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Survey of honeybee viruses in Syria

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Abstract

Beekeepers in Syria have reported higher-than-usual colony losses in the last 8 years. These elevated losses average is more than 20% nationally. This study aimed to detect seven honeybee viruses in some provinces in Syria. RT-PCR was used in 240 Samples, which collected from eight provinces. It is shown that there is presence of four-honey bee's virus (*Deformed wing virus* DWV, *Acute bee paralysis virus* ABPV, *Chronic bee paralysis virus* CBPV and *Sacbrood virus* SBV). The single viral infection rates were 100% (DWV), 18.89% (ABPV), 5.56% (CBPV) and 13.33% (SBV). DWV positivity prevalence in all studied regions, while the ABPV prevalence in four regions, and both CBPV and SBV prevalence in only two regions. This study is the first report of presence CBPV and SBV in Syria and adding a new recording of the ABPV in a new region.

Keywords: Honeybee viruses, RT-PCR, CCD

Introduction

Beekeepers in Syria have lost a lot of colonies abnormally during the last 8 years. These elevated losses average is more than 20%, nationally (Barhoum et al, 2017). In fact, colony losses were caused by many reasons reported, but the worst reason has been called "Colony Collapse Disorder" (CCD). Some beekeepers in the USA reporting CCD have lost 50-90% of their colonies (Van-Engelsdorp et al, 2007; 2008). Since 1963, twenty-four viruses have been identified and characterized from the genus *Apis*. According to the special correlation among some of these viruses, they are considered as a single species complex, so a total number of viruses were reduced to around 16-18 viruses (Ball and Bailey, 1991; de Mirand et al., 2013). Most of the honeybee viruses have been associated with a presence of *Varroa* mite (*Varroa destructor*), which is considered as a biological vector (Kevan et al., 2006). Recently some researchers have indicated that the viral diseases are responsible for CCD. Palacios et al. (2008) and Cox-foster et al. (2007) have considered that the *I. acute paralysis virus* (IAPV) is the pathogen responsible for

CCD, or at least as a co-assistant to those losses. On other hand, de Miranda et al. (2010) have declared a difficulty in confirming that IAPV is for sure the responsible virus for CCD, because there is a strong connection between IAPV and both of *Acute bee paralysis virus* (ABPV) and *Kashmir bee virus* (KBV). This connection is characterized by a high similarity and difficult to identify and distinguish each one from other one (Palacios et al., 2008).

Haddad et al. (2008) reported that three honeybee viruses (ABPV, *Sacbrood virus* (SBV) & *Deformed wing virus* (DWV)) were spread in Jordan, also Gulmez et al. (2009) and Gumusova et al. (2010) detected DWV, *Chronic bee paralysis virus* (CBPV) and *Black queen cell virus* (BQCV) in Turkey. In Syria, the presences of (DWV) and ABPV have been proved earlier (Mouhanna, 2016; Barhoum et al., 2017). The DWV has been recorded on four regions (Damascus, Homs, Tartous and Lattakia), whereas ABPV has been just recorded in Damascus, Homs and Hama regions. As a result of the decline in the number of colonies of bees in Syria and clearly observed the symptoms of infection of honeybee viruses by the beekeepers. We aimed in this study to survey and



detect of seven honeybee viruses in some provinces in Syria.

Material and Method

Samples collection

240 Samples were collected from stationary colonies in a selected apiaries from 8 Provinces (Damascus, Rif-Dimashq, Quneitra, Homs, Hama, As-Suwayda, Tartous and Lattakia) (Fig 1). These Samples were included just worker bees, pupae and larvae (drones and queen not included in this study) carrying one symptom at least such as deformation of the wings, abnormal trembling and paralyzing of wings and bodies, blackening, hairless, balding, dead insects, pupae and larvae, bees fail to fly and often crawl on the ground and up the stems of grass, dead bees in front of the cell in addition to apparently healthy bees. Samples were placed in tubes (15 ml) and immersed in nitrogen liquid to be used later on.

RNA Extraction

The QIAamp® Viral RNA Mini Kit (Qiagen, Germany) was used, and 15 insects were selected randomly from each sample, then grinded with mortar and pestle in nitrogen liquid, added 1ml of Phosphate-buffered Saline (PBS) then centrifuged at 5000 rpm for 15 min, after that, a 140µl had been taken and used to viral RNA extraction according to manufacturer's instructions. RNA viral was stored at -70 ° C until used.

