony, number of inner parts of the wired frame filled with sealed brood was counted and multiplied in 350 to get number of capped brood cells. Multiplying the percent of brood infestation with *Varroa* mites in total number of capped brood cells, was measured the infestation rate of each colony. In addition, multiplying the percent of infestation of adult bees with *Varroa* mites in number of adult bees of a colony, one can determine phoretic mite's population in each colony [14]. In order to determine number of the mites fallen down on the hive floor, first of all floors of the hives were set up with a 3 mm wire mesh and then a white waxy paper smeared with white grease was put under that. These waxy papers smeared with grease substitute once a week for 5 weeks and number of the mites on the papers counted. Length of two periods of *Varroa* destructor reproduction is five weeks [15].

**Statistical Analysis:** As the main purpose of the present study was to investigate the relationship between variable NFV and variables NVF, NVB, NAB and NBB to generalize the results, the experimental colonies were prepared from various regions of East Azarbayjan Province and these variables were recorded. In order to survey the relationship between the concerned major characteristic (NFV) with other variables, different statistical methods were selected. These four variables would predict had a relative strong correlation with another. Therefore, in the first step correlation coefficients calculated between the major trait and other variables as across all regions and by regionally (Tables 2 and 3). In addition, to do quantity survey of the effect of independent variables NVF, NVB, NAB and NBB on major variable NFV, applied a multiple regression model.

**Multiple Regressions:** Due to significance correlation coefficients among the variables, and in order to keep simplicity and fitness of the model, also to select the effective factors used the Forward method. To keep the variables in the model, selected meaningful level was 0.1, so designed the following model:

$$NFV_{ij} = \alpha + \beta_1 NAB_{ij} + \beta_2 NBB_{ij} + \beta_3 NVB_{ij} + \beta_4 NVF_{ij} + e_{ij}$$

Where:

$NFV_{ij}$ = number of *Varroa* mites fallen down in *j*th hive and *i*th region, $NAB_{ij}$ = number of adult bees in *j*th hive and *i*th region, $NBB_{ij}$ = number of capped brood cells in *j*th hive and *i*th region, $NVB_{ij}$ = number of the mites infesting the brood in *j*th hive and *i*th region, $NVF_{ij}$ = number of the mites in phoretic phase in *j*th hive and *i*th region, $\alpha$ = intercept, $\beta_i$ = *i*th regression coefficient, $e_{ij}$ = residual effects. Then, in order to complete the statistical survey and to investigate different regions' effects on the concerned variables, before doing analyze of variance method for regions, another statistical analysis i.e. Principal Component Analysis (PCA) was performed to variables reduction. Due to lack of correlation among variables NAB and NBB with NFV, these two variables (NBB, NAB) not used in Principal Components Analysis. Therefore, due to positive correlations among these three variables (NFV, NVB and NVF) principal component analysis was performed on these variables by SAS (9.1). Results obtained from the study indicate that the first principal component (Prin1) conveys 87% variance of total variables. Therefore, to survey the effects of a region and to get variance analysis table, the GLM method used to analyze data of the first principal component. To do so, used the following model:

$$y_{ij} = \mu + R_i + e_{ij}$$

Where:

$y_{ij}$ = the first principal component related to *j*th hive and *i*th region, $\mu$ = total mean, $R_i$ = the effect of the *i*th region, $e_{ij}$ = residual effects. After applying this method which included the region effect, analyses were performed on transformed amounts (Log transformation) of the first principal component due to lack of normal distribution of the model residuals ($p < 0.01$). Here, Shapiro-Wilk normality test confirmed the normality of residual effects.

## RESULTS AND DISCUSSION

The first of this study, NVF, NVB, NAB, NBB and NFV variables recorded in all experimental hives. Table 1 indicates the related statistical data. According to the calculated variation coefficient for all transformed variables, it was observed that variable NFV had the highest variation coefficient and hence the highest scattering.

**Table1. Descriptive statistics of recorded traits of NAB, NBB, NVF, NVB and NFV**

| Variable | Number of observations | Mean (n) | C.V. (%) | Min (n) | Max (n) |
|---|---|---|---|---|---|
| **NAB** | 50 | 16170 | 26.19 | 9000 | 24000 |
| **NBB** | 50 | 3840 | 39 | 1575 | 7000 |
| **NVF** | 50 | 801 | 112.43 | 0 | 4154 |
| **NVB** | 50 | 248 | 111.16 | 0 | 1470 |
| **NFV** | 50 | 371 | 114.02 | 40 | 2332 |

According to the results of data analysis, there is no significant correlation between NFV and NAB in colonies and NBB. However, between NVF and NVB, there is a positive and significant correlation ($p < 0.01$, $r = 0.818$). There is, also, a positive ($r = 0.575$) and significant ($p<0.01$) correlation between NVF and NFV. According to the calculation, there is a positive ($r = 0.625$) and significant ($p<0.01$) correlation between NVB and NFV (Table 2).

