An-Najah National University

Faculty of Graduated Studies

Detection and Identification of Viral and Viral-Like Diseases Infecting Citrus in the North of West Bank-Palestine

By

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

2017

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Dedication

To My Beloved Parents,

Who Always Picked Me Up On Time and Encouraged Me to Go On Achieving My Goals.

And Without Them None of My Successes Would Be Possible.

I Present This Work

Acknowledgments

I am thankful to Dr. Alkowni for his critical comments and suggestions for this study.

I greatly appreciate cooperation of faculty members of graduated studies and their support in doing applied part of my master research.

I am also highly grateful to my family for their support and I'm also grateful to people of NARC specially Shatela Jaradat and Maram Basem.

I take this opportunity to thank Nablus agricultural directorate, Qalqilya agricultural directorate and Tulkarm agricultural directorate for their support and cooperation.

I'd like to thank Ola Awwad the administrator of Qarwa Bani-Hassan Secondary Girls School (2015-2016),And Mo'yad Aqel the administrator of Zita-jmma'en Secondary Boys School (2014-2015) for their facilitations and elasticity in work.

الإقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

Detection and Identification of Viral and Viral-Like Diseases Infecting Citrus in the North of West Bank-Palestine

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 يُقدم لنيل أية درجة أو لقب 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:**

Signature: **التوقيع:**

Date: **التاريخ:**

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List of Abbreviations

%	Percentage
ARIJ	Applied Research Institute - Jerusalem
BrCA	Brown Citrus Aphids
BSA	Bovine Serum Albumin
CTV	*Citrus tristeza virus*
CPsV	*Citrus psorosis virus*
DAS-ELISA	Double Antibody Sandwich-ELISA
dsRNA	double stranded RiboNucleicAcid
DTBI- ELISA	Direct Tissue Blot Immunoassay- ELISA
ELISA	Enzyme-Linked Immunosorbent Assay
FAO	Food and Agriculture Organization
h	Height
H.U	Heat Units
KDa	kilodalton
MCAs	Monoclonal Antibodies
min	Minute
Mton	Millionton
NARC	National Agriculture Research Center
PBST	Phosphate Buffer Saline with Tween® 20
PCR	Polymerase Chain Reaction

pNPP para-Nitrophenylphosphate

PVP Polyvinylpyrrolidone

ss RNA single stranded Ribonucleic acid

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Abstract

Citrus is considered as one of the most fruit trees that are grown in West Bank, it is concentrated in the semi-coastal area specially Tulkarm and Qalqilyah and it is well grown in Nablus and Jenin areas. Due to water scarcity, citrus is counting for only 2.4% of the total fruit tree area, with production of 60,000 tons annually. Although citrus has small area but with larger economic importance.Citrus productivity is associated with many biotic stresses of which some fungi, bacteria, viruses and viriods, which cause several kinds of disease some of these diseases had high economical effect on citrus production.One of these diseases caused by a virus called *Citrus tristeza virus* (CTV), the virus that caused an epidemic effect on citrus trees leading to their decline. Usually found on those were grafted on sour orange and causes significant reduction of the total yield of the trees.

In this study, the prevalence of *Citrus tristeza virus* (CTV) among growing fields in northern of West Bank was assessed. A total number of 896 plant samples were collected from the four main districts. Serological assays

using Double Antibody Sandwich- ELISA (DAS-ELISA) to detect *Citrus tristesa virus* (CTV) and *Citrus psorosis virus*(CPsV) on 219 different citrus samlpes, while Direct Tissue Blot Immunoassay (DTBIA) to detect CTV only were applied on 677 of citrus samples. Besides that abiological assays were applied on10 selected samples.

CTV was detected in all districts with only 98 out of 869 samples, revealing an incidence of 10.9%. Surprisingly, the results showed that grapefruits and acid less Orange (Faransawi) were CTV free; while, the highest infection was recorded on Pomuelo ,Clementine and Orange cultivar(Valencia). The incidence of infection in each district was varied, Qalqilyah was showed the highest incidence of infectionwith a 23.5%, Nablus was came in the second rank of incidence of infection with a 12%, while Tulkarm was recorded a low incidence of infection with just 6%, the results also recorded a 4% of infection in nurseries, The differences between the four districts, reflecting the type of growing citrus varieties and farming activities. The bioassay (indexing) which was carried at the NARC facilities, were revealed of detection CTV and viriods symptoms by observing Vein corking yellowing and clear veining of the leaves. This study revealed that CTV is still a threat to citrus culture in Palestine. More care should be paid on dissemination and distribution of healthy propagating materials to farmers. The “Direct Tissue Blot Immunoassay” were applied for the first time here in Palestine to monitor the incidence of CTV, and proved its efficiency. Compared with classical DAS-ELISA, the biotechnique DTBIA had proved its simplicity and reliability in mass

diagnosis, and thus is highly recommended for nursery monitoring of the virus by the corresponding authorities.

Chapter One
Introduction

1.1 . Citrus physiology

Citrus trees are mostly evergreen plants, their leaves remain attached on the tree for one to two years, classified as anthophyta, angiosperm, which tend to produce a dicot seeds inside its fruits, and citrus trees are woody perennial trees, which not genetically programmed to die (Ferguson & Grafton-Cardwell, 2014).

Most citrus cultivars usually flower in the spring, while others tend to do that throughout the year like citron, limes and lemons cultivars, which usually have a three major growth periods, each flush period developing a new shoots and leaves parts ,the main blooming period appears in late February to March, additional growth may occur in September and October, each new shoot produces one to many complete flowers contains male structures and single female structure(see figure1.1) (Grafton-Cardwell & Ferguson , 2014).

Figure 1.1: complete citrus flower, with male structures and a single female structure in the middle**.** Source: http://www.backyardnature.net/yucatan/key-lime.htm.

Self-pollination usually happened in complete flower resulting in sexual reproduction which producing a zygote cell that typically tigers the development the ovule to produce seeds, which will surrounded by ovary tissue that will developed in to a fruit. Cross-pollination also happen among citrus cultivars, this process produce a new genetic material resulting in new offspring with hybrids characteristics, cross pollination could be happen between sweet orange(*Citrus sinensis*) and mandarin orage(*Citrus reticulata*) to yield tangors.

Figure1.2: Cross-pollination between sweet orange and mandarin to yield the Tangors hybrid species.

1.2. Citrus and Climate

Citrus cultivars are concentrated in tropical and subtropical regions between the latitude of 20-40 north and south of the equator, resent researches shows that were originated in Southeast Asia and some parts of India and China, and then transported worldwide (Spiegel-Roy& Goldschmidt, 1996).

Citrus growth is limited to several climatic conditions, which includes temperature, relative humidity, rainfall distribution and sunshine. All these factors influenced on quantity and quality of citrus production.

Excellent fruit quality could be produced in tropical and subtropical climate with average annual temperature about 20-30 °Cin tropical and 15-20 °C in subtropical. On the contrary, the poor- quality fruits can be produced in lowland tropics, like in some parts of Kenya and Sri Lanka. These regions have very high heat units (H.U.) and humidity make trees often flowering sparsely resulting in lower productivity, faster growth rate and increase respiration thus affecting the internal quality of fresh cultivars in low acidity content. In addition, the tropics climate condition encouraging pest's reproduction which disfiguring the fruit shape. While in subtropical such as the Mediterranean basin; which have a quite low H.U. due to the climatic condition with hot summer, cool winter, and low rainfall;is resulting in bright orange color, smooth skin and optimal blend of sweetness and acidity (Milind, 2007).

The minimum H.U. that needed for citrus growth is from 1000-1400, but it will mature faster with H.U. more than 3000.

World citrus production and consumption have grown strongly since the mid 1980s ,104 countries are considered to produce citrus, 70% of the total production are produced in the northern hemisphere and mainly concentrated in Mediterranean basin, China,USA, and Brazil. In 1996 citrus production reach 81.5 Mton/year, while 85.7 Mton/year in 2005(FAO, 2012).see figure 3.

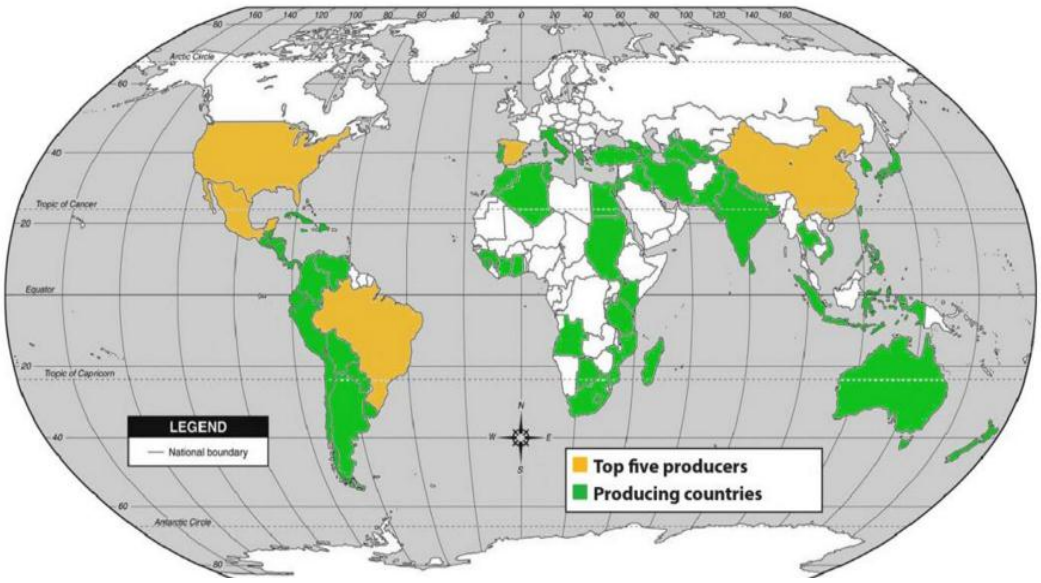


Figure1.3: Citrus producing counties worldwide (FAO, 2007).

West Bank of Palestine is located between the latitudes of 31.2-32 degree north of the equator, with an area about 5855.6 km^2, which divided into four different ecological agricultural zones these are: Jordan valley zone, eastern slopes, central highlands, and semi-costal region, by having the following percentage of area respectively :7.1%, 27.8%, 56.7%, and 8.4%,each region cultivated with crops that adapted with its climate. For example, central highlands, and semi-costal region cultivated mainly with fruit trees (olives, citrus, almonds, figs, grapes) and vegetables. Field crops also cultivated but in less manner. See table1:

Table 1: Distributions of agricultural-ecological system in West Bank.

	Jordan valley	Eastern slopes	Central highlands	Semi-costal	Summation
Jenin	-	-	343	230	573
Tulkarum	-	-	95.6	149.7	245.3
Qaliliya	-	-	88	86	174
Tubas	84	209	73	-	366
Nablus	-	157.5	456.1	-	613.6
Salfit	-	-	194	8	202
Ramallah	-	119	730	-	849
Jerico	316.1	293	-	-	609.1
Jerusalem	.2	101.3	252.1	-	353.6
Bethlehem	-	389	219	-	608
Hebron	-	308.2	759.3	-	1067.5
	source: Applied Research Institute - Jerusalem (ARIJ)				

Growing citrus in Palestine considered to be one of the most important sector of traditional agriculture, that has high contribution in increasing agricultural income, and meet the needs of local market.

Citrus agriculture begins in Palestine in nineteenth century when Palestinian farmers have evolved a new variety of citrus called Jaffa orange ‘Shamouti’, which originated as a bud mutation on Baladi tree near Jaffa. This later becomes the main oranges variety in the Middle East (Basan, G. 2007). By 1800s most of the inhabitants of the orange orchards are Palestinian Arabs, which tend to use the Sweet Lime (*CitrusLimetta*) and Sour Orange (Citrus *Aurantium*) as the primary rootstock in their farms.

In the period of 1850-1870 there were rapid expansions in citrus plantations as well as in citrus exportations to other countries especially to Europe region which documented in British consular reports in 1850s, Jaffa

Orange had became a very popular citrus variety used for export due to its tough skin(Krämer, G., & Harman, G.,2011; Issawi ,2016).

By the 1900s most of the inhabitants of the orange orchards wereJewish settlers, they owned approximately half of cultivated area with about 30,000 dunams of the country's 60,000 dunams of orange orchards (Issawi, 2016). Interestingly by 1930s citrus was recorded a 75 percent from the total yield of the exports, see figures: 1.4, 1.5.

Figure1.4: Sorting Jaffa's oranges, era from the British Occupation to the Great Palestine Rebellion, 1918-1935.http://btd.palestine-studies.org/content/jaffa-orange-palestinian-gift-world-7.

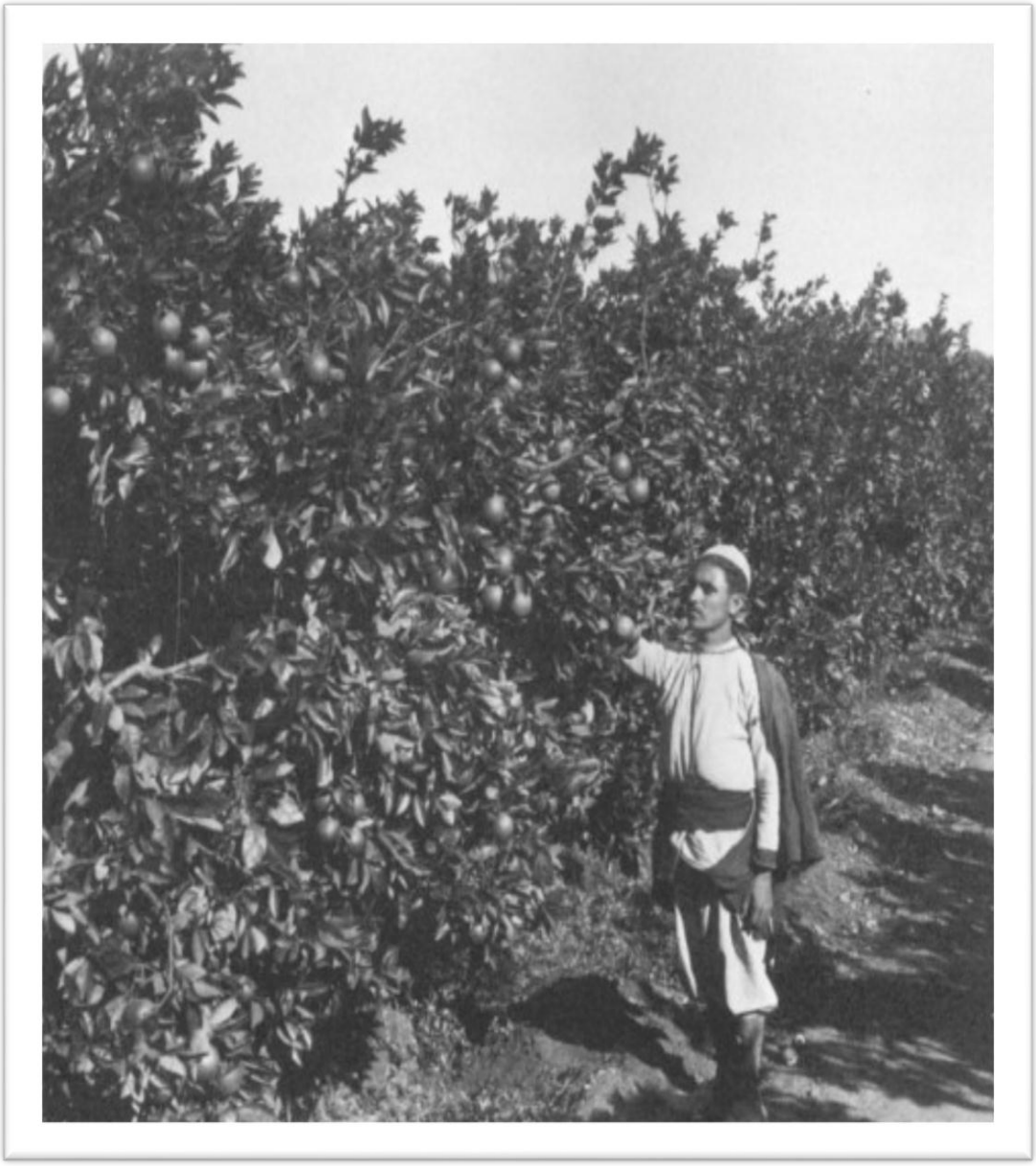


Figure 1.5: Palestinian farmer examining his citrus cultivar, era: From the British Occupation to the Great Palestine Rebellion, 1918-1935.http://btd.palestine-studies.org/content/jaffa-orange-palestinian-gift-world-2.

