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Survey of honeybee viruses in Syria and the role of plant extracts in stimulating the antimicrobial peptides in honeybee workers

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Introduction

- Economic importance (Pollination, Honey, Wax & Royal Food…)
- Infection by many pests and pathogens (insects, fungi, bacteria, and viruses)
- A decline in the number of colonies worldwide since 1940, due to the phenomenon called Colony Collapse Disorder (CCD).
- Interest in CCD has increased due to its association with viral infections.



- **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**
- **Four peptide families were found in the workerbee Hemolymph:**
  - ✓**Apidaecins (1a, 1b & 2)**
  - ✓**Abaecin**
  - ✓**Defensins (2 & 1)**
  - ✓**Hymenoptaecin**
  - ✓**Four different peptides "Jellins" produced by Royal Foods.**

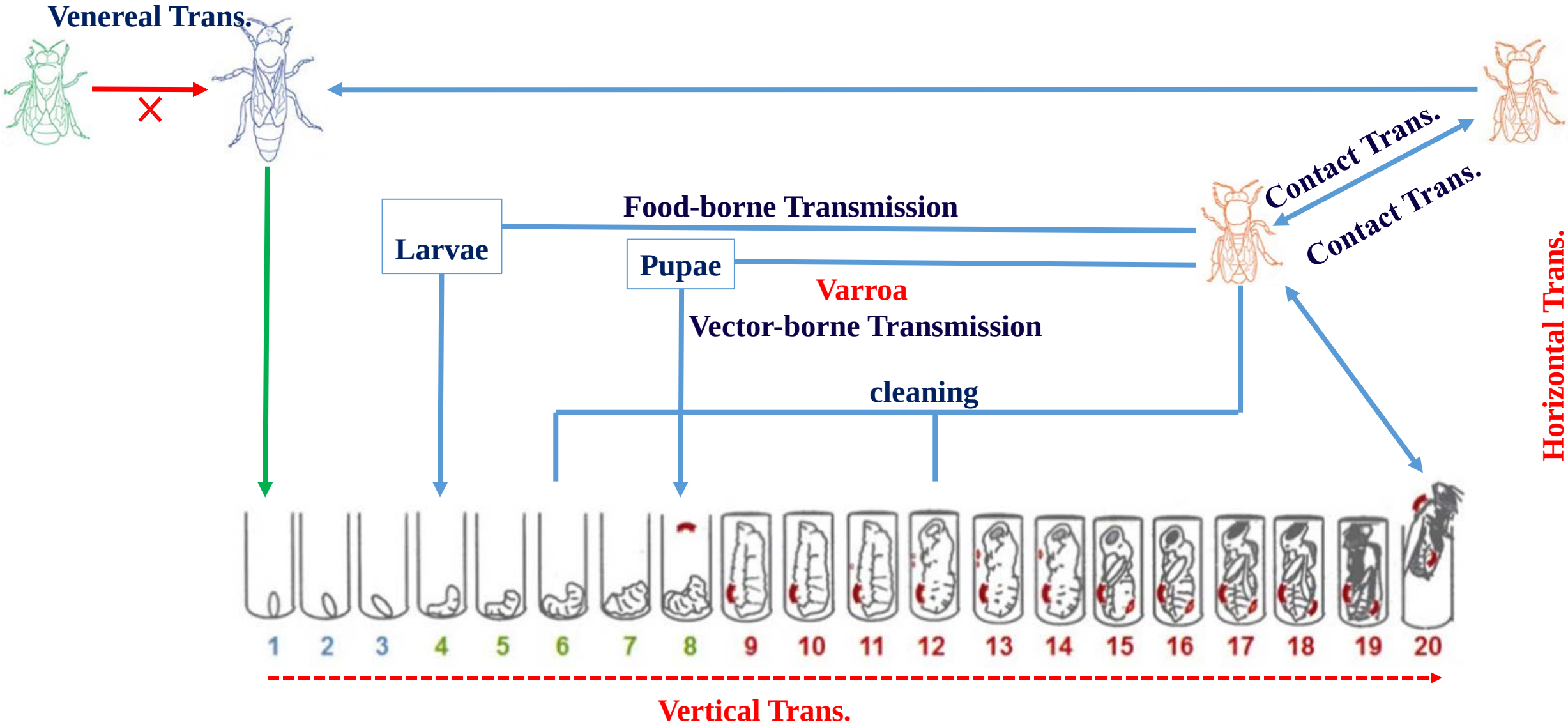
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List of the most important honey bee viruses in the world.

Family	Genus	Nucleic acid	Short name	VIRUS
undefined	*Iflavirus*	RNA	DWV	Deformed Wing Virus
undefined	*Iflavirus*	RNA	SBV	Sacbrood Virus
Dicistroviridae	*Cripavirus*	RNA	BQCV	Black Queen Cell Virus
Dicistroviridae	undefined	RNA	ABPV	Acute Bee Paralysis Virus
Dicistroviridae	undefined	RNA	KBV	Kashmir Bee Virus
undefined	undefined	RNA	CBPV	Chronic Bee Paralysis Virus
undefined	undefined	RNA	IAPV	I-acute Paralysis Virus
undefined	undefined	RNA	TSBV	Thai sacbrood virus
undefined	undefined	RNA	SPV	Slow paralysis virus
undefined	undefined	RNA	BVX	Bee virus X
undefined	undefined	RNA	BVY	Bee virus Y
Dicistroviridae	undefined	RNA	CWV	Cloudy wing virus
undefined	undefined	RNA	BBV	Berkeley bee virus
undefined	undefined	RNA	ABV	Arkansas beevVirus
undefined	undefined	RNA	EBV	Egypt bee virus
undefined	undefined	DNA	FV	Filamentous virus
Iridoviridae	*Iridovirus*	DNA	AIV	Apis iridescent virus

