

## ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

# EVALUATION OF THE RESISTANCE OF THE MITE *Varroa destructor* TO THE AMITRAZ IN COLONIES OF HONEY BEES (*Apis mellifera*) IN ALGERIA

## Cezayir'de Bal Arısı (*Apis mellifera*) Kolonilerinde Akarın (*Varroa destructor*) Amitraz'a Direncinin Değerlendirilmesi

(Genişletilmiş Özet Makalenin Sonunda Verilmiştir)

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### ABSTRACT

*Varroa* mite has become a major concern of beekeepers in Algeria since the discovery of the first cases of infestation in the year 1982. Amitraz is the predominant compound used in Algeria to control *V. destructor*, its constant application has caused the appearance of resistant mite populations to this product in several parts of the world. This study was conducted to detect the possible existence of populations of resistant mites to Amitraz in Algeria. To determine the mites mortality percentage to the Amitraz, they were exposed to a trips of 2.5 x 1.0 cm. *Varroa* mortality in apiaries treated with Amitraz was 39.23%, lower than the 87.40% mortality obtained in apiaries that only received an alternative treatment. A significant difference ( $P>0.05$ ) was found between two mortality of *Varroa*. Our results indicate a possible occurrence of *Varroa* resistance to Amitraz. The introduction of integrated programs for resistance management is required.

**Keywords:** Amitraz, *Varroa destructor*, Resistance, Algeria.

### ÖZ

Varroa akarı 1982 yılındaki ilk bulaşımın öğrenilmesinden sonra Cezayir'de arıcılar için önemli bir ilgi konusu olmuştur. Amitraz varroa için Cezayir'de sürekli kullanılan ana kimyasal olduğundan dünyanın bir çok yerinde olduğu gibi direnç gelişimine neden olmuştur. Bu çalışma Cezayir'de Amitraz'a karşı akar popülasyonlarının olabilecek direnç geliştirmesini araştırmak için yapılmıştır. *Varroa* ölümü sadece Amitraz kullanılan arılıklarda % 39.23 olurken ikinci bir alternative tedavi kullanılan arılıklarda ise % 87.40 olmuştur. İki varroa ölüm durumları arasında önemli bir fark ( $P>0.05$ ) bulunmuştur. Bu sonuçlar *Varroa*'nın Amitraz'a olabilecek bir direnç kazandığını göstermektedir. Bu durumda birleşik direnç yönetim programı uygulanması gerekli görülmüştür.

**Anahtar Kelimeler:** Amitraz, *Varroa destructor*, Direnç, Cezayir

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## INTRODUCTION

Honey bees, *Apis mellifera* (L.) are important agricultural assets, both for the direct production of commodities such as honey and beeswax, and for vital pollination services provided to support a wide range of wild and cultivated crops (Klein et al. 2007). The mites (Acari) that parasitize honey bees have become a global problem is one of the most serious diseases of honey bees (*Apis mellifera*). *Varroa destructor* mites are considered to be the reason for CCD (Colony Collapse Disorder). *Varroa* mites feed on the developing honey bee larvae, pupae and on the adult bees. Heavily infested colonies usually have large numbers of unsealed brood cells. Dead or dying newly emerged bees with malformed wings, legs, abdomen and thorax present at the entrance of affected colonies and heavily infected colonies produce little or no honey (Ritter, 1981).

*Varroa* disease is a parasitic disease of adult bees and brood, caused by an external parasitic mite, *Varroa destructor* (Anderson and Trueman, 2000); it is the most important parasite of *Apis mellifera* that influences the colony development and performance (Ball, 2003), and is considered as the most serious problem of the beekeeping industry worldwide (Haddad et al., 2007; Guzman-Novoa et al., 2010; Di Prisco et al., 2011; Adjlane et al., 2012). Parasitism of the bee by this mite causes deformation and weakness of the young workers. Heavy infestation causes death before the emergence of nymphs and the birth of mutilated bees (Boecking and Genersch, 2008). *Varroa* heavily decrease the general weight up to 30% of individuals (Bowen-Walker and Gunn, 2001), the total hemolymph volume and its protein content (Bowen-Walker et al., 1999). It was estimated to be between 10% and 50% reduction in total protein of parasitized nymphs (Dandeu et al., 1991). Infestation with the mite leads to quantitative and qualitative changes in hemolymph. Weinberg and Madel (1995) report that 1-3 mites feeding on a worker bee pupa cause a reduction in the host's hemolymph volume by 23.6%; in the case of a drone pupa, the volume of hemolymph decreases by 18.2%, on average. *V.destructor* is responsible for the transmission of viruses to their hosts (Rosenkranz et al., 2010). Varroosis is a high-incidence parasitic pathology, affecting colonies of *Apis mellifera* L. Varroosis causes serious production losses, both global and locally, as it