By 1950-1970 palestinain farmers tend to cultivate a new varieties like Mandarins, Tangarins, Valancia Orange and Grapefriut , in their orchards which mostly located in Qalqilyh , Nablus, Tulkarm ,Jordan Valley River groves and Gaza strip,See figure1.6 below)

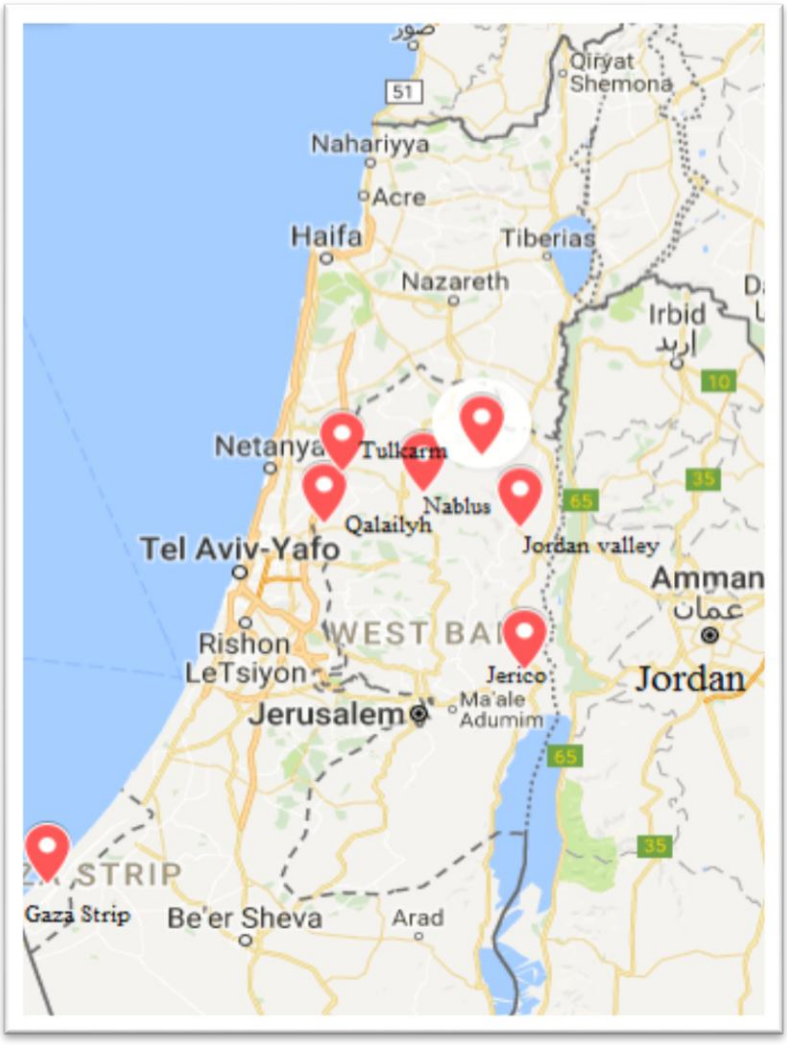


Figure 1.6: Cites where Palestinians grow citrus crops after 1965**.**

In 1970-1980 citrus cultivated area in Palestinian territories was approximately 72,000 dunams with a production of more than 200,000 ton.Palestinians are generally harvested citrus crops between November and March, and usually meet the needs of the local market, proximatelyhalf of the annual crop is exported to other near countries.

During the last three decades, there was an intrinsic change in Citrus agriculture, results in severe shortage in the size of the fields as well as the quantity of its production. The highest productivity years where recorded between 1955-1975; which there was up to 27,00 Hectares were cultivated

and its productivity were up to 200,000 ton, while between 1980-2002, there was a decline in its production with just 50,000 tons/year. This decline as mentioned earlier due to diminishing of area available for citrus growing due to political situation. Furthermore, the process of drying and cutting orchard continued till the beginning of the twenty first century. Actually the farmer cannot bears the high cost of workers, water used for irrigation, fertilizers, and pesticides. Thus the fruit prices cannot compete the prices in the foreign markets (Bitar,2011). This decline also associated with the decline in citrus trees in Israel and worldwide due to epidemic infection of sever virulent strain of Tristaza virus affecting the life cycle of citrus tree and thus its production.

The most productive citrus area located in north of West Bank with 14000 donum and its productivity about 30,000 ton, the most dominant type of citrus is lime 23% of the total area followed by 20% for (Shammoti) , 15% for Valancia ,1.5% Abu sora and 9% for the rest assets of Grapefruit ,Clementina and Mandarin (ARIJ, 2007).

This study we will assess the sanitary status of the citrus regarding the presence of the most dangerous virus that affect citrus crops in four different governorates in northern of West Bank, and record the incidences. In addition to that checking the seedlings in nurseries for their sanitary status as well as the germplasm collection of NARC in Jenin.

Chapter Two
Literature review

2.1. Citrus Diseases

Citrus trees usually exposed to abnormal condition that damaging the plant tissue, caused by pathogenic microorganisms and environmental stress, microorganisms could be bacteria, fungi, nematodes, viruses and viriods, which infect some parts or all parts of the tree(see table 2). Viruses and viriods considered to cause a systematic infection, that could spread through the vascular system to all parts and tissues (Dreistadt, 2012).

Table 2: Major diseases infecting citrus cropeworld wide.

disease	factor	description
Tristeza	Citrus tristeza virus (CTV)	decline (quick and slow) of the tree, stem-pitting, and seedling yellows.
Psorosis	Citrus psorosis virus (CPsV)	chlorotic flecks that are irregularly distributed,leaf mottling, and round chlorotic spots.
Cachexia	Citrus cachexia viroid (Hostuviroid)	gumming, bumps and projections on the bark.
Exocortis	Citrus exocortis viroid (CEVd)	stunted growth and reduced the yields.
Black rot	*Alternariacitri*	Fungal plant pathogen producing dark brown discoloration and decay in the leaves of fruit and vegetables.
Blue mold	*Penicilliumitalicum*	A fungal plant pathogen. It is a common post harvest disease.

The virus diseases of citrus trees usually identified by different detection methods (Roistacher, 1991), firstly field diagnosis and visual inspection in late spring and summer flush, secondly biological indexing , thirdly serological tests , and finally using molecular hybridization.

As mentioned earlier there are different type viruses and virus-like diseases that infect citrus trees affecting the life cycle of the tree or leading to affect its productivity, one of the most destructive disease is Tristeza.

2.1.1. Tristeza disease

Tristaza disease was likelihood emerge from china and then transported to other citrus producing countries by action of trade and propagation of bud-wood among citrus varieties. In fact Tristaza was first officially reported in South America in 1930s, the framers in Brazil named it "tristeza" which means sadness in Spanish, because it causes destruction of over 80 millions of citrus trees and renders other millions useless for production, thus damaging their economic. Its epidemic effect was detectedalso in Florida in 1951, many of researches were done between 1956-1959 on different citrus varieties which results in defining the main agent and sorting its isolates even from moderate one to quit sever one (knorr,1956; Grant,1959).the disease detected also inSpain in 1957, causing death more than 35 millions of citrus trees between 1962-1968 due to distribution of infected propagated budwood through out of country and importation of infected plant material from United State (Naqvi,2004).The disease continues to spread to a new areas all around the world,It has been isolated from different Mediterranean counties (Cyprus, Turkey, Lebanon, Morocco and Italy) where the sour orange is the predominant rootstock (Bar-Joseph & Lee, 1989 ; Roescher, 1991; Davino& Catara,1986; D 'On ghia, 1998).

Brown CitrusAphids(BrCA) (*Toxopteracitricida*),and other field aphids like: *Aphis gossypii*, *Myzuspersicae*. *T.citricida* was first officially discovered in Florida in 1995(Rocha-pena*et. Al* 1995; Halbert, 1998)see figure 2.1 ,and then considered to be the most efficient vector that transmit the disease inMediterranean region, these aphids transmit the disease through nearly all citrus species, hybrids and citrus relatives like: *Passifloragracilis* and *Pamburus missions* (Ahlawat& pant, 2003; Bar-Joseph & Lee, 1989).

Figure 2.1:A colony of brown citrus aphids (*Toxopteracitricida*, vectors of CTV) herded by ants on *Ladu tangerine* (Citrus *eticulateLadu*) fooiage.photograph: Michael Melze http://hawaiiplantdisease.net/cpg/displayimage.php?album=25&pid=681#top_display_media

http://www.ctahr.hawaii.edu/oc/freepubs/pdf/PD-77.pdf

Epidemiology studies indicate that *A.gossypii* the second effective victor that transmitted the disease to trees which are away from the sours tree by 8-10 trees, while *T.citricida* infect the nearest 1-2 trees from the sours, if both of aphid were found in the same area then *A.gossypii* transmit the disease for a big distances and *A.citricida*filling the gaps between these gapes, see figure 2.2. (Gottwald, 1996).

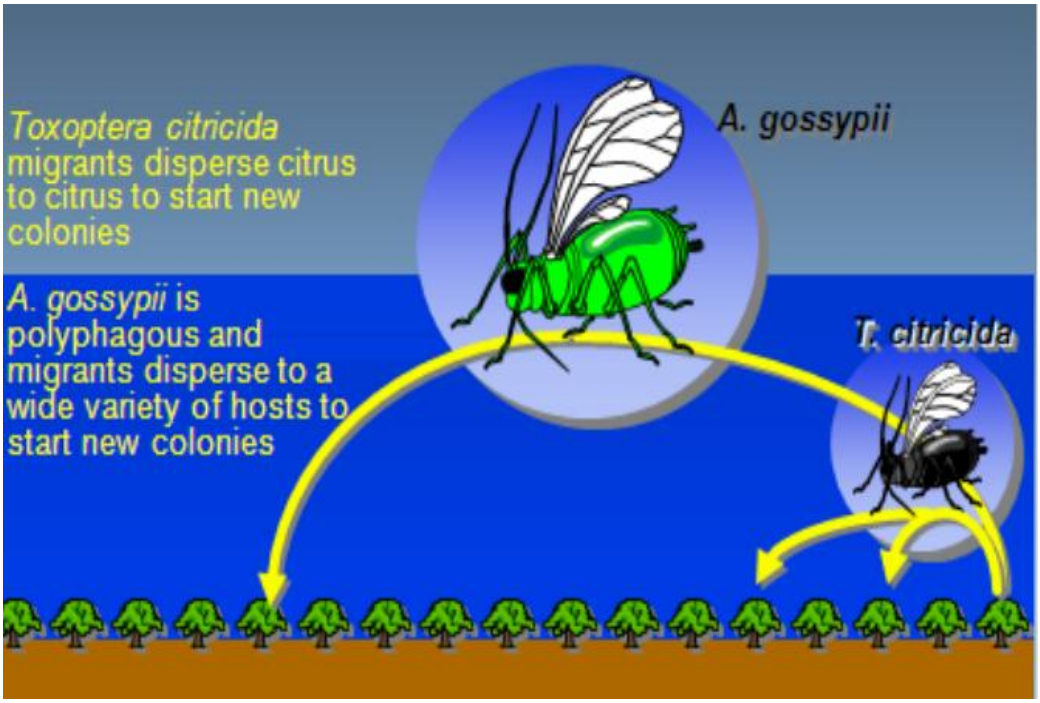


Figure 2.2: *T.citricida* and *A.gossypii* transmitting the infection within the same field. Source: http://citrusresearch.org/wp-content/uploads/Yokomi_Dr_Ray_Presence_CTV.pdf

Tristeza also could be spread by grafting on sour orang rootstock and mechanically by a knife cut, it was later shown to be caused by a virus and thus named as *Citrus tristeza virus* (CTV) (Bar-Joseph & Lee, 1989).

CTV isa member of the closterovirus group within the Closteroviridae, having a flexuous rod shape with 2000nm in long and 12 nm in diameter(figure 2.3), it is one of the largest RNA viruses known; that's due to its genome size typically between 19.2 and 19.3 kb long containing 12

open reading frames, which encoding at least 17 proteins from single stranded positive sense that enclosed by two types of capsid proteins (Karasev,2000; karasev*et al.* 1995; Bar-Joseph & Lee, 1989;Naqvi,2004).

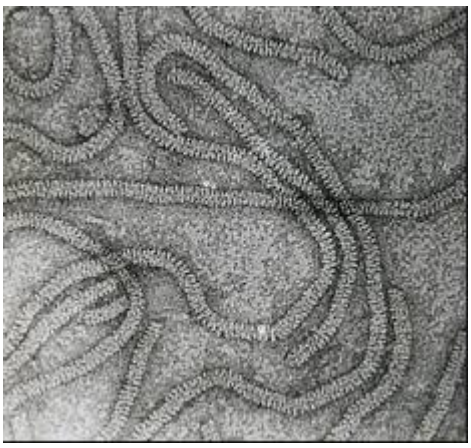


Figure2.3: Transmission electron micrograph of negatively stained, purified Citrus tristeza virus particles. Photograph courtesy of M. Bar-Joseph, Volcani Institute of Agricultural Research.

