T. C.

NİĞDE ÖMER HALİSDEMİR UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF PLANT PRODUCTION AND TECHNOLOGIES

EFFECTS OF POTATO VIRUS Y INFECTION AT DIFFERENT GROWTH STAGES ON YIELD OF LOCAL TOMATO GENOTYPE "SAZLICA"

MUSADIK ABDULLAHI AHMED

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Master Thesis

Supervisor

Assistant Professor Dr. EMİNUR ELÇİ

June 2019

Musadik Abdullahi AHMED tarafından **Dr. Öğr. Üyesi Eminur ELÇİ** danışmanlığında hazırlanan **"Effects of *Potato virus Y* infection at Different Growth Stages on Yield of Local Tomato Genotype "Sazlıca""** adlı bu çalışma jürimiz tarafından Niğde Ömer Halisdemir Üniversitesi, Fen Bilimleri Enstitüsü, Bitkisel Üretim Ana Bilim Dalı' nda Yüksek Lisans tezi olarak kabul edilmiştir.

(The study titled **"Effects of *Potato virus Y* infection at Different Growth Stages on Yield of Local Tomato Genotype "Sazlıca""** and presented by **Musadik Abdullahi Ahmed** with the help of supervisor **Asst. Prof. Dr. Eminur ELÇİ,** has been found as Master thesis by the jury at the Department of Plant Production and Technologies of Niğde Ömer Halisdemir University Graduate School of Natural and Applied Sciences.)

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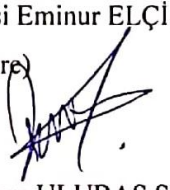

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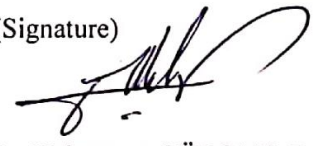

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THESIS CERTIFICATION

I certify that the research work presented in this thesis is entirely my own work and has not been taken from other contents and no part of this thesis has been submitted anywhere else for any other research or degree. The thesis has been prepared in accordance with the instructions issued by the institute regarding the format. All the assistance presented in preparing this thesis and sources have been acknowledged.

Musadik Abdullahi AHMED

ÖZET

FARKLI GELİŞİM DÖNEMLERİNDE PATATES Y VİRÜSÜ ENFEKSİYONUNUN YEREL ÇEŞİT "SAZLICA DOMATESİ" VERİMİ ÜZERİNE ETKİLERİ

Musadik Abdullahi AHMED
Niğde Ömer Halisdemir Universitesi
Fen Bilimleri Enstitüsü
Bitkisel Üretim Anabilim Dalı

Danışman : Dr. Öğr. Üyesi Eminur ELÇİ

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Bu çalışmanın amacı, Sazlıca domatesinde PVY virüsünün varlığını ve bazı ırklarının farklı bitki gelişim dönemlerinde meyve verim ve kalitesi üzerine etkilerini araştırmaktır. PVY varlığının tespiti için şüpheli domates örnekleri toplanmış, DAS-ELISA ile % 10 oranında PVY enfeksiyonu tespit edilmiştir. PVY ırklarının etkisinin tespiti için, Sazlıca domatesi farklı gelişim evrelerinde (7., 14., 21. ve 28. gün), PVY'nin iki ırkı ile üç farklı kombinasyonda ($PVY^{NW,\ NTN\ ve\ NW+NTN}$) mekanik olarak inokule edilmiştir. Kontrol olarak H2274 çeşidi kullanılmıştır. Sonuçlara göre, PVY^{NW} ırkının bütün denemelerde en yüksek enfeksiyon oranına sahip olduğu tespit edilmiştir. Bitki gelişim evreleri incelendiğinde, beklenildiği üzere en küçük bitkilerde en yüksek enfeksiyon oranı tespit edilirken, 28. gün inokulasyonlarında sadece bir bitkide enfeksiyon tespit edilmiştir. Denemelerin birinde domates meyvesi üzerinde simptomlara rastlanılmamıştır. Verim ve meyve kalite parametreleri incelendiğinde, en yüksek değerlerin (meyve sayısı, boyu, eni, ağırlığı, Briks değeri) PVY^{NW+NTN} ırklarıyla 21. günde inokule edilen Sazlıca domateslerinde olduğu tespit edilmiştir. Sonuç olarak, Sazlıca domateslerinin PVY ile enfekteli olduğu ve PVY^{NW} nin en etkili ırk olduğu tespit edilmiştir.

Anahtar kelimeler: Domates, Sörvey, ELISA, PVY ırkları, Inokulasyon, Meyve kalitesi.

SUMMARY

EFFECTS OF POTATO VIRUS Y INFECTION AT DIFFERENT GROWTH STAGES ON YIELD OF LOCAL TOMATO GENOTYPE "SAZLICA"

Musadik Abdullahi AHMED
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The aim of the study was to investigate incidence of PVY and the effect of PVY strains at different development stages on yield and fruit quality of local tomato genotype "Sazlıca" which has importance for local producers. To investigate PVY incidence, symptomatic samples were collected from Sazlıca and 10 % of samples were found to be positive by DAS-ELISA. For the evaluation of effects of PVY strains, seedlings were mechanically inoculated with two different PVY strains in three combinations ($PVY^{NW,\ NTN,\ and\ NW+NTN}$) at four different growth stages (7, 14, 21 and 28 days old). PVY susceptible commercial tomato cultivar H2274 was used as control. Among the PVY strains, PVY^{NW} has shown the maximum infection rates in the replications and varieties. For plant age, 7 days old inoculated plants have displayed the maximum PVY infections as expected, whereas, only one infection was observed in 28 days old plants. No any symptoms were detected on fruits for all replications. In the fruit quality and yield parameters, the highest fruit number, length, width, weight, and brix value were observed on PVY^{NW+NTN} in 21 days plants. It can be concluded that there are some PVY infections on Sazlıca tomatoes and among the tested strains, PVY^{NW} strain is the most effective strain on yield and fruit quality of Sazlıca tomatoes.

Keywords: Tomato, Survey, ELISA, PVY strains, Inoculation, Fruit quality.

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SYMBOLS AND ABBREVIATIONS

Abbreviations	Descriptions
PVY	Potato Virus Y
ELISA	Enzyme-Linked Immunosorbent
CVD	Cardiovascular Diseases
VPg	Viral Genomic Protein
ssRNA	Single Stranded Ribonucleic acid
NOHU	Niğde Ömer Halisdemir University
DAS	Double Antibody Sandwich Assay
CP	Coat Protein
PLRV	Potato leafroll luteovirus
AMV	Alfalfa Mosaic Virus
CMV	Cucumber Mosaic Virus
ToMV	Tomato Mosaic Virus
TSWV	Tomato Spotted Wilt Virus
PepYMV	Pepper yellow mosaic virus
R	Resistant
TAS	Triple-Antibody Sandwich
SAR	Systemic Acquired Resistance
TEV	Tobacco Etch Virus
TRV	Tobacco Rattle Virus
TBSV	Tomato Bushy Stunt Virus
3’NTR	3’-Nontranslated Region
MAbs	Monoclonal Antibodies
RFLP	Restriction Fragment Length Polymorphism
UTR	Untranslated Region

CHAPTER I

INTRODUCTION

Tomato (*Solanum lycopersicum L.*) plant is the family member of Solanaceae and is the biggest genus in the family comprising above 3000 species, reaching 1250 to 1700 species including many economically valuable plants such as potatoes, tobacco, peppers, eggplants, petunias, and Physalis. The Solanum species can be found on all warm and tropical continents and the interesting thing is because of their morphological and ecological difference (Véronique, 2014).

Tomato plant arisen from Andean land presently containing in part of Chile, Ecuador, Bolivia, Colombia and Peru. The time and exact location of tomato domestication is unknown surely. Before being brought to Europe in the 15th century, the tomato had achieved sufficiently developed stage of domestication and after reached to Europe in 18th and 19th centuries a great domestication had taken place in all Europe (Sims, 1980).

In 20th century, large arrays of morphologically different forms and cultivars from the single species *S. lycopersicum L.* through plant breeding have been conceived by human being. The scientist and breeders carried out breeding activities which they developed modern tomato varieties (mainly hybrids) with all sizes, colors and shapes (Bai and Lindhout, 2007).

In Turkey, Adana province was the first place of tomato growing at the beginning of the 19th century and this indicates how the tomato production plays an important role in Turkey's economy (Aksoy and Kaymak, 2016; Aybak and Kaygısız, 2004).

Tomato is universally confirmed as healthy diet and substantial factor avoiding chronic diseases, and weight management and energy balance. Researches have demonstrated strong adverse relationship between tomato intake and the risk of fixed cancer types,

age-related macular degeneration and cardiovascular diseases. Besides that, tomato is the most important vegetable after potato, this plant creates an outstanding source of health-advancing compounds owing to balanced mixture of minerals and antioxidants (Dorais et al; 2008).

Tomato provides many bioactive elements e.g. those that operate as antioxidants, such as the vitamins E and C, and carotenoids. The essential carotenoid in tomatoes is lycopene which usually expected to be caused for the positive health results visualized with raised tomato consumption. Especially, lycopene is the most effective carotenoid about scavenging singlet oxygen and reactive oxygen species. The antioxidant reaction of lycopene is possibly useful in diseases prevention for both prostate cancer and CVD (cardiovascular diseases). In consider to CVD, lycopene; tomatoes could potentially minimize the disease development by lowering inflammation, improving immune function or inhibiting cholesterol synthesis (Canene-Adams et al., 2005).

As it is an almost short duration crop. It can be cultivated with in a three to six months and profit can be earned instantly and also provides a high yield. In 2017 world tomato production was 182.302.395 million tons whereas world area harvested was 4.848.384 (FAOSTAT, 2017) (Figure 1). The significant of tomato is not only that is the most grown vegetable in the whole world but also is the superior producer of both fresh and paste tomatoes in USA, Italy followed by Turkey. In 2017 the productive potentiality of Turkey was of 187,070 tons (FAOSTAT, 2017), (Figure 2). Tomato productions have advanced in Turkey because of positive factors like appropriate ecological conditions, rising in public demand and growers want to make more income (Güney, 2007).

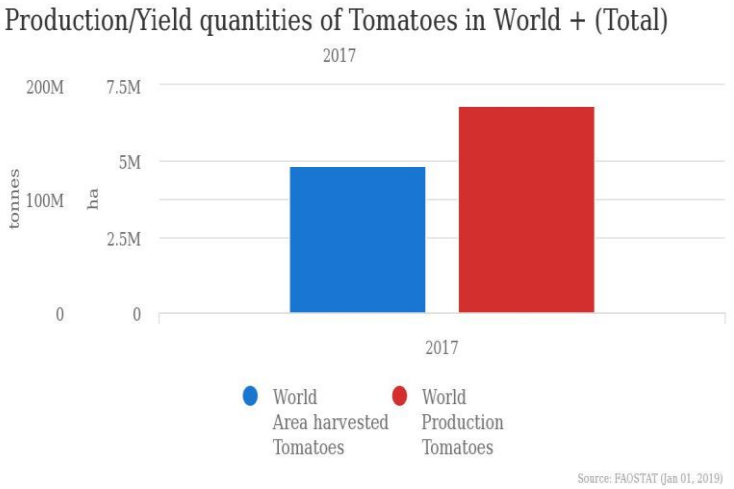

Figure 1. 1. World production of tomatoes in 2017 (Source: FAOSTAT, 2017: http://www.fao.org/faostat/en/#data)

Comparing the other vegetables and fruits processed, tomato paste furnishes the greatest amount of foreign exchange earned by this country. Tomato processing also created employment and making a positive reaction for the agricultural sector (Engindeniz, 2007). It has a significant economic value as frozen food, canned product, pickles, fruit juice, paste, ketchup, etc. (Yücel et al., 2008) and also it is a source of income and food security over the world especially in Turkey.

