An-Najah National University

Faculty of Graduate Studies

Assessment of Plum Pox Virus (PPV) and Apple Chlorotic Leaf Spot Virus (ACLSV) Infections on Stone Fruits in Palestine/West Bank

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This Thesis is Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology, Faculty of Graduate Studies, An-Najah National University, Nablus, Palestine.

2020

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Dedication

I am dedicating this thesis to four beloved people who have meant and continue to mean so much to me. Although they are no longer of this world, their memories continue to regulate my life. First and foremost, to my grandfather Ibrahim Nazzal whose love for me knew no bounds and, who taught me the value of hard work.

To my great parents for their endless love, support and encouragement/

To my Brothers who always stay by my side and encourage me to reach my goals.

to my dearest and closest friend (ayah sharsheer) that I've been fortunate enough to have been blessed with.

To the person who taught me my greatest lessons and helped me to believe that After every storm, there is a rainbow, to my inspiring Mr. Brain.

Acknowledgment

My deepest gratitude goes to God who has provided all that was needed to complete this research.

The person who teach and guide me to my great supervisor Dr. Raed Alkowni thanks a lot for your encourage, guiding and standing with me throughout this study.

Thanks for my doctors, teachers and all members of biology department at An-Najah national university.

Doctor Osama Alaballah thanks a lot for your guiding and helping.

Great thanks for the Palestinian Ministry of Agriculture engineers for their help during samples collecting.

All thanks for National Agriculture Research Center (NARC).

Great thanks to my friend ayah sharsheer for her love, understanding, prayers and continuing support to complete this research work.

I also thanks my beautiful family for standing with me in each step of my life.

My heartfelt thanks to Mr. Brain I am extremely grateful for what he has offered me.

الإقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل عنوان

Assessment of Plum Pox Virus (PPV) and Apple Chlorotic Leaf Spot Virus (ACLSV) Infections on Stone Fruits in Palestine/West Bank

أقر بأن ما اشتملت عليه هذه الرسالة، إنما هي نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم لنيل أي درجة علمية، أو بحث علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this Thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's name: **اسم الطالب:**

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Date: **التاريخ:**

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Table of Abbreviations

PPV	Plum Pox Virus
ACLSV	Apple Chlorotic Leaf Spot Virus
PNRSV	Prunus Necrotic Ring Spot Virus
PDV	Prune Dwarf Virus
APMV	Apple Mosaic Virus
Nm	Nanometer
K	Kilo
Mr	Moleculer mass
Nt	Nucleotide
ORFs	Open reading frames
DAS-ELISA	Double antibody sandwich enzyme-linked immunosorbent assay
RT-PCR	Reverse transcription polymerase chain reaction
T	Ton
NARC	National Agriculture Research Center
USD	*United States dollar*
Cm	Centimeter
Ml	Microliter
No.	Number
Ml	Milliliter
Mg	Milligram
Hr	Hour
Min	Minute
G	Gram
%	Percentage
CVA	Cherry virus A
MuVA	Mume virus A
ApLV	Apricot latent virus
CGRMV	Cherry green ring mottle virus
CNRMV	Cherry necrotic rusty mottle virus
ChVT	Cherry virus Turkey
ACLSV	Apple chlorotic leaf spot virus
APCLSV	Apricot pseudo-chlorotic leaf spot virus
CMLV	Cherry mottle leaf virus
PCLSV	Peach chlorotic leaf spot virus
PMV	Peach mosaic virus
ApMV	Apple mosaic virus
PDV	Prune dwarf virus

PNRSV	Prunus necrotic ringspot virus
PBNSPaV	Plum bark necrosis stem pitting-associated virus
LChV-2	Little cherry virus 2
LChV-1	Little cherry virus 1
PrGV	Prunusgeminivirus A
ChaLV	Cherry associated luteovirus
PaLV	Peach associated luteovirus
PPV	Plum pox virus
CVF	Cherry virus F
PLPaV	Peach leaf pitting-associated virus
PrVF	Prunus virus F
ALRSV	Apricot latent ringspot virus
CLRV	Cherry leaf roll virus
PEV	Peach enation virus
PRMV	Peach rosette mosaic virus
TBRV	Tomato black ring virus
ToRSV	Tomato ringspot virus
SLRSV	Strawberry latent ringspot virus
PeVD	Peach virus D
CGMMV	Cucumber green mottle mosaic virus
PYSaV	Prunus yellow spot-associated virus
CVT	Cherry virus Trakiya

Assessment of Plum Pox Virus (PPV) and Apple Chlorotic Leaf Spot Virus (ACLSV) Infections on Stone Fruits in Palestine/ West Bank

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Abstract

Palestine as many of developing countries; its economy depends on many factors including agriculture sector. Horticultural crops and mainly stone fruits were found infected with several pathogens and pests. This study was to assess the sanitary status of stone fruits regarding to Plum Pox Virus (PPV) and Apple chlorotic leaf spot virus (ACLSV) in West Bank / Palestine. Field surveys were conducted during growing season of 2019; where a total of 500 samples were collected from all districts. These samples were tested for PPV and ACLSV by double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA).ACLSV were found positive in 1.33% of tested samples particularly in samples collection plot at NARC in Jenin. All tested samples were confirmed negative for PPV; a quarantine virus in Europe and most of the world. This study was confirmed the Palestinian stone fruits as free from PPV. This destructive virus can be transmitted by a number of aphid species, monitoring its periodical check in order to protect the country from such dangerous disease should be a must.

Chapter One

Introduction

1.1 Overview

A stone fruit, or a drupe, is a fruit with large "stone" inside. The stone is not the seed, the fact is that the seed is inside the stone. The stones of the fruits can also be called a pit. Stone fruit examples are peaches, nectarines, plums, lychees, mangoes, and cherries. Some drupes have single large stones and others not just like raspberries.

