Detection of Sac Brood and Black Queen Cell Viruses from Honeybee Colonies of Jordan Using RT- PCR Technique

Amal A. Al-Abbadi [1*], Dhia S. Hassawi [2], Saida A. Abu-Mallouh [3] and Maher Obeidat [4]

ABSTRACT

The Reverse Transcription-polymerase Chain Reaction (RT-PCR) is an excellent technique for the detection of honeybee viruses. In this study, the presence of Sac Brood Virus (SBV) and Black Queen Cell Virus (BQCV) was demonstrated in 100 samples, collected from Jordanian honeybee colonies by employing RT-PCR. The collected samples represented infected (depopulation, paralysis, or dark coloring), dead, and apparently healthy honeybees of different developmental stages (adult and larvae). SBV was detected in 37% of the samples, whereas BQCV was detected in 5%. Nucleotide sequences of the PCR products from each virus was determined and found to be 433 and 309 nucleotides in SBV and BQCV, respectively. The identities to the Gene Bank were 95% for SBV and 91% for BQCV. This is the first record of BQCV in Jordan.

Keywords: *Apis mellifera*, Honeybee Viruses, Sac Brood Virus, Black Queen Cell Virus, RT-PCR.

INTRODUCTION

The native honeybee of Jordan (*Apis mellifera syriaca*) is prevailing in the east of Mediterranean region (Jordan, Palestine, Syria, and Lebanon) (Zaitoun *et al.* 2008). This honeybee is characterized by a bright yellow color, small size, and construction of several swarm cells. About 400000 honeybee colonies and 1000 beekeepers are existed in Jordan and the annual honey production reached about 200 tons (Agricultural Statistical Year Report, 2008). The honeybee has long been important for honey production and for the pollination of crops like all living organisms, honeybees can be infested with pests. Some of these are more deleterious to bee colonies than others; it is important for the beekeeper to be able to recognize the conditions that might be pests related and respond accordingly (Sanford, 2003).

Viral diseases of honeybee are of major economic consideration in apiculture. They have become increasingly important to the honeybee keepers and all related agricultural industries. At least 18 different viruses distributed worldwide have been detected in honeybees so far (Allen and Ball 1996), and the complete genome sequences of some honeybee viruses, namely Acute Bee Paralysis Virus (ABPV) (Govan *et al.* 2000), Black Queen Cell Virus (BQCV) (Leat *et al.* 2000), Sac Brood Virus (SBV) (Ghosh *et al.* 1999), Deformed Wing Virus (DWV) (Lanzi *et al.,* 2006), Kashmir bee virus (KBV) (de Miranda *et al.*, 2004), and

[1*]Corresponding author, Department of Plant Production and Protection, Faculty of Agricultural Technology, Al-Balqa' Applied University, Al-Salt, 19117 Jordan (E-mail: honeyqueen25@yahoo.com).
[2]Department of Biotechnology, Faculty of Agricultural Technology, Al-Balqa' Applied University, Al-Salt, 19117 Jordan (E-mail: dhassawi@yahoo.com).
[3]Department of Biotechnology, Faculty of Agricultural Technology, Al-Balqa' Applied University, Al-Salt, 19117 Jordan (E-mail: saida387439@yahoo.com).
[4]Department of Biotechnology, Faculty of Agricultural Technology, Al-Balqa' Applied University, Al-Salt, 19117 Jordan (E-mail: Obeidatgh@yahoo.com).
Received on 8/3/2011 and Accepted for Publication on 8/8/2012.

Israel Acute Bee Paralysis Virus (IAPV) (Maori *et al.*, 2007) have been determined. These viruses together with pollution and the use of insecticides resulting in high honeybee mortality rates pose a real threat to these industries (Morse and Calderone, 2000 and Spira, 2001).

SBV is so called due to the sac like appearance of the diseased larvae. It is a contagious disease that attacks the larvae of the brood and reduces their number, thus eventually reducing the population of adult bees. SBV can also affect adults, a characteristic which enables the virus to persist in bee colonies from year to year. SBV occurs most frequently in spring, when the colony is growing most rapidly and large numbers of susceptible larvae and young adults are available. SBV has a single positive strand RNA genome consisting of 8832 nucleotides (Ghosh *et al.*, 1999).