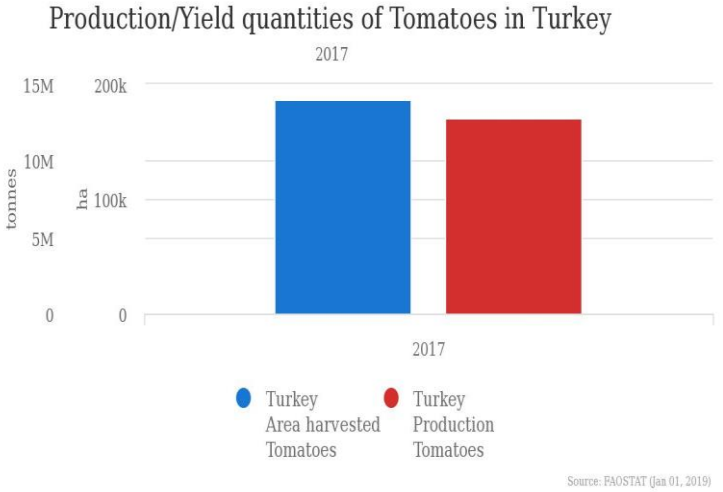

Figure 1. 2. 2017 the production of tomatoes in Turkey (Source: FAOSTAT, 2017: http://www.fao.org/faostat/en/#data)

1.1 *Potato virus Y* (PVY)

Potato virus Y (PVY) is the most familiar harmful virus found in potato and other *Solanaceous* crops production areas (Valkonen, 2007). The viral genome comprises of a single-stranded, positive sense RNA molecule of about 10 kb in length, with a viral genomic protein (VPg) attached covalently to the 59 end and a poly (A) tail at the 3 end. The virus of RNA encodes a single, big polypeptide which is cleaved by 3 virus-encoded proteases into nine products (Dougherty and Carrington, 1988; Tribodet et al., 2005).
PVY has a broad host range normally infecting plants of more than nine families, containing 14 genera of the *Solanaceae*, such as tomato, pepper, tobacco, and eggplant (Gray et al., 2010). Aphids are the most significant and the most damaging vectors of potato viruses and above 40 species of aphids are transmitting PVY in natural conditions (Sigvald and Hulle, 2004).

The current classification of PVY isolates based on primary hosts, symptoms caused in various plants and serological response to monoclonal antibodies. The isolates stated earlier, have been categorized in three major strains; PVY^N, PVY^O and PVY^C (Ramírez-Rodríguez et al., 2009). Strains of PVY are including PVY^N (Tobacco venial necrosis strains), PVY^O (Ordinary stains), and PVY^C (Stipple-streak strain, including potato virus C). The major diseases caused by PVY consist of mild to harsh leaf mottling, leaf-drop streak (PVY^O) with necrosis along the veins of underside the leaflets (PVY^N) and stipple streak (PVY^C) (Warren et al., 2005).

Table 1.1. PVY family, genus, host and vector

Virus name	Family	Genus	Host	Vector
Potato virus Y (PVY)	*Potyviridae*	Potyvirus	Potato, Tomato, Tobacco and Pepper	Transmitted by aphids, mechanical means or transmission by grafting

Making comparison to other *Solanaceous* crops, tomato appears to be insufficiently selective with consideration to symptoms caused by diverse PVY (isolates). (Abad and Jordan, 2000).

The different strains of PVY can infect to potato plant (Singh et al., 2008). The strains including PVY^{C}, PVY^{O} and PVY^{N} infect tomato plant (Comes et al., 2005) while PVY^{O} and PVY^{C} strains only infect to pepper (Cardin & Moury, 2008). PVY causes on potato severe mosaic, frequently followed by interveinal yellow spots and whitish spots on fruits, is identified with PVY^{N} strains. In the past 20 years, two new PVY variants were identified and assorted as subgroups of PVY^{N} strain, nominated PVY^{NTN} and PVY^{NW} (In North America named $PVY^{N:O}$). The first variant is PVY^{NTN} and it is the causal agent of potato tuber necrotic ringspot disease. The second variant is $PVY^{N}W$ and was found in Poland in 1984 (Chikh-Ali et al., 2007).

Control of the economic damages through this viral pathogen is a burning question in the agricultural practices of 21^{st} century. To ensure the efficient control of this pathogen information regarding its mode and site of infection, favorable environmental conditions, different biotic and abiotic factors and age of infection can be found a precious one. Infection of PVY in different growth age of tomato is hindrance the total quality-based production of tomato. From the small farmer's plots to larger field acreages are greatly affected by this notorious pathogenic viral agent. As a result of extreme nature and huge losses, PVY is ranked at 5th position in term of worldwide economic damages (Georgiev et al., 1988).

Sazlıca tomato is grown on small scale farming system and it is one of the most important and consumed tomatoes in Sazlıca town and Niğde province. It is also source of income and food security in Niğde. It is known to be rich in salt comparing with the other local tomato varieties. There is no any study on PVY incidence and effects on this local important tomato genotype Sazlıca. The purpose of this research is to investigate PVY incidence and the effect of PVY infection at different developmental stages on yield and fruit quality of local tomato genotype “Sazlıca” in Sazlıca town and Niğde region.

CHAPTER II

LITERATURE REVIEW

Potato virus Y (PVY) belongs to genus *Potyvirus* and family *Potyviridae*. The genome of PVY is single-stranded RNA (ssRNA) which is approximately 9.7 kb in size (Ward and Shukla, 1991). PVY is an economically important virus in the world. PVY has been ranked as one of the most important pathogens among tomato diseases and other Solanaceous crops such as potato (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.), and pepper (*Capsicum annuum* L.). This virus subjected to reduced yield and quality of the crops all over the world. Nowadays, it is the major challenge for agricultural production. The researchers and scientist are trying to find out alternative solutions to overcome this serious issue to increase the production of tomatoes worldwide.

2.1 World Distribution and Occurrence of PVY

2.1.1 World distribution of PVY

The PVY^{N} strains reported in Europe, South America, Asia and Africa (Weidemann, 1988), as well as, New Zealand (Fletcher, 1989), and the USA (Singh et al., 1993). The PVY^{NTN} strain has been found most potato producing countries all over the world, including the USA (McDonald and Singh, 1996). The PVY^{NW} recorded in a number of other countries Spain and France (Blanco-Urgoiti et al., 1998). The PVY^{O} strains in potato crops occur globally (Jeffries, 1998a). The strain of PVY^{C} is popular in North America, Europe, Australia, India, South Africa, Ecuador and New Zealand (Jeffries, 1998b), is apparently widespread than generally accepted in Japan (Ohshima et al., 2000). While $PVY^{N:O}$ reported in Manitoba (Canada) and Minnesota, Montana, North Dakota (USA) (Singh et al., 2003a). PVY has distributed globally in potato growing area, in outdoor tomato and pepper and in tobacco growing area in moderate climate countries (Tsedaley, 2015).

2.1.2 PVY occurrence

Thomas and McGrath (1988) reported a line of *Lycopersicon hirsutum* (P.I. 247087) became resistant to systemic infection by PVY isolates from Queenisland. The resistance inheritance to PVY virus was examined in a cross between P.I. 247087 and a susceptible tomato cultivar (*L. esculentum cv.* Floradade). The test of segregation data is from PVY inoculated parent lines of F1, F2 and backcross generations approved the theory that a single recessive gene conferred the resistance. Nevertheless, the reaction of this gene in some generations may be altered the extraordinarily susceptible genetic background of Floradade tomato. The authors concluded that a backcrossing program has started to link this resistance with commercially important tomato cultivar production.

According to Abad and Jordá (2000), PVY disease caused to affect the plantation of tomato with varying seriousness in Tenerife Island. These plants exhibited different symptoms including necrotic lesions mild-harsh in the leaves and causes whitish spots in pre-harvesting fruits that last after ripening. PVY isolates of tomato some potato and capsicum have been demonstrated based on serological, biological and molecular principles. PVY isolates totally reacted as positive to monoclonal antibodies specific for PVY^{N} or $PVY^{O/C}$ strains, and almost tomato PVY isolates 50% were identified by both. The authors concluded PVY strains accordance with inoculated observational plants was complicated due to the irregularity of viral aggression and symptomatology caused. Restriction Fragment Length Polymorphism (RFLP) investigation of the 3' untranslated region (UTR) and CP gene showed significant variability. Furthermore, different PVY strains with mixed infection exhibits the molecular properties and biological of those tomato PVY isolates which respond to both monoclonal antibodies were described for the result of RNA recombination among different PVY strains that infect the similar host plant.

Sikora (2004) reported that PVY is transmitted by many aphid species in a non-persistent manner, Aphids transmits the virus in less than 60 seconds from an infected plant to the healthy plant. Mr. Sikora also reported aphids may retain for longer 24 hours

if the feed is not appropriate. Potato is a significant virus source for tomato and other Solanaceous crops.

Crescenzi et al (2005) evaluated a severe viral disease of leaf necrotic including rachis distortion and unusual fruit ripening and necrotic mottling along leaflet veins in hydroponic production of miscellaneous tomato cultivars in the Calabria province of southern Italy. Electron microscopy detected a virus along with filamentous particles, about 750 nm in leaflet dips from naturally infected tomato plants from fifteen different plots. The virus mechanically transmitted from symptomatic plants to herbaceous plants. The serological and biological analysis confirmed that the symptoms are similar with PVY infection. ELISA using monoclonal antibodies analyzed symptomatic tomato samples for separation among PVY^{N}, PVY^{O} and PVY^{C} subgroups.

Aramburu et al (2006) examined a group of 39 PVY samples from tomato isolation, rising from several economical crops grown in north-east of Spain which characterized in biological, serological and molecular analysis. There were no coincide among three different analysis noticed. Biological characterization result by PVY inoculation isolates to *Nicotiana* spp. and pepper plants have not corresponded with the results given by ELISA using monoclonal antibodies specific to PVY^{N}, $PVY^{C,}$ and $PVY^{O/C}$ strains. The authors also reported in certain cases that a mixed infection of dissimilar PVY strains have been observed that selectively infected the various hosts as showed by the ELISA test. However, the most PVY strains obtained from similar tomato fields demonstrated a high degree of correspondence; this indicates that each tomato field has at least one-source of infection.

Ibaba (2009) examined a total of 39 isolates from different Solanaceous crops including 18 isolates infecting tomato, 9 infecting pepper and 12 infecting potatoes and were additionally separated into strains by means of RT-PCR using specific primers and ELISA using strain-specific antibodies to the various PVY strains found in the world. The author identified all PVY isolates infecting tomato and pepper as positive for the normal strain of PVY^{O} by both ELISA and RT-PCR whereas PVY isolates infecting

potato has more heterogeneous and consisted of PVY^{N}, PVY^{NTN} and $PVY^{N\,W}$ strains and some cases mixed infection have been observed.