The effect of Tristeza virus or Citrus quick decline virus on citrus trees has been variable, there are different strains of Teristaza can cause several field symptoms on grafted trees regardless of rootstock that was used .some isolated strains from trees grafted on sour orang often cause phloem necrosis at the bud-union which indicate bud-union failure , this causes starch depletion in the root system thus the death of the plant (Ahlawat, 2003).Others can cause vein-clearing or stem pittingreaction in stems of grapefruit, grapefruit hybrid and Mexican lime(*C. aurantifolia*)(see figure 2.4, see table 3), observations of infected Mexican lime indicated that stem pitting and vein-clearing in the leaves are strongly associated.While some

isolates of CTV from infected grapefruit trees showed that they are not associated (Roistacher, 1991).

Table3: Symptoms of CTV that persist and its natural host range (Brunt.Aet *al*.1996).

Natural host range	symptoms
*Citrus*ssp. Grafted onto *Citrus aurantifolia*(sour orange) root stock	Quick decline, pitted stem.
C.paradisi (grapefruit)	Stunt.
C. aurantifolia(lime)	Die-back.
C.aurantifolia (Seville orange)	Seedling yellows.

In the other hand, the susceptibility and the severity of the disease related to several factors these are: climatic condition, kind of species, virus strains and the grafted trees itself (D ‘On ghia , 1998).

Figure 2.4: Stem pitting (compare healthy versus CTV-infected)photograph: Scot C. Nelson

http://hawaiiplantdisease.net/cpg/displayimage.php?pid=633

2.1.1.1.Previous studies

The first record of CTV in Palestine was officially reported by Jarrar et al. (2000); the infected trees were found in the Western West Bank at the Israeli border(Tulkarm and Qalqilyah) but no infection was assessed in the Eastern zone(Nablus and Jenin).

Also a 154 samples of Different varieties and species were collected from eastern and western of west bank which grafted onto sour orange at the CIHEAM-MAIB lab in Valenzano,Italy . Also these samples were grafted onto Mexican lime and sweet orange as reported by Roistacher(1991) and analyzed. The results shows a 22 samples out of 154 was infected with CTV and all the infected samples were from the western area of West Bank which conferring the results of(Jarrar*et al.,* 2000); the results also indicate that 'Valencia' variety have the highest level of infection of the sweet orange followed by'Shamouti' and 'W. navel', whereas the local orange ‘Fransawi’ was virtually free from CTV (Djelouah K.&D’Onghia A.M. 2009).

In the other hand CTV was first detected in Israel in 1956 infecting Meyer lemon and some other citrus variety, and natural spred of CTV was noticed in 1970(Soroker*et al.,* 2009). During 1963-1978 many several studies conducted to detect the natural vector of CTV, the researchers didn’t detect *Toxopteracitricidus*(Swirski and Amitai, 1999) while others vectors like *A. spiraecola, A. gossypii* and *T. aurantii. A. gossypii* was imputed to transmit some strains of CTV in Israel (Raccah*et al.,* 1976).

2.1.1.2. Methods of detection

2.1.1.2.1. Field Diagnosis Method

The iodine test: the decline symptoms in trees of sour orange rootstock with scion sweet orange usually indicate a possible infection of CTV. The necrosis of the phloem resulting in preventing of the starch movement to the root from the canopy, thus the starch supplied in the root will depleted followed by sudden quick decline and wilting. a simple field test can be carried out to detect starch depletion in the rootstock of the sour orange and the scion of sweet orange, grapefruit or mandarin below the bud-union, the test simply done by applying the of iodine to the exposed cut surface of the root, the lake of development of a dark blue reaction indicate starch depletion thus possible presence of CTV, whereas the presence of blue color reaction indicates healthy phloem tissue to transport the carbohydrates from the canopy to the roots below the bud-union,(see figure2.5)(Bitancourt 1944; Fawcett, 1945;Wallace, 1978).

Figure 2.5: starch test for CTV. Source: http://citrusresearch.org/wp-content/uploads/Yokomi_Dr_Ray_Presence_CTV.pdf

2.1.1.2.2.Seedling Indexing Method

Seedlings of limes *(Citrus aurantifolia*) and citron (*Citrus medica*) are still a very powerful tool for detection of tristeza virus, which show vein clearing and stem pitting however, the Mexican lime is very sensitive to tristeza and it is the preferred indicator specially for the sever strains.

The process of indexing needing an indicator plant and inoculums tissue that used for grafting, usually two buds or leaf discs, or a minimum five to six leaf pieces can be used for each plant (see figure 2.6). Since CTV is phloem-limited, it is necessary that the inoculums tissue have a phloem part from donor to contact with phloem tissue of the receptor plant (indicator).the inoculums buds or leaf pieces placed in the lower parts of the test seedling after cleaning the stem from lower leaves. When the inoculums are observed for survival the seedling were cut for 20-25 cm from the soil surface and this step usually done after 2-3 weeks of inoculation. Then symptoms will be appears after 9 weeks of inoculations. The symptoms are vary from vein clearing, leaf cupping, vein corking and stem pitting of Mexican lime or other seedling indicator are highly diagnostic for CTV(Roistacher, 1991).

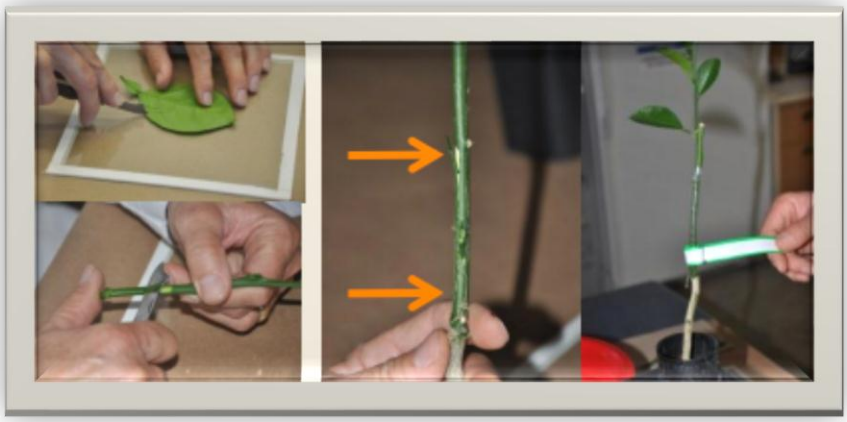


Figure 2.6: inoculums tissue that used for grafting: buds or leaf discs.

Source: http://citrusresearch.org/wp-content/uploads/Yokomi_Dr_Ray_Presence_CTV.pdf

2.1.1.2.3. Serological technique

Many serological technique have been evolved to diagnose CTV such as SDS-immunodiffusion techniques (Gonsalves*et al*., 1978), in situ immunoflorescence (Brlansky et al., 1984), and several enzyme-linked immunosorbent assay (ELISA) procedures (Bar-Joseph et al., 1979; Bar-Joseph &Malkinson, 1980).

The application of ELISA revolutionized the diagnosis by allowing a large number of samples to be tested in short period with a high level of sensitivity and low cost, it based on verifying the presence or absence of CTV isolates that are mild reacting in indicator plants. The production of monoclonal antibodies (MCAs) specific to CTV in 1982 helped in specify the isolates (Vela 1986).

The ELISA technique was used for large-scale surveys, CTV control in citrus nurseries, epidemiology, and other studies (Cambra, 1991; Gransey, 1991).

There are two famous technique of ELISA used to detect CTV : the DAS-ELISA and DTBI- ELISA, the first one is The double antibody sandwich ELISA (DAS) uses antibodies (IgG) which are bound to the surface of a microtitre plate to capture the antigen (A) of interest which may found in the plant extract. A specific antibody-enzyme conjugate (E) is then used to detect the trapped antigen. The presence of enzyme (usually alkaline phosphatase) is detected by a colorimetric substrate (S) reaction.

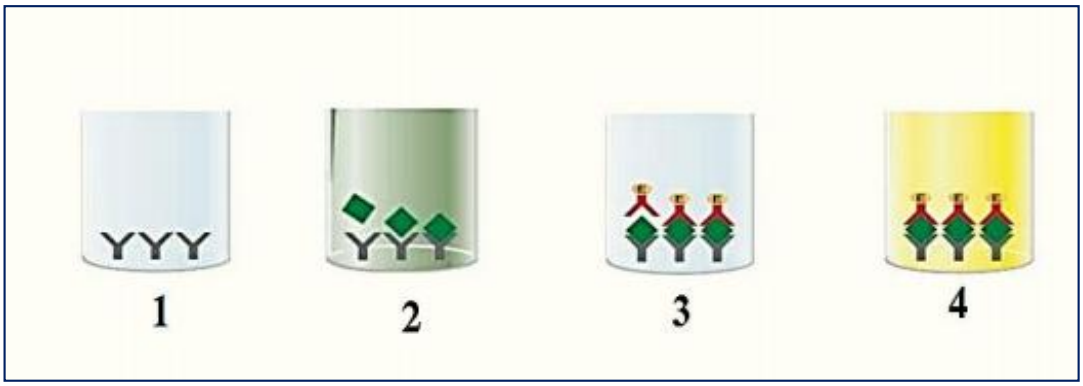


Figure 2.7: Illustration steps of DAS-ELISA technique: 1-coating of antibodies,2-adding antigen, 3-adding antibody-enzyme conjugate, 4-substrate reaction.

The most important limitation of DAS-ELISA is the necessity to prepare plant extract which is laborious and time-consuming and also enhances risk of contamination, and need an expensive equipment for reading the plates. The second one is the direct tissue blot immunoassay or tissue print - ELIZA, detect the virus in plant tissue that was imprinted on nitrocellulose membrane (Ravichandra, 2013).The use of membranes is a good alternative to extract preparation for detection of CTV thus revolutionized and simplified the detection of the virus (Lin,1990; Garnsey, 1993).It didn’t

need a specialized equipment and its considered to be as universal antiserum react to all strains.

2.1.1.2.4. Detection of CTV by dsRNAAnalysis Method

This technique based on detection of band migration of CTV dsRNA by polyacrylamide gel electrophoresis in plant extracts. (Dodds*et al.*,1983).The use of new methods to differentiate CTV strains and isolates may could help in providing rapid identification of isolates that took long time to be distinguished by indexing method (Bar-Joseph *et al.* , 1986).

Table 4: Brief summary about methods that used for detecting *Citrus tristezavirus* (CTV) (Nelson, *et al*.2011).

Method of detection	Comments
Biological indicator plants	Takes time to develop the symptoms,mild strain can't be detected
Serology-based methods (polyclonal and mono clonal antibodies)	Quick, inexpensive, reliable, easy to perform, and most commonly used (Rocha-Pena and Lee, 1991).
Reverse transcription polymerase chain reaction(RT-PCR)	Technically challenging, detect CTV when ELISA cannot (Mathews, D.M et al.1997).
Isolation of dsRNA from citrus	Complicated by variables like: strain of the virus or the host species, and the results may change by the time (Dodds, J.A *et al.*1987).

2.1.2. Psoriasis

It's a disease caused by *Citrus psorosis virus* , which is an infectious filamentous negative stranded tripartite virus located in the genus ophivirus, with coat protein (CP) of about 48 kDa, ss RNA genome is contained in short 300-500nm, and long1500-2500 nm and 10nm in diameter. It's economically important which causes annually decrease in yield of about 3% and decline in citrus trees by affecting conducting

system. So it is considered to be serious and most widespread disease in Mediterranean area (Loconsole*et al.* , 2006 ; Gary *et al.* ,1998).

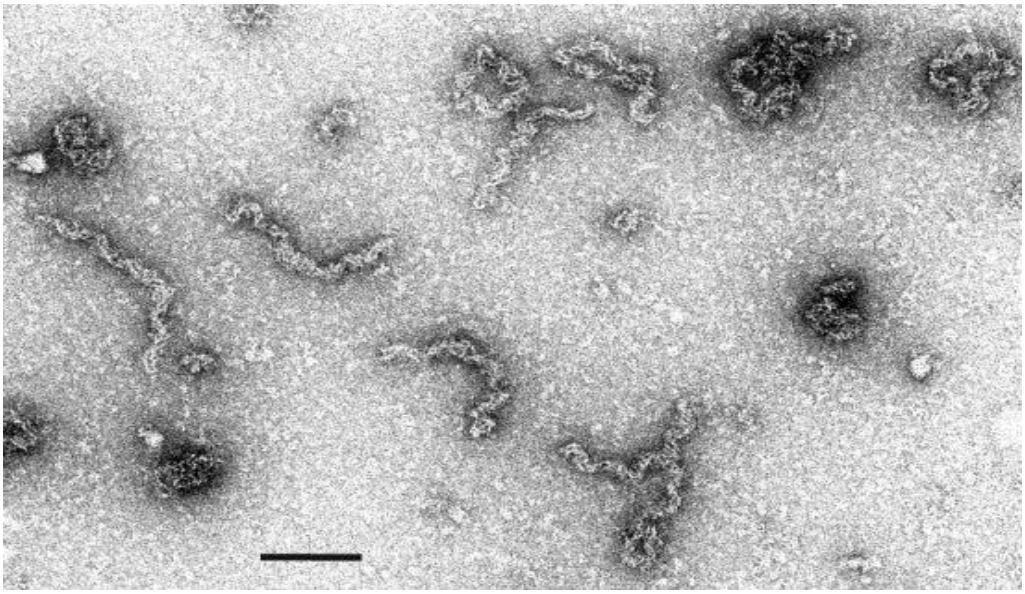


Figure (2.8): Electron Micrograph of Citrus psorosis virus, 100nm.

http://www.dpvweb.net/notes/showem.php?genus=Ophiovirus

Fawcett in 1933 observed a mosaic young leaves of citrus trees with bark scaling symptoms and suggest that cause of the disease is a virus (Gary *et al.*, 1998) see figure 2.9, which then named it as a citrus Psorosis A virus, in order to distinguish it from another severe form of Psoriasis called Psorosis B.

Figure (2.9): Mosaic symptom on young leaf. http://www.dpvweb.net/dpvfigs/d401f05.jpg.

Psorosis A complex has been linked with bark scaling of trunks and gum accumulates below the bark scales, wood staining and vessels occlusions. while the more virulent Psorosis B shows extensive bark-scaling, also affecting thin branches, appears earlier, with chlorotic blotches on old leaves, and sometimes ringspots on fruits (Roistacher,1991).

Psorosis A and B appear to be different strains, and may occur in the same tree but in different tissues, if Psorosis A occur in the same tissue with Psorosis B a mild symptoms will appear and the infection will diagnosed as psorosis A , this what called a cross-protection that used to decrease the effect of Psorosis B on trees in the field (Roistacher,1991). Identification of psorosis strains has become noticeably confused, since the bark scaling is the most characteristic symptom that appear on the tree and many related disease have the same features, for example citrus ringe spots have been describe to be different bark scaling disease, but later on it was suggested that ring spot and psorosis isolates are variants of the same disease (Achachi*et al.* ,2014).