**Table 2. Correlation coefficients among NFV, NAB, NBB, NVF, NVB**

| | NAB | NBB | NVF | NVB | NFV |
|---|---|---|---|---|---|
| **NAB** | 1 | 0.394** | 0.190 | 0.145 | 0.003 |
| **NBB** | 0.394** | 1 | -0.031 | 0.106 | 0.011 |
| **NVF** | 0.190 | -0.031 | 1 | 0.818** | 0.575** |
| **NVB** | 0.145 | 0.106 | 0.818** | 1 | 0.625** |
| **NFV** | 0.003 | 0.011 | 0.575** | 0.625** | 1 |

** *: significant at 0.01 probability level.*

**Table 3. Correlation coefficients among NFV, NAB, NBB, NVF, and NVB in experimental colonies prepared from different apiaries in different regions of the province**

| Region | Trait | NAB | NBB | NVF | NVB |
|---|---|---|---|---|---|
| **A** | NBB | 0.402 | | | |
| **G** | | 0.190 | | | |
| **K** | | 0.297 | | | |
| **N** | | 0.087 | | | |
| **S** | | -0.022 | | | |
| **A** | NVF | 0.292 | 0.286 | | |
| **G** | | -0.018 | -0.143 | | |
| **K** | | 0.512 | -0.351 | | |
| **N** | | -0.104 | -0.444 | | |
| **S** | | 0.071 | -0.543 | | |
| **A** | NVB | 0.597 | 0.802** | 0.685* | |
| **G** | | 0.699* | 0.170 | -0.226 | |
| **K** | | 0.205 | -0.210 | 0.815** | |
| **N** | | 0.047 | -0.278 | 0.878** | |
| **S** | | -0.293 | 0.469 | -0.168 | |
| **A** | NFV | 0.177 | 0.775 | 0.252 | 0.694* |
| **G** | | -0.023 | 0.176 | 0.039 | -0.024 |
| **K** | | -0.027 | -0.394 | 0.714* | 0.679* |
| **N** | | 0.094 | -0.288 | 0.569 | 0.385 |
| **S** | | -0.155 | 0.453 | -0.312 | 0.482 |

*Comments on abbr in the column related to region are as follows: A=Tabriz, G=Azarshahr, K=Ahar, N=Bostanabad, S=Shabestar, * and ** : significant at 0.05 and 0.01 probability level, respectively.*

Measurement of regional correlation coefficients among the major trait and four concerned variables confirmed total estimated correlation coefficients among these variables (Table 3).

According to results of multiple regression model analysis about lack of any effects of NAB and NBB variables on NFV, the transformed data of the first principle component of NFV and other two variables used as the dependent variable in variance analysis for different areas. Results indicated that there were significant differences among different regions in first principle component. Honeybee colonies prepared from different regions have significant differences in terms of the most principal component in 1% probability level (p= 0.003). After doing variance analysis, normality test was done on the residual effects and its normality was confirmed (p> 0.15) (Table 4).

**Table 4. Variance analysis of the most principal component of the three variables**

| Resources | Degree of freedom | Sum of square (Type III) | Mean square | F | Pr > F |
|---|---|---|---|---|---|
| **Regions** | 4 | 13476512 | 3369128 | 4.69 | 0.003** |
| **Residual** | 45 | 32358700 | 719082 | | |

** *: significant at 0.01 probability level.*

*In order to determine the region different from others, Duncan mean comparison test was done (Table 5).*

**Table 5. Comparison of means of apiculture regions by Duncan test**

| Apiculture Regions | Number of experimental hives | Mean |
|---|---|---|
| **Ahar** | 10 | 1799[a] |
| **Bostanabad** | 10 | 1052[a] |
| **Tabriz** | 10 | 897[a] |
| **Shabestar** | 10 | 496[ab] |
| **Azarshahr** | 10 | 306[b] |

*Numbers followed by the same letter are not significantly different based on Duncan's multiple range test (α= 0.05).*

As it is seen in Table 5, colonies in Azarshahr region have meaningful differences with colonies of other regions in terms of number of the mites fallen down on the hives floor.