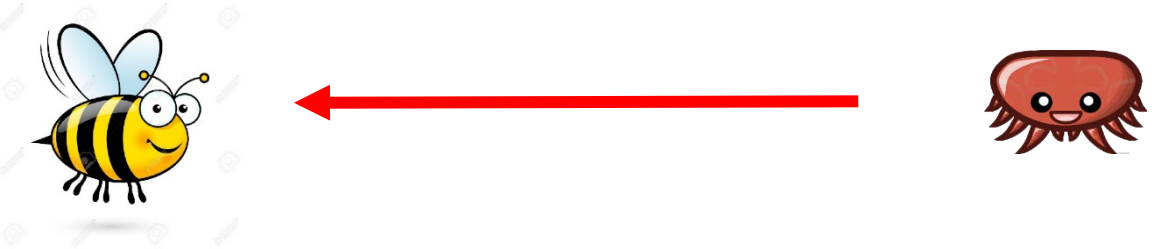
Methods of honeybee viruses transmission



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- **The relationship between Varroa & Viruses is not clearly understood yet.**

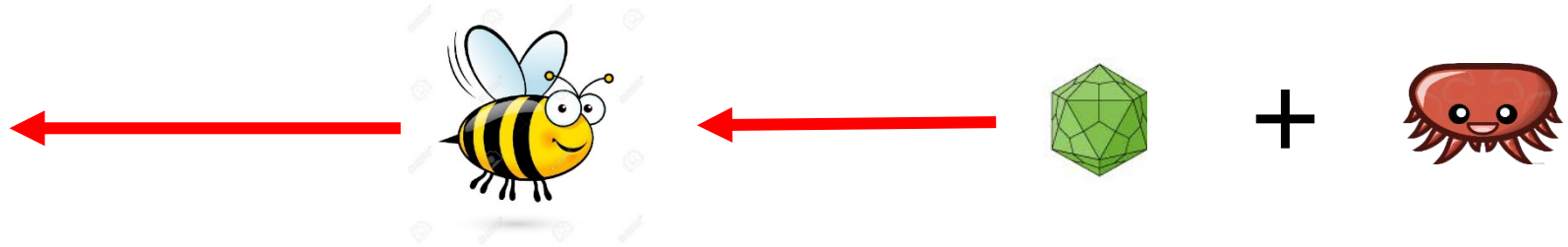
Hardly effects correl. with density
(Kuster *et al.*, 2014; Gregorc *et al.*, 2012)