The farmers often propagate infected bud from symptomless tree. That's because Symptoms often appeared on adult trees usually more than 10 years, thus the virus can be transmitted from symptomless host and induce symptoms in progeny trees (Aliotob*et al.* , 1999). The most susceptible trees to Psorosis are: sweet orange, mandarin and grapefruit, while soure orange, sour lemon and rough lemon less infected and show no bark scaly symptoms (Roistacher, 1991).

2.1.3. Viroids

viriods are small infectious agent that affect citrus by grafting or mechanically by tools, seed and vector transition have not been reported experimentally.

It classified into distinct groupbased on their biological and physical properties as follows:

Table 5: Classification of viriods that infect citrus (Kunta&Skaria, 2007):

Groups of viriods	Abbreviation
Citrus exocortis viroid	CEVd
Citrus bent leaf viroid	CBLVd
Hop stunt viroid	HSVd
Citrus dwarfing viroid	CVd-III
Citrus bark cracking viroid	CVd-IV

Recently, a new citrus viroid species, named Citrus viroid OS (CVd-OS),was reported (Ito et al., 2001).The exocortis viroid is the most destructive viriod on citrus whichcauses bark scaling and stunting of citrus plant(see figure 2.10), which mostly present where trifoliate orange is the primary rootstock,while if the rootstock is sour orange then the plant doesn't shows any symptoms for viroid infections, also it is may infect other non-citrus species such as tomato and cucumber and cause severe epinasty symptoms on leaves of these hosts.

Figure 2.10: Bark scaling extended up the bud union. http://betterbugs.com/blog/?p=114

Symptoms of CEVd was first reported by Fawcett and klotz in 1948 as bark cracking and peeling below the bud union, and stunting of infected trees(Ahlawat& pant, 2003) , by the time these symptoms show some of variation specially in the mode of scaling and the time of appearance , attributed to different strains of viriod for this plants.

Chapter Three
Materials and Method

3.1.Study samples

This study was conducted on citrus cultivars from different fields in the northern of West Bank of Palestine (see figure 3.1), with the collaboration work with national agriculture research center(NARC) that provided some of materials and instruments which were needed to complete the study.

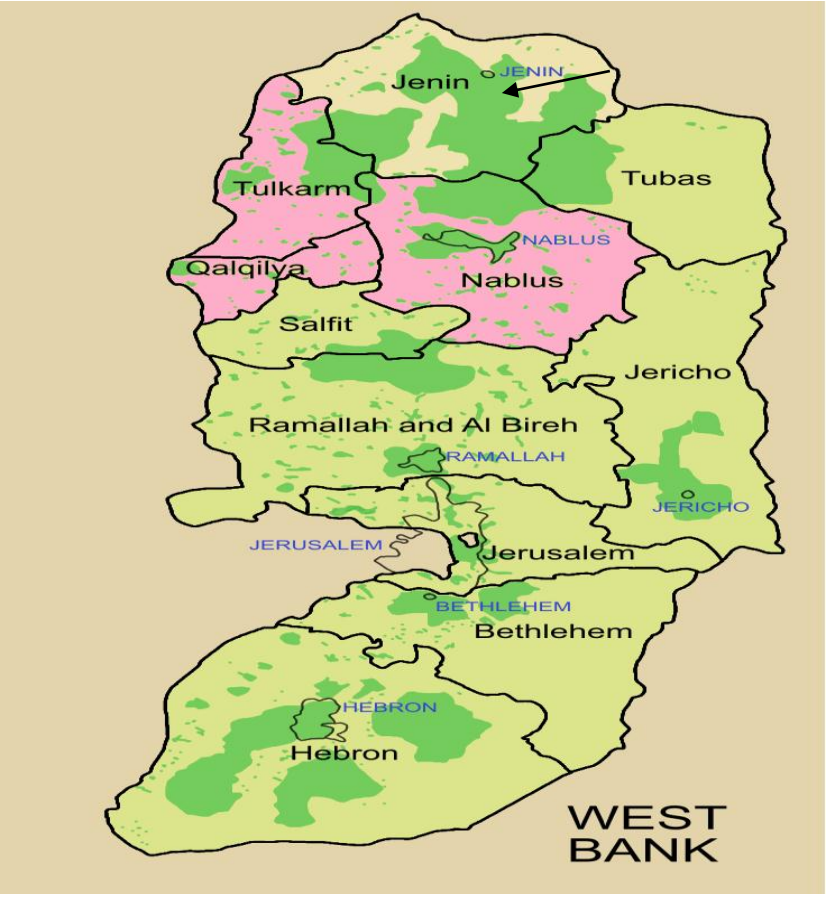
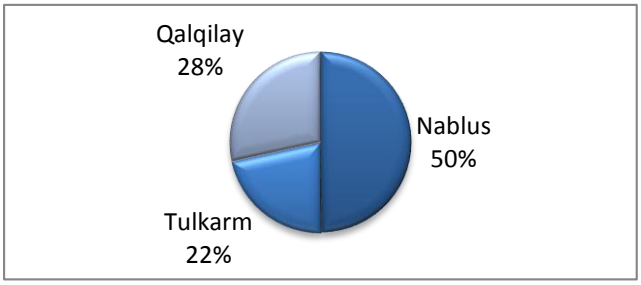


Figure 3.1: West Bank-Palestine, regions of collected samples appears in pink color.

source: https://upload.wikimedia.org/wikipedia/commons/f/f5/Palestine_election_map.PNG

The numbers of the samples from each governorate were in accordance with the citrus production, in which Nablus district was the most citrus

producing one in the northern of west bank followed by Qalqilya and Tulkarm. See digrame1 below:



Figurer 3.2: Distributions of collected samples among the three governorates.

3.2. Collection of citrus samples

Samples were collected from nurseries and commercial orchards of different citrus varieties and ages. Samples were collected during Septemberof 2015 and April of 2016 to givetotal of (896) plants from 35 fields, of size around (3-18 donum), and different nurseries.

DAS-ELISA detection for CTV was applied on 219 of the samples; of which 100 samplessubjected to CPsVdetection. While a total of 677 sampleswas applied to Direct Tissue Blot Immunoassay (DTBIA), see table (6) and (7).

Table 6: Distribution of the study samples in West Bank by city that used for DAS-ELISA, which were collected during 2015.

City	Number of samples
Nablus	79
Qalilay	46
Jenin- NARC	94
Totall number	**219**

Table 7: distribution of the study samples in west bank by governorate that used for DTBIA, which were collected during September of 2015 and February of 2016.

Governorate	Number of samples
Nablus	180
Qalqilya	162
Tulkarm	117
Other sours-plantations	218
Total number	**677**

The collected samples were prompted according to citrus agriculture distribution and economic value. Sampling process was obtained during autumn of 2015 and spring of 2016 and it was randomly collected from commercial orchards of different citrus cultivars.Three to five dormant cuttings of 10-20 cm long were collected from each plant, labeled and stored at 4°C.For DTBIA test samples were prepared by cutting tender shoots and leaf petioles with cleaned sharp blade and pressed immediately against the nitrocellulose membrane.

Table 8: types of main citrus crop that used in the study and their distribution among three main citrus economic areas according to Ministry of Agriculture.

Type of crop	Nablus		Tulkarm		Qalqilia	
	Area in dunom	Number of trees	Area in dunom	Number of trees	Area in dunom	Number of trees
Grapefruit	11.38	550	0.08	4	0.16	6
Polemo	24.5	1133	0.16	8	0.68	61
Lemon	844.2	38062	522.27	22047	754.89	33702
Valencia orange	524.44	23958	404.62	13993	195.92	8556
Shammoty orange	124.03	5380	9.7	453	41.80	1725
Navel orange	409.6	18371	1.36	63	52.5	2356
Francawy orange	9.5	450	2	100	15.2	890
Mandarin	92.41	4002	9.01	427	1.04	32
Clementine	322.27	14225	98.11	4471	233.63	8803
Poppy	21.51	809	12.61	538	37.25	1851
Other citrus	532.48	21809	211.29	8315	328.28	17211
Total	2916.32	128749	127.21	50419	1661.35	75193

Palestinian Central Bureau of Statistics and ministry of agriculture 2010.

3.3. DAS-ELISA

The followings were the main steps of double antibody sandwich enzyme-linked immunosorbent assay based on kit provided by (AGRITEST) for CPsV and BIOREBA for CTV detection:

First -coating wells with coating antibodies:the coating buffer was prepared by dissolving each tablet in 100 ml of double distilled water to have a 50mM of carbonate-bicarbonate buffer, PH with 9.6 and containing 0.02% of Sodium Azide (NaN3),then the antibody was diluted with coating buffer

at equal ratio, each plate consuming a 15 ml of coating buffer mixed with 15ul of IgG by loading a 200ul to each well. The plate was tightly covered with parafilm and placed in a humid box then incubated at 4-6 ˚C overnight.

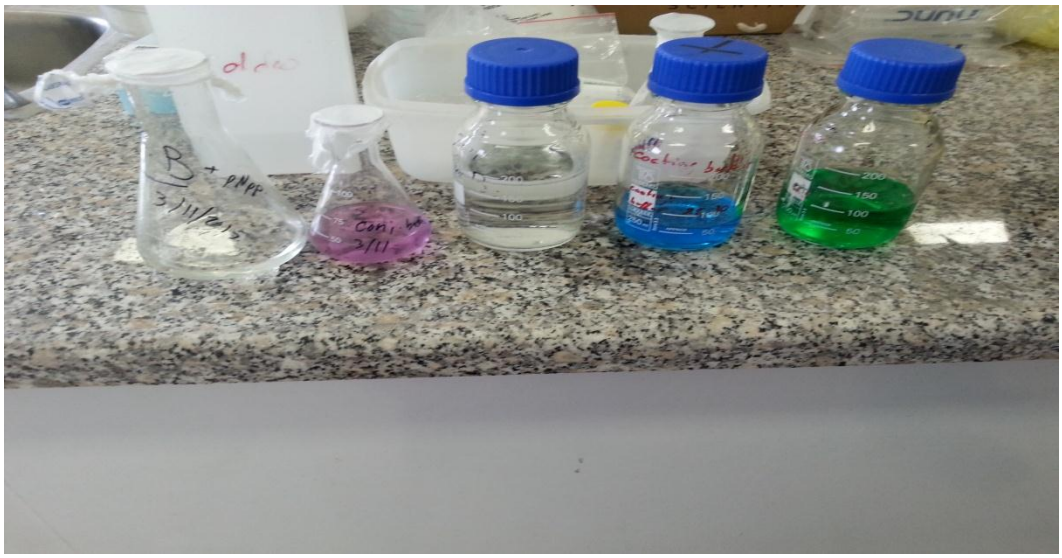


Figure3.3: Buffers that was used; extracting buffer in green color, coating buffer the blue one, conjugate buffer in pink and finally washing buffer.

Second-Preparing washing buffer: 10g of content of one pouch was dissolved in 1L of double distilled water, resulting in a 10mM of phosphate buffer (PH 7.4) and containing 140mM of NaCl, 3mM KCl, and 0.05% Tween 20 (PBST).After incubation getting rid of coating buffer and then the plates where washed three times with washing buffer, using 200ul/well of the washing solution, shaking and removing any liquid by blotting the plates on paper towels.

Third -sample preparation and deposition:The samples were prepared during the incubation period of coating step, by firstly preparing the general extraction buffer using 40ml of 10xof the stock solution adding it

to 360 ml of double distilled water. Secondly: 0.3g of plant tissue from each sample where weighted and mixed with 2ml of prepared extraction buffer using mortor and pestle. The extract was placed in ependroph and directly placed in the ice. And wait to have a clear extract. a 200ul of the extract where deposited in to each previously coated wells, with duplicated sample in considering the positive and negative controls, the plates where covered tightly ,placed in a humid box and incubated at 4-6^{o}C overnight.

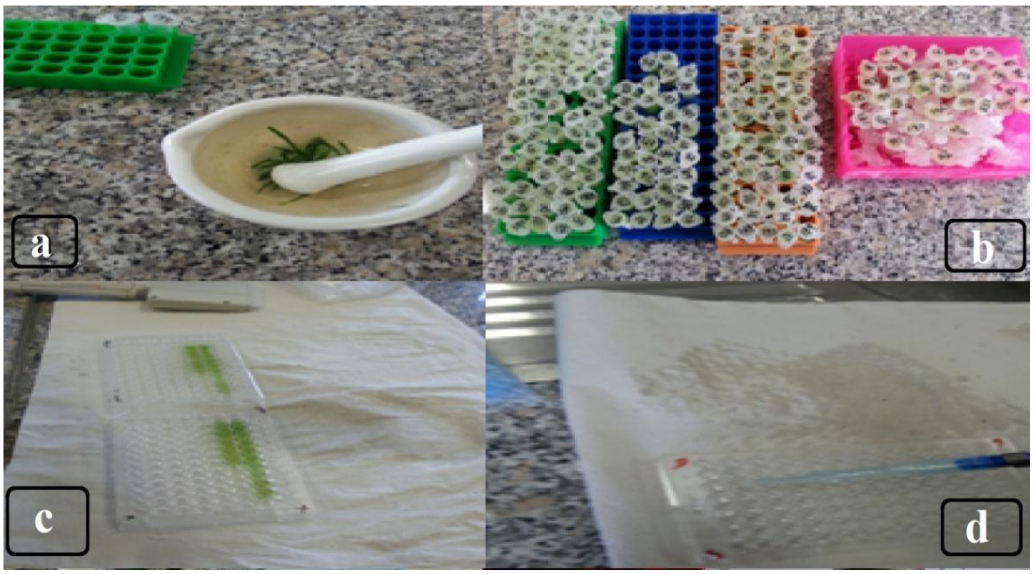


Figure3.4: Steps of DAS-ELISA: a) grinding plant tissue, b) plant extract after grinding, c) applying plant tissue in to wells, d) washing the plate.

Fourth-deposition of conjugated antibody: washing the plates as in b, then dilution of the conjugate buffer by adding 10ml of 10x concentrate to 90ml of double distilled water results in 20mM Tris buffer containing: 137mM NaCl, 3 mMKCl, 1 mM $MgCl_2$, 2% PVP 24 KD, 0.05% Tween 20, 0.2% BSA, and 0.02% NaN3.The diluted conjugate buffer were mixed with enzyme conjugate in equal ratios, 15ul:15ml for each plate, a 200ul of the mixture were used for each well.then the plates were covered and incubated

at 300C for 5 h.the plates washed out three times after the incubation period using washing buffer as in step b.

