*RESEARCH ARTICLE*

# DETECTION OF DEFORMED WING VIRUS IN THE LOCAL BEE COLONIES *APIS MELLIFERA INTERMISSA* IN ALGERIA AND ITS RELATIONSHIP WITH *VARROA DESTRUCTOR*

## CEZAYİR'DE BULUNAN *APIS MELLIFERA* TÜRÜNÜN LOKAL KOLONİLERİNDE DEFORME KANAT VİRÜSÜNÜN TESPİT EDİLMESİ

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***Summary:*** *Honeybees are threatened by over 18 viruses, nowadays deformed wing virus (DWV) is known to be one of the most prevalent virus worldwide.. This is the first study in Algeria and most North Africa region which is evaluating the prevalence of the DWV in beehives. The objective of this study is to evaluate the impact of this virus on honey bees mortality, and its relationship to infestation with the Varroa destrutor parasitic mite. We conducted this study on an apiary located in the central region of Algeria. PCR results showed the presence of DWV in Apis mellifera intermissa honey bee colonies, where 42% of the samples are infected with the virus. We highlighted the role of V. destructor and its association with the DWV and the mortality recorded in the same apiary.*

***Keywords****: Honey bee, Apis mellifera intermissa, DWV, Varroa destructor, mortality, prevalence, infestation*

***Özet****:Bal arıları günümüzde 18'in üzerinde virus tarafından tehdit edilmekte ve kıvrık kanat virüsü bunlar arasında dünyada en sıklıkla görülen virüslerden bir tanesidir. Bu çalışma, kıvrık kanat virüsünün kovanlardaki yaygınlığı ile ilgili Cezayir'de ve Kuzey Afrika'nın büyük bölümünde gerçekleştirilmiş olan ilk çalışmadır. Çalışmanın amacı bu virüsün bal arıları mortalitesi üzerindeki etkisini ve Varroa destructor parazitik akarı ile ile olan ilişkisini ortaya çıkarmaktır. Çalışma Cezayir'in merkezindeki bir arılıkta gerçekleştirilmiştir. PCR sonuçları, Apis mellifera intermissa bal arısı kolonilerinin %42'sinin bu virus ile enfekte olmuş olduğunu göstermiştir. Bu çalışma ile V. destructor'un kıvrık kanat virüsü ile ve arılıkta görülen ölüm vakaları arasındaki ilişkileri aydınlatmaktadır.*

***Anahtar Kelimeler:*** *Bal arısı, Apis mellifera intermissa, DWV, Varroa destructor, mortalite, prevalens, enfestasyon*

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## Introduction

Bees are essential elements of the environmental balance in the world for its role in the pollination of many plant species. It also important due to its production of honey, propolis, royal jelly,beeswax and bee venom. It is noteworthy that in recent years, impairments of colonies are reported in many countries. This situation leads to winter mortality of bee colonies than normal and population losses during the year and it was named "Colony Collapse Discorder" (CCD). It was estimated that there is a 20% to 30% decline between the years 1997 and 2009 (Genersch et al., 2010). Several studies are being conducted on the causes of these impairments involving a variety of pathogens (Boucher and Desjardin, 2005; Ellis and Munn, 2005; Oldroyd, 2007; Burgett et al. 2009;. Currie et al., 2010.; Guzman-Novoa et al., 2010;. Carreck and Neumann, 2010; Jabaji and Copley, 2012). In Algeria, the phenomena of abnormal mortalities and local bee disappearances are often reported by beekeepers.