Hardly effects



Often dramatically effects

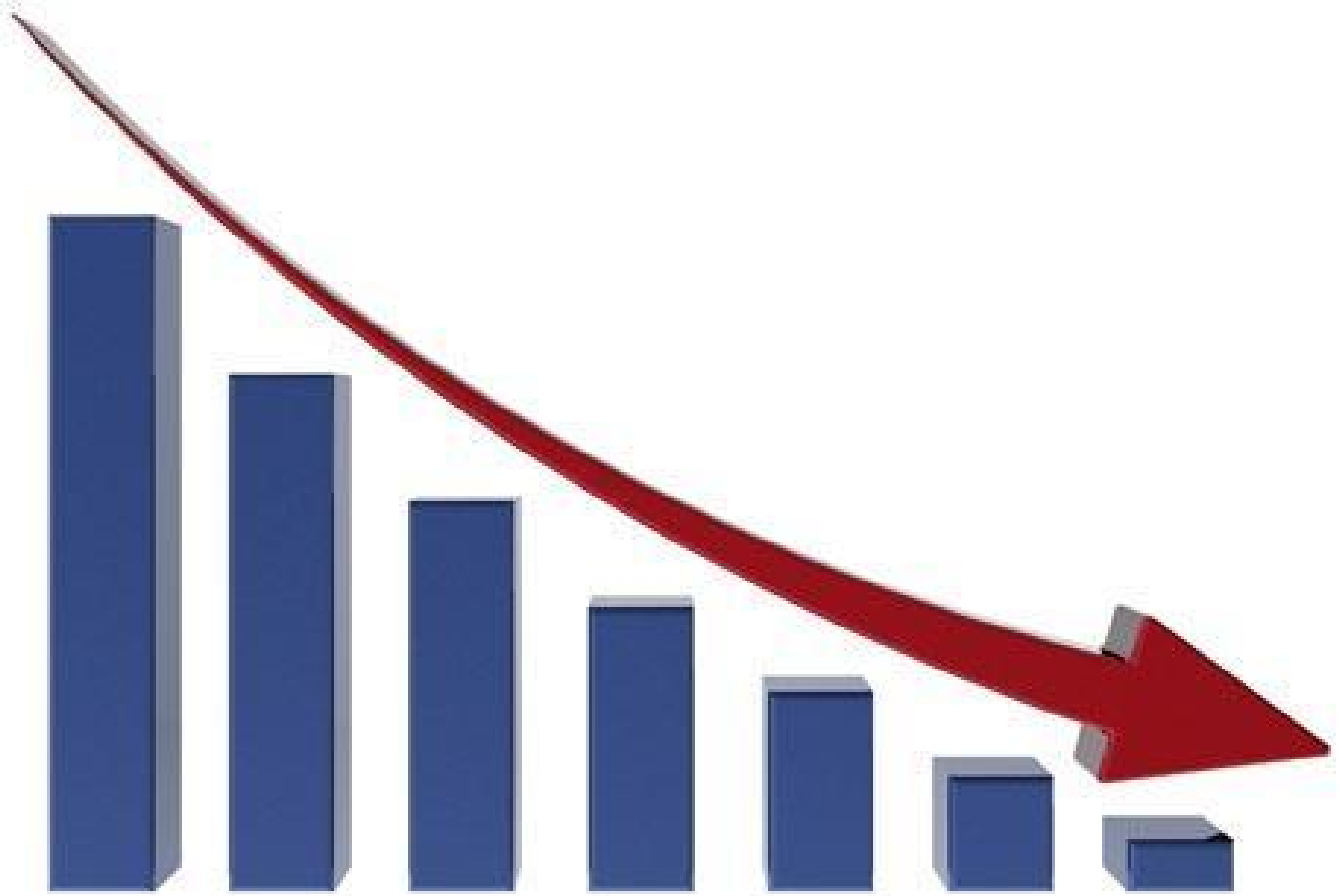


The role of Varroa

- **Transmit virus**
- **Activate virus**

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Honeybees have suffered significant losses in Syria, with a decreasing of bee colonies > 50%.



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The study aimed to:

1. Detection of honeybee viruses spread in Syria.
2. Determine the effect of some plant extracts on:
   - ✓ a survival life of worker bee.
   - ✓ stimulate honeybee's immune system.

Materials & Methods

Research region

- **Syrian Arab Republic**
- **Eight governorates: Damascus, Rif Dimashq, Quneitra, As-Suwayda, Homs, Hama, Tartous and Lattakia**



- **No. of samples: 407**
- **No. of apiaries: 35**
- **Study period: 2009 to 2017**

Materials & Methods

Specific Primers used for the detection of seven honey bee viruses

Virus	Nucleotide Sequence 5'-3'	Product size bp
ABPV	Forward:CATATTGGCGAGCCACTATG Reverse:CCACTTCCACACAACTATCG	398
BQCV	Forward:TGGTCAGCTCCCACTACCTTAAAC Reverse:GCAACAAGAAGAAACGTAAACCAC	700
	Forward:GGAGATGTATGCGCTTTATCGAG Reverse:CACCAACCGCATAATAGCGATTG	316
CBPV	Forward:AGTTGTCATGGTTAACAGGATACGAG Reverse:TCTAATCTTAGCACGAAAGCCGAG	455
DWV	Forward:TTTGCAAGATGCTGTATGTGG Reverse:GTCGTGCAGCTCGATAGGAT	395
KBV	Forward:GATGAACGTCGACCTATTGA Reverse:TGTGGGTTGGCTATGAGTCA	415
	Forward:GATGAACGTCGACCTATTGAA Reverse:TGTGGGTTGGCTATGAGTTCA	393
SBV	Forward:GGATGAAAGGAAATTACCAG Reverse:CCACTAGGTGATCCACACT	426
IAPV	Forward:AGACACCAATCACGGACCTCAC Reverse:AGATTTGTCTGTCTCCCAGTGCACAT	475
	Forward:GCGGAGAATATAAGGCTCAG Reverse:CTTGCAAGATAAGAAAGGGGG	588

- ✓ **RNA Extraction:** QIAamp® Viral RNA Mini Kit (No. 52904)
- ✓ **RT-PCR:** 2-steps RT-PCR Kit QIAamp® (RTPL12)
- ✓ **Primers for seven viruses**:
- ✓ Determine the effect of five plant extracts (*Artemisia herba-alba, Thymus serpyllum, Citrus aurantium, Laurus nobilis & Aloysia citriodora*) on:

  - A survival life of worker bee according to Diprisco *et al.*, 2013.
  - Stimulating honeybee's immune system according to Evans *et al.*, 2006 and Randolt *et al.*, 2008.