BQCV of *Apis mellifera* queen brood is caused by the black queen cell virus. The queen dies in the prepupal or pupal stage, and the dead brood changes the cell wall of the queen cell to dark brown or black. BQCV has a single positive strand RNA genome consisting of 8550 nucleotides excluding the poly (A) tail. It was classified within the *Picornavirales* order by the International Committee on Taxonomy of Viruses (http://www.ictvonline.org/virusTaxonomy.asp?version= 2009). There is no record for the presence of this Virus in Jordan till now.

The identification of bee viruses is of considerable importance, particularly when considering the lack of information on the natural incidence of virus infections in honeybee populations worldwide. Developing a sensitive diagnostic technique would help to identify viruses present in bees under natural conditions, and could be used to screen virus preparations, employed in research, to ensure that they are free of other contaminant viruses (Davison *et al.*, 2003).

The presence of bee viruses has traditionally been detected using ELISA and more recently, scientific interest has turned towards molecular techniques using PCR methods (Berenyi *et al.* 2006). Complete or partial sequencing of several RNA viruses of the honeybee has allowed the development of highly sensitive methods for viral detection, based on the amplification by reverse transcription-polymerase chain reaction (RT-PCR). This technique is considered as an excellent alternative for the detection of specific viral sequences. Several RT-PCR methods have been developed and applied for the diagnosis of different honeybee virus infections (Benjeddou *et al.*, 2001; Grabensteiner *et al.*, 2001; Ribière *et al.*, 2002; Tentcheva *et al.*, 2004; Chen *et al.*, 2004; Chen *et al.*, 2005; Topley *et al.*, 2005; Ward *et al.*, 2005; Chantawannakul *et al.*, 2006). The occurrence of IAPV, KBV, CBV and ABPV viruses have been proved in Jordan by Al- Abbadi et al, 2010a,b. ABPV, DWV, and SBV viruses were detected only in Ajloun area by Haddad *et al.*, 2008. This study aimed to detect the presence and frequency of two economically important honeybee viruses, SBV and BQCV viruses throughout Jordan using RT-PCR assay.

MATERIALS AND METHODS:

One hundred samples were collected from 45 colonies of ten different regions throughout Jordan. The randomly collected samples represent infected, dead, and apparently healthy honeybees of different developmental stages (larvae and adults). Each sample was consisted from 15 – 20 bees or larvae. The samples were kept in a separate test tube and stored at -80 °C until investigation. The frozen samples were homogenized in liquid nitrogen, and the RNA was then extracted by employing EZ10 spin column total RNA Minipreps Super Kit (BioBasic) according to the manufactures instructions.

The extracted RNA was used in the RT-PCR testes. For each test of SBV and BQCV, ten samples from each

of the ten different regions were used. Amplification was carried out in a total volume of 25 µl, where 1 µl of template RNA was added to 24 µl of the master mix that containing: 5 units of reverse transcriptase, 0.2 mM of dNTPs, 1 mM MgSO4, 5µl of 5X RT-PCR buffer, 5 units of Tfi polymerase, 1.0 µl of 10 µM of each forward and reverse primer. The SBV primer pair was SBVF (5'-GTGGCGCGCCCATTACTGTAGTGI-3') and SBVR (5'-CTCGACAATTCTCCCTAGTAGCCI-3') corresponding to nucleotides 8169-8191 and 8581-8603, respectively. The BQCV primer pair was BQCVF (5-'GGAGATGTATGCGCTTTATCGAG-3') and BQCVR (5'CACCAACCGCATAATAGCGATTG3'), corresponding to nucleotides 7882-7904 and 8176-8198, respectively (Benjeddou *et al.*, 2001). Doubled distilled water was added to the final volume.

Reverse transcription and amplification were conducted with a continuous RT-PCR method using the following program: RT for 45 min at 46 °C followed by one cycle of 2 min at 94°C, followed by 40 cycles of: 30 s at 94°C, 30 s at (56°C for SBV and 58°C for BQCV), and 45 s at 72°C, followed by 5 min at 72°C for final extension. The reactions samples were detected by agarose gel electrophoresis using 10µl of the PCR products, staining by ethidium bromide, and photographing under UV light.

The specificity of the RT-PCR assay was confirmed by sequence analysis. Samples from the PCR products were sent to Macrogen, Seoul, in South Korea for sequencing. Analysis of the sequence data of each virus fragment was performed using the DNAMAN ™ software (Iynnon Biosoft, Quebec, Canada, version 5.2.9) and the BLAST service provided by the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/BLAST/).