The stone fruit or the drupe mean just the fruit of the genus Prunus. Free stone refers to a drupe having a stone which can be removed easily from the flesh. Freestone plums are preferred for making homegrown prunes, and freestone sour cherries are preferred for making pies and cherry soup. There is some stone fruits seeds cannot be removed easily from the flesh and this type of stone fruits called clingstone. Tryma is difficult to categorize its nut-like drupes. Stone fruits in general prefer the warm climate, and because of this they are very susceptible to injury from very low temperatures. Blooming early in spring making the flowers of stone fruits frequently suffer damage from spring frost. Stone fruits should be planted on suitable site, with excellent air and water drainage and protection from high winds. They are susceptible to wide range of insects and disease pests. Stone fruits like a deep well drained soil rich in humus, heavy soil can be improved by incorporating gypsum and organic matter and mounding the soil before planting, the preferred PH is 6-7 (Savory 1967). About 40% of the world supply of stone fruits comes from the Mediterranean region. Almond and apricot contributing 50% and 45% of

the total production respectively, according to this percentage these tow crops is one of the most important crops. The cultivation of stone fruits in the Mediterranean depend on a high number of local differences because every country has its own germplasm, important especially for domestic market (Bassi and Pirazzoli, 1998). Agriculture is the power of the Palestinian economy, it contributes nearly 33 and 24% to the Gross National Product in the West Bank and Gaza Strip respectively (MoA, 2007). In Palestine olive trees, grapevines, almonds, figs and citrus are the major types of fruit trees. They occupy 90% of the total fruit tree area and produce 79% of total fruit production. Olive trees covering about 72% of areas devoted to fruit trees and contributing about 30% of the total fruit production. Grapevines are the second major fruit crops in the West Bank, with a total area of approximately 7,600 hectares and annual production of 43,000 tons in 1994. Approximately 68% of the total cultivated area of vineyards is found in the Hebron district. Almond trees occupy the third largest area among cultivated fruit trees, forming 7.1% of the fruit tree area and contributing 2.4% to the fruit production (ARIJ, 2004).

Table (1.1): Current status of stone fruits in Palestine according to Palestinian Central Bureau of Statistics 2010.

Stone fruit	Area (Dunums)	Number
Apricot	1,240.79	46,817
Cherry	1,011.19	26,855
Peach	3,541.85	176,864
Nectarine	22.87	956
Almond (hard)	12,649.28	454,502
Almond (soft)	595	3,425.06

1.2 Viruses Affecting Stone Fruits

Stone fruits affected by many viruses, which cause diseases with different effects in orchards around the world. They cause a lot of symptoms, ranging from symptomless (latent) to a general decline in productivity. Leaf symptoms include distortion or twisting, mottling, rolling, necrotic spots, shot holes, and unusual color patterns. Fruits size may show reduction and their quality may decrease, distortions in shape of the fruit, and alterations such as ring spots, mottling, and line patterns .Virus and virus like agent of stone fruits causes high economic losses to all sections of the production chain. For example Plumpox virus (PPV), the causal agent of Sharka disease, causes severe damage and has so many economic and social impacts. Tens of millions of Euros and dollars have been spent at least to control this virus without any results (Cambra et al., 2004). Other viruses like Illaviruses induce mild but significant losses in orchards which reduce the sustainability of many orchard operations. Stone fruits are hosts for large number of viruses that can cause substantial economic losses (Myrta et al., 2003), Prunus necrotic ringspot virus (PNRSV), Prune dwarf virus (PDV), and Plum pox virus (PPV) are the most significant with worldwide distribution. PNRSV, PDV and Apple mosaic virus (APMV) are members of the genus Ilavirus, family Bromoviridae (Roosinck et al., 1992). One of the most economically important viruses is Apple chlorotic leaf spot virus (ACLSV) it can spread rabidly and has a very harmful effects caused in some infected stone fruit species and rootstocks but these viruses have no effects on humans (Myrta etal., 1996).

Table (1.2): Viruses affecting stone fruits (Umer et al, 2019)

Virus name		Family	Genus	Group	Genome		Virion	
					organaization	Size(nt/bp)	Morphology	Size(nm)
1	CVA	Betaflexiviridae	Capillovirus	ssRNA(+)	Mono-	7383	Filamentous	640-700
2	MuVA	Betaflexiviridae	Capillovirus	ssRNA(+)	Mono-	7644	Filamentous	640×12
3	ApLV	Betaflexiviridae	Foveavirus	ssRNA(+)	Mono-	9311	Filamentous	/
4	CGRMV	Betaflexiviridae	Robigovirus	ssRNA(+)	Mono-	8372	Filamentous	1000-2000×5-6
5	CNRMV	Betaflexiviridae	Robigovirus	ssRNA(+)	Mono-	8432	Filamentous	/
6	ChVT	Betaflexiviridae	Robigovirus	ssRNA(+)	Mono-	8464	Filamentous	800×12-23
7	ACLSV	Betaflexiviridae	Trichovirus	ssRNA(+)	Mono-	7555	Filamentous	640-760×12
8	APCLSV	Betaflexiviridae	Trichovirus	ssRNA(+)	Mono-	7494	Filamentous	640-760×10-12
9	CMLV	Betaflexiviridae	Trichovirus	ssRNA(+)	Mono-	8018	Filamentous	760×11
10	PCLSV	Betaflexiviridae	Trichovirus	ssRNA(+)	Mono-	7465-7466	Filamentous	640-760×10-12
11	PMV	Betaflexiviridae	Trichovirus	ssRNA(+)	Mono-	7988	Filamentous	/
12	ApMV	Bromoviridae	Ilarvirus	ssRNA(+)	Tri-	3476; 2979; 2056	Icosahedral	25-29
13	PDV	Bromoviridae	Ilarvirus	ssRNA(+)	Tri-	3374; 2593; 2129	Isometric; Bacilliform	19-20; 73
14	PNRSV	Bromoviridae	Ilarvirus	ssRNA(+)	Tri-	3332; 2591; 1957	Isometric; Bacilliform	23-27

15	PBNSPaV	Closteroviridae	Ampelovirus	ssRNA(+)	Mono-	14214	Filamentous	1500×13
16	LChV-2	Closteroviridae	Ampelovirus	ssRNA(+)	Mono-	15050	Filamentous	1667×11
17	LChV-1	Closteroviridae	Velarivirus	ssRNA(+)	Mono-	16930	Filamentous	1786-1820
18	PrGV	Geminiviridae	Grablovirus	ssDNA	Mono-	3174 (circular)	Geminate	/
19	ChaLV	Luteoviridae	Luteovirus	ssRNA(+)	Mono-	5857	Icosahedral	25-30
20	PaLV	Luteoviridae	Unassigned	ssRNA(+)	Mono-	5819	Icosahedral	25-30
21	PPV	Potyviridae	Potyvirus	ssRNA(+)	Mono-	9741	Filamentous	750×15
22	CVF	Secoviridae	Fabavirus	ssRNA(+)	Bi-	5664 (partial); 2608	Icosahedral	/
23	PLPaV	Secoviridae	Fabavirus	ssRNA(+)	Bi-	6357; 3861	Icosahedral	30
24	PrVF	Secoviridae	Fabavirus	ssRNA(+)	Bi-	6165; 3622	Icosahedral	28-30
25	ALRSV	Secoviridae	Nepovirus	ssRNA(+)	Bi-	2218 (partial)	Icosahedral	30
26	CLRV	Secoviridae	Nepovirus	ssRNA(+)	Bi-	7918; 6360	Isometric	28
27	PEV	Secoviridae	Nepovirus	ssRNA(+)	/	/	Isometric	33
28	PRMV	Secoviridae	Nepovirus	ssRNA(+)	Bi-	8014; 5956	Icosahedral	28
29	TBRV	Secoviridae	Nepovirus	ssRNA(+)	Bi-	7358; 4633	Isometric	30
30	ToRSV	Secoviridae	Nepovirus	ssRNA(+)	Bi-	8214;	Icosahedral	28