weakens hives and leads to bee's death (Webster and Delaplane, 2001).

Different active ingredients (e.g. coumaphos, amitraz, bromopropylate, cymiazole, fluvalinate, flumethrin, etc) and substrates (e.g. plastic and cardboard strips, gels and sponges) (Webster and Delaplane, 2001) have been used to control varroosis. Amitraz acts on the target pest species interacting with the octopamine receptor of the central nervous system and is known as neurotoxic, sub-lethal miticide (Evans and Gee, 1980).

Amitraz (formamidine) has been also used for *V.destructor* control worldwide, and mite resistance to this acaricide was reported, too, although few cases were reported (Rodriguez-Dehaibes et al., 2005; Semkiw et al., 2013). Several chemical substances were used successfully to control mites, in recent years, resistance to acaricides has become a major problem in the control of varroa. *Varroa destructor* strains have been reported to be resistant to fluvalinate and flumethrin (Baxter et al., 1998), coumaphos (Spreafico et al., 2001), and to amitraz, (Elzen et al., 2000a).

The concept of resistance to natural toxins that is observed in animals is defined as the ability of the organism to tolerate a substance in toxic doses that may be lethal to most specimens in an ordinary population of the same species (Watkins, 1996). The ability of mites to develop drug resistance is believed to be at the root of a lower effectiveness of acaricides (Mathieu et Faucon, 2000). The objective of the research presented in this paper was to evaluate the efficacy of amitraz used as the contact varroacide (Apivar) to control *V.destructor* in the honeybee colonies.

## MATERIALS AND METHODS

Field experiment was initiated in July 2015 in 4 apiaries where the amitraz was applied in a continuous way for a period of 3 years for the control of *V.destructor* (Algeries), and 4 apiaries where some alternative method was applied (oxalic acid, fluvalinate, thymol) for a period of similar time (Blida), taking 20 colonies for each apiary.

The method of Elzen et al., (1999) was used to assess resistance levels in the eight apiaries. The assay was conducted as follows. Cut a 9mm by 25mm strip from an apivar strip and staple it to the centre of an index card. Place the card in a 500 ml

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jar with the strip facing inwards. Prepare a 2-3mm light metal mesh cover for the jar. Collect samples of 150 bees from each hive, place them in the jar, place a sugar cube in the jar and cover it with the mesh lid. Store upturned in the dark, at room temperature. After 24 hours, hit the upturned jar with your palm three times over white paper. Count the dislodged mites and place the upturned jar in a freezer until the bees are dead (4hrs). Count the remaining mites. Percent mortality was calculated as (number of mites killed in 24 h/total mites in the jar) × 100.

The data obtained was analysed with Statistical software version 5.0 using analysis of variance (ANOVA).