The amplicons were sequenced without sub cloning, and the sequences were compared with the complete genome sequence of both SBV and BQCV which are available in the gene bank to approve the presence of these viruses in Jordan.

RESULTS:

A total of 100 honeybee samples originating from different regions throughout Jordan, were investigated by RT-PCR for the presence of two important honeybee viruses (SBV and BQCV). The two bee viruses were detected in samples, but the frequencies varied; SBV was found to be the most prevalent, present in 37% of samples and detected in seven different regions including Irbid, Ajloun, Al-Salt, Amman, Al-Ghour, Al-Karak, and Al-Tafela. BQCV exhibited a prevalence of 5%, and it was present in samples from only two regions Irbid and Ajloun. However, samples collected from three regions Al-Mafraq, Jarash, and Humrat Al-sahen did not contain any of the two investigated viruses.

Simultaneous multiple infections have been detected in some colonies in two regions Irbid and Ajloun; the samples containing the two viruses at the same time represented 3% of the cases (Table1). The results also showed that high percentage of infection with SBV (40%) was found in adult bees in comparison with 31.4% in larval stage, while BQCV was found in 6.2% of adult bees in comparison with 2.9% in larval stage.

The main aim of this study was to detect SBV and BQCV on molecular level in samples of adults and larval honeybees. Two SBV and BQCV-specific primers were used to amplify different regions in the genome of the two viruses. Some samples tested positive in RT-PCR assays. PCR products of the expected sizes were observed as clear electrophoretic bands. In figure 1a, Lanes 2, 3, 5, 8, 9, and 10 shows that primer pair (SBVF, SBVR) amplified a fragment of 433 bp from the SBV genome. Figure 1b, (lanes 5, 6, and 9) shows that primer pair (BQCVF, BQCVR) amplified a fragment of 309 bp

from the BQCV genome.

Sequence similarity for SBV and BQCV was determined by alignment with the Genbank sequence as a reference. Figures 2, 3 and 4 present the results of this analysis and show all the nucleotide alignments for each region. Nucleotide sequence identities were 95% for SBV and 91% for BQCV, and deposited in the GenBank database under accession number FJ263282 for SBV and under accession number GU459315 for BQCV (http: //www.ncbi.nlm.nih.gov/Genbank/index.html). The 95% homology and the 5% divergence for SBV were found throughout the region amplified with primer pair (SBVF, SBVR) (nt 8169 to 8603). The 91% homology and the 9% divergence for BQCV were found throughout the region amplified with primer pair (BQCVF, BQCVR) (nt 7882 to 8198).

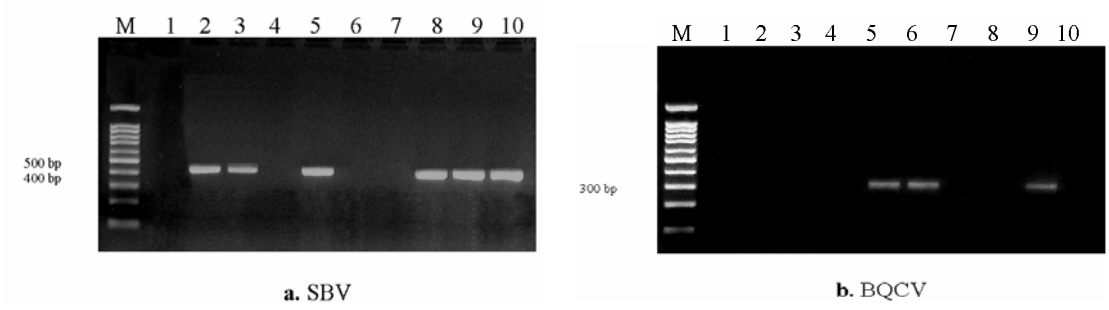

Figure 1. Agarose gel electrophoresis for detection of a. SBV and b. BQCV by RT-PCR in honeybee samples that collected from different regions, Where M: 100bp marker, lanes 1-10: adults and/or larval honeybee samples corresponded to that in table 1.

```
GTGGCGCGCC CATTACTGTA GTGGTAAATA CCCCCATTGG TAATTATATA TTTCGTAGCT
TGGGAGACGT TGGTAGGGAG TAAAGAAAGA GGGCAAACTT GGGAATCCTT CAAACAAAAT
GTAGAGTTAT TTTGCTACGG TGATGATTTG ATAATGTCAG TAACAGATAA ATATAAGGAT
GTTTTTAATG CATTAACAAT AAGTCAATTT TTGGCACAAT ATGGAATAGT AGCTACCGAT
GCGAATAAAG GAGATGAGGT TGAGGCTTAT ACAACGTTAT TAAATAGTAC GTTTTTAAAA
CATGGGTTTC GTCCTCACGA AGTGTATCCG CATTTGTGGC AATCTGCGCT GGCTTGGAGT
TCTATTAACG ATACTACGCA ATGGATATGG GAGTGTGCTG ATTTGAAGCC GGCTACTAGG
GAGAATTGTC GAG
```