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Results

Detection of honeybee viruses spread in Syria

1. *Deformed wing virus DWV*

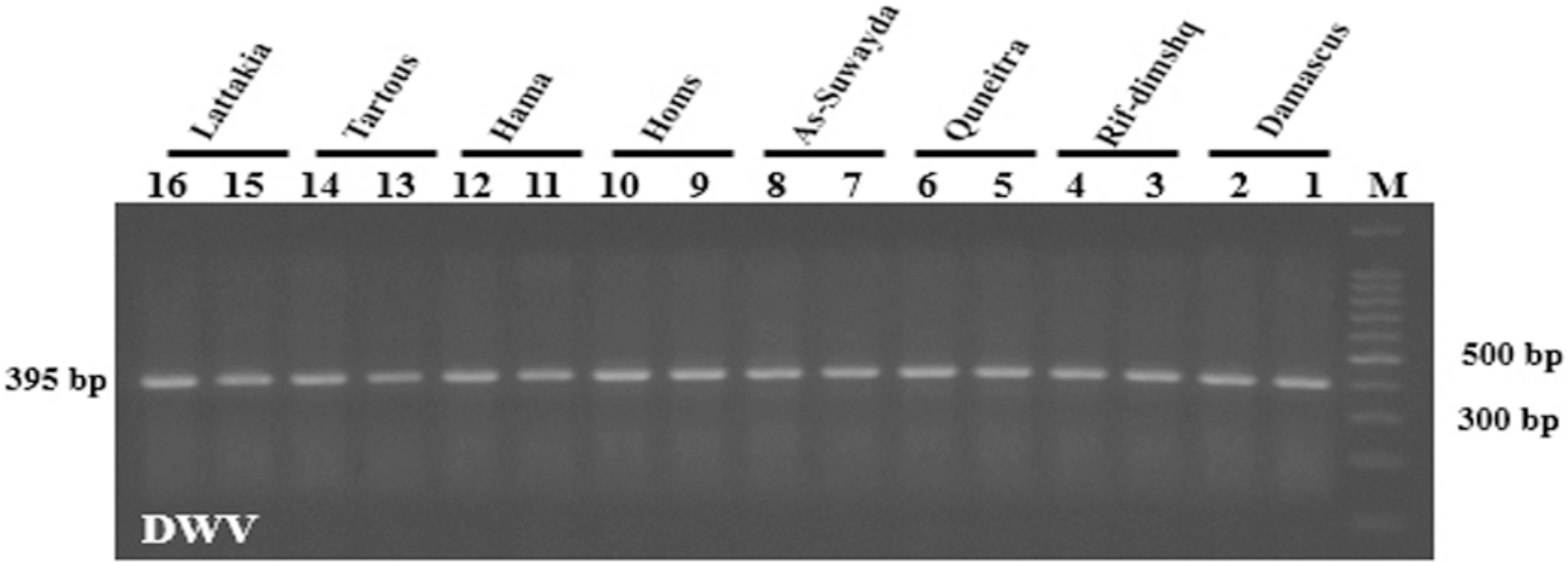




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Detection of honeybee viruses spread in Syria

1. *Deformed wing virus DWV*

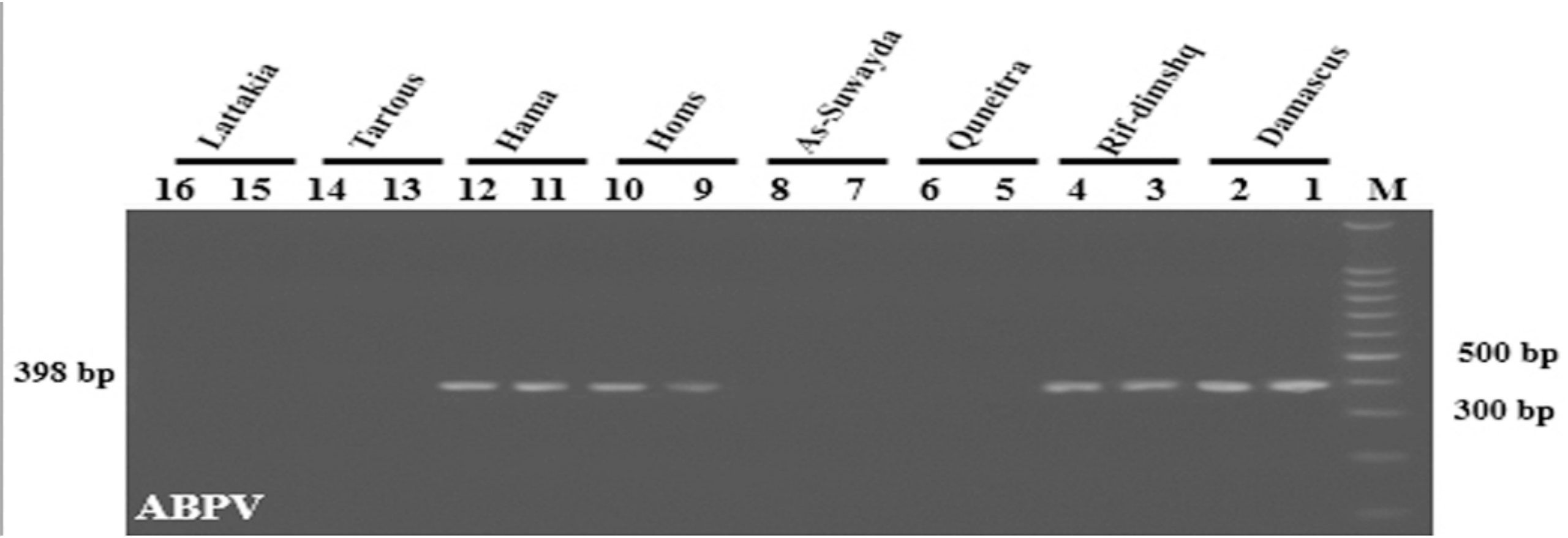


2.Acute bee paralysis virus ABPV



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2.Acute bee paralysis virus ABPV



