A PRELIMINARY STUDY ONDISCRIMINATION OFDIFFERENT INFESTATION LEVELS OF PARASITE (*Varroa destructor*)BY WING GEOMETRIC MORPHOMETRIC ANALYSIS ON HONEY BEES

Bal Arılarında Kanatların Geometrik Morfometrik Metodu ile Analiz Edilerek Farklı Seviyedeki Varroa (*Varroa Destructor*) Parazitinin Bulaşıklık Seviyesinin Belirlenmesi Konusunda Ön Çalışma

(Genişletilmiş Türkçe Özet Makalenin Sonunda Verilmiştir)

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Key Words: *Apis mellifera*, *Varroa destructor*level, geometric morphometrics

Anahtar Kelimeler: *Apis mellifera*, *Varroa destructor* seviyesi, geometrik morfometrik

ABSTRACT

The goal of this study was to investigate the possibility of using geometric morphometric method to discriminate different infestation level of Varroa mites (*Varroa destructor*) in honey bees. Three colonies of honeybees (*Apis mellifera anatoliaca*) were used as control, moderate and high infested with varroa mites. For geometric morphometric analysis of fore wings of worker honey bee, each left wing image of samples were archived and labelled with unique codes in the computer and a total of 139 wings of worker bee belonging to 3 colonies (2-*Varroa* infested colonies and 1-Control group) were used. All three groups of control, moderate and high infested colonies were discriminated clearly. The results here suggest that geometric morphometric analysis of honeybee wings can be used to discriminate different varroa infestation level.

INTRODUCTION

*Varroa destructor (*Anderson & Trueman)has been the main factor for *Apis mellifera* L. colony losses despite three decades of intensive research aimed at controlling this pest. It continues to be an important parasite of the western honeybee (*Apis mellifera*) throughout the world (Kevan et al., 2006; Vanengelsdorp et al., 2007). This is also true in Turkey where the Korean haplotype of *V. destructor* occurs (Warrit *et al.* 2004). Typically honeybee colonies left untreated die within 1-2 years of varroa infestation (Bailey and Ball 1991). In addition to the effect of the mite itself on honeybees, *V. destructor* is a vector for a number of viruses that also significantly affect honeybee population survival (Sumpter and Martin, 2004; Kevan et al., 2006). There are dozen of viruses such as DWV that is closely correlated with varroa mite and these viruses are tought to be the main cause of colony losses in recent years (Yang and Cox-Foster et al. 2005, 2007).

The first step to control this ectoparasite is to determine infestation level for each colony and decide to use effective control methods. In order to determine infestation level about 300 bees were collected from each colony and shaked in a jar with ethanol, detergent or other chemicals to detach varroa mited from bees (Webster 2001). Since honey bee colonies have low population in fall season this process affect each colony negatively. In recent years powder sugar method has been developed in order not to lose so many bees and used for researchers in this process (Webster and Delaplane 2001; Fakhimzadeh 2001; Çakmak et al., 2011).

Geometric morphometric has been gaining momentum mostly in taxonomy research including honey bee taxonomy and this method has been used for honey bee taxonomy studies in recent years. A few bees were used for identification of bee samples based on wing analysis (Adams et al. 2004; Francoy et al. 2006, 2008; Mitteroecker and Gunz 2009.). Miguel et al. (2011) suggested that wing geometric morphometric analysis is more appropriate than mitocondrial DNA and traditional morphometric analysis.

Also recent studies have focused on breeding varroa tolerant bee colonies and it is important to determine infestation level by sacrifying less worker bees. Therefore geometric morphometric method may provide an alternative for the determination of infestation level in honey bee colonies for both determining treatment and also breeding studies.

In our knowledge no study has been performed to use geometric morphometric to determine infestation level in honey bees. The aim of this study was to compare different infestation levels of varroa mites on wing shape of worker honey bees based on geometric morphometry.

MATERIALS AND METHODS

Anatolian bees (*Apis mellifera anatoliaca*) 3 colonies were used for the experiments in Uludag University Campus , Bursa, Turkey. Colonies were selected on based on falling varroa mites to the bottom. Control colony was treated with flumethrin two months ago to eliminate about %99 varroa mites. Left fore wings were taken from young adult worker bees from brood area and a digital camera (Nikon Coolpix 7600 with 7.1 megapixel) was used to take pictures of wings.