Figure 2. Nucleotide sequences (433 bp) of SBV that deposited in the GenBank database under accession number FJ263282.

```
GAGATGTATG CGCTTTATCG GGTGGAGTTC GAGTTAAGTA GATAGTGAGA AGGGTAGATT TCGTAGAGCT
ACGTGAGTCC TAACATACTT ACGGATGTGA TGCCGCTCCT ACGACTCATA TCAGTACTCC TTTGGCAATA
GAACAGATAC CTATAAAGGG AGTCGCAGAG TTCCAAATAC CGTACTATGC TCCATGTTTA TCATCTTCGT
TTACAGCGAA TTCGGAAACA TTTTATTATA GTTCAGGTCG GAATAATCTC CATATAGCCG CTTCACCTCC
AACCGCCAAT CGCTATTATG
CGGTTGGTG
```

Figure 3. Nucleotide sequences (309 bp) of BQCV that deposited in the GenBank database under accession number GU459315.

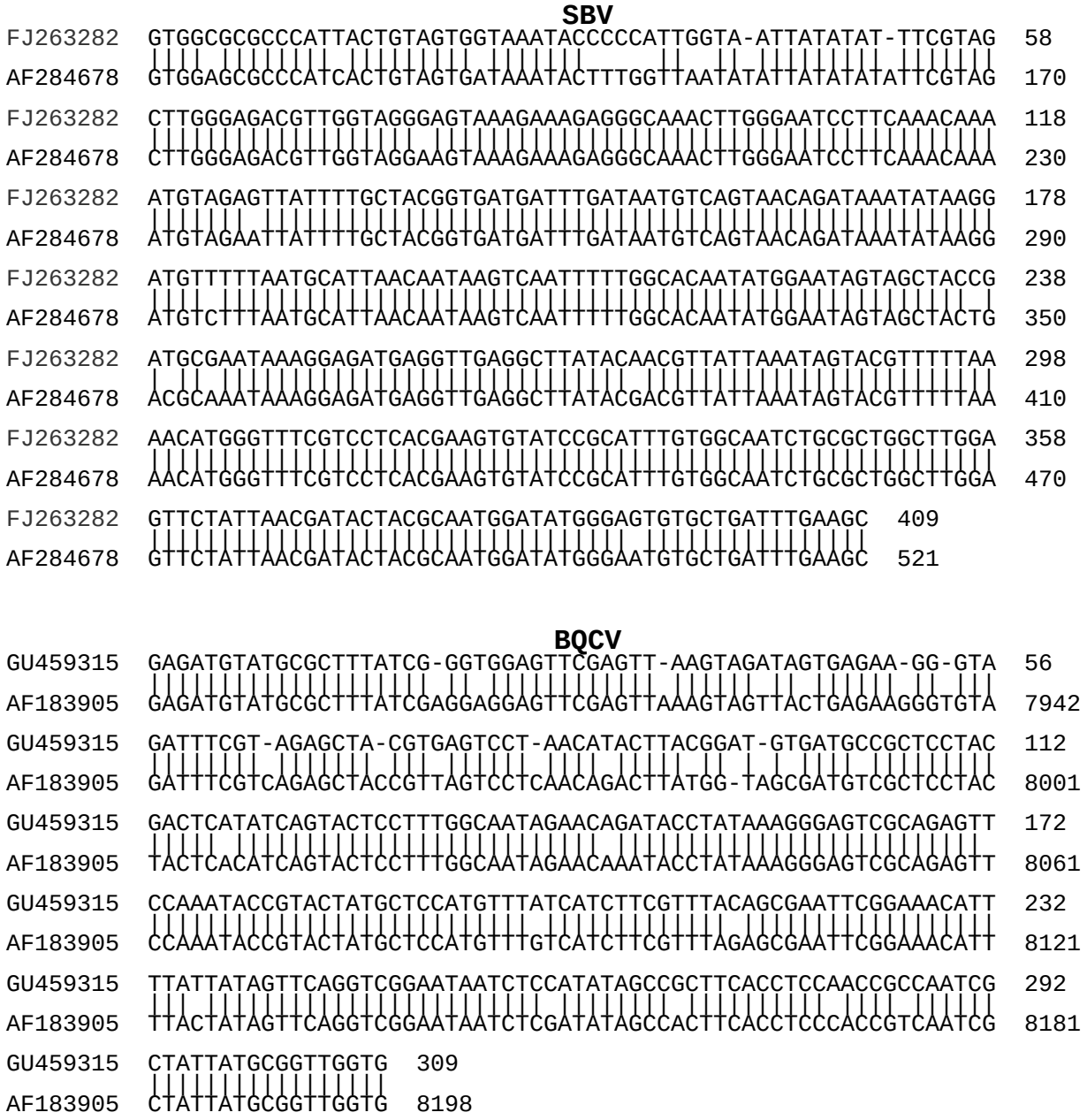

Figure 4. Alignment of the nucleotide sequences for BQCV and SBV that deposited in the GenBank database under accession number GU459315 and FJ263282 respectively.

Table 1. The presence of SBV and BQCV in adults and/or larval of honeybee samples that collected from the different regions of Jordan.

Sample no.	Irbid		Al Mafraq		Ajloun		Jarash		Al Salt		Amman		Homrat Asahen		Al Ghour		Al Karak		Al Tafela	
	SBV	BQCV	SBV	BQCV	SBV	BQCV	SBV	BQCV	SBV	BQCV	SBV	BQCV	SBV	BQCV	SBV	BQCV	SBV	BQCV	SBV	BQCV
1	+ L	- L	- L	- L	+ L	+ L	- L	- L	- L	- L	+ L	- L	- L	- L	- L	- L	- L	- L	- L	- L
2	+ L	- L	- L	- L	+ L	- L	- L	- L	- L	- L	- L	- L	- L	- L	- L	- L	- L	- L	+ L	- L
3	- L	- L	- L	- L	+ L	- L	- L	- L	+ L	- L	- L	- L	- L	- L	- A	- A	- L	- L	+ L	- L
4	- A	- A	- A	- A	+ L	- L	- L	- L	+ A	- A	- L	- L	- A	- A	+ A	- A	- A	- A	- L	- L
5	- A	+ A	- A	- A	- A	- A	- A	- A	+ A	- A	+ A	- A	- A	- A	+ A	- A	- A	- A	+ L	- L