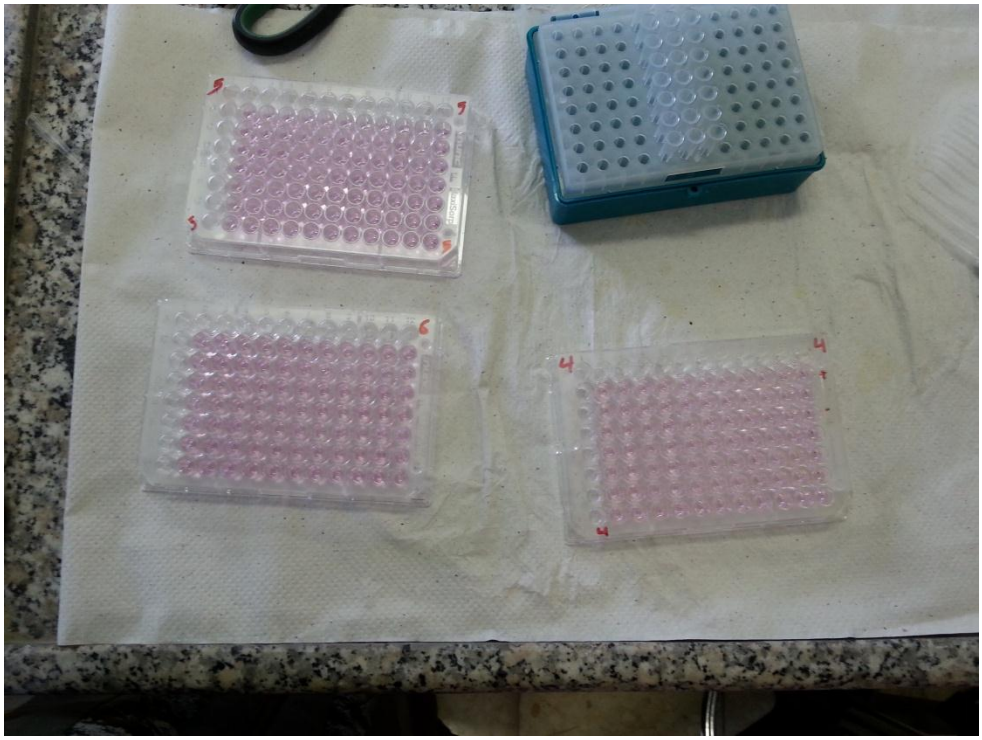


Figure 3.5: Applying the conjugate buffer to the wells.

Fifth-color reaction: 20ml of 5x substrate buffer where mixed with 80ml of double distilled water to results in 100ml of 1 M diethanolamine containing 0.02% NaN3.20mg of pNPP was dissolved in 20ml of the diluted substrate buffer to obtain a solution of 1mg/ml,and it should dissolved 15 min before used.the resulted mixter then will applied to wells as 200ul for each one.incubation at room temperature in the dark for 2h.followed with reading by an ELISA reader at 405 nm.

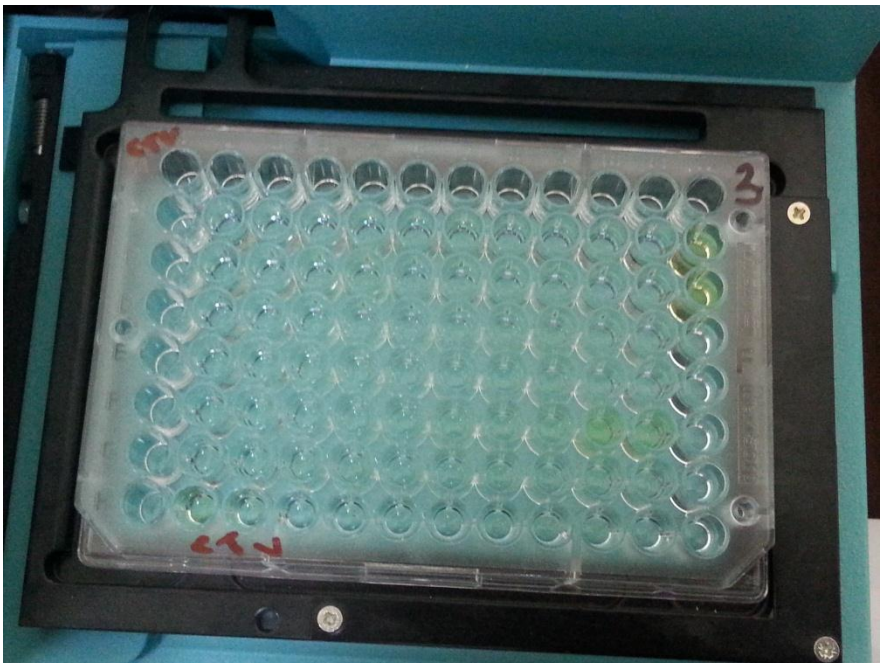


Figure 3.6: Color reaction and ELISA reading.

3.4. DTBIA

Direct tissue blot immunoprintingmain steps based oncompanykit from plantprint-Valencia, España is as follows:

First -membrane printing:a clean tender shoots and leave pedicels were cut freshly and pressed carefully against the nitrocellulose membranes that already divided in to squares and numbered to differentiate between the samples.

Second-membrane blocking:prepare the phosphate buffer saline by dilute the continent of each red tube 10ml of 10x PBS in 90ml of distilled water.0.2 g of bovine serum albumin (BSA) were dissolved in 20ml of PBSpervously prepared. To obtain a 1% solution. Then the printed membrane were placed in a plastic bag, submerging them completely by

1% of albumin solution with slight aggitation , then incubated it overnight at 4^{o}C.

Third-addition of monoclonal antibodies specific to CTV conjugated with phosphate alkaline:when the incubation time has been elapsed , the albumin solution were discarded, while keeping the membranes in the same container. To add the solution that contains the monoclonal antibodies, which prepared by diluting 0.2 ml of monoclonal antibodies in 20ml of previously prepared PBS. Then the membranes covered and incubated for 2-3 h at room temperature.

Fourth- washing of membranes:the conjugate solution were discarded ,and the membranes were washed out with washing buffer, which prepared by dissolving 1ml of Tween-20 and 10g of mineral salts in 1L of distilled water. Repeating the process three times with shaking the membranes manually for 5 min.

Fifth-membrane development and reading: the substrate buffer were prepared 10 min before use by dissolving the enclosed tablets (BCIP-NBT,Sigma Fast) in 20ml of distilled water. Then applied it on the membranes to incubate at room temperature till to the appearance of purple-violet color in the positive control, usually the color change takes 10-15 min, the reaction then was stopped by washing the membranes with tape water. The membranes were spread on an absorbent paper, and the printings were observed by using dissecting microscope, the presence of purple-violet precipitates in the vascular area of sections of plant material, reveals the presence of the CTV.

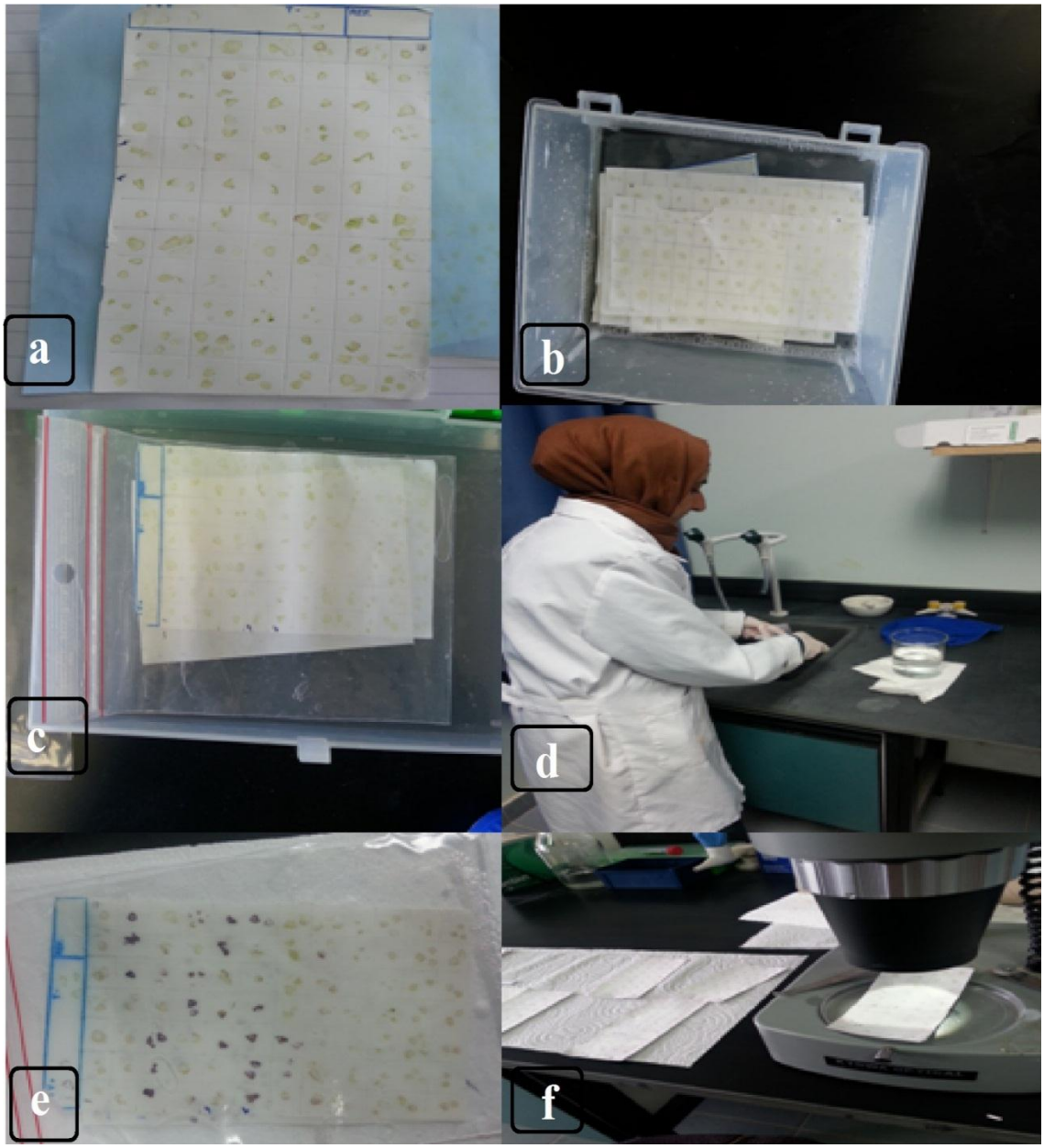


Figure 3.7: DTBIA steps a) membrane printing, b-c) membrane blocking, d) washing, e) membrane development, and f) membrane reading with dissecting microscope.

3.5. Bioassay (indexing)

Ten trees of Mexican lime and citronwere used as indicator plantsto detect CTV, where the inoculums tissue “buds” were obtained from three types of citrus(grapefruit, Polemo and sunrino) which were detected with CTV using DAS-ELISA.the grafted trees were placed in polycarbonate house in

NARC with adjusted temperature between 24-28 C^{o} maximum during the day and 17-21C^{o} in night. See picture 3.3 below.

Figure 3.8: Bio indexing were made for ten samples of citrus in NARC.

The trees were grafted in 23 of December 2015, after two weeks from inoculation the wrapping tapes were removed and the survival of the grafted inoculums was recorded.(see figure3.4 below) At this point the side shoots were trimmed in order to have a single shoot to examine the symptoms easily. Some of the symptoms usually appear after 4-5 weeks. While others needs about 4-6 months to be best evaluated.

Figure 3.9: the survival of the grafted inoculums**.**

The trees were examined at two periods for recording the developed symptoms one in the bingeing of March and the other in the beginning of May.

Chapter Four
Results and Discussion

4.1. Field observations

Among the thirty five different fields there were five fields showing a symptom based on visual screening for CTV and CPsV and viriods which affecting different varieties of citrus, the symptoms including yellowing and clear veining of the leaves, dwarfism, stem pitting and scaly bark (see pictures4.1 below):

Figure 4.1-a: Yellowing and clear veining of the leaves, dwarfism of the trees found in Nassariya-Nablus fields.

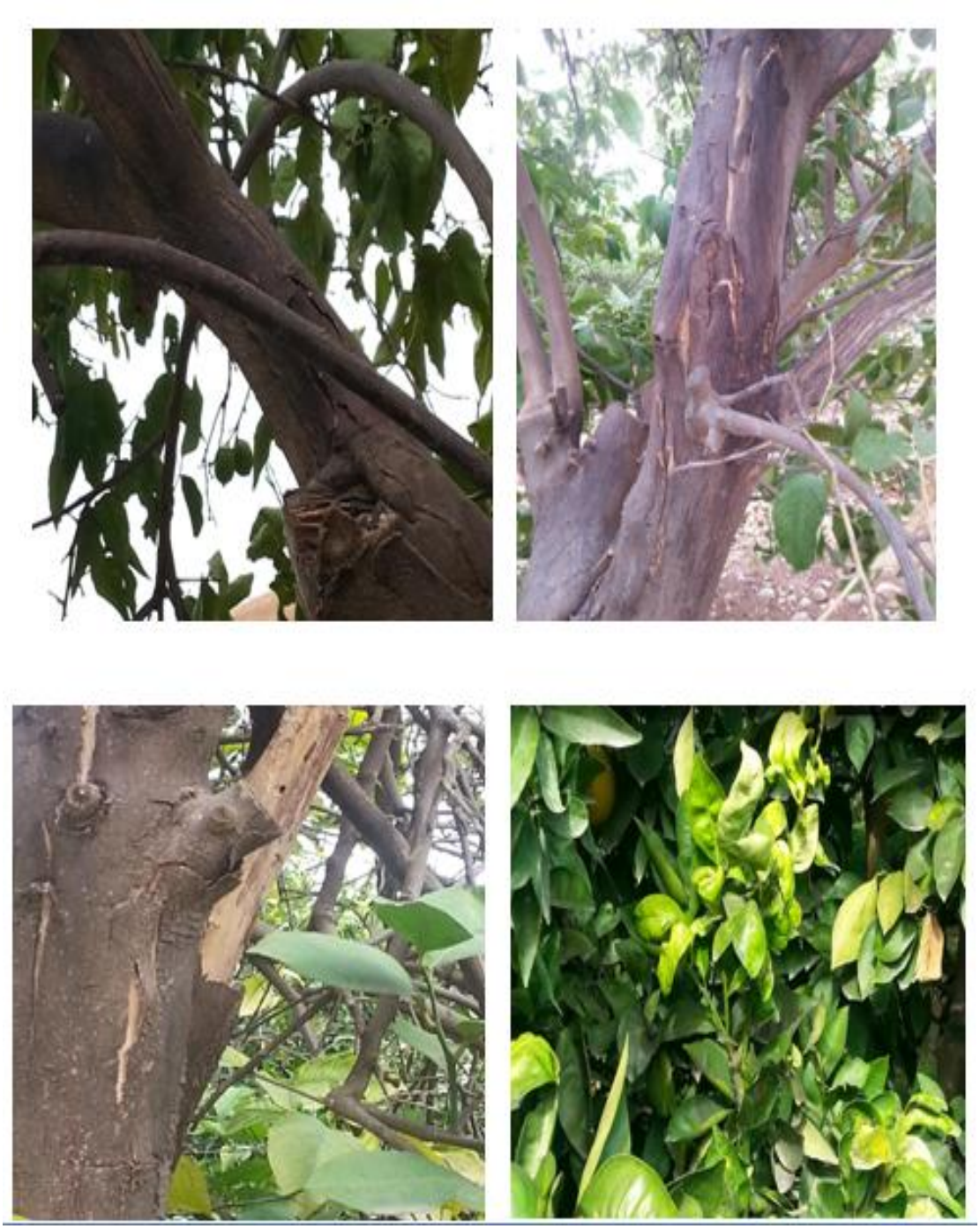


Figure 4.1-b: Symptoms of viriods were found in the fields of Nablus and TulkarmQalqiliya, scaly bark and leave corking.