Geometric morphometric analysis

For geometric morphometric analysis of fore wings of worker honey bee, each left wing image of samples were archived and labelled with unique codes in the computer and a total of 139 wings of worker bee belonging to 3 colonies (2-*Varroa* infested colonies and 1-Control group) were used. A total of 20 landmarks (venation intersections) on the fore wings of worker bee were identified according to Bookstein (1990) classification(Fig. 1) and landmarks were digitized using tpsDig 2.11 (Rohlf, 2008). Landmarks of samples were superimposed using a generalized least-square algorithm and landmark configurations were scaled, translated and rotated aganist the consensus configuration. A Multivariate analysis of variance (MANOVA) and pairwise tests were carried out on x, y coordinates data applied using Morpheus (Slice, 2002) in order to compare groups. The x, y coordinates data was also used as data set for discriminant function analysis of groups and cross validation test to check the accuracy of the equations in identifying the colonies. Differences in wing shape among *Varroa* infested honey bee colonies and control group were visualized by deformation grids using thin plate splines (Slice, 2002).

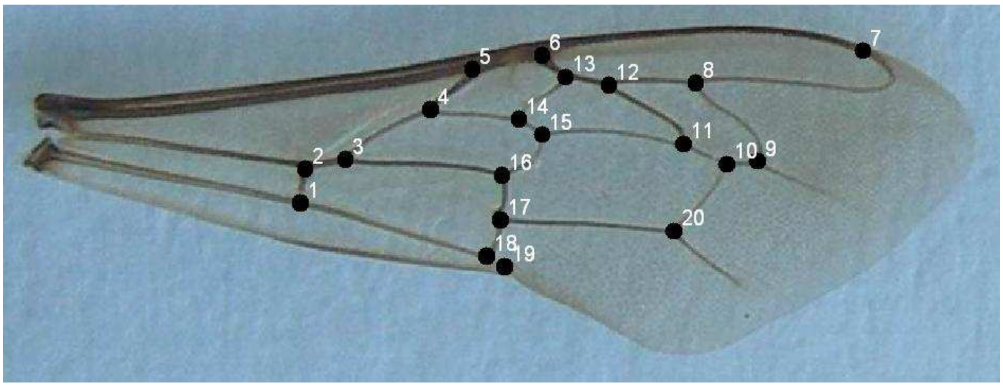

Figure 1. Location of landmarks on fore wing ofworker bee.

RESULTS

Honey bee colonies were allocated to three groups according to *Varroa* infested or not. Multivariate analysis of variance (MANOVA) and pairwise tests were used in order to compare *Varroa* infested colonies and control group. Discriminant function analysis was carried out on geometric morphometric data collected from the samples taken from the *Varroa* infested colonies and control group.

Using multivariate analysis of variance (MANOVA), we found significant differences between *Varroa* infested colonies and control group ($P<0.001$).Pairwise comparison were followed and significant differences between the groups matches were found ($P<0.001$). In discriminant analysis based on two discriminant function describing 56.5% and 43.5% of the total wing shape variation. Analysis of variance (ANOVA) of cartesian coordinates of the landmarks on fore wing showed that 29 out of 40 cartesian coordinates of landmarks, displayed statistical significant differences among colonies ($P<0.05$). All colonies were assigned to their original group with a high probability (96.4%) (Table 1).

Table 1. Classification results of colonies [N(%)] of groups based on geometric morphometry.

Groups	High number of *Varroa* (1)	Normal number of *Varroa* (2)	Control Group (3)
High number of *Varroa* (1)	43 (95.6)	0 (0.0)	2 (4.4)
Normal number of *Varroa*(2)	1 (2.1)	46 (95.8)	1 (2.1)
Control Group (3)	1 (2.2)	0 (0.0)	45 (97.8)

Cross validation tests based on discriminant functions correctly classified 87.8% of the colonies. Discriminant function analysis resulted in clear separation of groups when cartesian coordinates of landmarks were utilized as variables (Fig. 2).