Hosseini et al (2011) reported that cultivated fields in six (6) Iranian regions evaluated PVY survey between January 2005 to July 2007. Two hundred samples from tomato and potato were collected and examined using ELISA for Potyviruses. PVY, nearly one fourth (1/4) of the samples infected. Investigation of PVY positive samples using three monoclonal antibodies (MAbs) promoting the concurrent observation in the three main strains including PVY^{O} strains, PVY^{N} stains, and C (PVY^{C}) strains. Nevertheless, the fourth strain of PVY^{NTN} and few other recombinants isolates also recognized by using molecular procedures. The authors also identified that symptoms and host range investigation utilizing sap inoculation of four distinct PVY strains onto the extent of plants which the four strains presented biological characteristics which appeared to be constant with their molecular arrangement. Fourteen (14) PVY isolates selected based on geographical position and host, primer serology and specificity for more molecular and biological characterization. P1 genes, CP and 3'-nontranslated region (3'NTR) from fourteen representative isolates sequenced and examined with the sequences accessible in GenBank. Combination test of the CP, P1 and 3'-UTR sequences with complete full genome PVY sequences disclosed that PVY^{O}, PVY^{N} and PVY^{NTN} are the three available strains of PVY in Iran. The PVY^{NTN} strain isolates in Iran is more accurately similar to the European PVY^{NTN} isolate than North American one.

Abu-shirbi et al (2012) examined tomato viruses' occurrence in open field tomatoes in Jordan. An absolute 1647 samples were collected from different parts in Jordan particularly Northern Jordan, Central Jordan, Southern Jordan, and Badria. DAS-ELISA analysis demonstrated that one or more viruses infected about 39.6% of the collected samples. The findings showed the existence of PVY, *Tomato spotted wilt virus* (TSWV), *Tomato ring spot virus* (ToRSV), *Tomato yellow leaf curl virus* (TYLCV), *Tomato aspermy virus* (TAV), *Tomato bushy stunt virus* (TBSV), *Tobacco etch virus* (TEV) and *Tobacco rattle virus* (TRV). The authors concluded that the most important virus in tomato fields was TSWV as it found to be infected about 17.7% of the samples collected

with a single or mixed infection, Whereas PVY infection rate was 8.7%, then ToRSV with 7.8% infection. Nevertheless, *Alfalfa mosaic virus* (AMV), *Tomato mosaic virus* (ToMV), *Potato virus X* (PVX), *Cucumber mosaic virus* (CMV) and *Tobacco ring spot virus* (ToRSV) did not screen in any tomato sample.

Cuevas et al (2012) evaluated a different PVY host plants including economically significant crops such as tomato, tobacco, pepper and potato. A pool of 177 total PVY genomes from isolates taken around the world. After analyzing the recombination effect in their data set, they have used Bayesian techniques to analyze the influence of host species and geography in both the structure and dynamics of PVY population. The authors also carried out co-variation test and selection to recognize evolutionarily appropriate amino acid residues. The result demonstrated that both hosts driven adaptation and geographically defined PVY heterogeneousness. In addition, the main force driving PVY evolution is the purifying selection, even though some positive selection indications were accounted for the diverse strains. The authors concluded that the other important thing is that the P3N-PIPO analysis, a newly explained gene in Potyviruses, appears to present a variable length between the isolates evaluated, the host-driven adaptation part was explained in this variability.

Quenouille et al (2013) reported that PVY allocated all over the world and has a wide host range containing cultivated *Solanaceous* and non-*solanaceous* weeds. The genome analyses identified PVY species have five major clades. The C1(Common), O(ordinary) and N(necrotic) are the most widespread groups around the world while the Chilean and C2 groups are further restricted due to their limited geographical distribution or their small host range.

Celetti (2014) reported that The infected tomato leaves appear dark green bands along veins with slight mottling sometimes leaf distortion, nevertheless, these symptoms are depended upon situations, PVY strain, age and variety of the tomato plants, a greater harsh symptom might advance at the fields such as dark brown necrotic lesions among the veins in the leaves. The terminal leaflets might also be brown and dead. The infected

plants occasionally develop droopy as petioles and leaves curling downward. The author concluded there is no tomato fruit symptoms that have been stated.

Coutts and Jones (2015) identified *Solanum nigrum* plants without symptoms were infected by PVY^{O} after sap inoculation and seed transmission was not detected. Authors demonstrated their study that PVY^{O} can be transmitted into the plant by contact and needed to remove plant materials, machinery, decontaminating tools and clothing to keep the farm hygiene and to eradicate or minimize the spread of the PVY^{O} virus.

Sivaprasad et al (2015) reported PVY presence in tomato plants based on their symptoms and this process applied RT-PCR using Potyvirus primers arranged in the NIb gene (Zheng et al.,2008) and triple-antibody sandwich (TAS-ELISA with specific antiserum (Agdia USA). The resulting of 350 bp amplification purified sequenced (Macrogen, South Korea). Later, then sequences deposited in GenBank (accession Numbers. KT581015 (Ambato), KT581016 (Huachi Chico), KT581017 (Montalvo), KT581018 (Pelileo). The analysis of sequences (7.05 of BioEdit v.) presented 91.2-99.6% and 97.7-100 similarity with the NIb gene of the other PVY isolates at the amino acid levels and nucleotide. Phylogenetic tree constructed using MEGA version 4.1 formed two clades with Ecuadorian isolates (KT581018 ad KT581017) approximately were related to PVY isolates (KC634008, HQ912869, AB711146, JF927763, KF850513, KJ946936, JQ969037, KJ159976, KC296433, AB461453, AB714135, AY884984) in clade 1. Where the two other Ecuadorian isolates (KT581015 and KT581016) belonging to clade 2. The authors eliminated infected crops to eradicate the infection.

Liang et al (2015) investigated the tomato plants reaction particularly cultivar Rutgers for single and combined infections with PVY and PVX. Plant infected individually by PVY^{O}, $PVYN^{:O}$, PVY^{N} or PVY^{NTN} showing advanced leaf deformation and mosaic symptoms as plants infected with PVY advanced mild local lesion and differing degree of mosaic and leaf deformation symptoms. Combined PVY + PVX infection developed further serious symptoms, such as leaf drop and severe leaf deformation and local and

systemic leaf necrosis. The authors concluded in comparison to PVY or PVX with single infection, showing PVY+PVX is more interacted and serious for tomato production.

Nie and Molen (2015) reported the recovery phenomenon subsequent infection with PVY examined in tomato, tobacco and potato crops. The plant of tobacco, serious strains infection of PVY ($PVY^{N:O}$ or PVY^{N}) were induced obvious vein clearing and leaf deformation in the first three leaves higher the inoculated leaves, as the upper leaves had plenty milder symptoms. The recovery phenotype did not apparent infected with PVY strain in tobacco which caused mild symptoms (PVY^{O}). Nevertheless, disregarding of the virus strain, decreasing PVY RNA levels likewise evaluated in upper leaves of these plants. Discarding, the three leaves upper the inoculated leaves hinder with recovery development, indicating the signal (s) that mediating the improvement is probably caused in these leaves. In $PVY^{N:O}$ or PVY^{N} while not infected PVY^{O} in tobacco plants, the expression of the PR-1a transcripts were compared with the accretion level of PVY RNA. Decreased PVY RNA level in the above leaves also noticed infected tomato plants, while like this phenomenon did not notice in potato plants. PVY-derived small RNAs were discovered in both potato and tobacco plants and their accretion level were compared with the levels of PVY RNA. The authors concluded their result that the recovery of phenotype sub-sequencing the infection of PVY is host-specific and not certainly correlated with the expression of PR-1a and PVY small RNAs generation.

Chikh-Ali et al (2016) evaluated PVY isolates, PVY-H14 from tomato plants that have shown necrotic lesions and stunting on leaves and were collected from the island of Oahu in Hawaii. The PVY-H14 activated hypersensitive resistance reaction in potato varieties Maris Bard and King Edward, a conventional of a PVY^{C} strain and was not able to infect systematically the four investigated varieties, Maris Bard, Russet Norkotah, Desiree and King Edward. H14 and the whole genomes phylogenetic analysis of thirty-one PVY isolates from non-recombinant strains of PVY positioned PVY-H14 in the similar clade with PVY^{C} which is PVY isolates from tobacco and tomato.

Hussain et al (2016) described that PVY strains are allocated around the world. PVY Strains consist of PVY^N, PVY^O and PVY^C. The authors also reported the strains of PVY^N to arise in Europe, in South America and some African countries. PVY^O distribution is universal while PVY^C strains are found in Europe, Turkey, Australia, Pakistan, and India.

Khudiesh (2016) investigated the occurrence of PVY on potato plants and collected samples from main tomato growing fields in west-bank Palestine by utilizing serological methods and biological with molecular methods. Forty fields were surveyed and collect a total of 255 potato samples in the years of 2014 to 2015. the samples were analyzed for the presence of PVY by using DAS-ELISA (and only five samples were further tested by RT-PCR using degenerate primers. The author found a different symptom from some of the surveyed fields including stunting, rugosity, yellowish-green mosaic, wilting and general yellowing. In DAS-ELISA method, the occurrence of PVY virus was identified at an average of 15.29% and also confirmed by RT-PCR analysis and bioassay test. All infected samples were belonged by Spunta variety excluding one sample from Mondial. In the meantime, the largest area where the virus had been recognized in its fields found in Nablus region with the percentage of (48%).The author concluded that PVY virus is exists in the fields of main growing land and recommended to take action to prevent the spread of this virus and finally regarded the result as significant in supporting a helpful stage for further studies to create suitable management way to control the viral disease of the plants in Palestine.

Lacomme et al (2017) have reported almost 765 species of aphids (Hemiptera: *Aphididae*) transmitted to PVY. The PVY has a wide host rage infecting *Solanaceous* and other host plants species including ornamentals and weeds. Authors also reported that PVY can be transmitted by mechanical ways such as grafting and wounding.

Ahmad et al (2017) examined a collection of 595 tomato samples that have shown the symptoms of vein chlorosis, mosaic, and mild molting symptoms from fields of Pakistan. DAS-ELISA screened all samples for the presence of PVY and used specific

polyclonal antiserum (Bioreba AG, Switzerland). As the result of symptomatic samples, 104 became positive for PVY infection, only eight in symptomatic samples additionally screened for the existence of PVY by RT- PCR used primer pair PVYPK-F/R, which developed in 1050 bp fragments amplification. Sequencing each amplicon comprising a full-length |CP inclusive of 300 bases of UTR, a complete of 1050 nucleotides obtained. Two isolates sequences submitted to Genbank under accession No: KX816570 and KX816568. The isolate of AARTPK (KX816568) used in screening of 11 tomato cultivars. Kalam, NSC-92, and Yaqui cultivars found resistant (R), while Rio-grandi neutrally resistant (MR) and Super-SPC and Giant-cluster as result of neutrally susceptible (MS). Likewise, the reaction of BSS-30 and Gala recorded as susceptible (S) whereas CKD-267, Junny-2144, and Jagular as extremely susceptible (HS). The authors identified resistant cultivars can be benefited in the future as a genetic source for improving resistant varieties against PVY.