Viruses are obligate intracellular parasites. They use the host cell machinery to replicate infested and thereby cause damage. They are composed only of nucleic acid surrounded by a protein capsid. In the bee, about 18 viruses are known so far. With the exception of filamentous virus (FV, filamentous virus) which is considered as a virus genome DNA, all of the honeybee viruses identified so far are small RNA virus positive single-stranded and non-enveloped capsids. The presence of viruses and their relationships with bee mortality is a concern that is being studied worldwide (Benjaddou et al., 2001; Grabensteiner et al., 2000; Bakonyi et al., 2002; Tentcheva et al., 2004, Chen et al., 2005; Genersch and Yue, 2005; Chantawannakul et al., 2006; Siede and Chen, 2007; Weistein et al., 2008; Genersch and Aubert, 2010). Deformed wing virus (DWV) is the virus most often cited in research (Tentcheva et al., 2004; Antunez et al., 2006; Berenyi et al., 2006; Cox-Foster et al., 2007; Gisder et al., 2010, Martin et al., 2010; Zioni et al., 2011). This virus was originally isolated from adults in Japan colonies infested with *V. destructor* (Ball, 1985). It was later identified as a cause of death of bee colonies in many countries (Ball, 1985; Ball, 1987; Bailey and Ball, 1991). This is the most prevalent and most dangerous viruse currently (Kajobe et al., 2010;. Mockel et al., 2011;. Hongxia et al., 2012.). The name given to the virus comes from the characteristic symptom of the deformed wing or poorly developed in newly hatched bees from infected colonies (Ball, 1993). Deformed wing virus affects eggs, larvae, pupae and adult bees (Allen and Ball, 1996). The same virus can be detected in all parts of the body of the bee (Chen et al., 2004. Genersch and Yue, 2005). Nurses infected transmit the virus to young larvae through the larval jelly (Ball, 1987). Adult bees transmit the virus during trophallaxis (Bowen-Walker et al., 1999. Norsdtrom et al., 1999).

Only one study about the DWV and its identification was published in Algerian and most North Africa Region (Loucif-Ayad et al., 2013). Therefore the question about the relationship between DWV, Varroa mite and the mortality recorded had, to answer this question, we conducted this study.

## Materials and Methods

### *Location of study*

Sampling is carried out in an apiary located in the region of Bougara near Blida. Blida region is considered one of the main regions of the honey

### *Biological Material*

The apiary study consisted of 45 colonies. It suffered heavy losses during the winter of 2011-2012. Therefore, at the end of winter, there were only 19 colonies left. The biological material used in this study is represented by adult worker bees *Apis mellifera intermissa* of indeterminate age. The colonies were already treated against *V. destructor* in June 2011 with the amitraz drops in two parts separated by an interval of one week time application.

### *Collection of samples*

The adult bee samples were taken randomly within the colonies stored in RNAlate,in plastic tubes and and stored at –25°C until their use in the detection of the DWV using PCR and biotechnology techniques. A bigger sample of approximately 100 bees from each colony was collected in plastic bags and stored at –25°C until their usage to determine the *Varroa* infestation level.

***Experimental method for estimating the rate of infestation of brood varroa***

A sample of 100 emerging brood cells was kept open in order to determine the level of infestation of the brood to determined the *V. destructor* and its immature stages.

The infestation rate of brood (IRB) is determined by the following equation:

$$\text{I.R.B (\%)} = 100 \times \frac{\text{Number of cells infested with } V.\ destructor}{\text{Number of open cells}}$$

***Experimental method for estimating the rate of infestation of Adult bees with V. destructor***

It was necessary to shake two frames of brood over a plastic film before getting bees in a glass jar material. Alcohol was added and samples were shaken inside the jar for one minute. Then the jar was poured on a double screen. The first screen allowed only the mites without bees. As for the 2nd, it prevented mites to pass. Thorough rinsing with water was carried out on bees. Mites present in the second screen following the previous operation was counted. Once counting mites fact, the percentage of infection is determined by sample (OIE, 2005). It is a rule of three that lets you know the bee infestation rate (B.I.R).

$$\text{B.I.R (\%)} = 100 \times \frac{\text{Number of } V.\ destructor.\ \text{Phoretic found}}{\text{Number of bees collected}}$$

***Methodology of virus detection DWV***

For the analysis of virus, 10-12 bees were taken at random within each sample and were placed in sterile plastic bags using sterile forceps containing 10 ml of phosphate buffer saline (PBS). Bees were overwritten during 2 minutes at high speed in a blender. The resulting homogenate was centrifuged at 1500g or revolutions per minute for 10 minutes. The supernatant was collected and centrifuged again at 12,000 g for 15 minutes. An amount of 140 ml of the supernatant was removed with a view to the extraction of viral RNA. RNA was extracted using a commercial kit produced in Germany: NucleoSpin ® RNA II (ACHEREY-NAGEL) according to the manufacturer's instructions. Reverse transcription of RNA and DNA amplification were performed using a continuous RT-PCR method with Kit (Qiagen) RT-PCR kit according to the manufacturer's process. The primers used to amplify the desired virus are shown in Table 1. The program includes an RT-PCR reverse transcription step at 50°C for 30 minutes, followed by a PCR initial activation phase at 95°C for 15 minutes. This was followed by 40 cycles at 94°C for 1 minute at 55°C for 1 min, and 72°C for 1 minute. An extension step at 72°C for 10 minutes occured. Products were visualized by electrophoresis in 0.6% (weight / volume) agarose stained with ethidium bromide (Gauthier et al., 2007) gels.