Figure 4.1-c: Symptoms of CPsV were found in the fields of Shwakeh-Tulkarm**.**

All citrus varieties which including to this study mainly are:Lemons,Clementine, Poppy, Pomelo , Shammoti, Naval-orange, Valencia orange, Mandarin, Grapefruit, Acid less(Faransawi) and others.Only 98 out of 896 samples are detected with CTV, with 10.9% of incidence. See figure 4.2 below:

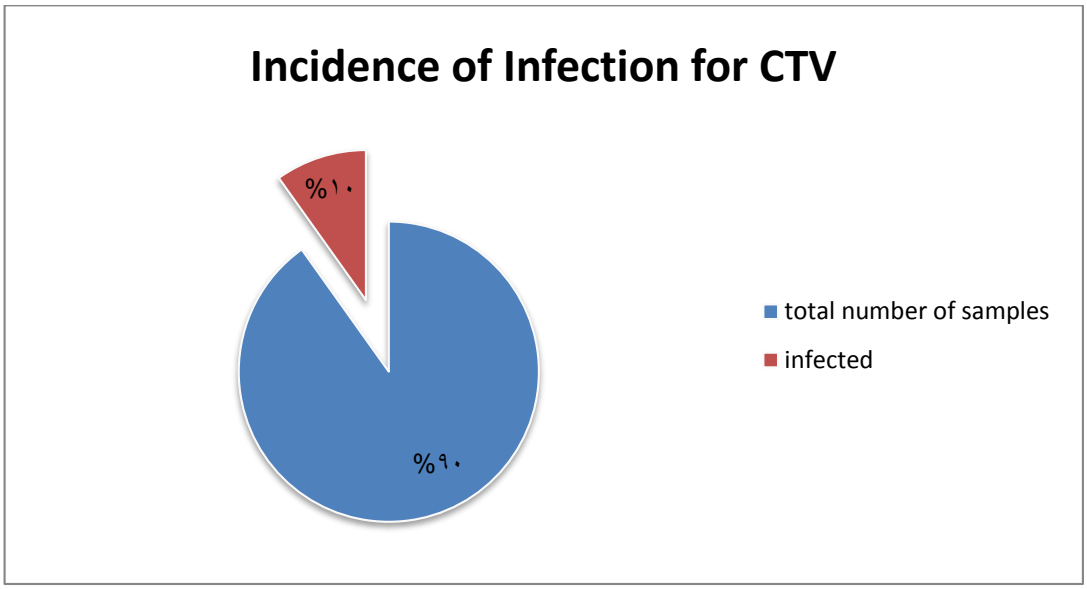


Figure 4.2: The incidence of infection of CTV among the collected samples.

The incidence of infection in nurseries was 4% which shows a remarkable improvement in choosing the right root stock and flowing the rules that the ministry of agriculture emphasize on it for testing the trees before getting sold to the farmers, in fact most nurseries nowadays using different citrus root stock (see table 9) mostly used Volkameriana ,which can tolerate most of virus diseases like CTV and CPsV and some viriods like xyloporosis, it is also Moderate tolerance to salinity. In contrast using Volkameriana yields a poor fruit quality for most of citrus varieties.

Table 9: Different citrus root stockand their susceptible to infect with most destructive disease of citrus.

Citrus root stock / **Disease**	**Sour orange *C.Aurantium***	**Volkameriana**	**Rough lemon (C.Jambhiri)**	**Cleopatra**
CTV	Sensitive	tolerate	tolerate	tolerate
CPsV	Tolerate	tolerate	tolerate	sensitive
Exocorisviriods	Tolerate	tolerate	tolerate	tolerate

In another hand the incidence of infection in commercial orchards still high its nearly the same percentage of Jarrar (2000), it was roughly 14%this steady state referred to the situation of most orchards doesn't change very much, this means most of orchard which farmers sill depending on it were planted before 20 years at least, thus these trees may had been infected with CTV and CPsV or even infected with a typical viriods. In addition there is less interest of the farmers towered their lands, and less supporting and educating from Ministry of Agriculture about the disease itself.

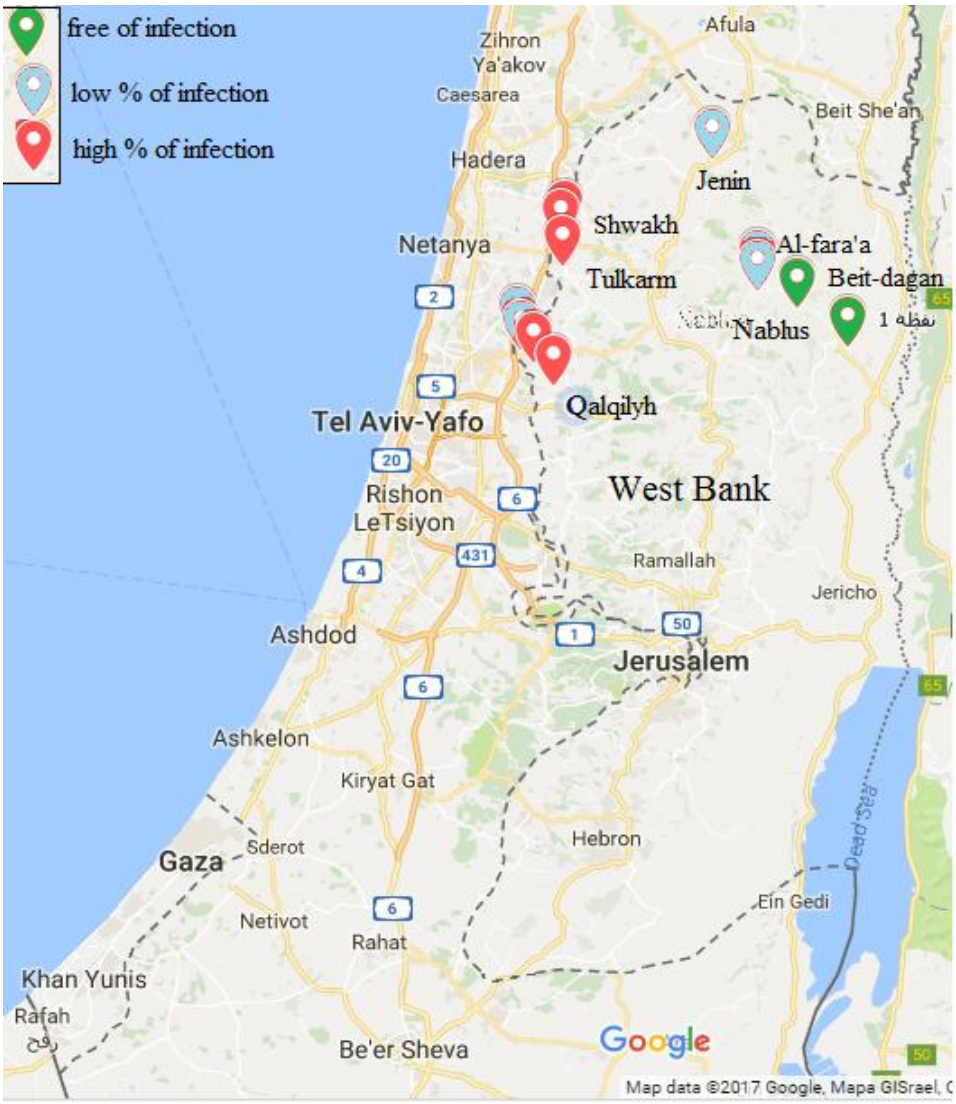


Figure 4.3: Locations and status of tested fields in West Bank –Palestine.

It was expected to have a CTV in western part of northern West Bank (Tulkarm and Qalqilyh) which confirms the results of Jarrar Study(Jarrar et al., 2000), but also shows that the eastern part (Nablus and Jenin) have been recorded with CTV infection which Jarrar doesn't have it.

Some of infections were recorded in orchards nearby Israeli borders especially in Shwakh in Tulkarm(see figure 4.3)were also many pest were observed. Other infection recorded in Qalqilyah area in spite of noticing a good care for citrus fields. In Nablus area some tested fields were totally

free infection, located in Al-forush –beitdagan, and An-nassaryh village which is located in North-East of West Bank. (See figure 4.3), most citrus trees in this area aged from 10-15 years. In the other hand other fields in Nablus were shown symptoms of CTV and viriods which confirmed serologically, these fields relatively aged more than 30 years, which located in Al-far'a and Wadi al-badan area, see figure 4.3).

By analyzing the samples according to their varieties, we found that grapefruit and Acid less orange (Farancawi orange) have no infection which classified as sweet orange which typically tolerate the CTV disease, while each of Pomelo, Lemon, ValanciaOrange, Shammoti, Naval orange, Celementine and Poppy have recorded an infection which represented in the flowing figure 4.4:

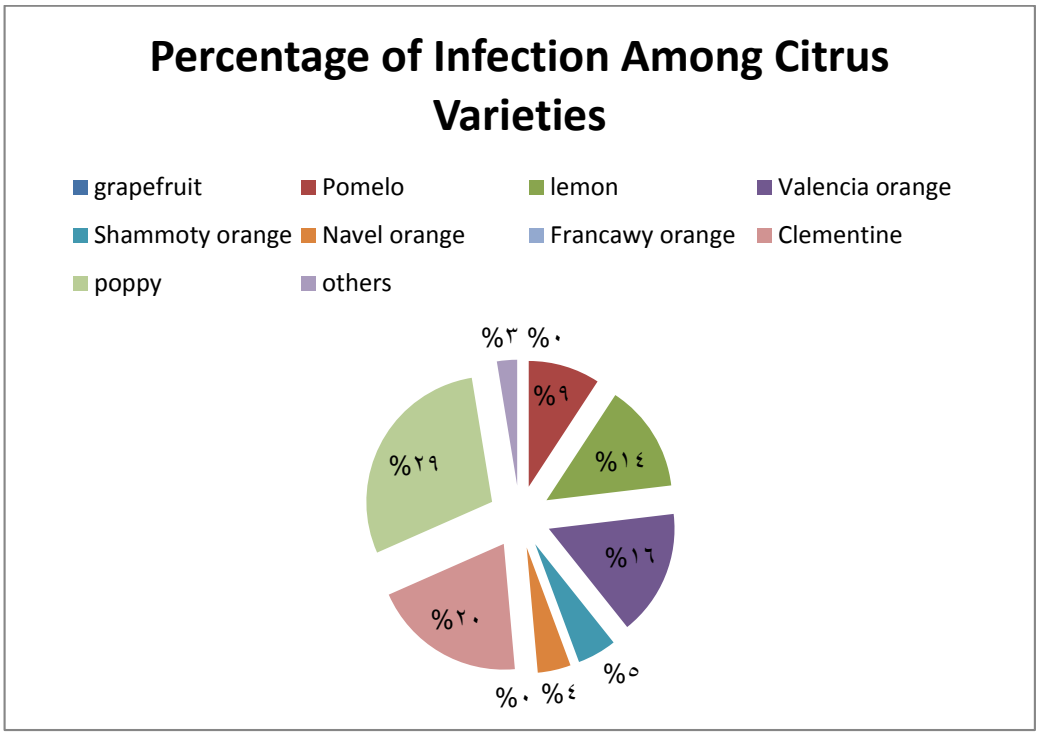


Figure 4.4: All citrus varieties used in the study and its incidence of CTV infection.

Figure 4.4shows the highest infection were recorded for poppy with 29% followed by celementine with 20% while valencia orange and lemone represented the third and fourth rank with 16% and 14% respectively. Pomelo goes in to fifth rank with roughly 10% ,shammotti and navel orange only recorded approximately 4%. Interestingly Valencia orange still proved to be one of the most infected variety with CTV, while local fransawi orange was totally free from CTV which indicate that this variety is excellent resistance of the disease (Jarrar etal.,2000; Djelouah K.,&D'Onghia A.M. 2009).

Nablus and Qalqilyh were reported the same percentage of infection for lemon variety, while Tulkarm was recorded a 21% of infection for naval orange, at the same time Nablus and Tulkarm were reported no infection among that variety. In addition Nablus was recorded an infection more than a 9 % of that recorded for Qalqilia for poppy variety. Also Qalqilia was reported a high infection of Clementine with a 31.5 %.

4.2 Serological detection of CTV and CPsV:

If we looke at the samples according to the detected methodology, then we find that DAS-ELISA method recorded less infection compared with tissue print (see table 10 below), there was a 9 infected samples out of 200 samples that tested with DAS-ELISA, while in tissue print there was a 9 infected out of 78 samples, this differences are due to that DAS- ELISA is more efficient and accurate test than tissue print.

Table 10: Number of infected samples in each test and its percentage.

Typeof crop	DAS-ELISA samples	Tissue print samples#1	Tissue print samples #2	Total number of Infected samples	Total number of all samples	% of total infection
Grapefruit	0	0	0	0	55	0
Pomelo	2	4	0	6	67	8.9
Lemon	0	2	33	35	261	13.4
Valencia	0	5	5	10	64	15.6
Shammoty	1	0	1	2	41	4.87
Navel	0	6	0	6	146	4.11
Francawy	0	0	0	0	6	0
Clementine	4	0	17	21	110	19.1
Poppy	0	6	10	16	57	28
Others	2	0	0	2	79	2.5

For Psorosis virus we don't detect the virus in 200 sample using DAS-ELISA, even the positive control doesn't react with the substrate, and that's may because the expired date of the using kit was in August but we use it in November.

Under 10x magnification the printed samples were clearly visible, the outline of the printed stem referred to the phloem area, percent of strong deep purple staining shows CTV infected stems, while the absence of strong stained in the blots indicate healthy tissue and usually appeared in green color.See figures(4.5,4.6).