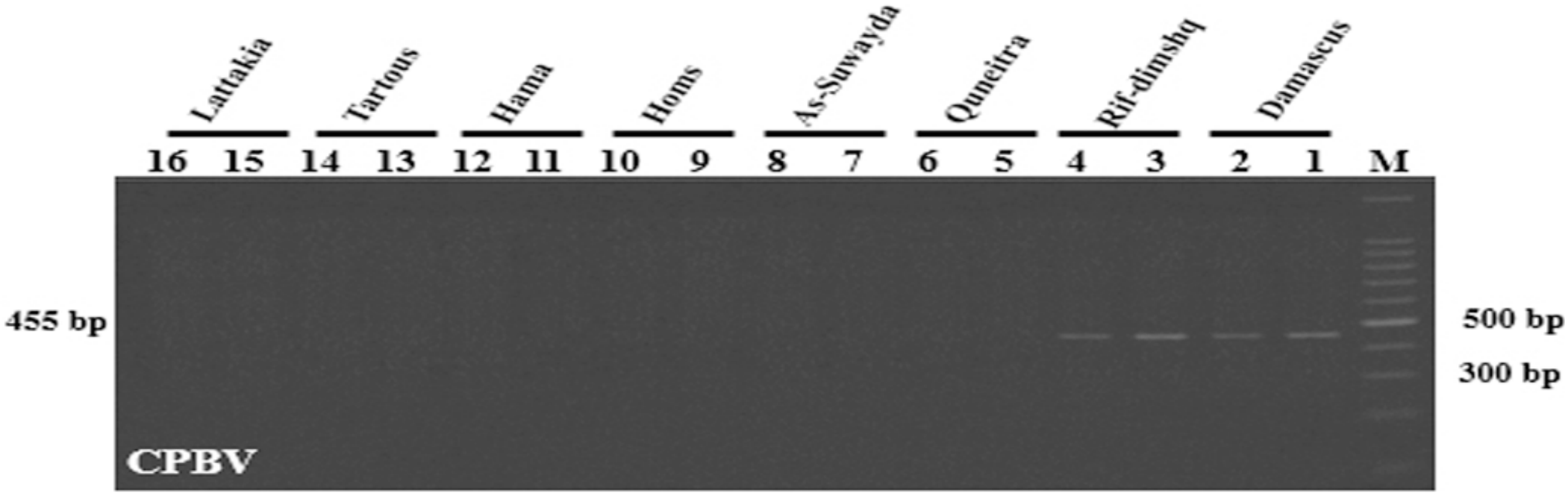
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3.Chronic bee paralysis virus CBPV



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3. Chronic bee paralysis virus CBPV