Nikolic et al (2018) examined and collected 3220 samples from tomato crops from 56 areas of 18 districts in Serbia. This survey was conducted in 2011-2012. Among 12 viruses were tested, including PVY, CMV, AMV, TSWV, ToMV) and TMV discovered in 40%, 42.1%, 11%, 8.6%, 2.3% and 1.3% of all _investigated samples. The result showed that PVY was common in 2012 and CMV in 2011. Single infection was the most type of repeated infections whereas the double infections were the most common infections and the most wide-spread association was PVY and CMV. In 2011, the diseases occurrence and the total percentage of infection types were necessarily higher than in 2012. The authors also reported that tomato has naturally a wide host range for more than 200 pathogens and the plant viruses are one of the most challenges of tomato production around the world. The harsh economic losses of tomato production caused by virus based on many factors including; age of the plant at the time of infection, virus strain, and plant genotype in addition to temperature during pathogen development, nearly 146 viruses infecting tomato have reported and some of them including PVY, which caused a huge reduction the quality and the quantity of the fruits.

Oliveira et al (2018) reported that PepYMV (*Pepper yellow mosaic virus*) initially described as a resistance-breaking PVY separate on the cultivars of *Capsicum annuum*

L., this virus also infecting the tomato plants in Brazil. The authors tested the resistance source of both PepYMV and PVY were investigated in a 119 collection accessions owned by seven Solanum species. At first, germplasm assessed to PepYMV response by mechanical inoculation along by evaluation of symptoms and ELISA. For the first time,

the resistance source of PepYMV recognized in *S. peruvianum, S. habrochaites, S. corneliomuelleri, S. chilense, S. pimpinellifolium,* and one addition of derivative from an interspecific cross (*S. lycopersicum x S. peruvianum*). A 24 accessions sub-group with negative serology of PepYMV also questioned with a PVY isolate, along with by molecular and serological discovery with global primers. *Solanum habrochaites* 'L.03683' and 'L.03684' only accessions achieved with stable resistance to both viruses. The authors concluded that results confirmed *S. habrochaites* are the most substantial source of various resistance factors apparent in Potyvirus species.

2.2 PVY Distribution in Turkey

Bostan and Haliloğlu (2004) studied to find out the distribution and percentage of PVY (PVY^N, PVY^O, PVY^C), PLRV and PVS and seed tubers were used for sowing materials in the significant potato producing provinces in Turkey. The symptoms induced by single or mixed infection were observed under field conditions. Primarily, virus-specific polyclonal antibodies were used to analyze a total of 880 leaf samples. Secondly, the samples of 83 that were detected the presence of PVY infection of the first result were re-analyzed by utilizing PVY^O, PVY^N -PVY^C-virus-specific monoclonal antibodies. The ELISA result displayed seed potato tubers utilized for the planting materials were infected with the percentage of PVY (17.7%), PLRV (14.2%), PVS (4.6%) and PVX (11.8%). On the contrary, the outcome monoclonal antibodies for PVY strains displayed the rate of PVY^O and PVY^N were (4.3%, 14.4%) while PVY^C did not find any result Under the field condition, a plant found to be infected with PLRV shown young leaf rolling, upright growth and pinky color but PVS have not induced any apparent symptoms. Single PVX or the association of PVX with PLRV, PVY with PVS induced mild or serious mosaic symptoms on whole cultivars. PVY caused leaf drop streak, yellowing of leaves, and venial necrosis on view plants from whole plants, nevertheless,

some plants were not shown any visible symptoms whether infected with PVY. The mixed of PVX and PVY induced more serious mosaic, wrinkling and decreased leaf size. Plant infected with PLRV and PVY shown yellowing of leaves, leaf dwarfing, leaf drop, and leaf rolling and wrinkling. On the other side, the symptoms on a plant infected with PLRV and PVS or PVY and PVS did the same to single infection of PLRV and PVY.

Arli-Sökmen et al (2005) analyzed a total of 313 samples from field-grown pepper in Samsun, Turkey to determine the characteristics of several viruses that affect pepper plants. The sample surveys were collected between 1998 and 1999 and ELISA (enzyme-linked immunosorbent assay) were used for analyzing. Six viruses including, PVY, AMV, CMV, ToMV, TMV, and TSWV were identified in the samples. 42 of 313 plant samples were double infected, while the highest common double infection was PVY+TMV (15.4%). In addition, this was the first report of AMV in pepper fields of Turkey. The authors also evaluated the effect of some weed species that may operate as a source of these viruses were also studied in the province and twenty-four (24) weed species owned by 14 families were examined, at least one virus discovered to infect the 16 weed species out of the 24. *Amaranthus retroflexus* (Redroot pigweed) seemed to be a common host of PVY, CMV, ToMV, TMV, and TSWV, while *Hibiscus trionum* (Venice mallow) registered as a new weed host of TSWV and PVY. The authors summarized that the majority of weed species found to be infected by the virus were common the growing areas of the pepper in the region. They pointed out the pepper fields were contaminated by these weeds and they are under viral infection risk.

Güner and Yorgancı (2006) studied to investigate the viral pathogens mainly occur in potato growing area of Niğde and Nevşehir regions in between 2003-2004 by using biological and serological methods to test the virus effectiveness. The plant materials of the survey were tuber and leaf samples that were collected randomly from symptomatic and non-symptomatic plants. The presence of PVY, PVX, PVA, PVS, PVM and PLRV tested by using mechanical inoculation and DAS-ELISA. The viruses including PVY, PVS, PVX, PVA and PLRV were observed in both tubers and leaves by using DAS-ELISA method in the regions of Niğde and Nevşehir. The authors also identified these

viruses as single or combined infection. In addition, the single infections such as PVX, PVY, PVS and PLRV while combined infections such as PVY+PVS, PVY+PLRV, PVY+PVS+PLRV, PVS+PVA, and PVY+PVA were also found as a result of complex infections. Though PVS inoculated on to *Chenopodium amaranticolor, C. quinoa, Nicotiana tabacum* cv. *Maden, N.t.* cv *White Burley, N.t.* cv. *Xanthi, N. glutinosa, Capsicum annuum, Datura stramonium* examine plants, view symptoms like; mosaic (with bubble mosaic), mottling, vein clearing, vein banding, chlorotic, leaf distortion and necrotic local lesion were noticed on the leaves. The general disease occurrence was calculated as 32.35 % for 2003 and 34.05 % the year 2004.

Çelebi-Toprak et al (2009) examined nearly 20 various tomato accessions that stand for 6 diverse species which mechanically inoculated with PVY^O. The plants were arranged visibly for the symptoms and after inoculation for 2-4 weeks the existence of the virus was analyzed by ELISA. The results were varying. The majority of the wild species of tomato maintained PVY^O duplications on inoculated leaves. Some of the wilds displayed an immune reaction, whereas, some others were systemically infected. The authors concluded the analysis and inoculation of population F_2 proposed that a single recessive gene controlled the resistance in different wild species.

Deligöz and Arli-Sökmen (2014) evaluated PVY stains infecting pepper vary from strains on tobacco or potato serological and biologically. Even though the techniques based on monoclonal antibodies and coat protein (CP) sequence analysis can be utilized to distinguish PVY of pepper strains from other PVY strains detected in diverse types of hosts, these methods did not succeed to differentiate even PVY strains the pepper itself. The isolation of the peppers was sorted into three groups in accordance with the response of the pepper genotypes having *pvr21* and *pvr22* resistance genes in pvr2 locus. In this research, the PVY pathotypes infecting the pepper of the Samsun region were analyzed. Almost 502 and 510 leaf samples were collected from pepper growing range of years 2010 and 2011, respectively, DAS-ELISA using virus-specific polyclonal antiserum analyzed these samples. The ratio of PVY infections was 11.9% (in 2010) and % 5.8 (in 2011). Furthermore, an overall of 20 PVY isolates was chosen from infected ones, and the pathotypes of PVY-0 and PVY-1 were identified using sap inoculation

method practiced to leaves of various hosts that including diverse genotypes of pepper, on the other hand, PVY-1, 2 did not determine in PVY positive samples. The authors concluded that among the isolates examined, PVY-0 (80%) was more common than PVY-1 (20%) in Samsun.

Yardımcı et al (2015) examined and diagnosed in a different Potato virus including PVY, PVA, PVX, PVS and PLRV on leaves and tubers of diverse potato varieties i.e. Safran, Solea, Proventa, Floris, Milva, Universal, Lady Olympia, Vangogh and Marabel grow in Afyon, Turkey. From 2009 to 2010, different varieties of tomato tubers were collected from producers of Afyon province and sowed in experimental plots in Isparta province of Turkey. About one hundred sixty-nine (169) samples that collected from the leaves demonstrated the symptoms of the virus during vegetation period whereas one hundred nine (109) samples collected from tubers of suspicious plants during the period of harvest. DAS-ELISA method was used to test the complete 278 samples. The test of DAS-ELISA showed that both leaves and tubers infected with PVY, PVS, PVX, PVA and PLRV. The authors concluded that 87.45% (244 samples) of tested plant samples infected with one or more viruses and the rest number (12.54%) (34 samples) of the tested samples provided a negative response with DAS-ELISA. Concerning the virus popularity among the potato varieties in this research, it was detected that all samples characterized to Milva and Safrane varieties were infected by one or more viruses.

Furthermore, the other varieties of potato demonstrated various rates of virus infection. The analysis of mechanical inoculation, systemic chlorosis, and serious stunt in, and leaf deformation symptoms noticed on *N. glutinosa* whereas symptoms including leaf distortion, mottling, chlorotic and necrotic local lesions were noticed on the leaves of other investigated plants.

Karataș et al (2017) tested the response of 43 red pepper lines with PVY pathotypes of (0), (0,1) and (0,1,2). The PVY isolates LYE84, CAA16 and SON41P of PVY pathotypes (0), (0,1) and (0,1,2) were manifold in *Nicotiana tabacum L*. "Samsun" and the virus existence was corresponded by ELISA tests. The connection between pvr loci and pathotypes were investigated by serologically and biologically onto Yolo Wonder,

Yolo Y, Florida VR2, and W4 pepper genotypes. The red pepper lines were mechanically inoculated with PVY pathotypes. The virus amplification and expansion respecting with three pathotypes tested by DAS-ELISA for three weeks after initial symptom characteristics on positive controls. PVY (0) induced leaf mosaic whereas PVY (0, 1) caused downward leaf rolling, deformation on the leaf surface, stem deformation and elongated color deformation on fruits. PVY (0, 1, 2) induced defoliation afterward and necrosis along the veins. The Authors concluded that the three lines did not show any symptoms owing to virus infection. In accordance with results of serological examines, these can be used as a resistant nominee to the three of PVY pathotypes.

Yıldırım et al (2018) tested almost 120.000 seeds from diverse genetic background collected and examined in 2008 and 2016 in order to progress new varieties. The objective of the study was to advance excellent potato varieties tolerant to PVY and PVX, which have immense agronomic tuber traits. PVY and PVX are the most substantial viruses, inducing commercial crop losses in potato plant around the world. The best productive method to control these viruses is to use resistance genes. By using molecular markers, resistance genes were transferred to new varieties which were tightly linked to resistance genes (*Rx1 and Rx2 for PVX and* Rya_{dg} *for PVY*). Replicated trials of potato lines were analyzed in various potato provinces of Turkey including Niğde, Nevşehir, Adana, İzmir, Afyonkarahisar, and Kütahya in 8 years to assess the genotype x of environmental interaction and 85 higher lines. The authors concluded the analysis 4 early seasons and 3 major seasons higher lines accepted for registration as economical varieties whereas 4 of 7 competitor commercial varieties decided as appropriate for French fries and were commercialize it.