						7271		
31	SLRSV	Secoviridae	Unassigned	ssRNA(+)	Bi-	7496; 3842	Icosahedral	30
32	PeVD	Tymoviridae	Marafivirus	ssRNA(+)	Mono-	6612	Icosahedral	30
33	CGMMV	Virgaviridae	Tobamovirus	ssRNA(+)	Mono-	6424	Straight rod	300×18
34	PYSaV	Unassigned	Gratylivirus	ssRNA(+)	Mono-	6072	Isometric	30
35	CVT	Unassigned	Unassigned	ssRNA(+)	Mono-	8620	/	/
Sum	35	**9 + 2 unassigned**	**15 + 3 unassigned**	2	3	**6424-16930**	**6**	**/**

1.3 Apple Chlorotic Leaf Spot Virus (ACLSV)

ACLSV is widely distributed which was first reported in malus (Watpade et al., 2012). ACLSV is a member of the genus Trichovirus and it is one of the important latent virus due to its worldwide distribution and broad host range. The infection rate of this virus ranges up to 80-100 % in many commercial apple cultivars with yield losses of the order of 30-40 %. ACLSV appeared as a major virus on apple with disease incidence of 85-90 % (Al Rwahnih et al., 2001). The complete genome sequence of several ACLSV isolates has been determined during last year's, its particle size is 720 x 12nm containing a single coat protein species with an Mr of about 22K (Yoshikawa & Takahashi, 1988) it encapsulate a single-stranded positive sense genomic RNA of Mr 2'5 x 106 (Lister & Bar-Joseph, 1981). the nucleotide (nt) sequence of the genomic RNA of the p863 isolate of ACLSV Recently determined. The length of ACLSV genomic RNA is 7555 nt excluding the poly(A) tail and possess three overlapping open reading frames(ORFs) coding for proteins of 216-5K, 50K and 28K. The 28K ORF contains in frame a 21.5K ORF encoding the capsid protein (Candresse et al., unpublished results).

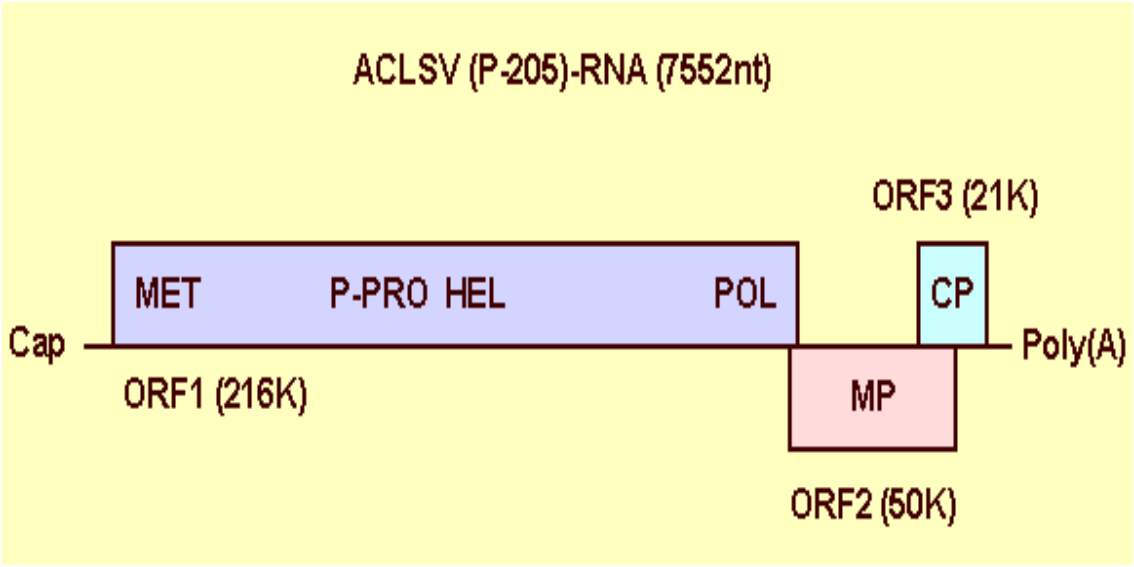

Figure (1.1): ACLSV Genome (Yanase et al., 1974)

1.4 Symptoms, Detection and Determination of Incidence ACLSV

ACLSV transmitted by mechanical inoculation and by grafting, so it is not seed or pollen transmitted in any of its hosts. No natural vector has been identified for ACLSV (Yoshikawa, 2001). According to this the virus host range have to be well known for effective pathogen management. DAS-ELISA and RT-PCR are the most commonly used assays for the detection of ACLSV (Myrta et al., 2011). Although ELISA testing is rapid, cheap and easy, it can often be problematic because of the uneven distribution of the virus in the trees and the very low virus titers sometimes observed, which may be below the detection limit of the method. Different RT-PCR assays have been developed for the specific detection of ACLSV using various primer pairs (Mathioudakis et al., 2010). However, not all ACLSV isolates can be detected due to the high molecular variability of the virus (Al Rwahnih et al., 2004; Malinowski, 2005; Yaegashi et al., 2007). Nevertheless, PCR-based assays have been demonstrated to prevail over

other detection techniques in terms of polyvalence and sensitivity. And depending on the virus strain, the host species and cultivar infected Symptoms can be highly variable. Symptoms can include cholortic leaf spots and line patterns on the tree leaves, asymmetric leaf distortion, premature leaf drop, stunting, terminal dieback, inner bark necrosis and xylem pitting, and local bark necrosis surrounding the inoculums buds (Yaegashi et al., 2007). Plumpseudopox which caused by ACLSV infection decrease the market value of fruit from infected trees (Nemeth, 1986).

The disease symptoms are usually sunken spots, bands or rings on the skin of the fruit, leaf symptoms may occur sometimes. The disease known as Apple ringspot caused by dual infections of ACLSV and severe strain of Apple stem spitting virus. Most varieties of sweet cherry and sour cherry are infected by ACLSV (Nemeth, 1986). In peach the virus often causes graft incompatibility leading to necrosis and early decline. It can also cause dark green, sunken spots or wavy lines on peach leaves and the same occurs with apricot (Nemeth, 1986).