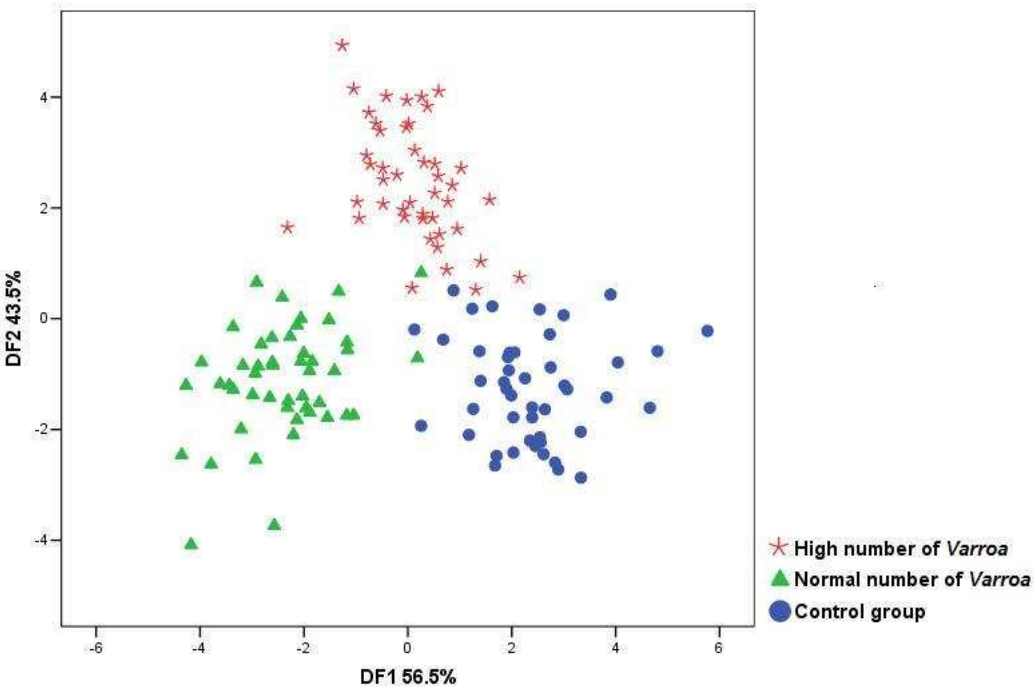

Fig. 2 A scatter plot of discriminant analysis of groups based on geometric morphometry.

Differences between groups were illustrated by deformation grids on the thin plate spline. The thin plate spline representations (Fig. 3A, 3B, 3C) show that the highest differences were seen in pairs with control group.