CHAPTER III

MATERIALS AND METHODS

3.1 Experimental Site Description

The experiment was conducted between July 2018 – December 2018 on a greenhouse of The Faculty of Agricultural Science and Technologies at Niğde Ömer Halisdemir University. Niğde Ömer Halisdemir University (former known as Niğde University) located in Niğde, Central Anatolia in Turkey, 6 km from southwest of Niğde. The area has the following climatic conditions; sunshine the average of 13hrs, 22 min /day and the average temperature is 21°C/69.8F, the experiment was done under a greenhouse which is hotter than outside at around 25°C/77F.

3.2 Materials for the Study

3.2.1. Pots

The study was conducted in both pots and trays (Figure 3.2.1). First, the seeds were sown in trays and their numbers were (32 cells seedling viyols ×13) =416. The pots were used later for transplanting and their numbers were (100×4) = 400. The total germinated plants that were used for inoculation were (379) plants.

Figure 3. 1. Left-side figure is shown viols and right-side figure is for pots

3.2.2 Plant materials

The local genotype “Sazlıca” is one of the most consumed variety in Niğde region and especially in Sazlıca town. To evaluate the effects of PVY infection at different growth stages on this local genotype “Sazlıca” a total of 379 plants of genotype “Sazlıca” along with PVY susceptible H2274 commercial tomato seeds were tested. The seeds of the Sazlıca were collected from various private companies in Niğde town, while the seeds of H2274 commercial tomato were obtained from the faculty of Agricultural Science particularly the Department of Plant Production and Technologies. The following (Table 3.1) is summarized the total tomato plants that were used in the experiment.

Table 3.1. The total plants used during the current study

No	Replications	Sazlıca Inoculated plants	H2274 Inoculated plants	Sazlıca Controls	H2274 Controls	Total samples
1	1st	33	39	5	5	82
2	2nd	45	45	5	5	100
3	3rd	45	45	5	5	100
4	4th	45	42	5	5	97

3.2.3 Virus isolates

PVY isolates from *Potato virus* Y^{NW} (pat3-8) and *Potato virus* Y^{NTN} (pat 25-62) strains were used in the experiment. Tobacco plants inoculated with these isolates which were obtained from Prof. Dr. Çiğdem Ulubaş Serçe (NOHU) which are identified under project Number: TUBITAK-1140153 were mechanically inoculated onto the leaves of the tomatoes respectively (Figure 3.2).

Figure 3.2. Tobacco plants that were used as inoculum source

3.3 Location and Field Survey

3.3.1 Location

Sazlıca village is where the survey was conducted and locates in the central district of Niğde region in Turkey (Figure 3.3.). At 37°54′N 34°38′E it lays on the Turkish state highway which connects Niğde to Çukurova It is 8 kilometers from south of Niğde and its population number was 3,411 in 2011 (Source: Wikipedia)

Figure 3.3. X; Location where the tomato samples were collected (source: google map).

3.3.2 Field survey

The survey was conducted during the summer growing season especially between June and July in 2018. A total of 50 leaf samples showing virus symptoms were randomly collected from different fields in Sazlıca town. Each field a number of samples were obtained; Field 1 (7 samples), Field 2 (11), Field 3 (14 samples) and Field 4 (18 samples). The samples were collected by plastic bags and instantly stored in a cooling box and later transferred in to laboratory refrigerator under -80 °C until testing time.

3.4 Experimental Design

To evaluate the effects of virus infection time at different growth stages on local cultivar Sazlıca tomato, seeds were germinated in plastic pods at greenhouse condition. As a control, commercial tomato variety H2274 was used. Each plot was included fifteen plants with three replications. After 7 days, 14 days, 21 days and 28 days of planting, two virus strains were used for inoculation in three different combinations; PVY^{NW}, PVY^{NTN} and PVY^{NTN+NW} with uninfected controls (Table 3.2).

Table 3.2 The details of PVY strains used for the inoculation during the study

Treatment code	Treatment description
T1	Control
T2	PVY^{NW} (pat 3-8)
T3	PVY^{NTN} (pat 25-62)
T4	PVY^{NW+NTN} (pat 3-8) + (pat 25-62)

3.5 Biological Assay (Mechanical Inoculation)

3.5.1 Plant-transplanting

At first, the tomato seeds were sown in small pots (viols) and waited until they reached the transplanting time. Before one day the tomatoes were watered well to keep the soil moist that will cling to roots and protect them from drying before started to repot then the seedlings were transplanted into pots (Figure 3.4). The transplantations were done in different times based on the four replications of the experiment.

Figure 3.4. Before transplanting (left-side) and after transplanting (right-side).

3.5.2 Plant inoculation

During the growing stages of tomato plants, the PVY virus strains (PVY^{NW} and PVY^{NTN}) were inoculated on tobacco plants and these plants were later used for inoculation source on tomato plants. Virus inoculation for tomato plants was carried out by a mechanical virus inoculation method. Leaf extract was added, PVY inoculation buffer (pH: 7.4) including 0.199 g/l KH_2PO_4, 1.14 g/l Na_2HPO_4 and 0.1% Na_2SO_3 and 1% PVP-40 were used. Infected tobacco plant materials were ground in mortal and pestle to macerate the tissue and it was the initiating step in the preparation of plant leaf extract for inoculation. Before one day the inoculation, the plants were kept in a shade place in order plants to be susceptible for the virus and the next day were started inoculating virus on plants' leaves. At the starting point, carborundum was sprinkled on to the leaves and the virus preparation was rubbed on to the surface of tomato leaf in such a way as to break the surface cells without making too much mechanical damage (Figure 3.5). Tap water was rinsed the leaves of the plants soon after 2-3 hours of inoculation. After virus spread on plant leaves, the inoculated plant leaf samples were collected representing whole plant part. Details concerning the names of the varieties/genotype and PVY strains used in this study based on the 4 different inoculation time (after 7, 14, 21, and 28 days inoculation) are summarized in (Table 3.5).

Figure 3.5. PVY inoculating on tomato leaves

Table 3.3. The details of 7, 14, 21 and 28 days inoculated plants (both Sazlıca and H2274) used in the current stud

No	Virus strains	Variety	Inoculation number	Inoculation date
1	Control	Local genotype "Sazlıca"	1-5 (mocks)	31.07.18
2	PVY^{NW}		1 to 11	
3	$PVY^{NW/NTN}$		1 to 11	
4	PVY^{NTN}		1 to 11	
1	Control	H2274	1-5 (mocks)	
2	PVY^{NW}		1 to 13	
3	$PVY^{NW/NTN}$		1 to 13	
4	PVY^{NTN}			
1	Control	Local genotype "Sazlıca"	1-5 (mocks)	16.08.18
2	PVY^{NW}		1 to 15	
3	$PVY^{NW/NTN}$		1 to 15	
4	PVY^{NTN}		1 to 15	
1	Control	H2274	1-5 (mocks)	
2	PVY^{NW}		1 to 15	
3	$PVY^{NW/NTN}$		1 to 15	
4	PVY^{NTN}		1 to 15	
1	Control	Local genotype "Sazlıca"	1-5 (mocks)	29.08.18
2	PVY^{NW}		1 to 15	
3	$PVY^{NW/NTN}$		1 to 15	
4	PVY^{NTN}		1 to 15	
1	Control	H2274	1-5 (mocks)	
2	PVY^{NW}		1 to 15	
3	$PVY^{NW/NTN}$		1 to 15	
4	PVY^{NTN}		1 to 15	

Table 3.3. (Continue) the details of 7, 14, 21 and 28 days inoculated plants (both Sazlıca and H2274) during the current study.

1	Control	Local genotype "Sazlıca"	1-5 (mocks)	30.08.18 and 15.01.19
2	PVY^{NW}		1 to 15	
3	$PVY^{NW/NTN}$		1 to 15	
4	PVY^{NTN}		1 to 15	
1	Control	H2274	1-5 (mocks)	
2	PVY^{NW}		1 to 15	
3	$PVY^{NW/NTN}$		1 to 15	
4	PVY^{NTN}		1 to 15	

3.6 Serological Analysis

3.6.1 DAS-ELISA

PVY were tested in the samples collected from the department's greenhouse and the survey samples collected from Sazlıca town by using DAS-ELISA (Double antibody sandwich–enzyme linked immunosorbent assay), according to Clark and Adams (1977) and instructions of the antisera's manufacturer (Bioreba AG, Switzerland) for the polyclonal antisera of PVY. Six plates were used for conducting this test (See Tables 3.7, 3.8, 3.9, and 3.10). Before samples analyzed, each sample was transferred into new 2 ml tube and added extraction buffer; 200 µl of sample juice and 200 µl of extraction were added. In first step of DAS-ELISA which is coating were done; 40 µl of IgG in 40 ml of coating buffer were diluted and 100 µl were added to each well. The plates were covered tightly and incubated 30 °*C* for 4 hours. After 4 hours plates were washed with washing buffer in three (3) times then each well 100 µl of sample were added, covered tightly and placed them in a humid box and incubated at 4 °C overnight. The next morning plates were washed as in step one, then 40 µl of enzyme conjugate in 40 ml of conjugate buffer were diluted and 100 µl were added to each well. Plates were covered tightly, placed them humid pox and incubated at 30 °C for 5 hours. After 5 hours the

plates were washed as in step one then dissolved pNPP (Para-nitrophenyl-phosphate) at 0.04 mg in 40 ml of substrate buffer and 100 µl were added to each well and finally the positive, negative and extraction buffer were added and were repeated two times in the wells (Figure 3.6). The plates were incubated at room temperature (20-25 ℃). ELISA result was observed the reaction and read yellow color development in the plates after 90 minutes visually and was applied and measured at 405 nm on Biotek el.800 ELISA reader.

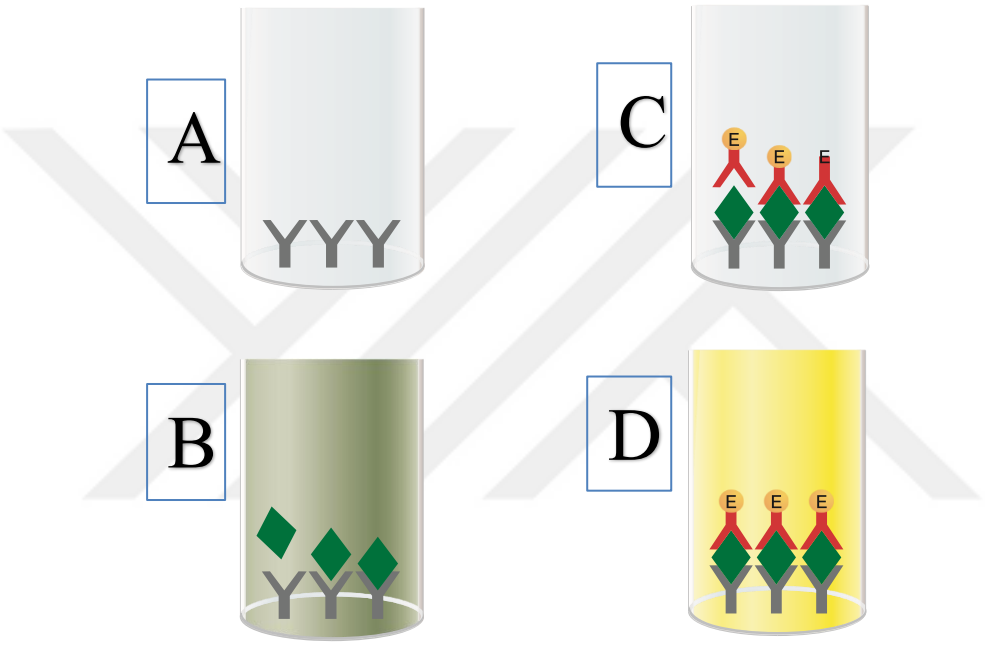

Figure 3.6. DAS-ELISA procedure. A- Coating: specific antibody adsorbed to surface of microtiter wells. B- Antigen: incubation of plant extract. C- Conjugate: Incubation of enzyme -labeled antibody D-Substrate: color reaction indicates infected samples (source: BIOREBA AG).