ACLSV is likely distributed worldwide in locations where natural hosts are cultivated. A high infection level of ACLSV in apple cultivars was reported from England93.5% (Campbell 1961), Moldova 86% (Verderevskaja & Marinescu 1985), USA 60% (Waterworth 1993), Czech Republic 69% (Karesová & Paprstein 2001), Turkey 21.28% (Çağlayan et al. 2006) and Tunisia 34% (Mahfoudhi et al. 2013).

Figure (1.2): ACLSV Symptoms (Németh, 1986).

1.5 Plum Pox Virus (PPV)

Plum pox potyvirus, which cause of the most destructive viral disease of prunus, it has been established in North America and has occurred in almost all European countries but with different degrees (Roy and Smith, 1994). It was first detected in Eastern Europe (Bulgaria) (Atanassov, 1932) from where it has spread. It is an RNA virus with a flexuous filament particle of approximately 12.5-20 nm which contain a positive-sense single stranded RNA genome of about 9.8 kb (Kegler and Hartmann 1998). It is the only known potyvirus infecting stone fruits Prunus.

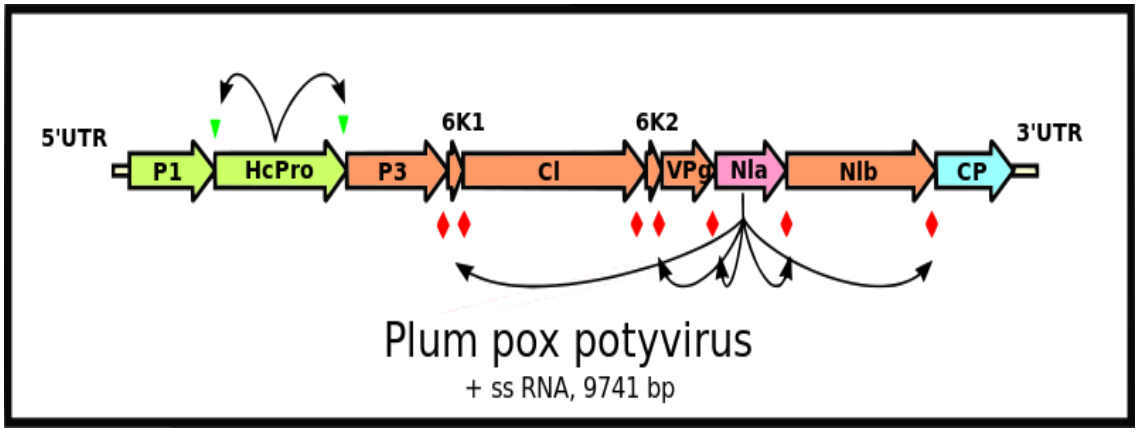

Figure (1.3): Genomic map of plum pox virus (Dolgov et al., 2010).

PPV is divided into six strains: PPV-D, PPV-M, PPV-El Amar, PPV-C, PPV-W and PPV-Rec (Candresse and Cambra, 2006; James and Glasa, 2006). The PPV-D strain was detected in North America for the first time in peach and plum orchards in Pennsylvania in 1999. PPV-D also was detected in Canada in June of 2000, and subsequently, positive trees have been located in all major peach/nectarine production areas of the Niagara peninsula (Ontario, Canada). PPV-D was first detected in 1970 in France, mainly in apricot orchards and was maintained at a very low incidence by strict sanitation programs. The severity of the symptoms changes with the Prunus species and cultivar, the virus strain, season and location. In previous studies carried in turkey the incidence of sharka disease was 71.1% in apricot, 60.5% in plums and 48.8 in peaches (Elibuyuket al., 2004) The disease causes blotches and/or deformations in the fruits of apricot, and peach, and severe fruit dropping in susceptible plum and apricot varieties. Four strains of the virus, with differences in biological, serological, molecular and epidemiological behavior have been identified: Marcus (PPV-M), Dideron (PPV-D), El Amar (PPV-EA) and Cherry (PPV-

C). The virus strains recorded most frequently in the region are PPV-D and PPV-M, whereas PPV-EA has been recorded only from Egypt (Wetzel et al., 1991). Strain PPV-M, which has been present for long time only in Eastern- European countries, became established during the 1990s in Southern France and Northern Italy causing severe damages to the peach industry. PPV-M was reported recently from Southern countries such as Jordan and Syria (Al Rwahnihet al., 2000)

1.6 Symptoms, Detection and Determination of Incidence PPV

From the 1930s when sharka began to spread methods for detection and diagnosis of PPV where based on visual observation of symptoms (Nemeth,1994). Enzyme linked Immunosorbent Assay (ELISA) has been applied to PPV since its introduction in plant pathology (Voller et al., 1976). ELISA is a simple, rapid, sensitive, and adaptable method which can be used for large scale analyses using crude plant extracts resulting in a low cost per sample (Cambra et al., 2011). Recently, monoclonal antibodies have been developed for the accurate detection of all PPV strains (5B-IVIA) (Cambra et al., 1994) and for the four serogroups, M, D, C, and EA strains (Crecenzi et al., 1998; Boscia et al, 1997; Myrta et al, 1998). In the 1980s application of cDNA and cRNA probes used to detect PPV, this sensitive and accurate method helped to overcome the problem of low concentration of the virus (Varveri et al., 1987, 1988).Plum pox virus has been transmitted by nearly 20 aphid species, although only Brachycauduscardui, B. helichrysi, Myzuspersicae, and

Phorodonhumuliare the most important aphid vectors. Virus strain, time of the year, host cultivar, and age of the host cultivar all together affecting and controlling the efficiency of transmission of the virus (Gottwald et al., 1995). Aphids can acquire the virus in probes as short as 30 seconds, and can transmit for up to 1 hour. Aphids that have been starved before feeding can transmit for up to 3 hours after acquisition. There is no correlation between the ability to transmit PPV and the ability to colonize Prunus. PPV can be spread in orchards by transient aphids as efficiently as aphids colonizing Prunus (Labonne et al., 1994).