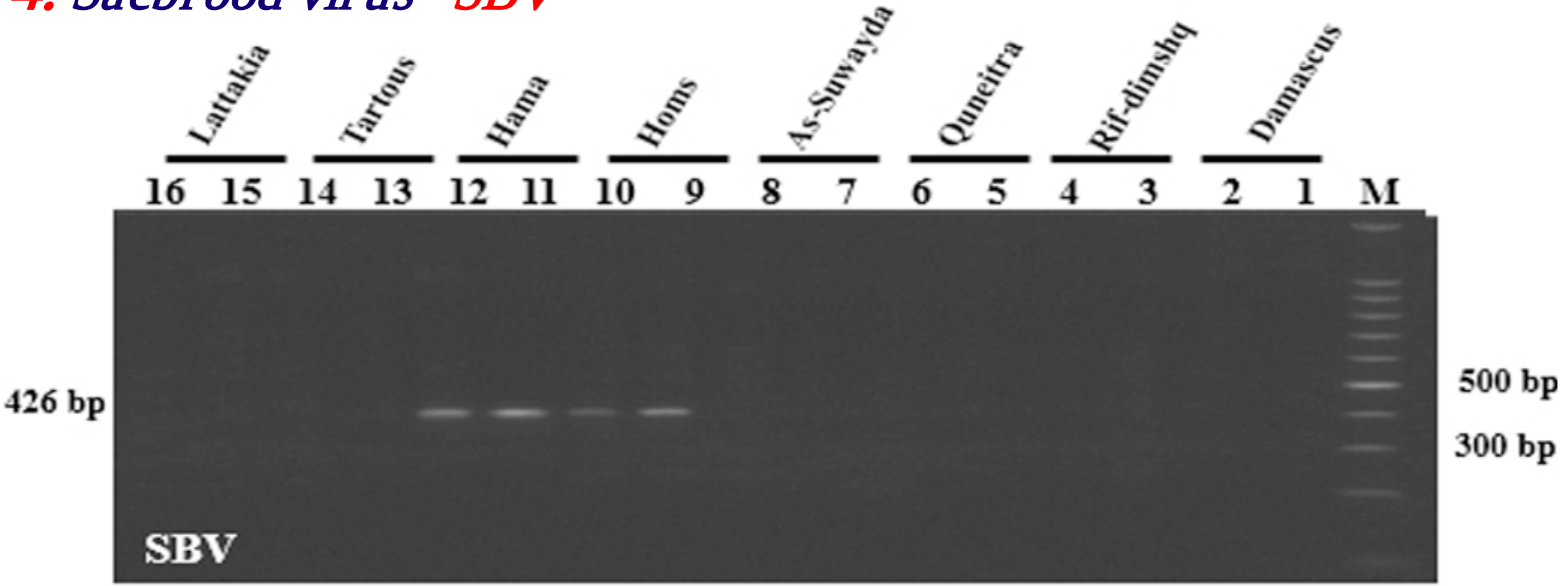


4. Sacbrood virus SBV



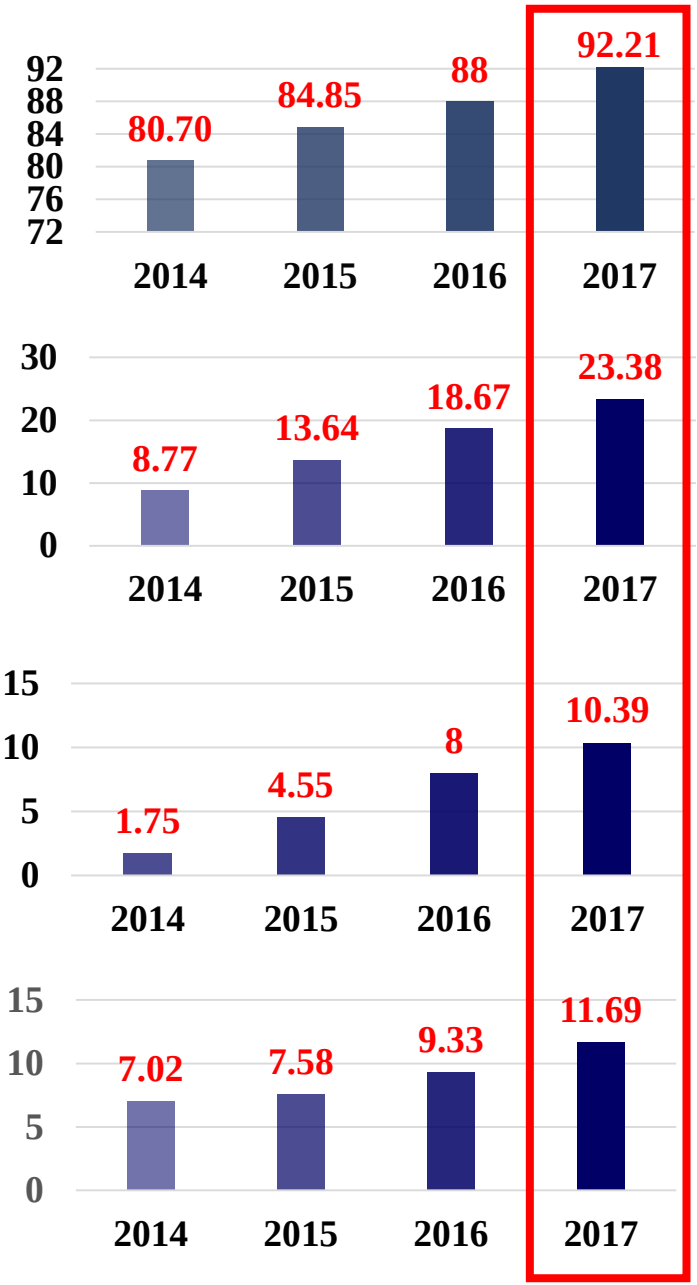


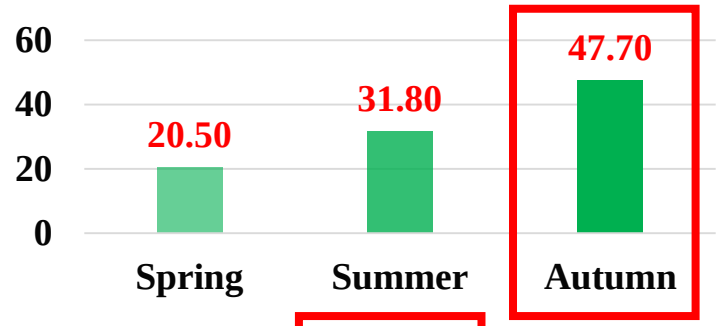
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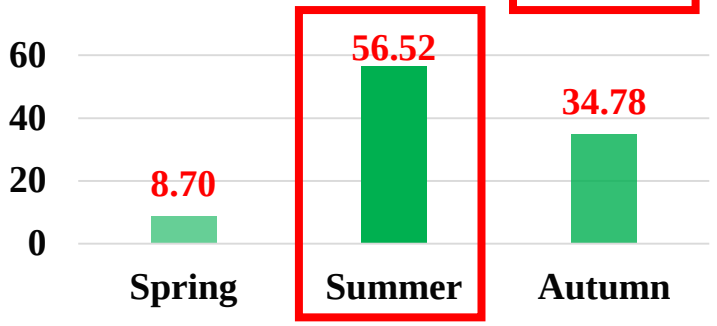
Annual and yearly changes in rate of infections of honey bee during the study period