3.7 Calculation of PVY Infection Rate (Percentage)

In order to determine the percentage of PVY in inoculated tomato plants, the following formula were used:

$$\textbf{\% PVY incidence} = \frac{\textbf{Tomato samples confirmed positive by ELISA}}{\textbf{Total tomato samples tested}} \times \mathbf{100}$$

3.8 Tomato Phenotypic Records

As the inoculated tomato plant became mature and started fruit production, the plants were measured in different ways. Firstly, the plant's heights and leaf length (LL) were measured with ruler and measures were noted down. The plants also were counted the number of fruits (NF) and number of branches (NB).

3.9 Yield and Fruit Quality Parameters

Ripen fruits from each sampling in the four different replication (stages) were collected with plastic bags by hand and were stored in a refrigerator at +4 °C until study was conducted. These fruits different parameters were observed such as fruit length (FL), fruit width (FWth) and fruit weight. The fruits length and fruit width were measured with the rulers while the fruit weight (FW) were measured by digital weight scale. The fruits were also measured the soluble-solid content (Brix content) by using A. KRÜSS Optronic GmbH, AR-2008 (Figure 3.7).

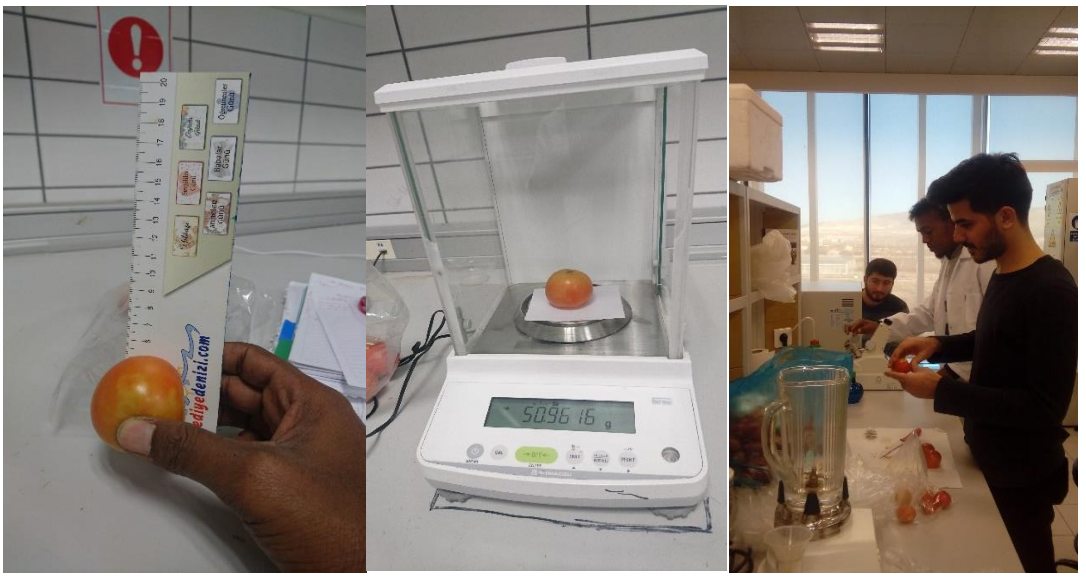

Figure: 3.7. (A); Measuring fruit width with ruler, **(B);** Weighting fruit(g) with digital weight scale, **(C);** Measuring soluble solid content (brix) by using A. KRÜSS Optronic GmbH, AR-2008.

3.10 Data Analysis

DAS-ELISA was used to detect PVY**.** The samples were also compared as tables to present the variance among replications and strains. The fruit quality parameters were analyzed by using post hoc, Duncan, the software IBM SPSS statistical 25 version.

CHAPTER IV

RESULTS

This section shows the results and data achieved from the field experimentation. The results are divided into four sections which are; I. Field survey, II. Mechanical inoculation, III. Serological analysis, and IV. Yield and fruit quality parameters.

4.1 Field Survey

During the survey a different virus symptom were observed in the tomato fields at Sazlıca town. The tomato plants seemed to be infected by the virus were collected and tested by DAS-ELISA. The symptoms observed were including; leaf rolling, necrotic, yellow leaf curl and stunting (Figure 4.1). As shown ELISA plate 4, some of the samples in the fields located in Sazlıca have shown positive results. The highest PVY positive reaction was observed the samples collected from Field 1 which was (14%) in comparison to samples collected from Field 4 (11%), Field 2 (9%) and Field 3(7%) in Sazlıca town. In general, the percentage of PVY positive samples from the fields in Sazlıca town were 10% which means that five (5) samples were positive out of fifty (50) samples.

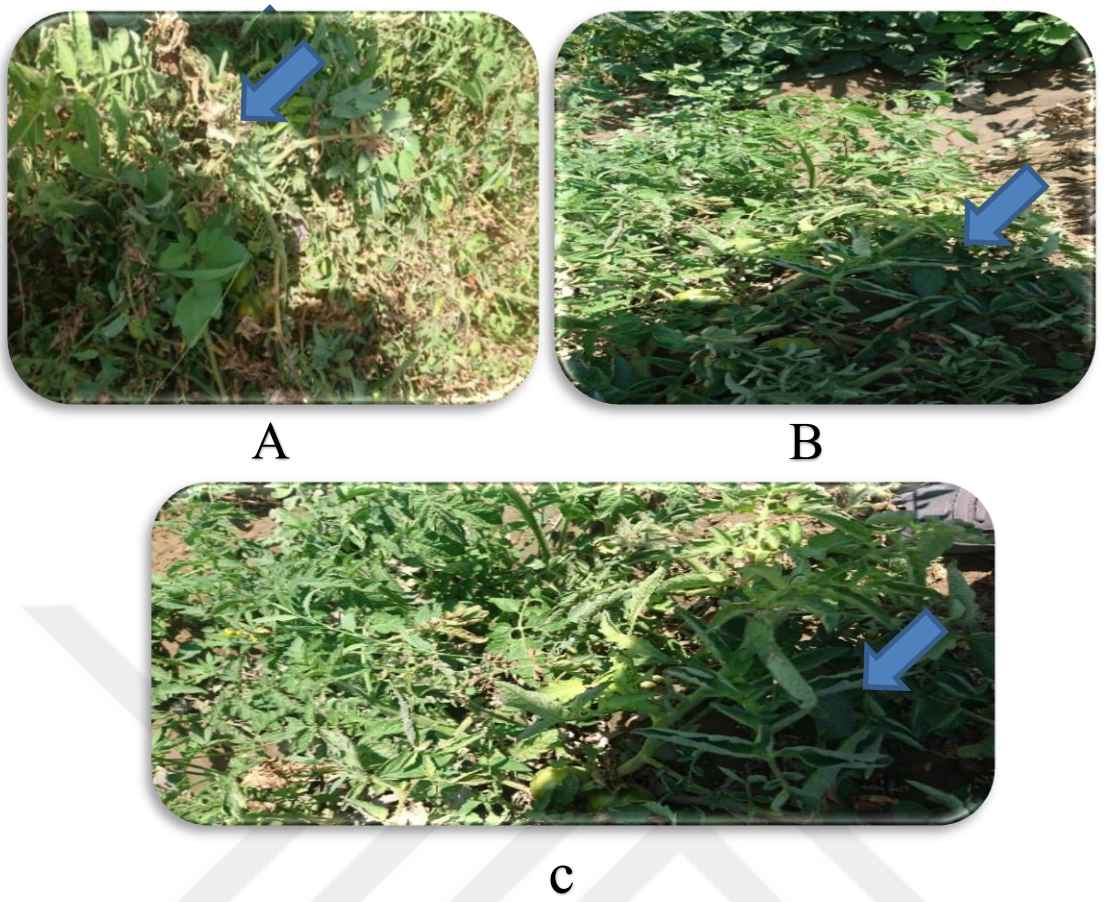

Figure 4.1. PVY symptoms recorded during the field surveys. (A): necrotic. (B): leaf rolling. (C): yellow leaf curling.

4.2 Mechanical Inoculation

The symptoms appearance on tomato leaves varied according to inoculated time, PVY strain, plant age and environmental conditions. PVY symptoms usually became visible four to five weeks after inoculation (Figure 4.2). Samples were collected from all inoculated plants along with controls, and were tested the presence of PVY using DAS-ELISA. The main symptoms observed on the plants during infection development are shown in the following Figure 4.2.

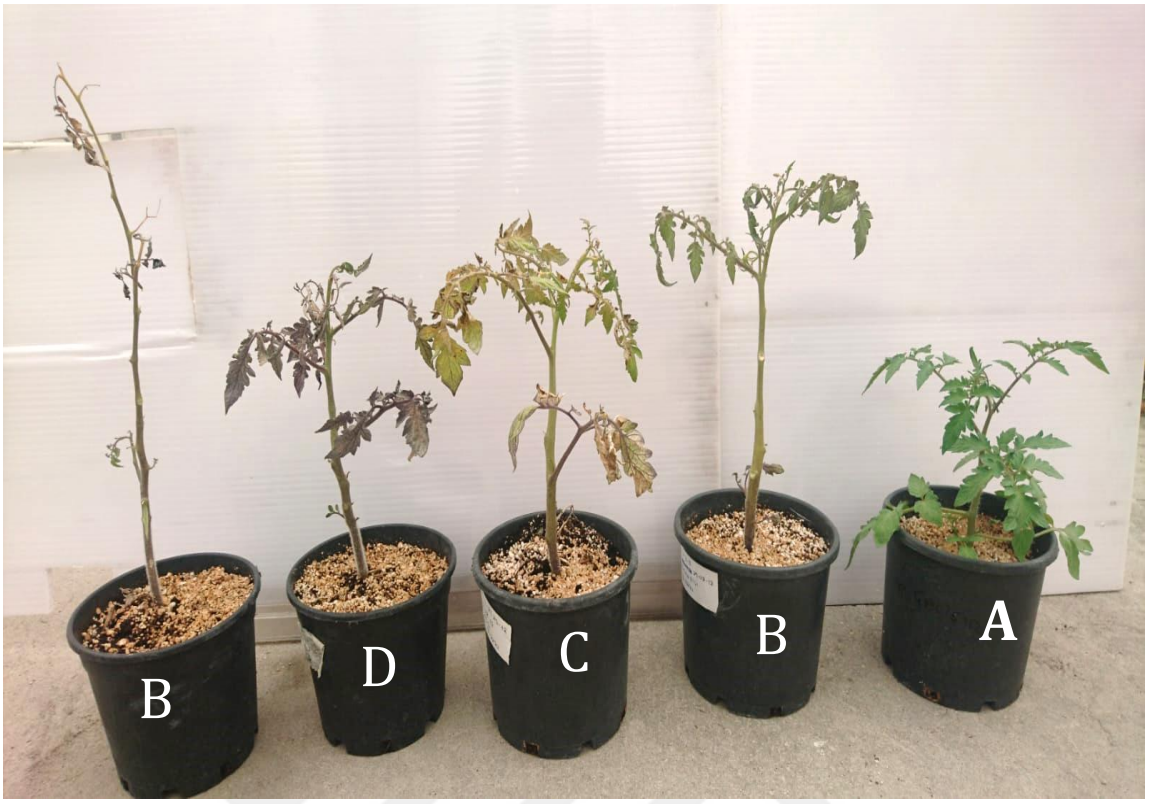

Figure 4.2. (A)Healthy plant, (B)leaf rolling and slight yellowing (C)Vast yellowing and terminal leaves dying (D)severe yellowing and slight dark brown color (E) Dark brown color and all leaves dying.