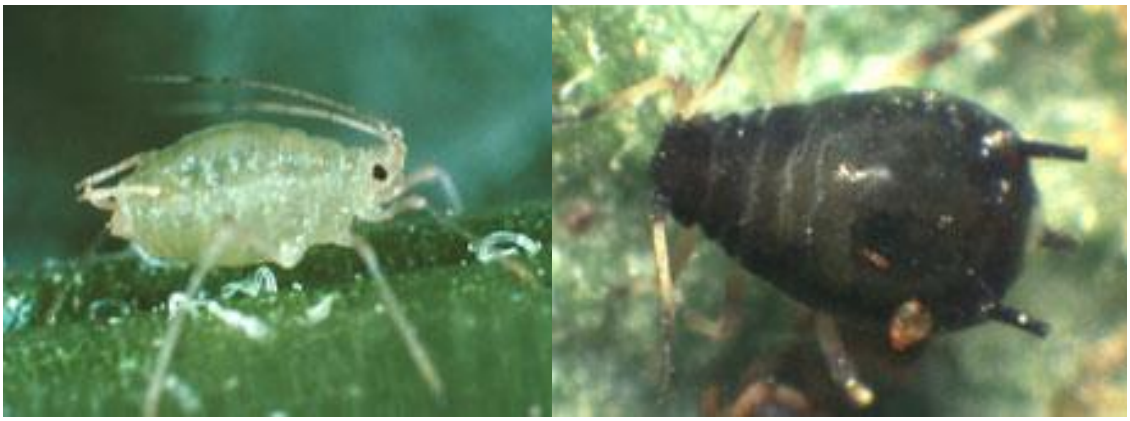

Figure (1.4): Aphid vectors of Plum pox virus: the black bean aphid

(Aphisfabae) (Photo by B. Nault) and the green peach aphid (Myzuspersicae) (photo by J. Ogrodnick) are major vectors.

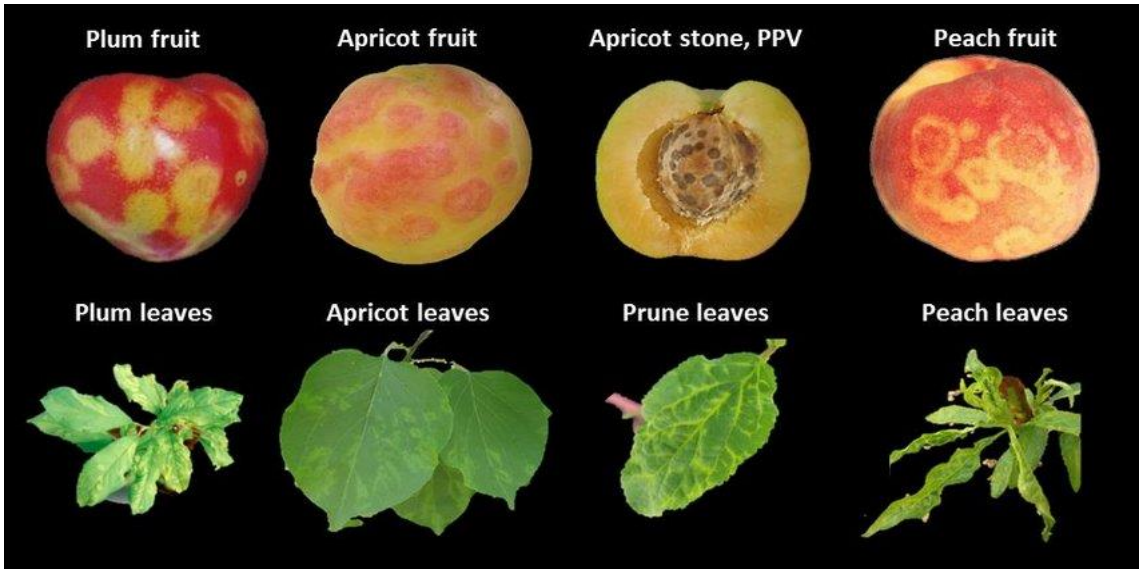

Figure (1.5): Plum pox virus symptoms ((Dunez, 1987)

Infected trees may or may not produce visual symptoms on leaves and fruits, inprunus, the symptoms of Plum pox virus appear on seeds, leaves, flowers, and fruits. Leaves and fruits show chlorotic and necrotic ring patterns, and chlorotic bands or blotches, sometimes the fruit can be misshapen and deformed, or rings may be present on their stones. Not all parts of an infected tree will show symptoms. The virus can often be detected at the bottom of a branch but not the tip; however, once a branch shows symptoms, it will continue to display them in subsequent years. prunus species and cultivar, PPV strain, season and location determine the severity of the symptoms (kegler, 1998).

1.7 Economic importance of Plum Pox Potyvirus in Stone-Fruits

PPV has a great economic importance, this virus can spread rabidly and cause a really severe damage (Cambra et al. 2006). The diseased fruit weight may decrease by 0.2-3.7% in the case of tolerant, but in the case of

susceptible cultivarsit will decrease by 2.8-345, and the sugar content also may decrease by 0.14-2.1 Oh. At the same time, acid content increase (Pielka et al., 1971). anthocyanin content of the diseased fruits decrease, and this cause a problem in industrial processing (Gyorgy, 1976). In Bulgaria, almost all fruits dropped before harvest (Trifonov, 1974). From year to year loses changed, but in some years it's more than 60,000 t fresh weight. In 1955a large scale survey showed 16 million diseased trees (Novakovic, 1995). The average fruit yield per treedropped by 83.4% in severely affected districts in Czechoslovakia, according to Blattny & Heger (1965). The production of Slivowitz brandy decreasedby 85% between 1950 and 1953. In the Brno area in 4 years losses were estimated at 120,000USD**,** due exclusively to PPV. In 1961 alone, 90,000 nursery trees were destroyed (Kralikova, 1963).In Romania, a 12-77% yield decrease was observed in different cultivars, together with a 21-38% growth and trunk circumference decrease and simultaneous decrease in the carbohydrate content and increase in the nitrogen and amino-acid content of the trees (Minoiuet al., 1979).

Apricot trees grew more slowly, the average trunk circumference of 5-year-old infected trees was 5.7 cm, while that of the healthy trees was 7.2 cm. Mortality among diseased trees wasl6.6%, while none of the control trees died . PPV enhances the effect of other viruses occurring in stone fruits. Severe growth reduction, bark canker and trunk malformation may be observed in case of mixed infections by PPV and Prune dwarf Ilarvirus (PDV) on some plum cultivars, and this lead to death. The synergistic

effect of PPV with other viruses was confirmed in Hungary on GF305 peach seedlings by inoculation with PDV, PNRSV, Apple chlorotic ringspot clostero virus and Strawberry latent ringspot nepovirus in various combinations. According to the combinations of viruses, the growth of 2-years old seedlings was reduced by 9.2-69.1% (Nemeth, 1992).