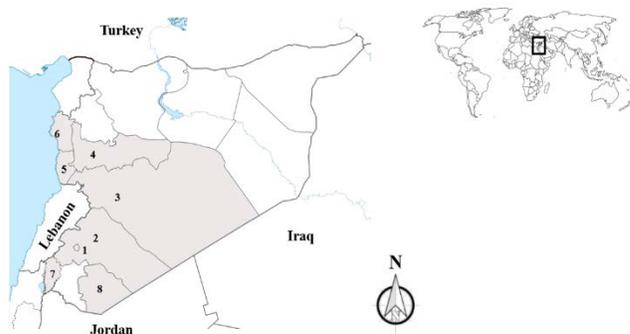


Figure - 1: Locations of samples
1: Damascus, 2: Rif-Dimashq, 3: Homs, 4: Hama, 5: Tartous, 6: Lattakia, 7: Quneitra, 8: As-Suwayda

RT-PCR to detect viruses

Reverse transcriptase was performed with the one-step RT-PCR kit (Qiagen, Germany), by adding 2.5µl viral RNA sample to a final 50µl reaction mixture (manufacturer's guidelines 30 m/50°C reverse transcription; 15 m/95°C denaturation and HotStarTaq activation, and PCR conditions were 35 cycles of amplification with 30s/94 °C; 45s/ 60°C; 1 m/ 72 °C and a final elongation step for 7 m/ 72°C). Specific primers had been used to detect seven honeybee viruses were obtained from Alpha DNA (Table1).The RT-PCR products were electrophoresed on a 1.5% TAE agarose gel and then stained with ethidium bromide, and 100bp ladder was used as a reference for fragment sizes. Bands were photographed by a MicroDOC System with UV Tran's illuminator (Clever Scientific Ltd).

Table - 1: Primers used for the detection of seven bee viruses.

Virus	Sequence 5' → 3'	Length (bp)	Position in the genome (nt)	Reference
ABPV	CATATTGGCGAGCCACTATG CCACTTCCACACAACCTATCG	398	8115-8513	Bakonyi et al., 2002
BQCV	GGAGATGTATGCGCTTTATCGAG CACCAACCGCATAATAGCGATTG	316	7882-8198	Topley et al.,2005
CBPV	AGTTGTCATGGTTAACAGGATACGAG TCTAATCTTAGCACGAAAGCCGAG	455	2580-3035	Welch et al., 2009
DWV	TTTGCAAGATGCTGTATGTGG GTCGTGCAGCTCGATAGGAT	395	8561-8956	Tentcheva et al., 2004
KBV	GATGAACGTCGACCTATTGAA TGTGGGTTGGCTATGAGTTCA	393	5406-5799	Stoltz et al., 1995
SBV	GGATGAAAGGAAATTACCAG CCACTAGGTGATCCACACT	426	7747-8173	Tentcheva et al., 2004
IAPV	AGACACCAATCACGGACCTCAC AGATTTGTCTGTCTCCCAGTGCACAT	475	8860-9335	Maori et al., 2007

Results

Symptoms of viral infection of honeybee were not often apparent at the colony level until the virus concentration rises to high levels. Nevertheless, it has a negative impact on all bee products. During the visits to the apiaries, many symptoms observed at the individual level. Figure 2 showed some of these symptoms, A) Dead and black color pupae with deformation of the wings. B) Crawler bee, abnormal trembling and paralyzing of wings and bodies. C) Dead larvae, look like a distended sac containing a fluid with a thin cuticle wall.