## Results and Discussion

The infestation rate of colonies with DWV is given in Table 2. On all 19 hives, 8 are characterized by the presence of DWV virus; in other words 42.1% of samples are contaminated with the virus. The bee viruses usually cause subclinical infections that can not be detected by beekeepers for many years. Being one of the most dangerous viruses, deformed wing virus

***Table 1:*** *Primers used in the detection of DWV*

| Primers | Sequences | Length (bp) | Reference |
|---|---|---|---|
| DWV 1<br>DWV2 | TTTGCAAGATGCTGTATGTG<br>GTCGTGCAGCTCGATAGGAT | 395 | Tentcheva *et al*. (2004) |

*DWV 1 and 2 primers (primers) used in this study, each primer is a short sequence of single-stranded DNA of 20 bases.*
*bp: base pairs*

(DWV) is a positive-strand RNA virus that was originally isolated for the first time from adult bees (*Apis mellifera*) and *Varroa destructor* in Japan (Bailey and Ball, 1991). The main host of DWV is *Apis mellifera*, which is present around the world (Allen and Ball, 1996; Nordstrom et al., 1999. Calderon et al., 2003. Tentcheva et al., 2004. Berenyi et al., 2006. Chantawannakul et al., 2006. Forgach et al., 2008. Sanpa and Chantawannakul, 2009). DWV is already widely studied in recent years (Grabensteiner et al., 2000.. Huang, 2000. Benjeddou et al., 2001; Bakonyi et al., 2002. Tentcheva et al., 2004. Chen et al., 2005. Genersch and Yue, 2005; Chantawannukul et al., 2006. Williams et al., 2009; Martin et al., 2010; Zioni et al., 2011; Yanez et al., 2012). It is the virus most frequently found in the samples analyzed. Its frequency increases in the hives, from spring to autumn. It is closely related to *V. destructor*, which plays both the role of the vector and viral activator. Nearly 98% of the colonies analyzed contain *V. destructor* (Tentcheva et al., 2004).

The present study demonstrates that this is probably one of the causes of the collapse of bee colonies in the apiary studied. The manager of the apiary beekeeper had lost about 58% of his hives, which highlights the negative role played by viruses. Haddad et. al (2008) show through a study that DWV is the most common virus responsible for colony losses in Jordan. The same observation is made in other countries by several studies (Topley et al., 2005. Autunez et al., 2006. Berenyi et al., 2006.). In Switzerland, DWV seems to be the most prevalent virus. It is present in all apiaries (13/13 or 100%) according Dainat et al., (2008) and 65% of the colonies analyzed in 2004 after Berthoud et al., (2005). A 2006 study by Grabensteiner et al., (2007) noted that among the apiaries that have suffered significant losses, ABPV virus (Acute Bee Paralysis Virus, responsible for

***Table 2:*** *Detection of DWV virus in samples of adult bees, and evaluation the rate of infestation of adult bees and brood*

| No. of Colonies | Presence DWV | Rate infestation by Varroa Phoretic | Rate infestation in the brood |
|---|---|---|---|
| 1 | + | 3,58 | 7 |
| 2 | – | 4,65 | 12 |
| 3 | + | 2,80 | 15 |
| 4 | + | 3,67 | 9 |
| 5 | + | 5,35 | 11 |
| 6 | – | 3,35 | 9 |
| 7 | + | 4,66 | 12 |
| 8 | + | 3,25 | 10 |
| 9 | – | 4,90 | 13 |
| 10 | + | 7,55 | 7 |
| 11 | – | 5,60 | 3 |
| 12 | + | 6,45 | 12 |
| 13 | – | 7,85 | 14 |
| 14 | + | 8,1 | 16 |
| 15 | – | 3,26 | 13 |
| 16 | – | 2,85 | 13 |
| 17 | – | 5,10 | 9 |
| 18 | – | 2,05 | 8 |
| 19 | – | 6,85 | 7 |

acute bee paralysis) is detected in 60% of severely infected colonies, while DWV is present in almost all colonies. By cons on 60 samples from colonies having wintered well, none of PWA has virus and 13 with mild infestation DWV. The same is reported in France by the report Tentcheva et al., (2004) which showed that 97 apiaries were infected with DWV. All these studies are recently confirmed by Martin et al., (2012). These authors noted that the association between the virus and *V. destructor* is responsible for the death of thousands of colonies in the region of Hawaii. *V. destructor* is a parasitic mite that feeds on bees as to the state of nymphs than that of adults. It can serve as a vector to transmit the virus (Bailey and Ball, 1991; Bowen-Walker et al., 1999.).