1.8 Aims of the Study

The main aim of this study was to evaluate the sanitary status of the stone fruits based on their viral infections. The incidences of two most economical important viruses (PPV and ACLSV) were assessed on stone fruits using the available biotechnological tools (serological and/or molecular) for their detection.

Chapter Two

Materials and Methods

2.1 Field Surveys

Field inspections and sample collection was carried out in early spring 2019 from eight Governorates (Jenin, Tulkarm, Tubas, Nablus, Hebron, Bethlehem, Ramallah, Salfeet). A total of 500 leaf sample (sample represents a set of fully-expanded young leaves were collected from different branches) was collected randomly from stone fruit trees. Samples were then stored at 4°C prior to grinding before testing.

Samples were collected from young leaves and various leaf symptoms were observed. Samples were picked up from leaves with symptoms and symptomless ones to be tested for the two viruses as seen in the following tables.

Table (2.1): The samples taken from NARC in Jenin.

Stone fruit tree	No. of samples
Laroda	3
Appricot	8
Methlii	3
Almond	6
Lorban	3
BozElijel	3
Black diamond	3
Fermoza	3
Appricot	3
Kelsi	3
Green cherry	3
Red cherry	3
Red golden	3
Nectarine	3
Peach	3
Total	53

Table (2.2): The samples taken from Tulkarm.

Stone fruit tree	No. of samples
Almond	11
Green cherry	10
Red cherry	9
Nectarine	3
Peach	5
Apricot	18
Kelsi	3
Boz Elijel	3
Total	62

Table (2.3): The samples taken from Jenin.

Stone fruit tree	No. of samples
Almond	18
Green cherry	13
Red cherry	10
Nectarine	1
Peach	5
Apricot	16
Total	63

Table (2.4): The samples taken from Hebron.

Stone fruit tree	No. of samples
Apricot	16
330	3
Almond	10
Laroda	4
Kelsi	5
Wedson	4
Santa roza	5
Total	47

Table (2.5): The samples taken from Tubas.

Stone fruit tree	No. of samples
Almond	33
Green cherry	7
apricot	6
Red cherry	8
Peach	5
Total	59

Table (2.6): The samples taken from Nablus.

Stone fruit tree	No. of samples
Almond	22
Green cherry	9
Apricot	16
Red cherry	5
Peach	1
Total	53

Table (2.7): The samples taken from Ram-Alla.

Stone fruit tree	No. of samples
Almond	18
Green cherry	10
Apricot	13
Red cherry	9
Peach	3
Layla	4
Nectarine	3
Total	60

Table (2.8): The samples taken from Bet-lahem.

Stone fruit tree	No. of samples
almond	20
Green cherry	7
apricot	10
Red cherry	9
Peach	3
Nectarine	3
Total	52

Table (2.9): The samples taken from Salfet.

Stone fruit tree	No. of samples
Almond	19
Green cherry	5
apricot	22
Red cherry	3
Peach	1
Nectarine	1
Total	51

Table (2.10): The samples taken from each city and tested for ACLSV.

NARC (Jenin)	53
Tulkarm	62
Hebron	47
Jenin	63
Total	250

2.2 Laboratory Tests

For sample preparation, a 20 g. of leaves (including Pituls and green parts), were grinding in mortar using 2ml of extraction buffer that was prepared according to manufacturer instruction. Homogenous grounded leaf solution was then put in small Eppendorf tubes and then submerged in the ice till use.

Serological Detection (ELISA Test).

Collected extracts were tested by Double Antibody Sandwich ELISA (DAS-ELISA) (Cambra, et al., 1994).

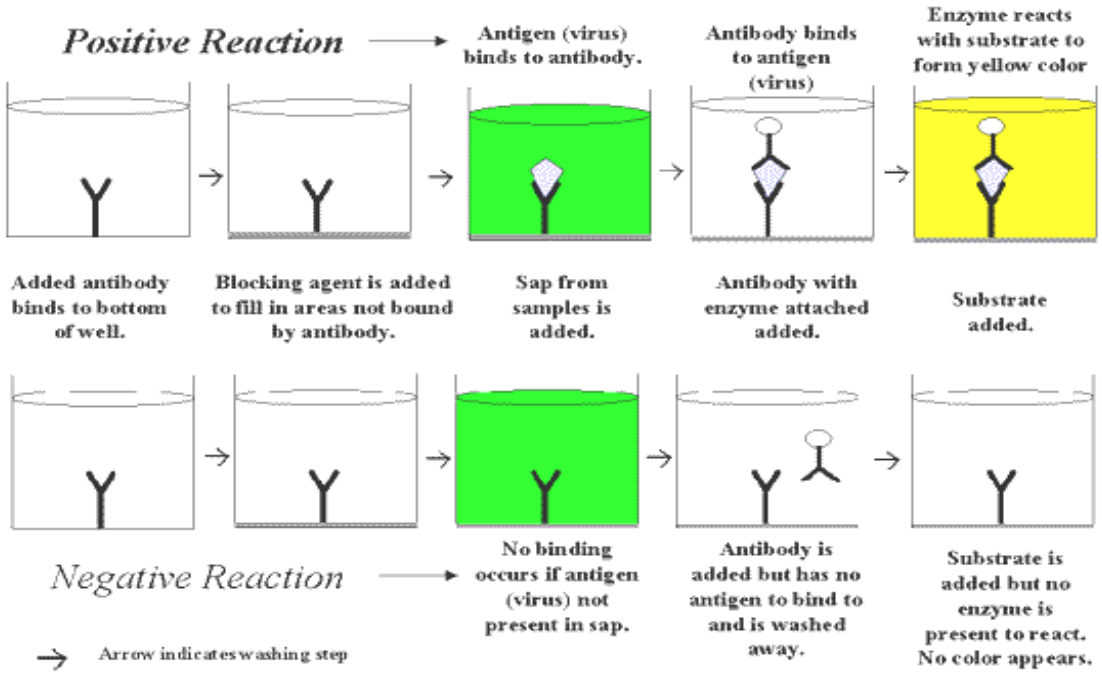

Figure (2.1): The general steps for DAS-ELISA method

For the detection of Apple Chlorotic leaf Spot Virus by Bioreba ACLSV complete kit 96.

Figure (2.2): Bioreba ACLSV complete kit 96.