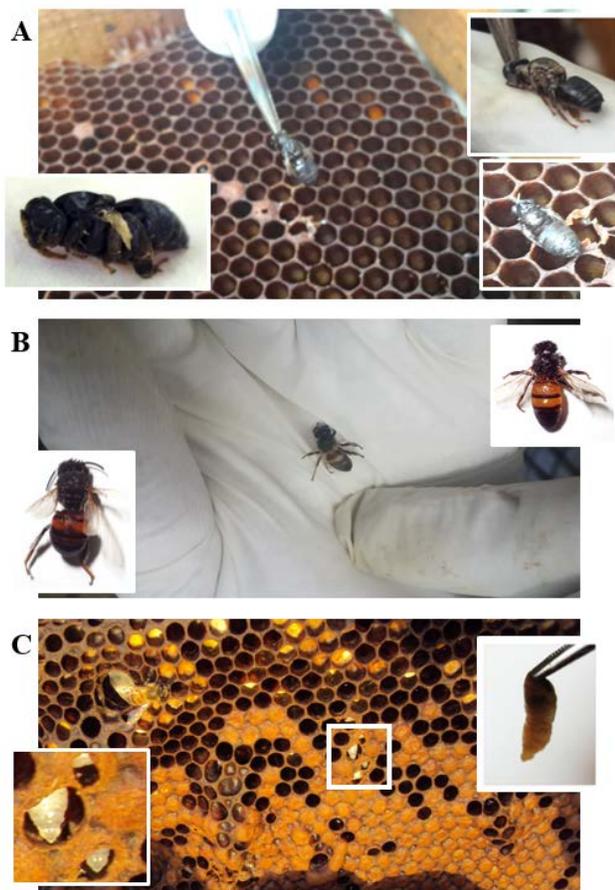


Figure - 2: Some symptoms of honeybee viruses

In this study a 240 samples were examined for seven honeybee viruses. Moreover, the RT-PCR products showed the presence of four honey bee's virus: DWV, ABPV, CBPV and SBV as we see in (Fig 3).

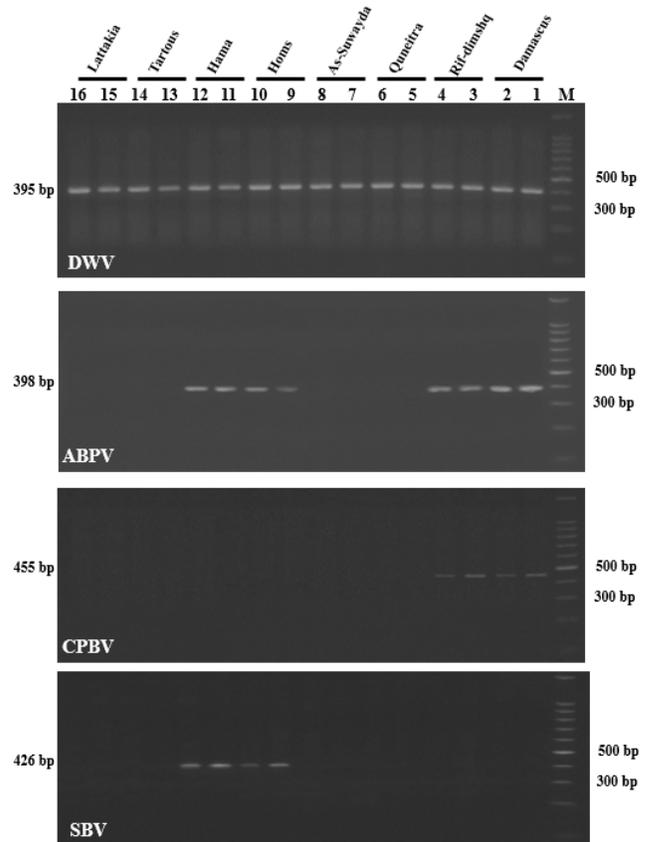


Figure - 3: Detection of DWV, ABPV, CBPV and SBV in honeybee by RT-PCR

Results showed that the single viral infection rates were 100% (DWV), 18.89% (ABPV), 5.56% (CBPV) and 13.33% (SBV). While the highest rates of dual viral infection were 18.89% (DWV+ABPV) and 13.33% (DWV+SBV) and lower rate when the infection occurred by (ABPV+CBPV) with 2.78% value, otherwise not dual infection occurred by (CBPV+SBV). For triple viral infection of (DWV+ABPV+CBPV) the rate was 8.33% (Fig 4).