6	+ A	+ A	- A	- A	+ A	+ A	- A	- A	+ A	- A	+ A	- A	- A	- A	- A	- A	+ A	- A	- L	- L
7	- A	- A	- A	- A	+ A	- A	- A	- A	+ A	- A	+ A	- A	- A	- A	- A	- A	- A	- A	- A	- A
8	- A	- A	- A	- A	+ A	- A	- A	- A	+ A	- A	+ A	- A	- A	- A	- A	- A	- A	- A	+ A	- A
9	- A	+ A	- A	- A	+ A	- A	- A	- A	+ A	- A	+ A	- A	- A	-A	- A	- A	- A	- A	+ A	- A
10	+ A	- A	- A	- A	+ A	- A	- A	- A	+ A	- A	+ A	- A	- A	- A	- A	- A	- A	- A	+ A	- A

L: Larval stage
A: Adult stage
(+): Infected sample with virus
(-): Healthy sample

DISCUSSION:

Viral diseases of bees (*Apis mellifera*) are a major economic consideration in apiculture. In this study, the occurrence of SBV and BQCV was investigated in bee samples collected from different geographic regions in Jordan. Clear differences were identified in the distribution pattern of the two viruses. These differences may be partly explained by differences in climate and density of the bee populations; however, contaminated equipment, and bee products between apiaries, regions, may be of greater importance in the spread of viruses. Therefore, virological and parasitological investigations should be considered by beekeepers before they purchase and import possibly infected bees and bee products. Several studies established that bee viruses are widespread and often cause inapparent, multiple infections in seemingly healthy bee colonies (Allen and Ball, 1996; Nordstom *et al.*, 1999; Ribie`re *et al.* 2000; Bakonyi *et al.*, 2002; Gauthier *et al.*, 2004).