Testing procedure was carried according to manufacturer instruction as follows:

Coating polyclonal antibody was diluted with coating buffer at 1:1000 dilution, dispensed 200 µlinto the wells of an ELISA plate (certified Nunc-Immuno Plates MaxiSorp F96), the plate was covered tightly and placed in a humid box, then incubated at 4°C over night. The washing buffer, extraction buffer and coating buffer were prepared according to the manufacturer's instructions. The ELISA plate were washed 3 times with washing buffer at room temperature, 3 minutes soaking each time and blotted the plate on paper towels. While incubating the ELISA plates, stone fruit leaf extraction were prepared. 200 µlof plant extraction were added into each well. The plate was covered tightly, placed in a humid box and incubated overnight at 4°C. ELISA plates were then washed 3 times with

washing buffer at room temperature, 3 minutes soaking each time and blotted dry. Detection polyclonal reagent was diluted at 1:1000 in conjugate buffer and dispensed 200 µlper well, the plates was covered tightly, placed in a humid box and incubated for 5 hours at 30°C. ELISA plates were then washed 3 times with washing buffer at room temperature, 3 minutes soaking each time and blotted dry. The freshly prepared para-nitrophenyl-phpsphate substrate (pNPP) was dissolved at 1 mg/ml in substrate buffer, then added 200 µlinto test wells and incubated at room temperature (20 -25°C) in the dark. ELISA results were recorded visually (color changing) or photo metrically using ELISA reader with dual filters at 405 nm.

Agri-test kit was used for the detection of Plum Pox Virus. The kit designed for 500 samples but as it shown in figure (2.3) the puffers were not sent with the kit so I prepared them in the laboratory of NARC using chemicals and materials from ANU store.

Figure (2.3): PPV Agri-test kit.

Table (2.11): Buffers were prepared according to the following instructions

	Chemicals	Quantities per one liter
PBS	In distilled water - adjusted pH 7,4 NaCl KH2PO4 Na2HPO4 KCl NaN3	8 g 0.2 g 1.15 g 0.2 g 0.2 g
Coating buffer	In distilled water pH 9.6 Na2CO3 NaHCO3 NaN3	1.59 g 2.93 g 0.2 g
Conjugate buffer	In PBS pH 7.4 PVP MW 24000 BSA Tween 20	20 g 2.0 g 0.5 ml
Substrate buffer	In distilled water - adjusted pH 9.8 Diethanolamine NaN3 MgCl 2	97 ml 0.2 g 0.095 g
Washing buffer	In PBS pH 7.4 Tween 20	0.5 ml
General extraction buffer	InPBS pH 7.4 PVP MW 24000 Tween 20	20 g 0.5 ml

Figure (2.4): Buffers prepared in laboratory to detect PPV in leaves extracts samples.

For the detection of Plum Pox Virus; testing procedure was carried according to manufacturer instruction as follows:

Coating polyclonal antibody was diluted with coating buffer at 1:1000 dilution, dispensed 200 µlinto the wells of an ELISA plate (certified Nunc-Immuno Plates MaxiSorp F96), the plate was covered tightly and placed in a humid box, then incubated for 2 hours at 37°C. The washing buffer, extraction buffer and coating buffer were prepared according to the manufacturer's instructions. The ELISA plate were washed 3 times with washing buffer at room temperature, 3 minutes soaking each timeand blotted the plate on paper towels. While incubating the ELISA plates, stone fruit leaf extraction were prepared. 200 µlof plant extraction were added into each well. The plate was covered tightly, placed in a humid box and incubated 2 hours at 37°C. ELISA plates were then washed 3 times with washing buffer at room temperature, 3 minutes soaking each time and

blotted dry. Enzyme conjugate (protein A alkaline phosphatase) was diluted at 1:500 dilution in conjugate buffer, dispense 200 µlper well, the plates was covered tightly, placed in a humid box and incubated for overnight at 4°C. ELISA plates were then washed 3 times with washing buffer at room temperature, 3 minutes soaking each time and blotted dry. The freshly prepared para-nitrophenyl-phpsphate substrate (pNPP) was dissolved at 1 mg/ml in substrate buffer, then added 200 µl into test wells and incubated at room temperature (20 -25°C) in the dark for 2 hours. ELISA results were recorded visually (color changing) or photo metrically using ELISA reader with dual filters at 405 nm.

Chapter Three

Results and Discussion

Palestine is one of the developing countries which depends on the agriculture sector to drive the Palestinian economy, the agricultural sector's has been decreasing gradually, due to growth in other sectors in addition to the continuous Israeli policies that has led to the marginalization of the Palestinian agricultural sector through land confiscation. Harsh weather conditions have resulted in fluctuations in the production of some of the main Palestinian agro products such as olives, vegetable**,** fruit trees. One of the largest challenges that faces agriculture sector is viral diseases which affects the fruit trees production and fruits quality. According to this, current study used Double Antibody Sandwich ELISA (DAS-ELISA) to detect the presence of two economically important stone fruit viruses in Palestine.

3.1 Disease Symptoms

The symptoms of the two diseases that were described in the first chapter of this research were verified in the field surveys. Various leaves were noticed to have some necrosis and mosaic yellow dots as shown in Figure (3.1).

Figure (3.1): Viral diseases symptoms found on leaves of studied stone fruit trees from various districts.

3.2 ELISA Assay for ACLSV

Double Antibody Sandwich ELISA (DAS-ELISA) assay was used for detection of the two selected viruses: Apple Chlorotic Leaf Spot Virus and Plum Pox Virus.

The kit of ACLSV was tested positively 3 samples out of 225 (1.33%) samples belonged to Fermosa variety (2 samples), and Nectarine. Positive ELISA result for viral disease was found in samples taken from NARC germplasm collection in Jenin. These samples which gave positive

results were symptomless. The results of ELISA was visualized after signal development indicated by the appearance of yellow color in samples; and were further confirmed by using ELISA reader at 405nm.

The incidence rate of ACLSV viral infection in stone fruit trees in the current study was lower than that reported from England 93.5% (Campbell 1961), Moldova 86% (Verderevskaja & Marinescu 1985), USA 60%(Waterworth, 1993), Czech Republic 69% (Karesová & Paprstein 2001), Turkey 21.28% (Çağlayan et al. 2006) and Tunisia 34% (Mahfoudhi et al. 2013).

Table (3.1): The disease distribution in the various studied districts of the West Bank area of Palestine.