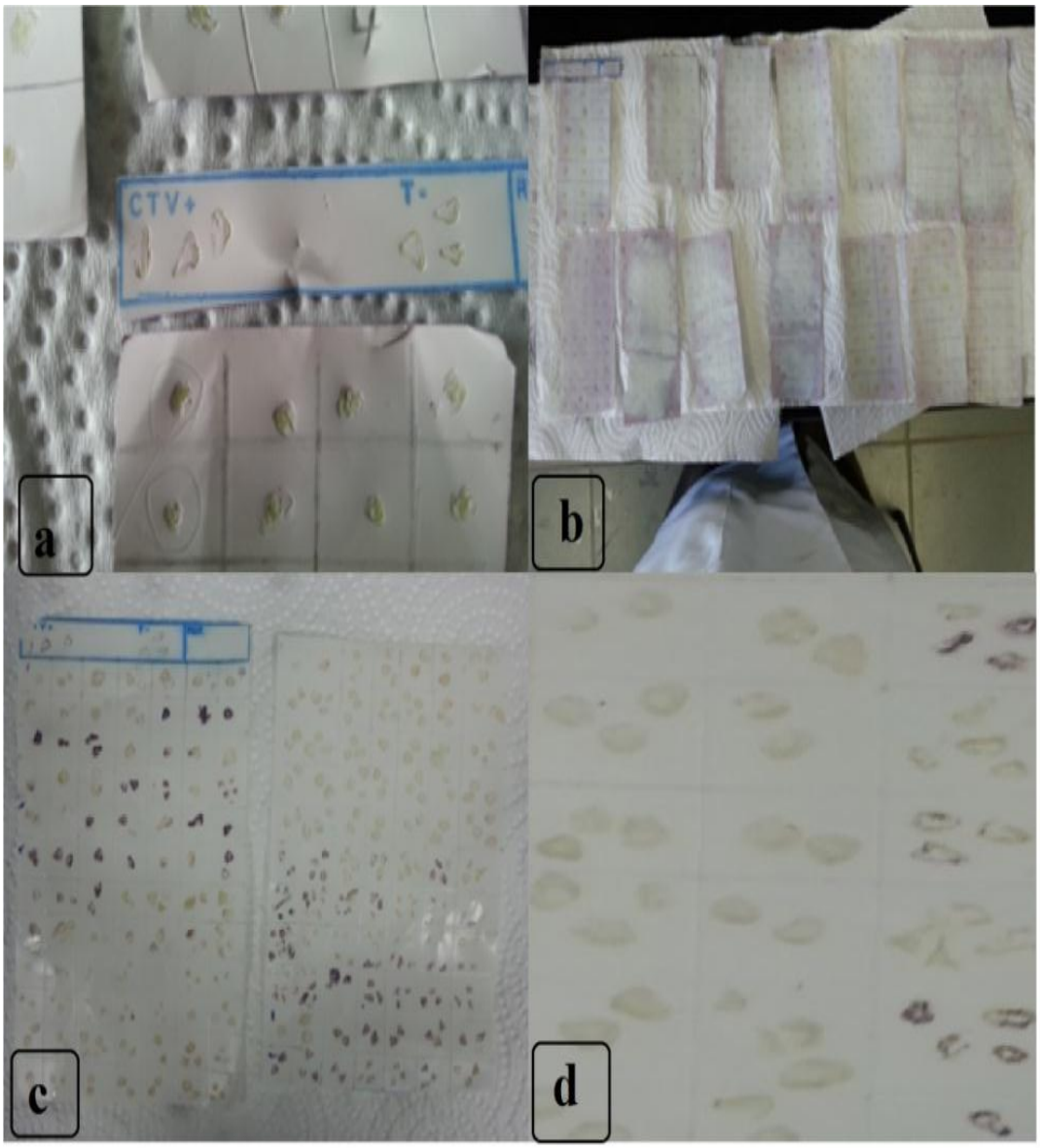


Figure 4.5: a) negative and positive control placed near one printing membrane in order to detect the differences visually, b-c) all the printed membrane, d) a printed membrane with positive purple print and negative print with green color.

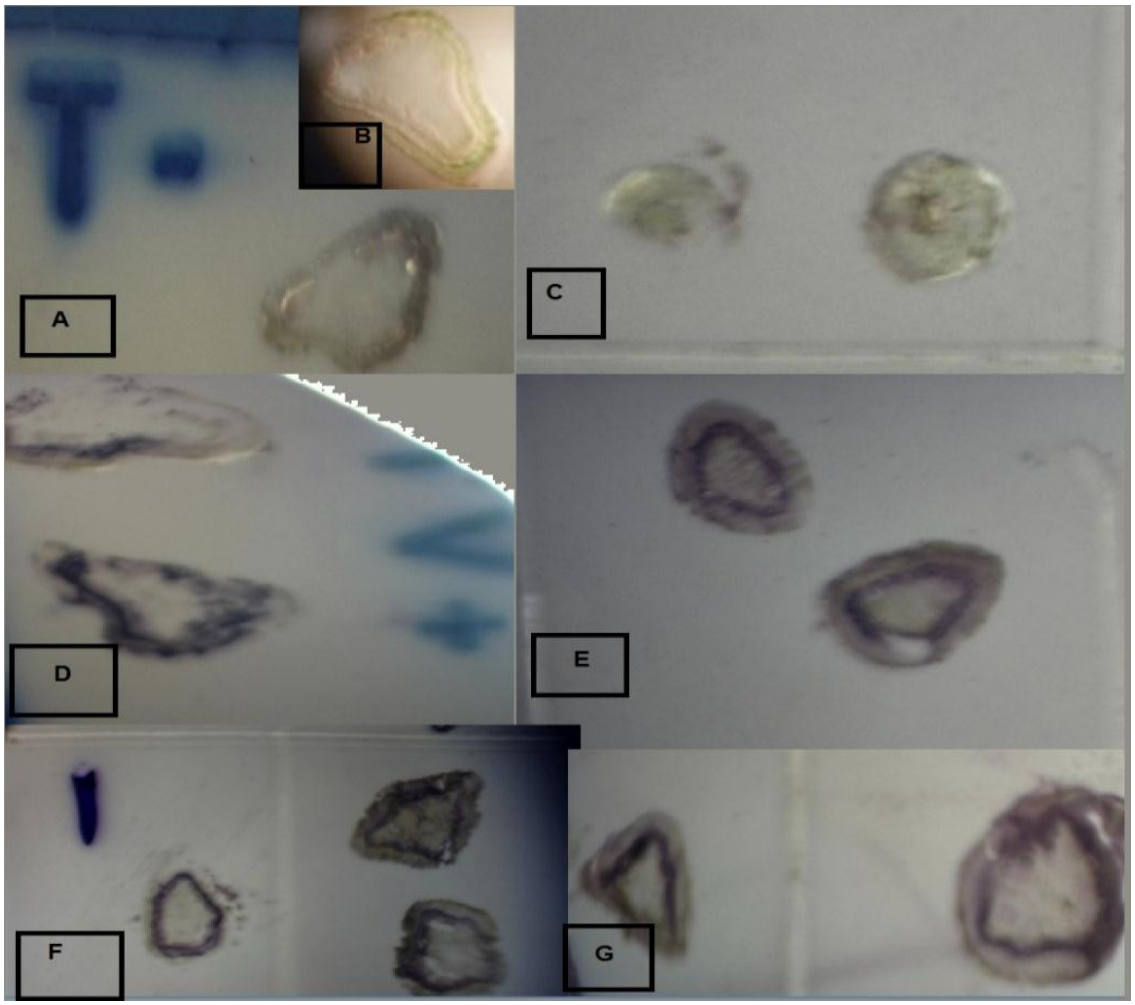


Figure 4.6: A,B) cross section in stem of negative control print.C) negative petiole print sample. D, E, F, G) positive printing stem samples, which shows a purple color, locate in phloem part of the cross section.

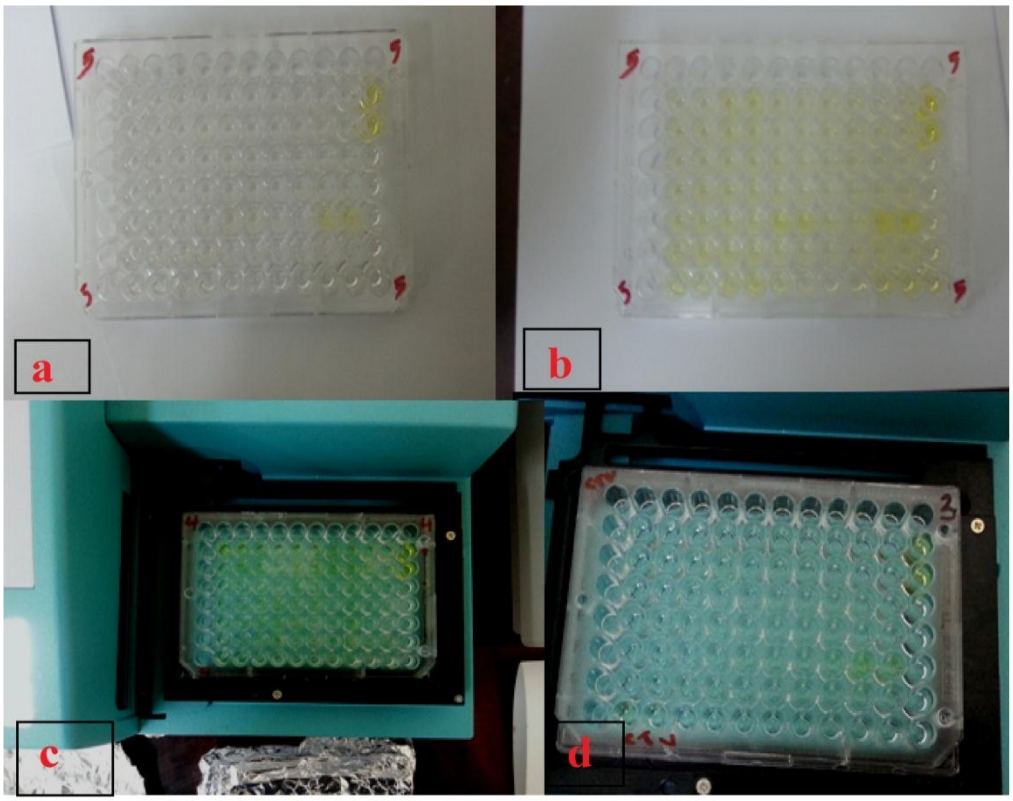


Figure 4.7: a) plate after 2 hours of incubation, yellow color indicate for positive control and in the middle possible extract CTV infection. B) The same plate after 24 hours of incubation, which the yellow color becomes dark, while the negative turned in to pale yellow. c-d) shows anther plates prepared for reading the results.

4.3. Bioassays

One out of ten trees that were grafted and monitored were shown a CTV symptoms, 3 out of ten were found *Aphid gossypii* on the grafted inoculum area.

Figure 4.8: Bio indexing result: a-Two healthy plants, no symptoms were observed.

Figure 4.8: Bioindexing result: b- infected plant shows cupping leave symptoms (grapefruit grafted on citron).

4.4. Conclusion remarks

The presence of CTV in northern of west bank is not surprising thing, because it was detected before in Israel (19) and in Palestinian territory in 2000 by Jarrar. The importance of this study was to assess the sanitary status of the citrus after 15 years of the last study that was carried out in IAM-B., and opening the way for other researchers on citrus related diseases that will done in future. This study considered a research that accomplished for the first time in west bank on citrus, in addition it

supplements the Palestinian ministry of agriculture the locations of fields that were detected with CTV to manage the situation before spreading more infection to other areas by wrong propagation or by natural vectors, and supporting the farmers and retrieve them by offering healthy plants, and showing them the symptoms of the disease thus do not propagate the infected one and destroy it .it may also encouraging them to grow a tolerate varieties of citrus for CTV .

Its recommended to do further molecular analysis depending on PCR and sequencing , in order to establish a full genome profile for the strains of CTV and other viriods that infect local citrus cultivars.

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Appendix A:

An-Najah
National University
Faculty of Graduate Studies

جامعة
النجـــاح الوطنية
كلية الدراسات العليا

التاريخ: 2015/9/6

حضرة الدكتور محمد ابو عيد المحترم
مدير مركز البحوث الزراعية الوطني/ جنين

الموضوع: تسهيل مهمة الطالبة/ نهى جاسر حسني شلبي، رقم تسجيل (11255711)
تخصص ماجستير العلوم الحياتية

تحية طيبة وبعد،

الطالبة نهى جاسر حسني شلبي/ رقم تسجيل 11255711 تخصص ماجستير العلوم الحياتية في كلية الدراسات العليا، وهي بصدد اعداد الاطروحة الخاصة بها والتي عنوانها:

(التقييم المصلي والجزئي للامراض الفيروسية وشبه الفيروسية التي تصيب الحمضيات في شمال الضفة الغربية)

يرجى من حضرتكم تسهيل مهمتها من خلال مركزكم لانجاز جزء من مشروع الرسالة الخاص بها.

شاكرين لكم حسن تعاونكم.