Deformed wing virus **DWV**

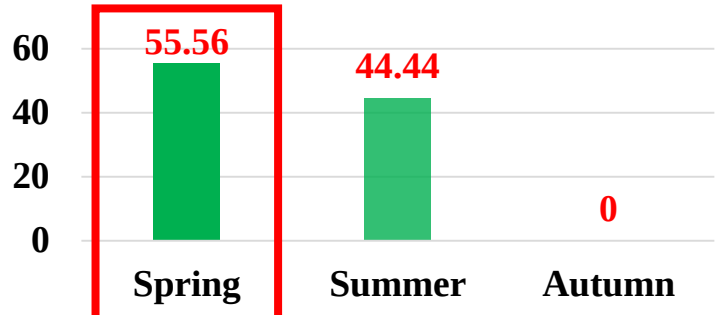




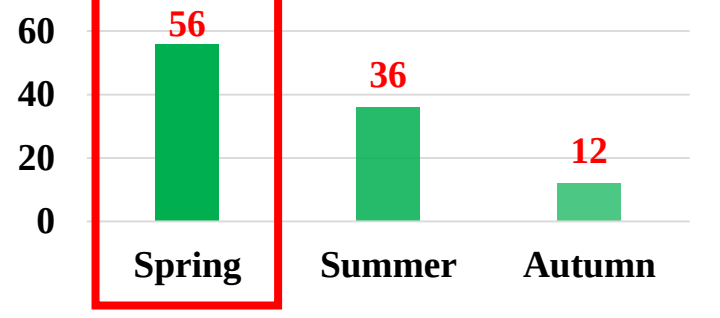
Acute bee paralysis virus **ABPV**



Chronic bee paralysis virus **CBPV**



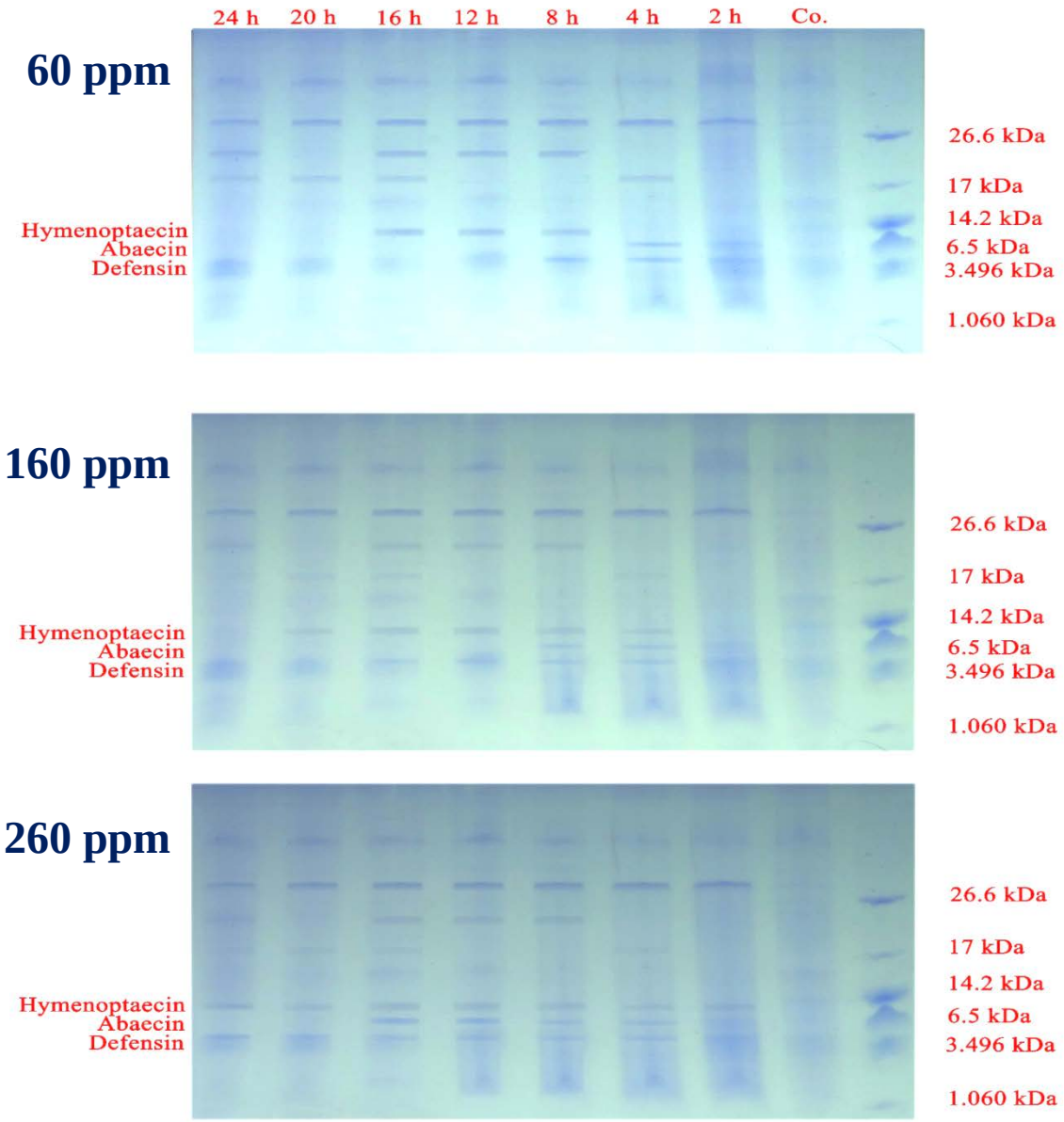
Sacbrood virus **SBV**



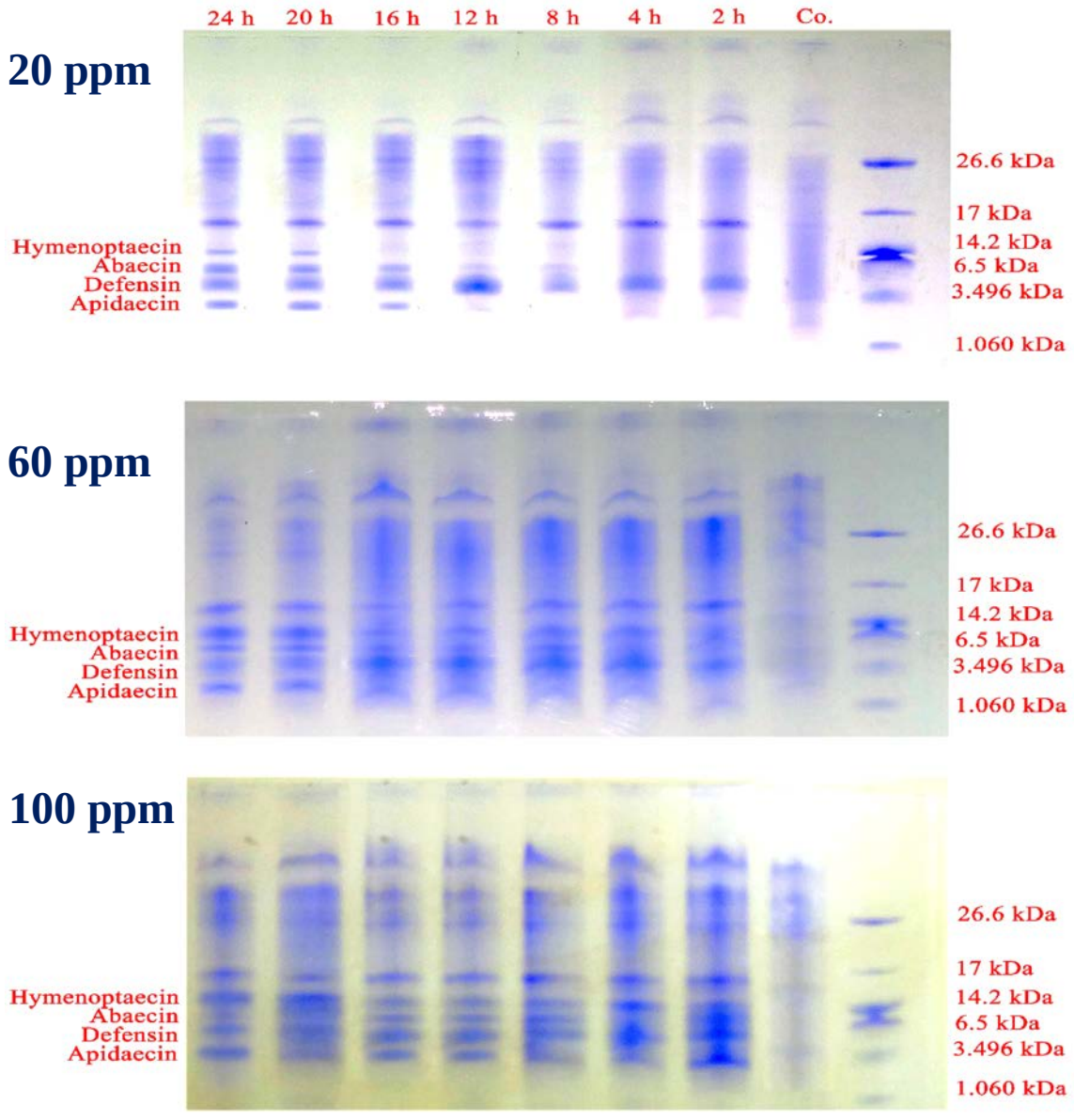
Effect of some plant extracts on Mean and Median survival time of honeybee.

Median survival time (Hour)	Mean survival time (Hour/worker bee)	Plant extracts
27.780 a	18.260 ab	*A. herba-alba*
18.550 b	16.743 b	*T. serpyllum*
22.373 ab	18.157 ab	*C. aurantium*
22.163 ab	17.600 ab	*L. nobilis*
25.412 a	18.551 a	*A. citriodora*
7.200 c	8.710 c	**Control**
6.47	**1.54**	**LSD**

A. citriodora aqueous extract



A. herba-alba plant oil



Ability of some plant extracts to stimulating AntiMicrobial Peptides AMPs

Apidaecin	Defensin	Abaecin	Hymenoptaecin	PPM	Plant extracts
+	+	+	+	20	
+	+	+	+	60	*A. herba-alba*
+	+	+	+	100	
+	-	+	-	50	
+	+	+	-	150	*C. aurantium*
+	+	+	-	250	
-	+	-	-	40	
-	+	+	-	120	*T. serpyllum*
-	+	-	-	200	
-	+	+	-	100	
-	+	+	-	200	*L. nobilis*
-	-	+	-	300	
-	+	+	+	60	
-	+	+	+	160	*A. citriodora*
-	+	+	+	260	
-	-	-	-	0	
-	-	-	-	0	Control
-	-	-	-	0	

Conclusions

These results are:

- ✓ **First report of honeybee viruses in Syria (DWV, APBV, CPBV & SBV).**
- ✓ **The most distribute virus was DWV, followed by ABPV, then CBPV, and the least one SBV virus.**
- ✓ **External influences have contributed to the increase in rate of infection of honeybee viruses in Syria, such as the illegal import of queens and others. Rate of infection of viruses increased during the years of study (2014 to 2017), while annually was the highest rate of infection during the Autum by DWV, and summer by ABPV, and spring by both CBPV and SBV.**

Conclusions

- ✓ **First study about the effect of some plant extracts on both a survival life of worker bee and stimulate of honeybee's immune system.**

- ✓ **The role of plant extracts not only in reducing the spread of viral infections, but also appeared clearly in:**

  - ➢ **Significant effect on mean and median survival life of honeybee worker was r = 0.86 *.**
  - ➢ **Stimulating the honeybee's immune system, where the response of the immune system of bee was 46.2% at 20 ppm of *A. herba-alba* oil, 30.6% at 200 ppm for the extract of the *A. citriodora* leaves, and 24.6% at 150 ppm of *C. aurantium* oil.**

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Thank you for listening



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