4.3 Serological Analysis

4.3.1 DAS-ELISA

The samples of local genotype "Sazlıca" and H2274 plants inoculated by $PVY^{NW,}$ PVY^{NTN} and mixed of PVY^{NW}+PVY^{NTN} have shown negative and positive reactions. All control plants have shown negative. The samples of inoculated plants, PVY have been identified in 112 out of 379 samples. The result of DAS-ELISA was visually observed the reaction and read yellow color development in the wells of ELISA plates which are shown in Figures 4.3 and 4.4. On seven days inoculated plants, the maximum ELISA positive result was recorded for H2274 with mixed PVY strains (PVY^{NW+} $PVY^{NTN)}$ (85%), followed by Sazlıca with PVY^{NW} strain (73%). In fourteen days inoculated plants, the maximum ELISA positive results were achieved by Sazlıca with the PVY^{NW}

strain (53%). In twenty-one days inoculated plants, the maximum ELISA positive results were obtained by H2274 with PVY^{NTN} (40%). In twenty-eight days inoculated plants

only one positive sample was found and it was Sazlıca with PVY^{NW}. The details of all ELISA positive results are also summarized in Table 4.1.

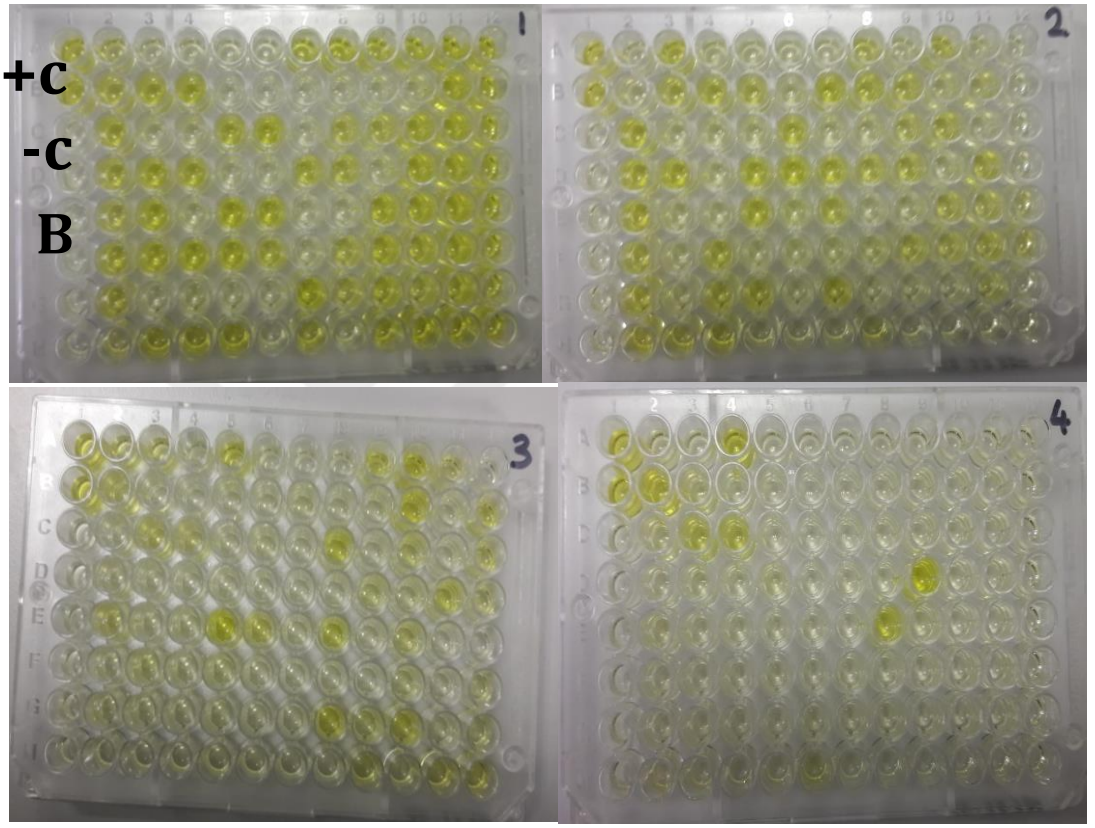

Figure 4.3. ELISA plates showing the positive and negative reactions. Samples showing positive reaction are same to the positive control (+C) which displaying by the yellow color. The negative control (-C) and Extraction buffer (B) are without color indicating a negative reaction. The above plates are samples from the plants of 7 days, 14days, 21 days, inoculum sources, survey samples and negative controls.

Table 4. 1. The details of ELISA result in Sazlıca and H2274 varieties and PVY strains in 7 days, 14 days, 21 days and 28 days during the current study

Variety	Inoculation date	Total # of tested samples	Total infected samples	PVY strains	Infection rate (%)
Sazlıca	7 days	33	8	PVY^{NW}	73%
			7	PVY^{NTN}	64%
			5	PVY^{NW+NTN}	45%
H2274		39	9	PVY^{NW}	69%
			5	PVY^{NTN}	38%
			11	PVY^{NW+NTN}	85%
Sazlıca	14 days	45	8	PVY^{NW}	53%
			7	PVY^{NTN}	47%
			7	PVY^{NW+NTN}	47%
H2274		45	8	PVY^{NW}	53%
			7	PVY^{NTN}	47%
			6	PVY^{NW+NTN}	40%
Sazlıca	21 days	45	3	PVY^{NW}	20%
			2	PVY^{NTN}	13%
			2	PVY^{NW+NTN}	13%
H2274		45	7	PVY^{NW}	47%
			6	PVY^{NTN}	40%
			3	PVY^{NW+NTN}	20%
Sazlıca	28 days	45	1	PVY^{NW}	7%
			0	PVY^{NTN}	0%
			0	PVY^{NW+NTN}	0%
H2274		45	0	PVY^{NW}	0%
			0	PVY^{NTN}	0%
			0	PVY^{NW+NTN}	0%

4.4 Yield and Fruit Quality Parameters

Knowing the effect of PVY at different growth stages on yield of local tomato genotype "Sazlıca" and H2274 variety on fruit length, weight, width and brix were analyzed. The result was obtained by performing variance test in post hoc, Duncan, SPSS. The result is summarized both tables and figures (Table 4.2, 4.3 and 4.4; Figure 4.2.-4.15).

Table 4. 2. Fruit quality parameters results of seven days inoculated plants (the average of infected plants along with control plants).

Tomato variety/virus strain	Fruit length (cm)	Fruit weight (g)	Fruit width (cm)	Brix (%)
H2274 PVY^{NTN}	3.6429	27.0843	4.0357	5.9143
H2274 PVY^{NW}	4.0385	34.2983	3.3077	5.2462
H2274 $PVY^{NTN+}PVY^{NW}$	3.7857	18.8074	4.0000	5.4214
Sazlıca PVY^{NTN}	1.7778	17.2171	2.3929	3.2714
Sazlıca PVY^{NW}	2.3182	18.1190	2.9000	3.9400
Sazlıca $PVY^{NTN+}PVY^{NW}$	2.4000	26.3600	3.7000	5.1600
H2274 Control	4.3500	39.4460	4.0000	6.320
Sazlıca Control	4.6000	30.6020	3.8500	5.560

For fruit length of seven days inoculated plants, the maximum length is recorded for H2274 PVY^{NW} and PVY^{NW+NTN} with the similar average of 5.5 cm whereas the highest length of Sazlıca is obtained by Sazlıca PVY^{NW} with average of 4.75 cm. The average length of H2274 negative control is 5 cm while Sazlıca negative control is recorded for 6 cm and this indicates us that Sazlıca negative control is higher than the positive one while H2274 negative control is less then and close to positive control. For the fruit width of seven days inoculated plants, the maximum fruit width is achieved by H2274 PVY^{NW} with the number of 5 cm and the highest Sazlıca fruit width is obtained by Sazlıca PVY^{NTN} with average of 5 cm. The highest H2274 negative control is 5.5 cm while Sazlıca negative control is 5.75 cm. For the fruit weight of seven days inoculated plants, the maximum fruit weight of Sazlıca is recorded for Sazlıca PVY^{NW+NTN} as 42.09 g/plant whereas the maximum fruit weight of H2274 is obtained by H2274 PVY^{NW} as 55.80 g/plant. The optimum fruit weight of Sazlıca negative control is 60.54 g/plant while H2274 is 75.32 g/plant. In the fruit sugar soluble content (brix) of seven days inoculated plants, H2274 PVY^{NW+NTN} has the maximum fruit brix of 6.9% while the highest sugar soluble content of Sazlıca is belonged by Sazlıca PVY^{NW} with the average of 6.6%. On the other hand, the maximum H2274 negative control is 7% whereas Sazlıca maximum negative control is 7.2%.

Post hoc, Duncan analysis showed significant differences between control and infected seven days inoculated plants and non-significant effect in the fruit length (Sazlıca; P=0.494, H2274; p=0.075), fruit weight (Sazlıca; P=0.243, H2274; P=0.056), width (Sazlıca; P=0.110, H2274; P=0. 164) and total soluble solid content (brix) which became (Sazlıca; P=0.065, H2274; p=0.090), between varieties, respectively.