Hygienic behavior of the bees in resistant colonies against *Varroa* mites showed that hygienic behavior ability depends on relative number of these bees in the colony [16]. In a hygiene colony, relative number of those bees that do hygienic activities is just a few, but those bees do discovery activities, uncapping and removing infested brood very well [17]. In a study, percent of mites that infest the bee's brood and number of the mites that infest the adult bees (mites in phoretic stage) considered as a symbol of hygienic behavior; and while they showed that both these characteristics play a meaningful role, they estimated regression coefficient of brood infesting mites more than phoretic ones [18].

## CONCLUSION

Our findings, show that there is not any meaningful correlation between bees population of the colonies and number of the mites fallen down the hives floor. It means that there is no relationship between total number of worker bees in a colony and resistance against *Varroa*, rather the important factor is presence or lack of the bees that do hygienic activity. According to our calculations, there is a positive and significant correlation between NFV and NVF ($r = 0.575$, $p<0.01$); and NFV and NVB ($r = 0.625$, $p<0.01$) (Table1). It means, while worker bees are cleaning up the cells infested with *Varroa* destructor, they uncap the cells and kill the mites inside and/or hurt them, therefore the mites fall down on the hives floor. In addition, wherever frequency of hygienic behavior in worker bees is high, number of the mites cleaned up from adult bee bodies and fallen down on the hive floor is increased. Fitting model by forward method confirmed the relationship between number of *Varroa* brood and number of the mites fallen down on the hive floor. The basis of breeding bees with resistance ability against diseases and parasites, and selection in terms of the mentioned characteristics is to survey about variety in honeybee's population. Therefore, the present research surveys hygienic behavior in limited populations of *Apis mellifera* meda and examines variety amount of the concerned characteristics in this mass. To do so, it analyzed its data by PCA method and then analyzed variance on the transformed amounts of the first principal component. Results of means comparison test showed that there is a significant difference among various apiculture regions in terms of number of the mites fallen down on the hives floor. 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 *Apis mellifera* meda has potential of resistance against *Varroa* mites without any drugs and chemicals; and one-can produce resistant bees for beekeepers through selection and breeding.

## REFERENCES

[1] Anderson, D. L., and J. W. H. Trueman. **2000**. *Experimental and Applied Acarology*, 24: 165–189.
[2] Anderson, D. L. **2006**. RIRDC Publication No 06/007. RIRDC Project No CSE-87A.
[3] Ibrahim, A. M. PhD doctorate Tesis. University of Minnesota. USA. **2005**.
[4] Martin, S.J., and D. Kemp. **1997**. *Journal of Apicultural Research* 36(3/4): 113–123.
[5] Peng, Y. S., Y. Xu. Fang, and L. Ge. **1987**. *J Invert Pathol*. 49: 54–60.
[6] Ruttner, F., and H. Hänel. **1992**. *Apidologie* 23: 173–187.
[7] Rosenkranz, P., I. Fries, O. Boecking, and M. Stürmer. **1997**. *Apidologie*, 28: 427–437.
[8] Moosbeckhofer, R. **1992**. *Apidologie*, 23, 523–531.
[9] Fires, I., W. Huazhen, S. Wei, and C. S. Jin. **1996**. *Apidologie*, 27: 3-11.
[10] Spivak, M. **1996**. *Apidologie*, 27:245–260.
[11] Davis, A. R. **2009**. *Apidologie* 40: 151-162.
[12] Harris, J. W. **2007**. *Journal of Apicultural Research*, 46(3): 134-139.
[13] Harbo, J.R., and J.W. Harris. **2005**. *Journal of Apicultural Research*, 44: 21-23.
[14] Mondragón, L., M. Spivak, and R. Vandame. **2005**. *Apidologie*, 36: 345–358.
[15] Arechavaleta-Velasco, M.E., and E. Guzman-Novo. **2001**. *Apidologie*, 32: 157-174.
[16] Arathi, H., and M. Spivak. **2001**. *Animal Behavior*, 62: 57–66.
[17] Palacio, M., E. Rodriguez, L. Goncalves, E. Bedascarrasbure, and M. Spivak. **2010**. *Apidologie*, 41: 602–612.
[18] Ibrahim, A., G. Reuter, and M. Spivak. **2007**. *Apidologie*, 38: 67–76.