مع وافر الاحترام ،،،

رئيس قسم الدراسات العليا للعلوم الطبيعية
د. عوني ابو حجلة
التوقيع
د. عوني ابو حجلة

جامعة النجاح الوطنية
كلية الدراسات العليا
نابلس - فلسطين

فلسطين، نابلس، ص.ب 707، هاتف /2345115، 2345114، 2345113 (09)(972)* فاكسميل 2342907(09)(972)
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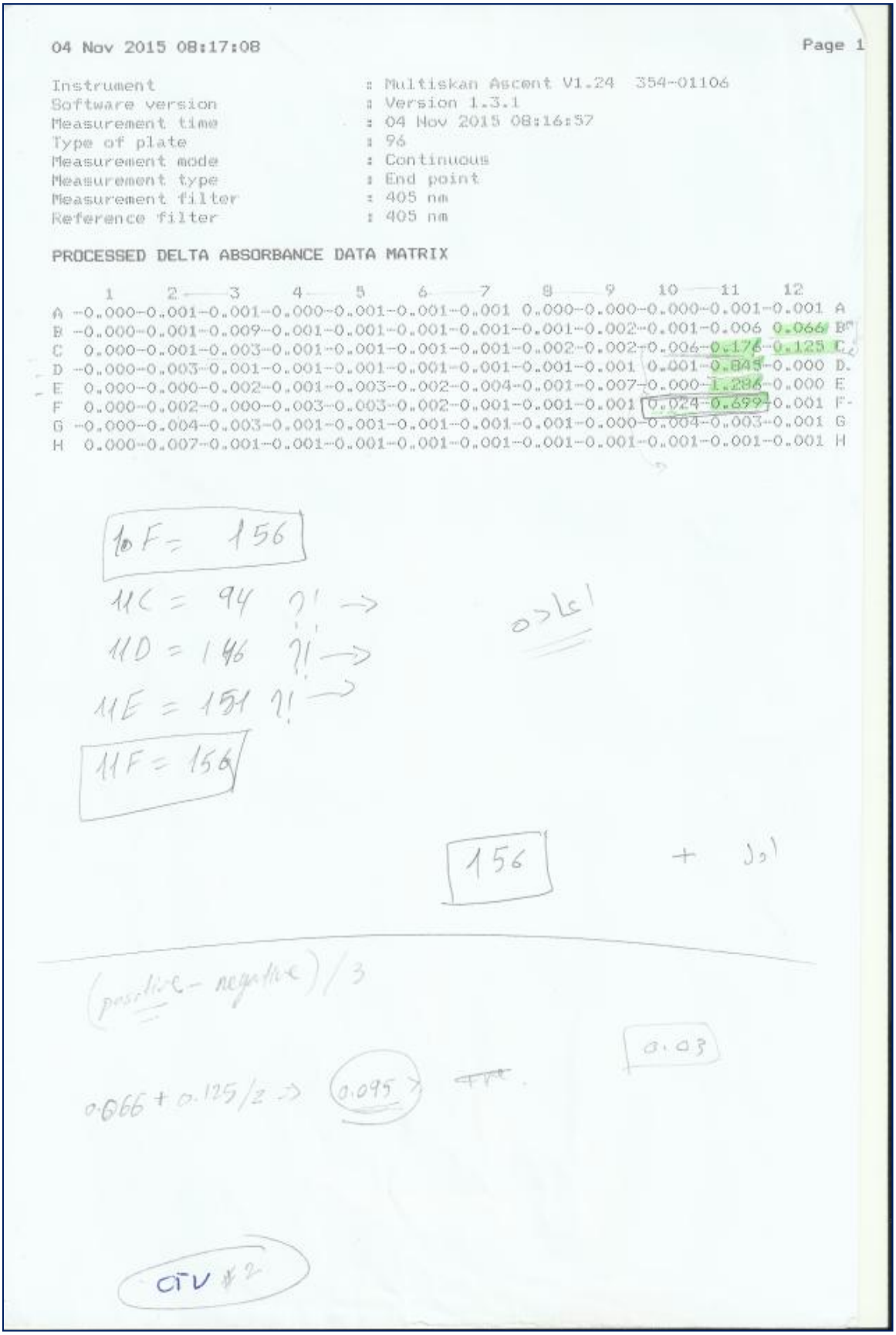
Appendix B: DAS-ELISA readings:



04 Nov 2015 08:17:08 Page 1

Instrument	: Multiskan Ascent V1.24 354-01106
Software version	: Version 1.3.1
Measurement time	: 04 Nov 2015 08:16:57
Type of plate	: 96
Measurement mode	: Continuous
Measurement type	: End point
Measurement filter	: 405 nm
Reference filter	: 405 nm

PROCESSED DELTA ABSORBANCE DATA MATRIX

	1	2	3	4	5	6	7	8	9	10	11	12	
A	-0.000	-0.001	-0.001	-0.000	-0.001	-0.001	-0.001	0.000	-0.000	-0.000	-0.001	-0.001	A
B	-0.000	-0.001	-0.009	-0.001	-0.001	-0.001	-0.001	-0.001	-0.002	-0.001	-0.006	0.066	B
C	0.000	-0.001	-0.003	-0.001	-0.001	-0.001	-0.001	-0.002	-0.002	-0.006	-0.176	-0.125	C
D	-0.000	-0.003	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	0.001	-0.845	-0.000	D
E	0.000	-0.000	-0.002	-0.001	-0.003	-0.002	-0.004	-0.001	-0.007	-0.000	-1.286	-0.000	E
F	0.000	-0.002	-0.000	-0.003	-0.003	-0.002	-0.001	-0.001	-0.001	0.024	-0.699	-0.001	F
G	-0.000	-0.004	-0.003	-0.001	-0.001	-0.001	-0.001	-0.001	-0.000	-0.004	-0.003	-0.001	G
H	0.000	-0.007	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	H

10F = 156

11C = 94 ?! →

11D = 146 ?! →

11E = 151 ?! →

11F = 156

>1<

156 + 3>1

(positive - negative) / 3

0.066 + 0.125 / 2 → 0.095 ~~FF~~

0.03

CTV #2

08 Nov 2015 08:30:41

Instrument	: Multiskan Ascent V1.24 354-01106
Software version	: Version 1.3.1
Measurement time	: 08 Nov 2015 08:30:30
Type of plate	: 96
Measurement mode	: Continuous
Measurement type	: End point
Measurement filter	: 405 nm
Reference filter	: 405 nm

PROCESSED DELTA ABSORBANCE DATA MATRIX

	1	2	3	4	5	6	7	8	9	10	11	12	
A	-0.000	0.000	0.001	0.000	0.001	0.001	0.000	0.000	0.000	0.000	0.001	-0.000	A
B	-0.000	-0.015	-0.029	-0.018	-0.011	-0.047	-0.042	-0.026	-0.015	-0.002	-0.002	0.000	B
C	0.001	-0.019	-0.030	-0.006	-0.038	-0.022	-0.015	-0.016	-0.013	-0.010	-0.001	0.680	C
D	0.000	-0.016	-0.024	-0.022	-0.020	-0.021	-0.013	-0.016	0.000	-0.001	-0.006	-0.010	D
E	0.001	-0.045	-0.033	-0.012	-0.014	-0.014	-0.010	-0.001	-0.005	-0.006	-0.004	-0.008	E
F	0.001	-0.016	-0.030	-0.016	-0.017	-0.013	-0.021	-0.012	-0.013	1.158	1.526	-0.007	F
G	0.000	-0.030	-0.012	-0.009	-0.029	-0.008	-0.010	-0.012	-0.003	-0.004	-0.011	-0.011	G
H	-0.001	0.929	0.002	-0.014	-0.002	-0.013	-0.019	-0.007	-0.007	-0.014	-0.013	-0.006	H

CTV. #3

positive - negative → 0.680 - 0.009 → 0.671/3 = 0223 .

suspected → $H_{(2,3)}$, $F_{(10,11)}$

103 → Q

93 → Q

2015 08:32:16

rument	: Multiskan Ascent V1.24 354-01106
tware version	: Version 1.3.1
easurement time	: 08 Nov 2015 08:32:04
Type of plate	: 96
Measurement mode	: Continuous
Measurement type	: End point
Measurement filter	: 405 nm
Reference filter	: 405 nm

PROCESSED DELTA ABSORBANCE DATA MATRIX

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.000	0.000	-0.000	0.000	0.000	0.000	0.001	0.004	0.000	-0.000	0.001	0.000	A
B	-0.006	0.343	-0.050	-0.015	-0.019	-0.009	-0.012	-0.006	-0.002	-0.008	-0.015	1.629	B
C	0.000	-0.024	-0.015	-0.023	-0.015	-0.018	-0.011	-0.004	-0.003	-0.004	-0.009	1.675	C
D	-0.001	-0.019	-0.011	-0.016	-0.019	-0.019	-0.011	-0.005	-0.020	-0.012	-0.001	-0.004	D
E	-0.002	-0.019	-0.013	-0.013	-0.012	-0.011	-0.009	-0.003	-0.008	-0.013	-0.014	-0.009	E
F	-0.004	-0.027	-0.009	-0.006	-0.008	-0.013	-0.007	-0.011	-0.006	-0.010	-0.010	-0.007	F
G	-0.000	-0.015	-0.005	-0.006	-0.023	-0.015	-0.011	-0.028	-0.002	-0.004	-0.010	-0.014	G
H	0.001	-0.014	-0.007	-0.008	-0.001	-0.005	-0.008	-0.007	-0.009	-0.010	-0.010	-0.009	H

plate # 4 - CTV.

تخزين

+ve ⇒ 1.629 + 1.675/2 ⇒ 1.652

-ve ⇒ 0.0065

+ve -(-ve) ⇒ 1.652 - 0.0065 = 1.6455 +ve.

no susbected

2015 08:34:27

trument	: Multiskan Ascent V1.24 354-01106
ftware version	: Version 1.3.1
easurement time	: 08 Nov 2015 08:34:16
Type of plate	: 96
Measurement mode	: Continuous
Measurement type	: End point
Measurement filter	: 405 nm
Reference filter	: 405 nm

PROCESSED DELTA ABSORBANCE DATA MATRIX

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.000	0.001	0.000	0.000	0.002	0.012	0.008	0.002	0.000	0.001	0.000	0.000	A
B	-0.001	-0.024	-0.038	-0.022	-0.028	-0.020	-0.013	-0.015	-0.029	-0.031	-0.033	0.201	B
C	0.000	-0.002	-0.032	-0.015	-0.011	-0.012	-0.013	-0.012	-0.014	-0.013	-0.014	0.347	C
D	0.001	-0.015	-0.016	-0.018	-0.008	-0.014	-0.012	-0.005	-0.006	-0.001	0.001	-0.003	D
E	-0.000	-0.032	-0.036	-0.011	-0.016	-0.017	-0.008	-0.013	-0.012	-0.011	-0.007	0.000	E
F	0.000	-0.008	-0.022	-0.010	-0.016	0.019	0.041	-0.015	-0.017	0.000	-0.295	-0.012	F
G	-0.001	-0.022	-0.005	-0.023	-0.010	-0.015	-0.012	-0.012	-0.011	-0.024	-0.026	-0.005	G
H	0.000	-0.011	-0.003	-0.017	-0.015	-0.010	-0.004	-0.005	-0.010	-0.019	-0.007	-0.001	H

plate # 5. CTV.

+ve ⇒ 0.201 + 0.347/2 ⇒ 0.2740

+ve - neg ⇒ 0.270 - 0.003 ⇒ 0.271/3

0.0903

$F_{(10,11)} \Rightarrow (88) \rightarrow Q$

08 Nov 2015 08:36:18

Instrument	: Multiskan Ascent V1.24 354-01106
Software version	: Version 1.3.1
Measurement time	: 08 Nov 2015 08:36:06
Type of plate	: 96
Measurement mode	: Continuous
Measurement type	: End point
Measurement filter	: 405 nm
Reference filter	: 405 nm

PROCESSED DELTA ABSORBANCE DATA MATRIX

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.000	0.000	0.000	0.001	0.000	0.001	0.001	0.000	0.003	0.000	0.001	0.000	A
B	0.000	-0.021	-0.012	-0.014	-0.018	-0.018	-0.014	-0.010	-0.013	-0.024	-0.009	-0.250	B
C	0.000	-0.041	-0.029	-0.011	-0.057	-0.012	-0.006	[illegible]		-0.033	-0.019	-0.217	C
D	0.000	0.017	-0.027	-0.013	-0.016	-0.015	0.017	0.110	-0.075	-0.015	-0.012	-0.007	D
E	-0.000	-0.008	-0.028	-0.016	-0.021	0.132	0.062	0.284	-0.065	0.669	-0.222	-0.003	E
F	0.000	0.007	0.228	-0.010	-0.001	-0.032	0.019	0.033	-0.026	-0.019	-0.066	-0.008	F
G	0.001	1.267	0.006	0.021	-0.041	0.000	1.674	-0.005	-0.006	0.305	0.149	-0.023	G
H	0.000	-0.009	-0.028	-0.015	-0.012	-0.006	-0.005	-0.006	-0.003	-0.005	-0.001	0.203	H

plate * 6. CTV.

0.250 + 0.217 ⇒ .467/2 ⇒ 0.2335.

0.2335 - 0.005 ⇒ 0.2285 /3 ⇒ 0.076.

⁂ ⇒ C (8,9). 90 Q.

D (8,9) 116 J.

E (6,7) 118 J.

E (8,9). 54 F.

E (10,11). 95 Q.

F (2,3). 53 F

G (2,3). 13 B

G (6,7) 35 F

G (10,11). 57 F

جامعة النجاح الوطنية

كلية الدراسات العليا

كشف وتحديد الأمراض الفيروسية وشبه الفيروسية التي تصيب الحمضيات في شمال الضفة الغربية

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قدمت هذه الاطروحة استكمالا لمتطلبات درجة الماجستير في العلوم الحياتية بكلية الدراسات العليا في جامعة النجاح الوطنية، نابلس–فلسطين.

2017

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الملخص

تعتبر زراعة أشجار الحمضيات بجانب زراعة الزيتون والعنب من أهم الزراعات المعتمدة على كمية هطول الأمطار في الضفة الغربية، وتتركز زراعة الحمضيات في المناطق شبه الساحلية والتي تتمثل في كل من محافظات طولكرم وقلقيلية، كما ويتم زراعتها بمناطق مختلفة من محافظة نابلس وجنين. و تشكل هذه الزراعة ما نسبته 2.4 % من مجموع الأراضي المزروعة بالأشجار المثمرة و يتعدى الإنتاج السنوي لها 60.000 طن، و بالرغم من صغر المساحة المزروعة إلا أن لها مردود اقتصادي جيد إذا ما قورنت بالزراعات الأخرى.

ويرتبط كمية الإنتاج الزراعي من الحمضيات بالكثير من مسببات الأمراض الحيوية و التي تتمثل في الفطريات والبكتيريا بالإضافة إلى الفيروسات و أشباه الفيروسات. و التي تسبب الكثير من الأمراض التي لها تأثير سلبي على كمية الحمضيات المنتجة، و لعل من أهم مسببات الأمراض تلك التي تنتج عن أمراض فيروسية و أهمها مرض التدهور السريع (Tristeza) الذي يسببه فيروس CTV و هذا لفيروس له تأثير تدميري على أشجار الحمضيات والذي يسبب موتها بوقت قصير.

ففي هذه الدراسة تشخيص لنسبة انتشار المرض في الحقول الواقعة شمال الضفة الغربية بحيث تم جمع 896 عينة من أربع محافظات، حيث تم استخدام تقنية الفحص المصلي (DAS-ELISA) على 219 عينة لفحص فيروس CTV و فيروس CPsV ، في حين تم

استخدام تقنية البصمة النسيجية المباشرة DTBIA على العينات المتبقية لفحص فيروس CTV فقط.

أظهرت النتائج وجود فيروس CTV في كلفة المحافظات التي تم اخذ العينات منها، اذ كان هناك 98 عينة مصابة من أصل 896 عينة، بنسبة بلغت 10.9 %، بينما بلغت نسبة الإصابة في المشاتل 4%. كما أظهرت ان أصناف الجريب فروت والفرنساوي لم تسجل اية إصابة بالمرض، لكن بقابل ذلك سجلت أصناف أشجار البوملي والكلمنتينا والبلنسي نسبة عالية من الإصابة على الترتيب. وعلى هذا فإن نسبة الإصابة في كل منطقة تختلف عن الأخرى بحكم عوامل منها نوع الأصناف المزروعة وقابليتها للإصابة ودرجة نشاط المزارعين واهتمامهم بالأشجار المزروعة.