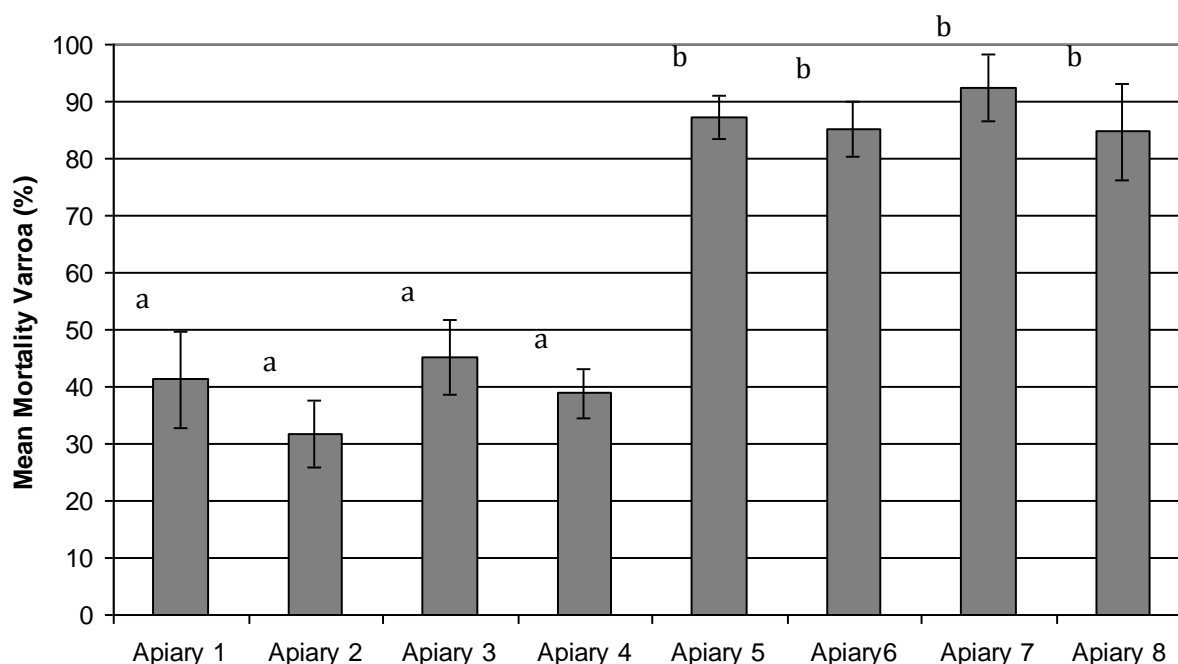
### RESULTS

The results of the treatments carried out on the eight apiaries are indicated in Figure 1. The

average efficacy of the treatment with fluralinate in Algiers was 39,23%. In the region of Blida, the efficacy of the treatments was 87,40%. Significantly lower mite mortality was observed in Algriescmpared to the other apiaries in Blida. The analyses of variance showed no difference in effectiveness ( $P > 0.0$ ) among the three apiaries of Algriess and Blida.

Our results indicate a possible occurrence of *varroa* resistance to amitraz. The reduction in the effectiveness is due to the continuous and inadequate use of the amitraz, because some beekeepers do not remove the plastic ribbons of Apivar®, which should only remain for six weeks inside the beehive. However, these ribbons of the product usually remain around one year inside of the beehives; therefore the mites are exposed to smaller dose to those recommended.

**Figure 1.** Percentage of *Varroa* mortality obtained with the amitraz application (letters indicate significant differences between treatments ( $P < 0.01$ )).



Apiary 1, 2, 3 and 4 : location of Algriess

Apiary 5, 6, 7 and 8: location of Blida

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### DISCUSSION

The mites populations resistance to amitraz was also confirmed in the USA, Italy, Portugal, Argentina, Mexico and France (Elzen et al, 1999; Milani, 1999; Elzen et al., 2000; Mathieu and Faucon, 2000; Pires et al., 2005; Rodríguez-Dehaibes et al., 2005; Maggi et al., 2010).

Floris et al. (2001) found that the efficacy of amitraz in plasticstrips against *V. destructor* in colonies with broodwas only  $74.90 \pm 5.90$  %.

Resistance of mites to Apivar has been reported in Minnesota and other areas in the United States of America (Elzen et al., 2000). In 17% of the colonies examined in Portugal, the Apivar efficacy did not exceed 60.1% (Pires et al., 2007). In a field test in eastern Algeria, Loucif-Ayad et al (2010) recorded a very low efficiency for Apivar (82-88%).

Elzen and Westervelt (2002) reported the first case of resistance of *varroa* to coumaphos in Florida after only 4 years of use as a *varroa* treatment. *Varroa destructor* resistance to amitraz has been described by several authors (Elzen et al., 1999; Mathieu and Falcon 2000; Rodríguez-Dehaibes et al., 2005). Floris et al., (2001) have found 75% efficacy using amitraz in Italy. Faucon et al. (2007) conclude that Apivar ND preserved treated colonies against 99,5% of the population found in control colonies. The tests carried out in Ghazvin in Iran against *Varroa destructor* showed a good effectiveness, of Apivar® 96.68%, for Bayvarol® 96,59% (Shahrouzi 2009). Marinelli et al., (2002) also used Apivar® and obtained an average efficiency of 67%. Migratory apiary management constitutes an additional factor contributing to the proliferation of resistant Varroa mites.