District	No. of samples	Positive No. (%)
NARC(Jenin)	53	1.33
Tulkarm	61	0
Hebron	47	0
Jenin	63	0
Total	225	1.33

The outcome of this study spot light on the necessity of testing seedlings for these viruses before they will be disseminated all over the country. Germplasm collection plot. ACLSV which was detected in 1.33 % of the samples which in fact a low but alarming result to be aware of the spread of such economical important virus. The absence of any symptoms in most of the ACLSV infected plant samples exemplifies the need to test more samples to determine the infection rate of the virus in Palestine. The three samples of positive DAS-ELISA gave a negative RT-PCR results the

problem of this result was that the primer which used was old and didn't work correctly. The other expectation of this result that new variations of the virus could be appeared in this study and need other molecular technique to be detected. The three samples kept to be tested later on by c-DNA. Serological assays and nucleic acid based assays, including reverse transcription polymerase chain reaction (RT PCR), are the most used methods to identify ACLSV in stone fruits and help in the identification of clean propagation material. There is no cure and no anti-virus treatment for ACLSV in an established orchard and the only way to prevent its spared is removing the infected trees. The most effective way to control the virus and protect the stone fruit trees is to prevent its introduction in new orchards. Since ACLSV is latent in many cultivars and can be transmitted via grafting, the use of infected propagation material is the initial step to help the virus to spread more and more. Clean, virus-tested (negative) planting materials have to be selected carefully to prevent the introduction of ACLSV in new orchards. Therapeutic methodologies such as heat- and chemotherapy can be used in the laboratory to regenerate clean propagation material.

3.3 ELISA Assay for PPV

The detection of Plum Pox Virus (PPV); a quarantine dangerous virus, was carried on 500 leaf extracts and all samples gave negative results. Samples were collected from different areas as seen in Table (3.2)

Table (3.2): No. of samples tested for PPV and their districts.

The city	No. of samples
NARC(Jenin)	53
Jenin	63
Tubas	59
Nablus	53
Tulkarm	62
Hebron	47
Ram-Alla	60
Bet-Lahem	52
Salfet	51
Total	500

Although positive result appeared in one test plate but it gave a negative result on ELISA reader and this plate the only one which showed color change in some leaf extract samples and the rest gave no change.

PPV one of the most destructive viruses around the world and fortunately all the samples gave a negative ELISA results The first detection of PPV was in Eastern Europe (Bulgaria) (Atanassov, 1932) and it spread from there to most countries of the continent (OEPP/EPPO, 2006). No occurrence had been reported from outside the Euro-Mediterranean area until 1992. A report on finding PPV in India (Thakur et al., 1994) has not yet been confirmed. PPV was detected in many countries all over recent years. It was detected in Chile in 1992 (Herrera, 1994; Rosales et al., 1998), the USA in 1999 (Levy et al., 2000; ProMED, 2006; Snover-Clift et al., 2007), Canada in 2000 (Thompson et al., 2001), China in 2004 (Navratil et al., 2005) and Argentina in 2005 (Dal Zotto et al., 2006).

Fortunately this study approved the absence of PPV in west bank districts. This virus also does not occur in some neighboring countries i.e. Lebanon (Jawhar et al., 1996), it has been reported from others, i.e. Egypt and Syria (Dunez, 1988) and recently Jordan (Al Rwahnih et al., 2000).According to this researches results efforts should be serious to prevent the introduction of this dangerous virus in our country.

The positive result which appeared in some wells didn't give a positive result on ELISA reader and this due to low titer of the virus. This virus recently detected in very close countries like Jordan, and Syria (Jarrar et al., 2001) which mean that it will be hard to stop its spread and keep it away from our country. Management strategies of plum pox virus aim to prevent the introduction of the virus by using virus-tested clean nursery stock. Aphids are effective for spreading PPV within a tree and to adjacent trees. Aphids have several generations per year and have winged forms for movement from tree to tree. Insecticide management strategies to keep aphid populations low may help to slow PPV spread in areas where PPV is rare. Insecticide treatment can sometimes cause winged forms of the aphids to leave treated areas, taking the virus with them to infect new hosts. There is no way to make the infected tree free of virus.

Chapter Four

Conclusion and Recommendations

The current study used DAS-ELISA to detect two of most dangerous viruses infecting stone fruits in the districts of the West Bank in Palestine. DAS-ELISA was considered as simple, easy to perform, high specificity, high efficiency, and overall safe. It is not time consuming and give reliable results.

Fortunately PPV was not detected in our stone fruits growing fields; which must be under monitoring and observation. This thesis recommend to periodically testing randomly collected samples from the field every growing season to keep our country free of this virus.

ACLSV was one of the most growing threaten to stone fruit industry. the economic importance of ACLSV is largely due to its worldwide distribution and its capacity to induce severe graft incompatibilities in some Prunus combinations, causing major problems in nurseries. This study confirmed what was known about low or absence infection in our orchards. Even though the newly collected germplasm plot showed low incidence of the virus 1.3%; which indicate the possibility of the virus to be spread in nurseries continue to disseminate untested seedling to farmers. This study recommends keeping testing the virus at nurseries to make sure that distributed stone fruit samples are free from that putatively new emerged virus in Palestine.

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تقييم الاصابة بفيروس جدري البرقوق وفيروس البقع الورقية الشاحبة على أشجار اللوزيات في فلسطين

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قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في العلوم الحياتية بكليّة الدراسات العليا في جامعة النّجاح الوطنيّة في نابلس، فلسطين.
2020م

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الملخص

فلسطين شأنها شأن معظم الدول النامية تعتمد في اقتصادها على المحاصيل الزراعية بشكل كبير. بعض المحاصيل الزراعية وخصوصاً اللوزيات تصاب سنوياً بالعديد من الأمراض الفيروسية والبكتيرية والطفيليات. هذه الدراسة تهدف تقييم الإصابة بفايروس جدري البرقوق وفايروس البقع الورقيّة الشاحبة لأشجار اللوزيات في مناطق الضفة الغربية في فلسطين.

تمّ جمع ٥٠٠ عيّنة من مدن فلسطينية مختلفة في الضفة الغربية وتم فحص العيّنات للكشف عن وجود هذين الفايروسين من خلال الفحص السيرولوجي المصلي.

أظهر هذا البحث أنّ نسبة انتشار فايروس البقع الورقية الشاحبة ١,٣٣٪ في العيّنات التي تم فحصها من جنين في المركز الوطني الفلسطيني للبحوث الزراعية. جميع العيّنات كانت سليمة ولا تحمل فايروس جدري البرقوق الذي لم يسبق أن تم تأكيد وجوده في فلسطين على الرغم من انتشاره الواسع في العديد من الدول في أوروبا وبقية العالم ونظراً لأهمية هذا الفايروس الاقتصادية وقدرته على إحداث أضرار جسيمة وبأنه من الممكن أن ينتقل وينتشر عن طريق ناقل خاص به يجب على الباحثين أن يستمروا في فحص اللوزيات بشكل دوري للتأكد من عدم دخوله إلى البلاد وحماية المحاصيل منه.