The virus can be transmitted horizontally when the mite feeds on uninfected bees (Bowen-Walker et al., 1999). The second mode of viral transmission is vertical. In fact, when a queen is infected with DWV, it is located in the ovaries and spermatheca. In this case the queen can lay eggs infested. The virus can also be transmitted vertically from infected larvae to direct contact queen (Chen et al., 2006; De Miranda and Fries, 2008).

The analysis of the rate of infestation of colonies by *V. destructor* clearly explains its association with DWV virus and mortalities are recorded at the same apiary. Regarding infestation by *V. destructor* colonies, all colonies were infested with *V. destructor* at different rates. The phoretic *V. destructor* infestation rates were between 2.5 and 7.9% with an average of %4,5 for all colonies. During the phase of phoresy, while feeding on the adult female, *V. destructor* performs regular punctures on the host hemolymph of the parasite. Akimov et al., (1988) indicate that following the period of phoresy the first oviposition of *V. destructor* begins between 5-14 days after the release of the young female of the brood cell where she was born. From the second reproductive cycle, phase of phoresy seems more obligatory (De Ruijter, 1987).

The *V. destructor* infestation rate of brood was between 3 and 16% with an average infestation of 10.9%. Ritter et al., (1984) noted that, in heavily infested colonies with *V. destructor*, workers abandoned the old and heavily infested brood and therefore the brood perished due to cooling caused by a reduction in the strength of the colony. According to two independent studies, the production of brood and bee population were compared between colonies infested with *V. destructor* or not (Downey and Winston, 2001; Maurilhas, 2002). The results state that heavily infested colonies with *V. destructor* had less brood and bees, which corresponds to a general weakening. The high infection rate recorded in this study may be explained by the wrong choice of treatment used by the beekeeper. Amitraz is a drop in non-approved product and it is characterized by its very low efficiency (Adjlane, 2009).

When mite infestation of bees between colonies with or without DWV, is compared, the rates show that the hives infected with the virus had a higher rate of 5.04% against 4.46%. The analysis of variance between the two groups showed no significant difference ($P = 0.21$). Regarding the rate of infestation of the brood, the group of infested colonies with DWV recorded had the highest percentage of 11% against 10.1% for non-infested colonies. ANOVA showed no significant difference between the two groups ($p = 0.56$).

According to Chen et al., (2005) observation of a positive correlation between the level of infestation with *V. destructor* and the level of viral concentration in infested bees would suggest that *V. destructor* plays, in addition to its role as a vector, the activator of viral replication in the honeybee. Parasitism would decrease the immunity of the bee, which would promote viral replication.

The association of DWV with honeybee colonies infested with mites is widely reported (Bailey et al., 1981; Ball and Allen, 1988; Chen et al., 2004). Mortality caused by DWV is often reported as being associated with high rates of infestation with *V. destructor* (Martin et al., 1998; Nordstrom, 2000; Martin, 2001; Santillan–Galicia et al., 2008; Martin et al., 2010). In the presence of *V. destructor* and DWV causes distinctive symptoms, such as malformations of the wings, swelling of the abdomen, reduction of size, emerging bee paralysis and eventually death of bees (Bailey and Ball, 1991 Bowen-Walker et al., 1999. Lanzil et

al., 2006). Viral particles are especially present in the salivary glands of the mite (Cicero and Sammataro, 2010). This is demonstrated for DWV (Bpwen-Walker et al., 1999). Bowen-Walker et al., (1999) concluded from field data that the mite is a very efficient vector of the virus. Dainat et al., (2008) reported that when DWV performs a host change, that is to say, bees infected with *V. destructor* become more virulent. There is no systematic correlation between the infection rate by the presence of *V. destructor* and DWV virus (Highifiel et al., 2009)

## Conclusion

The present study confirms the presence of DWV in Algeria and to points out the relationship between DWV, varroa infestation and hive mortality which is estimated to be approximately %60. Therefore, an epidemiological survey on DWV and other viruses in relation to *V. destructor* covering most of the country is necessary to show the spatial and temporal dynamics of the distribution of the virus.

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