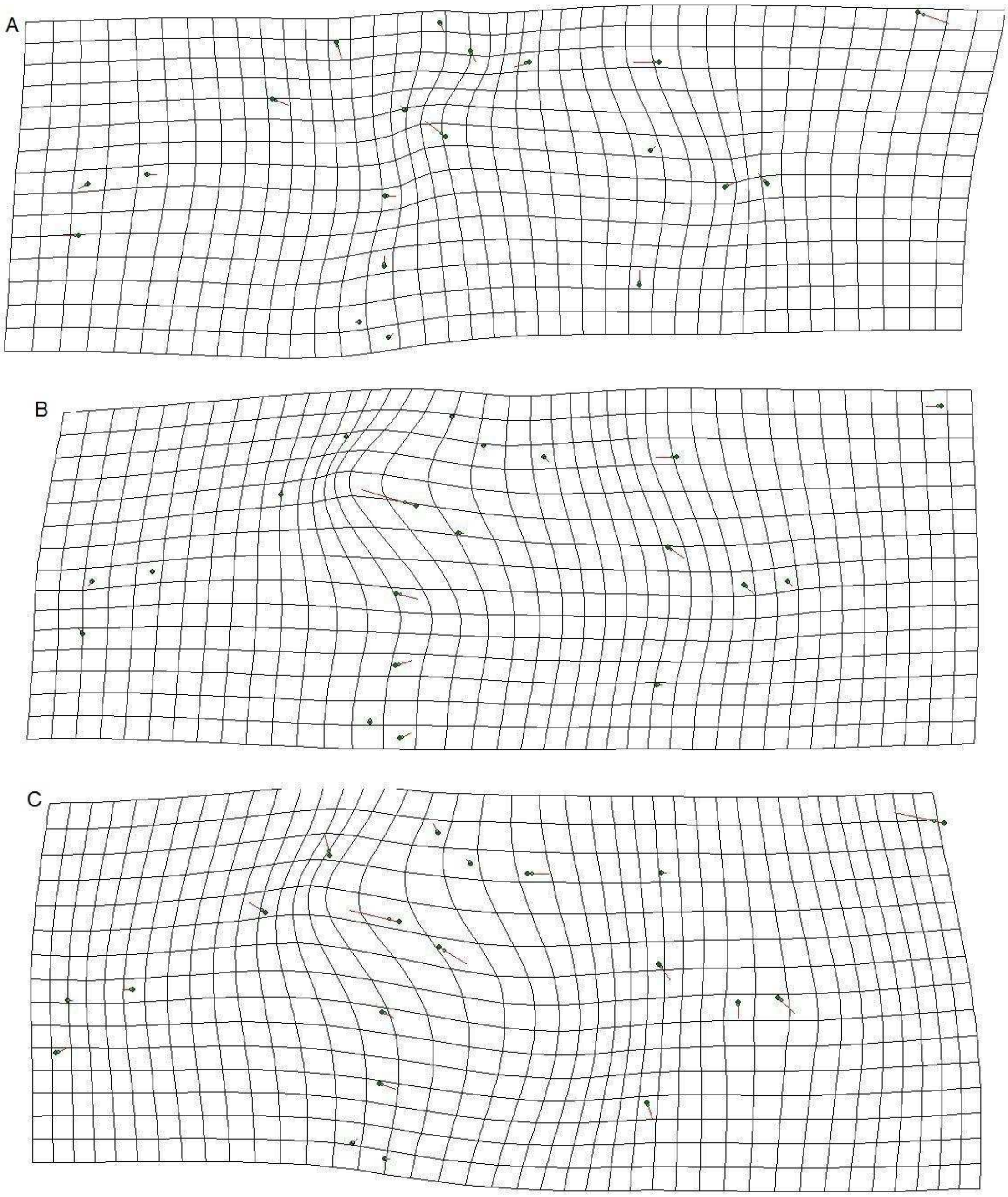

Fig. 3 Comparision of deformation grids of honey bee colonies: 1 with 2 (A), 1 with 3 (B), 2 with 3 (C).

DISCUSSION

Geometric morphometric method might be useful tool to differenciate varroa infestation level and also virus infections. This study results show that parasites/pathogens affect wing shape of honey bees even with moderate infestations of varroa mites. Particularly deformed wing virus (DWV) with low infection might be determined by wing geometric morphometric method. Beekeepers if know varroa infestations and the virus infections in advance may take precautaions e.g. to treat and feed bees and reduce stress factors to combat better with these parasites and viruses.

Since all groups; high and moderate infestation and control groups are clearly separated even a small sample size can be used such as 10 bees from each colony to determine parasite infestation level instead of sacrifying 200-300 bees when common methods used such as ETOH, Ether or Detergent with the exception of powder sugar shaking method in which no bees are sacrified to determine varroa level for each colony (Webster and Deplane 2001; Çakmak et al. 2011).

Geometric morphometric wing analysis of honey bees may also open a door for selection studies. For example; If varroa tolerant and varroa sensitive colonies are differentiated by this method (According to this study high and low varroa infested honey bee colonies had already been discriminated by wing analysis). It is worth to compare varroa tolerant and sensitive colonies. If a threshold is set for varroa tolerant bee colonies in north hemisphere the discrimination and selection between two groups will be possible. Because geometric morphometric is so sensitive to small changes if there are more varroa mites in some colonies means more differences in wing shape will be captured. This is expected since mites feed bee larva and affect body size, shape including wings.

However, in practice geometric morphometric method requires a lof time to remove wings, to capture very clear pictures in close distance and to analyse to make conclusion about varroa infestation. Therefore, this method is time consuming and labor intensive when compared to common methods with chemicals such as ETOH, Ether, or Detergent. In addition it can not be done in the field. This makes this method unpractical for beekeepers and researchers in varroa research.

REFERENCES

Adams D.C., Rohlf F.J., Slice D.E. 2004. Geometric morphometrics: ten years of progress following the revolution. *Italian Journal of Zoology*, 71: 5-16.