Due to difficulties with the classical diagnostic methods in bee virology, scientific interest has turned towards molecular techniques. In the last few years the diagnostic methods employed for the investigation of viruses in honeybee samples changed to PCR-based techniques (Benjeddou *et al.*, 2001; Grabensteiner *et al.*, 2001; Ribière *et al.*, 2002; Tentcheva *et al.*, 2004; Chen *et al.*, 2004; Chen *et al.*, 2005; Topley *et al.*, 2005). The results of this study provide evidence for the first time for the existence of BQCV in Jordan; the results also confirmed the existence of SBV in seven regions; Haddad *et al.*, 2008 mentioned the existing of SBV in Jordan in one region (Ajloun) only. The RT-PCR assay used in this study could become a standard method in the health certification for honeybee imports and exports, and in the screening of virus preparations used in research.

Our findings indicating the co-existence of the two bee viruses (SBV and BQCV) in a single bee colony; this agrees with the findings of (Hung *et al.*, 1996; Tentcheva *et al.*, 2004; Chantawannakul *et al.*, 2006). The question raised is how the viruses may compete or suppress each other to exhibit their abilities to transmit and cause disease in honeybees. The simultaneous detection of BQCV and SBV is not unusual; it was mentioned by other researchers such as (Topley *et al.*, 2005; Leat *et al.*, 2000). The later one mentioned that the simultaneous infection of these two viruses could be due to their wide distribution rather than a specific relationship between them.

Sac brood is a condition affecting the brood of the honeybee, resulting in larval death (Grabensteiner *et al.*, 2001); these dead larvae are thrown away by the cleaning activity of honeybee workers. For that the highest percentage of infection that was found in adult bee in comparison with larval ones, it is worthy to

mention that SBV may affect the adult bee, and the viruses are able to propagate inside them, but in this case obvious signs of disease are lacking (apparently healthy). BQCV is common in adult bees; however, it clinically affects mainly prepupae or pupae of the honeybee queen (Berényi, *et al.*, 2006).

Since the complete genome sequence information of SBV and BQCV is available in gene bank databases, random samples of the PCR product for each pair of the primers used in this study were sequenced. The sequence analysis proved that PCR fragments representing SBV and BQCV viruses matched the corresponding virus sequences published in the GenBank, indicating that amplification bands in this study were virus specific. This study also shows that RT-PCR is a powerful tool for studying virus infection of honeybees. It proved to be a rapid, specific, and sensitive diagnostic tool for the direct detection of SBV and BQCV nucleic acid in samples of honeybees and brood regardless of geographic region. The emergence of these viruses in certain areas of Jordan is probably due to the result of trade and exchange of infected bees, contaminated equipment, and bee products between regions.

ACKNOWLEDGEMENTS

The authors would like thank Al-Balqa Applied University for the financial support of this study and the beekeepers for their cooperation. Special thank to Mr. Sufian Al-Hanbali for his help in sample collection.

REFERENCES

Agricultural Statistical Year Report 2008: Ministry of Agriculture, Jordan, Amman.

Al- Abbadi A, Hassawi D and Abu-Mallouh S. 2010a. Detection of chronic and acute bee paralysis viruses from Jordanian honeybee apiaries by reverse transcriptase PCR. ***Journal of Food, Agriculture & Environment*** **8** (3&4): 1016-1019.

Al- Abbadi A, Hassawi D, Abu-Mallouh S and Al-Mazra'awi M. 2010b. Novel detection of Israel acute paralysis virus and Kashmir bee virus from honeybees *Apis mellifera* L. (Hymenoptera: Apidae) of Jordan using reverse transcriptase PCR technique**. *J. Applied Entomology and Zoology*** **45** (1): 183 – 190.

Allen M and Ball B. 1996. The incidence and world distribution of honeybee viruses. ***Bee World*** **77**: 141-162.

Bakonyi T, Farkas R, Szendroi A, Dobos-Kovacs M and Rusvai M. 2002. Detection of acute bee paralysis virus by RT-PCR in honeybee and varroa destructor field samples: screening of representative Hungarian apiaries. ***Apidologie*** **33**: 63–74.

Benjeddou M, Leat N, Allsopp M and Davison S. 2001. Detection of acute bee paralysis virus and black queen cell virus from honeybees by reverse transcriptase PCR. ***Applied and Environal Microbiology*** **67**: 2384–2387.