Marinelli et al., (2002) noted the operational and climatic conditions of central Italy where the acaricide was evaluated may have had some effect on amitraz management and therefore may be the cause of discrepancies in the results. Resistance is not limited to the Amitraz varroacides, it has also been reported for coumaphos in Italy (Abed and Ducos de Lahitte, 1993; Milani and Della Vedoiva, 1996; Spreafico et al., 2001). Resistant genotypes usually are at some fitness disadvantage in the absence of pesticides (Denholm and Rowland, 1992), because of unbalanced or unregulated physiological processes. This makes the frequency

of resistant mites decline when the acaricide is not used (a phenomenon usually called reversion).

According to Sammataro et al. (2005), the presence of resistant mites (in colonies where no strong acaricides pressures are obvious) may be due to (1) bees robbing honey from a weak or dying hive (with resistant mites) within the flight range of the apiary and, as a consequence, acquiring those mites; (2) introduction of packaged bees and queens from other states already parasitized by resistant mites; or (3) drifting bees, a common phenomenon in large apiaries where phoretic mites can be swiftly distributed throughout the whole apiary in a short time.

### Conclusion

In apiaries where Varroa mites are resistant, the introduction of integrated programs for resistance management is required. This includes the selection of mite-tolerant bees, monitoring of mite populations, nonchemical control methods, and pesticides rotation, whether natural or synthesized.

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### GENİŞLETİLMİŞ ÖZET

Bal arıları hem arı ürünleri ve hem de tozlaşma ile sağladığı katkılardan dolayı önemli bir konu olarak dünyada ilgi alanı içindedir. Bal arısı kolonilerinde hem ölümlere ve hemde ürün kaybına nedne olan en önemli faktör olarak varroa akarı karşımız çıkmaktadır. Bu yüzden varroa akarına karşı çok çeşitli tedavi ve ilaçlar kullanılmaktadır. Bu ilaçlardan Amitraz (formamidine) octopamine

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alicına bađlanarak nörotoksik etki gösteren bir yapıya sahiptir. Amitraz dünyada yaygın olarak kullanıldığından son yıllarda bu akar ilacına karşı direnç mekanizması rapor edilmiştir. Bazı varroa hatları için ise fluvalinate, flumethrin, coumaphos'a karşı ilaç dirençleri de rapor edilmiştir.

Varroa akarı 1982 yılındaki ilk bulaşımın öğrenilmesinden sonra Cezayir'de arıcılar için önemli bir ilgi konusu olmuştur. Amitraz varroa için Cezayir'de sürekli kullanılan ana kimyasal olduğundan dünyanın bir çok yerinde olduğu gibi direnç gelişimine neden olmuştur. Bu çalışma Cezayir'de Amitraz'a karşı akar popülasyonlarının olabilecek direnç geliřtirmesini arařtırmak için yapılmıştır.

Bu çalışma 2015 Temmuz ayında başlatılmış ve Cezayir'de 4 arılıkta sürekli 3 yıl boyunca Algerias bölgesinde yapılmıştır. Blida bölgesinde 4 arılıkta ise alternative tedavi okzalik asit, timol ve fluvaliante aynı süre için ayrılan zamanda her arılıktan alınan 20 kolonide kullanılmıştır.

Varroa ölümü sadece Amitraz kullanılan arılıklarda Algerias bölgesinde % 39.23 olurken ikinci bir alternative tedavi kullanılan Blida bölgesindeki arılıklarda ise % 87.40 olmuştur. İki varroa ölüm durumları arasında önemli bir fark ( $P>0.05$ ) bulunmuştur. Bu sonuçlar Varroa'nın Amitraz'a olabilecek bir direnç kazandığını göstermektedir. Bu durumda birleşik direnç yönetim programı uygulanması gerekli görülmüştür.