Bailey L., Ball B. 1991. Honey bee pathology. Academic Press.

Bookstein F.L. 1990. Introduction to methods for landmark data. In: Rohlf F. J. &F. L. Bookstein (Eds.), Proceedings, Michigan Morphometrics Workshop, 1988–The University of Michigan Museum of Zoology, Special Publication No. 2, AnnArbor, MI, pp. 216-225.

Çakmak I., Seven-Cakmak, S., Fuchs, S., Yeninar, H. 2011. Comparison of Powdered Sugar And Detergent Methods to Determine Varroa Infestation Level in Honey Bee Colonies. *U. Bee J./U. Arı Drg.* 11: 63-68.

Frankoy T.A., Wittmann D., Drauschke M., Müller S., Steinhage V., Bezerra-Laure M., DeJong D., Gonçalves L.S. 2008. Identification of Africanized honey bees through wing morphometrics: two fast and efficient procedures. *Apidologie* 39: 488-494.

Francoy T.M., Prado P.R.R., Gonçalves L.S., Costa L.F., DeJong D. 2006. Morphometric differences in a single cell can discriminate Apis mellifera racial types. *Apidologie* 37: 91-97.

Fakhimzadeh, K. 2001. Effectiveness of confectioner sugar dusting to knock down *Varroa destructor* from adult honey bees in laboratory trials. Apidologie 32: 139-148.

Kevan P.G., Hannan M.A., Ostiguy N., Guzman E. 2006. A summary of the Varroa-virus disease complex in honeybees. *American Bee Journal*, 146: 694-697.

Miguel I., Baylac M., Iriondo M., Manzano C., Garnery L., Estonba A. 2011. Both geometic morphometric and microsatellite data consistently support the differentiation of the *Apis mellifera* M evolutionary branch. *Apidologie* 42: 150-161.

Mitteroecker P., Gunz P. 2009. Advances in Geometric morphometrics. *Evol Biol*., 36: 235-247.

Rohlf F.J. 2008. tpsDIG, version 2.11. – Department of Ecology and Evolution, State University of New York, Stony Brook, New York.

Slice D.E. 2002. Morpheus, For morphometric research software. Department of Biomedical Engineering Wake Forest University School of Medicine, Winston, Salem.

Sumpter D.J.T., Martin, S.J. 2004. The dynamics of virus epidemics in Varroa-infested honey bee colonies. *J. Anim. Ecol.,* 73: 51-63.

Yang X., Cox-Foster D. 2005.Impact of an ectoparasite on the immunity and pathology of an invertebrate: Evidence for host immunosuppression and viral amplification. *Proceedings of National Academy of Sciences of USA,* 102: 7470-7475.

Yang X., Cox-Foster D. 2007. Effects of parasitization by *Varroa destructor* on survivorship and physiological traits of *Apis mellifera* in correlation with viral incidence and microbial challenge*. Parasitology,* 134: 405-412.

Vanengelsdorp D., Underwood R., Caron D., Hayes J. 2007. An estimate of managed colony losses in the winter of 2006-2007: a report commissioned by the apiary inspectors of America. *American Bee Journal,*147: 599–603.

Warrit N., Hagen T.A.R., Smith D.R., Cakmak I. 2004. A survey of V*arroa destructor* strains on *Apis mellifera* in Turkey. *J. Apic. Res,.* 43: 190-191.

Webster T.C., Deplane K.S. 2001. Detection and measurement of varroa mite populations. In “Mites of the Honey Bee” Ed. By T. Webster and K. Delaplane, Dadant Publication, Pp. 163-178.

GENİŞLETİLMİŞ ÖZET

Amaç: Bu çalışmada geometrik morfometrik metodu kullanılarak Anadolu bal arılarında (*Apis mellifera anatoliaca*) Varroa (*Varroa destructor*) bulaşıklık seviyesinin belirlenmesi ve koloniler arasında bulaşıklık seviyesine göre farklılıkların belirlenebileceği konusu araştırılmıştır. Son yıllarda dünyada ve özellikle bazı ülkelerde gözlenen yüksek seviyedeki koloni kayıplarının nedenlerinden birisi olarak karşımıza çıkan Varroa parazitinin kolonilerdeki bulaşıklık seviyelerinin doğru olarak belirlenmesi daha önemli hale gelecektir.