Berényi O, Bakonyi T, Derakhshifar I, Kőglberger H and Nowotny H. 2006. Occurrence of six honeybee viruses in diseased Austrian apiaries. ***Applied and Environal Microbiology*** **72**(4): 2414–2420.

Chantawannakul P, Ward L, Boonham N and Brown M. 2006. A scientific note on the detection of honeybee viruses using real-time PCR (TaqMan) in varroa mites collected from a Thai honeybee (*Apis mellifera*) apiary. ***Journal of Invertebrate Pathology*** **91**: 69–73.

Chen Y P, Higgins J A and Feldlaufer M F. 2005. Quantitative analysis by real-time reverse transcription-PCR of deformed wing virus infection in the honeybee (*Apis mellifera* L.). ***Appl. Environ. Microbiol*** **71**:436–441.

Chen Y P, Zhao Y, Hammond J, Hsu H T, Evans J D and Feldlaufer M F. 2004. Multiple virus infections in the honeybee and genome divergence of honeybee viruses. ***J.***

***Invertebr. Pathol* 87**:84–93.

Davison S, Leat N and Benjeddou M. 2003. Development of molecular tools for honeybee virus research: the South African contribution. ***African Journal of Biotechnology* 2**(12): 698-713.

De Miranda J R, Drebot M, Tyler S, Shen M, Cameron C E, Stoltz D B and Camazine S M. 2004. Complete nucleotide sequence of Kashmir bee virus and comparison with acute bee paralysis virus. ***Journal of General Virology* 85**: 2263–2270.

Gauthier L, Tentcheva D, Cousserans F, Colin M E and Bergoin M. 2004. A survey of six bee viruses in France using quantitative PCR, p. 97–98. *In* Abstracts of the First European Conference of Apidology, 19–24 September 2004, Udine, Italy.

Ghosh R C, Ball B V, Willcocks M M and Carter M J. 1999. The nucleotide sequence of sac brood virus of the honeybee: an insect picorna-like virus. ***Journal of General Virology* 80**: 1541-1549.

Govan V A, Leat N, Allsopp M and Davison S. 2000. Analysis of the complete genome sequence of acute bee paralysis virus shows that it belongs to the novel group of insect-infecting RNA viruses. ***Journal of Virology* 277**: 457-463.

Grabensteiner E, Ritter W, Carter M J, Davison S, Pechhacker H, Kolodziejek J, Boecking O, Derakshifar I, Moosbeckhofer R, Licek E and Nowotny N. 2001. Sac brood virus of the honeybee (*Apis mellifera*): Rapid identification and phylogenetic analysis using reverse transcription-PCR. Clin. Diagn. Lab. ***Journal of Immunology* 8**: 93–104.

Haddad N, Mohammad B, Migdadi H and de Miranda J R. 2008. First detection of honeybee viruses in Jordan by RT-PCR. ***Jordan Journal of Agricultural Sciences* 4** (3): 242-247.

Hung A C F, Ball B V, Adam J R, Shimanuki H and Know D A. 1996. A scientific note on the detection of American strains of acute paralysis virus and Kashmir bee virus in dead bees in one US honeybee (*Apis mellifera* L) colony. ***Apidologie* 27**: 55–56.

Lanzi G, de Miranda J R, Boniotti M B, Cameron C E, Lavazza A, Capucci L, Camazine S M and Rossi C. 2006. Molecular and biological characterization of deformed wing virus of honeybees (Apis mellifera L.). ***Journal of General Virology* 80** (10): 4998-5009.

Leat N, Ball B, Govan V and Davison S. 2000. Analysis of the complete genome sequence of black queen-cell virus, a picorna-like virus from honeybees. ***Journal of General Virology* 81**: 2111-2119.

Maori E, Lavi S, Mozes-Koch R, Gantman Y, Peretz Y, Edelbaum O, Tanne E and Sela I. 2007. Isolation and characterization of Israeli acute paralysis virus, a dicistrovirus affecting honeybees in Israel: evidence for diversity due to intra- and inter-species recombination. ***Journal of General Virology* 88**: 3428-3438.

Morse R A and Calderone N W. 2000. The value of the honeybee pollination in the United States. ***Bee Culture* 128**: 1-15.

Nordstom S, Fries I, Aarhus A, Hansen H and Korpela S. 1999. Virus infection in Nordic honeybee colonies with no, low or severe *Varroa jacobsoni* infestations. ***Apidologie* 30**:475–484.