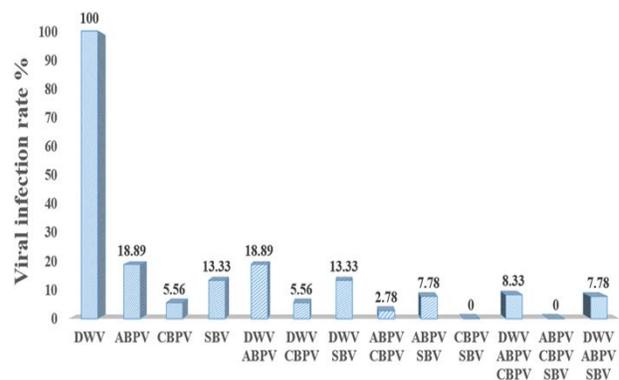


Figure - 4: Infection rates of honeybee viruses

The obtained data revealed positive prevalence of DWV in all studied region, the ABPV prevalence was detected in four regions (Damascus, Rif-Dimashq, Homs & Hama), the CBPV was notified in two regions Damascus and Rif-Dimashq, and SBV was presented only in Homs & Hama (Table 2).

Table – 1: Prevalence of honeybee viruses in Syria.

Regions	Honeybee’s viruses			
	DWV	ABPV	CBPV	SBV
Damascus	+	+	+	
Rif Dimashq	+	+	+	
Quneitra	+			
As-Suwayda	+			
Homs	+	+		+
Hama	+	+		+
Tartous	+			
Lattakia	+			

Discussion

The apiculture does not has any significant economy roles as supposed to be in agriculture field even for that last 20 years, and for years and years, all the bees colonies created to produce the honey, wax, pollen, and royal food, without recognizing the valuable and essential role in the pollination of cultivated and natural plants, which is the worth part of the food chain (Morse, 1997). The honeybee colonies losses worldwide increase the interest in bee pathology especially the viral diseases, which is considered a major economic in apiculture.

In the recent years, a significant evolution has occurred in the diagnosis method for honeybee viruses. It has changed from serological to molecular DNA tests (PCR-based method) (Berenyi et al. 2006; De Miranda et Al., 2010). The RT-PCR is considered as a good method and many researchers have used and developed it for the detection of specific honey bee viruses (Tentcheva et al., 2004; Maori et al., 2007; Welch et al., 2009). This technique allows rapid analysis of large samples number while maintaining a high degree of both sensitivity and specificity (Freeman et al., 1999; Bustin and Nolan, 2004).

In this research, we depended on the molecular-genetic evidence of seven viruses in the bee samples collected throughout Syria between 2014-2016, and we identified by RT-PCR four out of seven viruses . DWV has the highest viral infection rate value of

100% and recorded on all regions studied, a previous study has indicated that DWV is widespread among bee colonies in Syria (Mouhanna and Barhoum, 2016). DWV has previously detected in Turkey (Gulmez et al. 2009) and Jordan (Haddad et al. 2009).Several studies have declared that DWV is likely the responsible of CCD (Nordstrom et al., 1999), whilst others noted that DWV is a secondary pathogen (Bowen-Walker and Martin, 1999; Tentcheva et al., 2004). ABVP was spreads in four provinces (Damascus, Rif-Dimashq, Homs and Hama) and the viral infection rate was 18.89%. Mouhanna (2016) has confirmed our finding that ABPV was spread in three regions (Damascus, Homs and Hama). ABPV and SBV were recorded in Jordan (Haddad et el. 2009), whereas just CBPV was recorded in Turkey (Gumusova et al. 2010). The dual viral infection was the highest level (18.89%) by DWV and ABPV, whereas the triple viral infection was (8.33%) by infections with DWV, ABPV and CBPV. The results showed a different occurrence of honeybee viruses in the studied regions (Table 2), which are probably caused by climate differences and lack of clean equipment used, which play a major role in the spread of honeybee viruses. Therefore, preventive healthcare should be considered by beekeepers because several studies have confirmed that honeybee viruses are often cause latent symptoms in the infected colonies (Nordstom et al., 1999; Ribie`re et al. 2000; Bakonyi et al., 2002; Gauthier et al., 2004).

In conclusion, these results are the first report of presence CBPV and SBV in Syria and add a new record of the ABPV in a new region. This study reveals that the RT-PCR is a simple, rapid, and specific genetic marker for studying of honeybees viruses.

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