Araç ve Yöntem: Bu çalışma sonbaharda kontrol, yüksek ve orta düzey Varroa bulaşıklık seviyesi olmak üzere genetik olarak akraba 3 adet Anadolu bal arısı (*Apis mellifera anatoliaca*) kolonilerinde yürütülmüştür. Araştırmada; Varroa bulaşıklık seviyeleri ızgaralı kovanlarda zemine düşen Varroa sayılarına göre belirlenmiştir. Kontrol grubu olarak kullanılan koloni, örnek toplama tarihinden 2 ay önce flumethrin ile Varroa mücadelesi yapılarak ergin arılar üzerindeki Varroa'lar kolonide çok düşük seviyelere indirilmiştir. Bu tedavi uygulamasından yaklaşık 2 ay sonra her bir koloniden 50 adet işçi arının sol kanatlarının resimleri çekilerek dijitalize edilmiş ve kodlanarak arşivlenmiştir. Araştırmada yeterince net ve ayrıntılı görüntü alınamayan resimler iptal edilerek toplam 139 kanat resmi kullanılmıştır. Kanat damarlarının birleştiği 20 nokta kanatlar üzerinde belirlenmiş (Bookstein 1990), gruplandırılması (Rafl 2008) ve grupların istatistiki karşılaştırılması diskriminant analiz yöntemi (MANOVA) ile sağlanmıştır. Geometrik morfometrik yöntemi ile kanatlarda bu belirlenen 20 nokta kullanılarak bu bölgelerdeki deformasyonların farklı Varroa bulaşıklık seviyeleri ile kontrol grubu arasındaki farkların belirlenmesinde kullanılıp kullanılamayacağı araştırılmıştır.

Bulgu ve Sonuçlar: Tüm koloniler birbirinden %96.4 oranında ayrılmış ve Varroa bulaşık ve kontrol kolonisi arasındaki farklılık önemli ($P<0,01$) olarak belirlenmiştir. Bu durumda geometrik morfometrik yöntemi Varroa bulaşıklık seviyesinin koloniler arasında belirlenmesinde kullanılabilir. Ayrıca kanatlardaki bu deformasyon kanat deformasyon virüsü enfeksiyonunun düşük olduğu durumların belirlenmesinde de kullanılabilir. Çünkü Varroa'nın taşıyıcı olduğu en önemli virüslerden biri budur. Bu yöntemde yaygın olarak kullanılan kimyasallar (Ör: Etil alkol, Eter, Deterjan gibi) ile 200-300 arı numunesi alınarak Varroa bulaşıklık seviyesinin belirlenmesi testlerine oranla 50 arı numunesi alınarak daha az arı telef edilmektedir. Fakat bunun yanında bu yöntem kanatların alınması, resimlerin çekilmesi ve bu numunelerin analiz edilmesi ile daha zor bir süreci gerektirdiğinden uygulamada pratik değildir. Bunun yerine son yıllarda pudra şekeri ile yapılan çalışmalar ön plana çıkmaya başlamış, pudra şekeri ile daha fazla sayıda arı numunesi alınıp arılar telef edilmeden yani arı kaybı olmadan yaklaşık 300 arı numunesi ile deterjan benzeri sonuçlar alınabilmektedir (Çakmak ve diğ. 2011).

Sonuç olarak bal arılarında Varroa bulaşıklık seviyesinin sağlıklı ve doğru bir şekilde belirlenebilmesi arıcılar ve Varroa konusunda çalışan araştırmacılar için uzun süre ciddi bir sorun olarak kalmıştır. Kimyasal belirleme yöntemleri ile doğru sonuçlar alınabilmekle birlikte özellikle erken ilkbahar ve geç sonbahar dönemleri gibi ergin işçi arı populasyonunun az olduğu dönemlerde 200-300 adet ergin arı kaybı önemli bir sorun teşkil etmektedir. Varroa bulaşıklık oranlarının tesbitine yönelik çeşitli uygulamalara geometrik morfometrik yöntemi eklenmiş olmakla birlikte arazi uygulamalarında arıcılar ve Varroa konusunda çalışan araştırmacılar için pratik bir uygulama değildir.