Ribiére M, Faucon J P and Pépin M. 2000. Detection of chronic honeybee (*Apis mellifera* L.) paralysis virus infection: application to a field survey. ***Apidologie* 31**: 567–577.

Ribiére M, Triboulot C, Mathieu L, Aurieres C, Faucon J P and Pepin M. 2002. Molecular diagnostic of chronic bee paralysis virus infection. ***Apidologie* 33**: 339–351.

Sanford M T. 2003. "Diseases and pests of the honeybee," ***Florida Cooperative Extension Service Circular*** *766*, University of Florida.

Spira T P. 2001. Plant pollinator interactions: a threatened mutualism with implications for the ecology and management of rare plants. ***Natural Areas Journal* 21**: 78-88.

Tentcheva D, Gauthier L, Jouve S, Canabady-Rochelle L, Dainat B, Cousserans F, Colin M E, Ball B V and Bergoin M. 2004. Polymerase chain reaction detection of deformed wing virus (DWV) in *Apis mellifera* and *Varroa*

***destructor*. *Apidologie* 35**: 431–439.

Topley E, Davison S, Leat N and Benjeddou M. 2005. Detection of three honeybee viruses simultaneously by a single multiplex reverse transcriptase PCR. ***African Journal of Biotechnology* 4**(7): 763-767.

Ward L, Chantawannakul P, Tomkies V, Fisher T, Boonha N and Brown M. 2005. Generic technologies for the detection of bee pests and diseases in the field and the laboratory. ***XXXIX Apimondia***, Dublin, Ireland (August 21st–26th 2005).

Zaitoun S, Hassawi DS and Shahrour W. 2008. Origin of Jordanian honeybees *Apis mellifera* (Hymenoptera: Apidae) using amplified mitochondrial DNA. ***European Journal of Entomology* 105**(1): 41–44.

تشخيص فيروس تكيس الحضنة وفيروس اسوداد بيت الملكة اللاذان يصيبان نحل العسل في الأردن باستخدام تقنية RT-PCR

أمل العبادي[1]، ضياء حساوي[2]، سائدة أبو ملوح[3]، و ماهر عبيدات[4]

ملخـص

هدفت الدراسة إلى تحديد نسب وجود وتوزيع كل من فيروسي تكيس الحضنة (SBV) واسوداد بيت الملكة (BQCV) التي تصيب نحل العسل في الأردن وذلك باستخدام تقنية Rt-PCR. ولتحقيق ذلك تم جمع 100 عينة عشوائية لنحل العسل من خلايا النحل المنتشرة في مناحل المملكة جميعها. العينات التي تم جمعها كانت إما عينات تبدو سليمة وخالية من الأمراض أو عينات من خلايا تظهر عليها علامات الإصابة. وتبين من خلال النتائج وجود فيروس تكيس الحضنة بنسبة 37% من مجموع عينات الدراسة، بينما يوجد فيروس اسوداد بيت الملكة فقط بنسبة 5% من العينات المدروسة. وكان طول قطعة PCR يساوي 433 نيكليوتيد لفيروس SBV وهذا الطول مطابق بنسبة 95% لجينوم الفيروس المسجل في بنك الجينات، بينما كان طول قطعة PCR يساوي 309 نيكيلوتيد لفيروس BQCV وهذا الطول مطابق بنسبة 91% لجينوم الفيروس المسجل في بنك الجينات . والجدير بالذكر أن هذا البحث يعد أول تسجيل لفيروس BQCV في الأردن.

الكلمات الدالة: *Apis mellifera*، فيروسات نحل العسل، فيروس تكيس الحضنة، فيروس اسوداد بيت الملكة،RT-PCR .

---

[1] قسم وقاية إنتاج النبات، كلية الزراعة التكنولوجية، جامعة البلقاء التطبيقية، السلط، الأردن

[2] قسم التقنيات الحيوية، كلية الزراعة التكنولوجية، جامعة البلقاء التطبيقية، السلط، الأردن

[3] قسم التقنيات الحيوية، كلية الزراعة التكنولوجية، جامعة البلقاء التطبيقية، السلط، الأردن

[4] قسم التقنيات الحيوية، كلية الزراعة التكنولوجية، جامعة البلقاء التطبيقية، السلط، الأردن

تاريخ استلام البحث 2011/3/8 وتاريخ قبوله 2012/8/8.