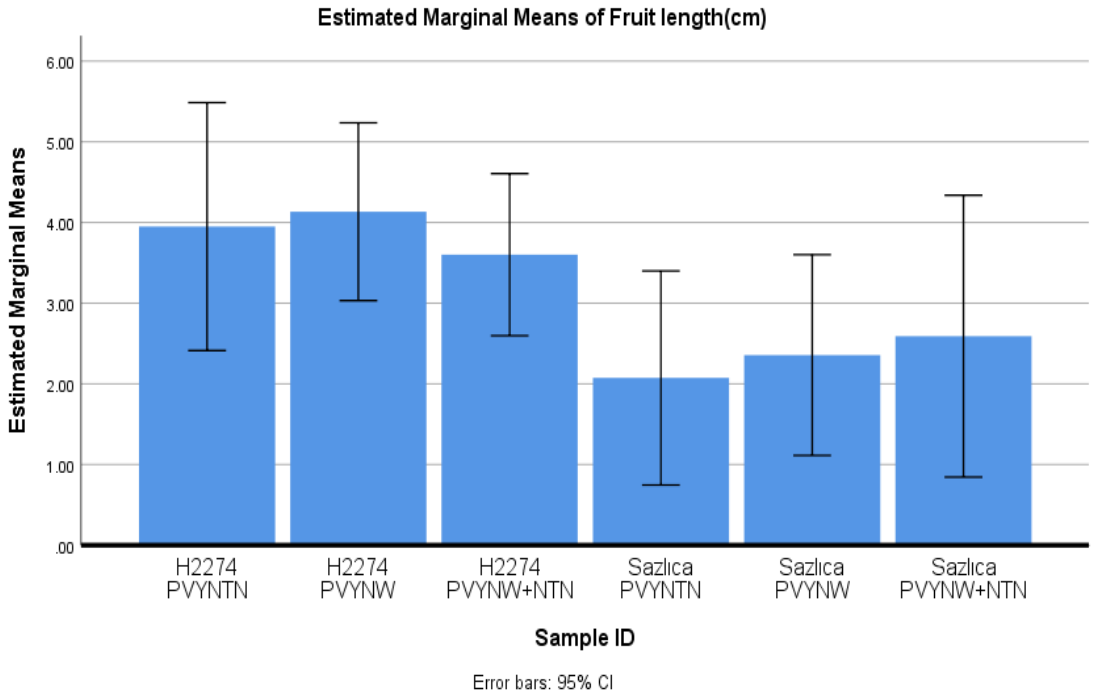

Figure 4.4. Average fruit length of 7 days inoculations

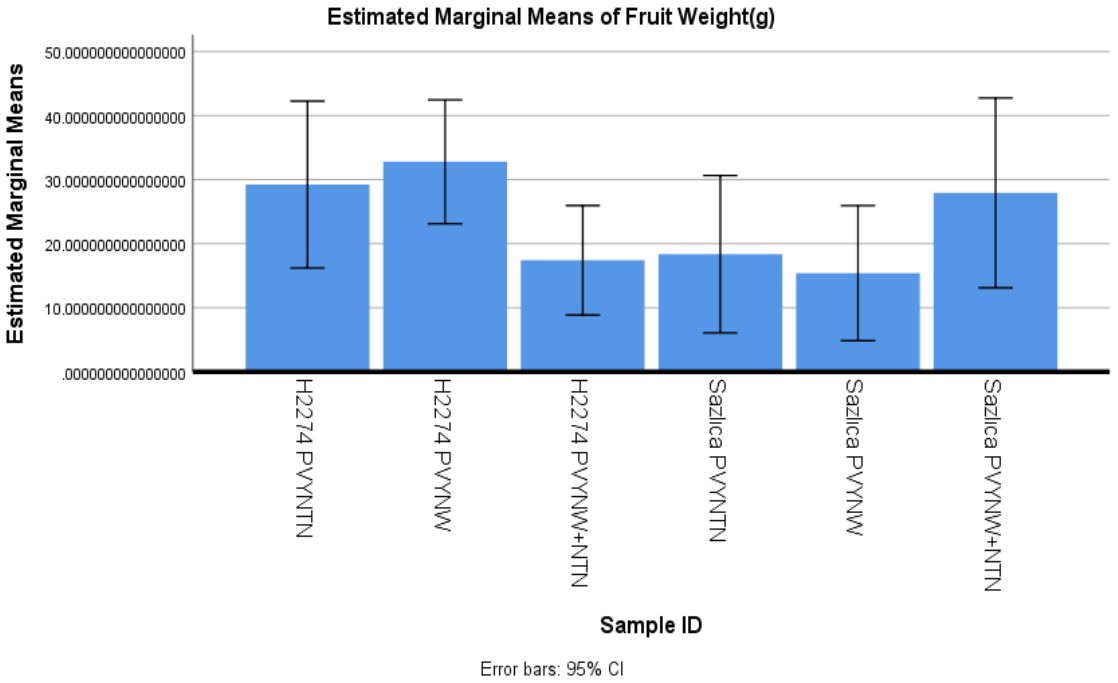

Figure 4.5. Average fruit weight of 7 days inoculations

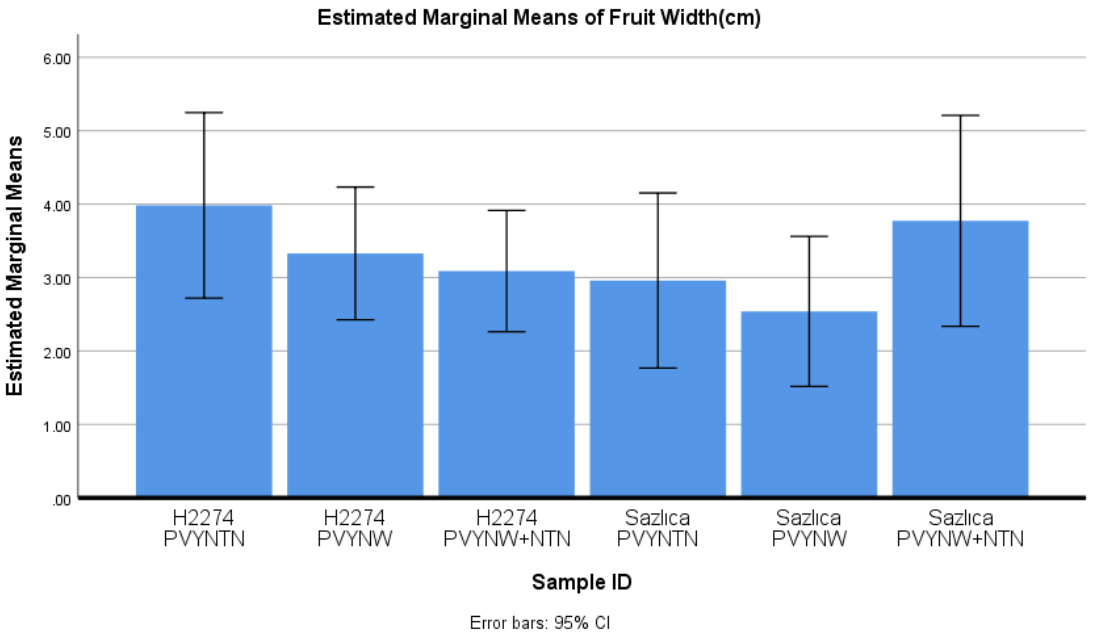

Figure 4.6. Average fruit width of 7 days inoculations

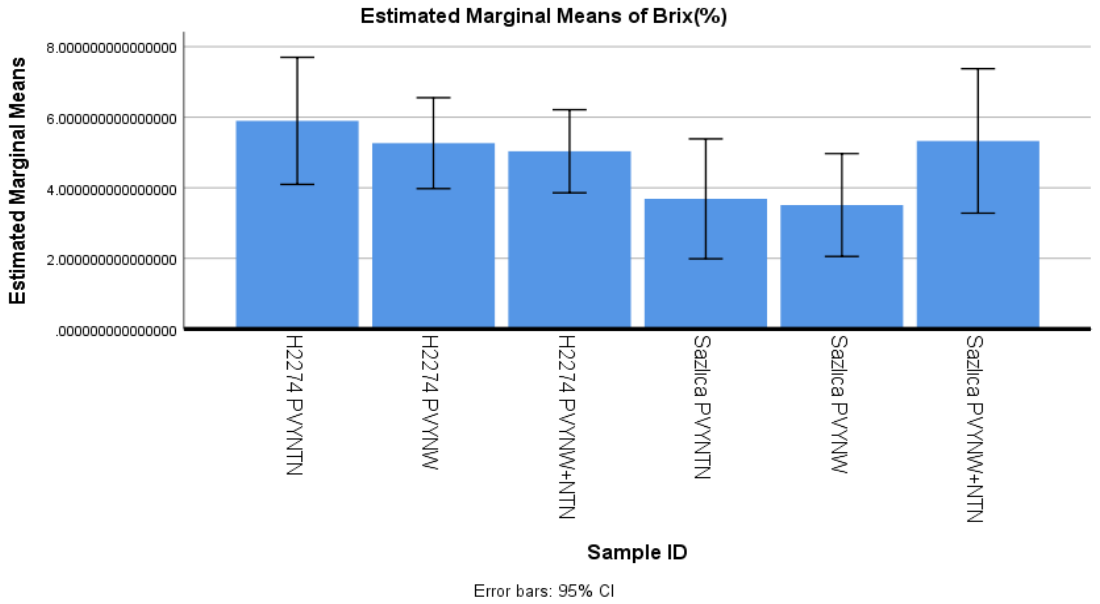

Figure 4.7. Average fruit brix of 7 days inoculations

Table 4.3 Fruit quality parameters results of fourteen days inoculated plants (the average of infected plants along with control plants).

Tomato variety/virus strain	Fruit length (cm)	Fruit weight (g)	Fruit width (cm)	Brix (%)
H2274 PVY^{NW}	2.4750	17.8360	2.3250	3.500
H2274 PVY^{NW}+PVY^{NTN}	3.3571	28.5828	3.5714	4.585
H2274 PVY^{NTN}	3.5500	27.6420	4.0500	5.730
Sazlıca PVY^{NTN}	3.3437	27.3287	3.5625	4.487
Sazlıca PVY^{NW}	2.9722	20.5533	2.9444	4.562
Sazlıca PVY^{NW}+PVY^{NTN}	3.8864	26.3890	4.2727	5.580
H2274 Control	4.7000	45.3800	4.5000	6.260
Sazlıca Control	4.5000	42.6600	3.9000	6.300

For fourteen days inoculated plants, the maximum fruit length of H2274 is achieved by H2274 PVY^{NW+NTN} and PVY^{NW} with the same number of 5.0 cm while the maximum length of Sazlıca is obtained by Sazlıca PVY^{NTN} and PVY^{NW+NTN} with an average of 5 cm. The maximum length of H2274 negative control is 5 cm while Sazlıca negative

control is recorded for 6 cm. For the fruit width of fourteen days inoculated plants, the highest H2274 fruit width is reached by H2274 PVY^{NTN} with the number of 5.50 cm and the highest Sazlıca fruit width is recorded for Sazlıca PVY^{NTN} and $PVY^{NW+}PVY^{NTN}$ with the same average of 5 cm. The maximum H2274 negative control is 5 cm while Sazlıca negative control is 4.50 cm. For the fruit weight of fourteen days inoculated plants, the highest fruit weight of Sazlıca is obtained by Sazlıca PVY^{NTN} as 51.08 g whereas the maximum fruit weight of H2274 is obtained by H2274 PVY^{NW+NTN} with 42.90 g/plant. The optimum fruit weight of Sazlıca negative control is 49.29 g/plant while H2274 is 52.30 g/plant. In the fruit sugar soluble content (brix) of fourteen days inoculated plants, H2274 PVY^{NTN} has the maximum fruit brix of 8.1% whereas the highest sugar soluble content of Sazlıca is achieved by Sazlıca PVY^{NW} with the 6.8% value. On the other side, the maximum H2274 negative control is 6.8 % whereas Sazlıca maximum negative control is 6.9%.

Post hoc, Duncan analysis showed significant differences between control and infected fourteen days inoculated plants and non-significant effect in the fruit length Sazlıca and H2274; P=0.62, fruit weight Sazlıca and H2274; P=0.221, width Sazlıca; P=0.105, H2274; P=0.091 and total soluble solid content (brix) are Sazlıca and H2274; P=0.060, between varieties, respectively.

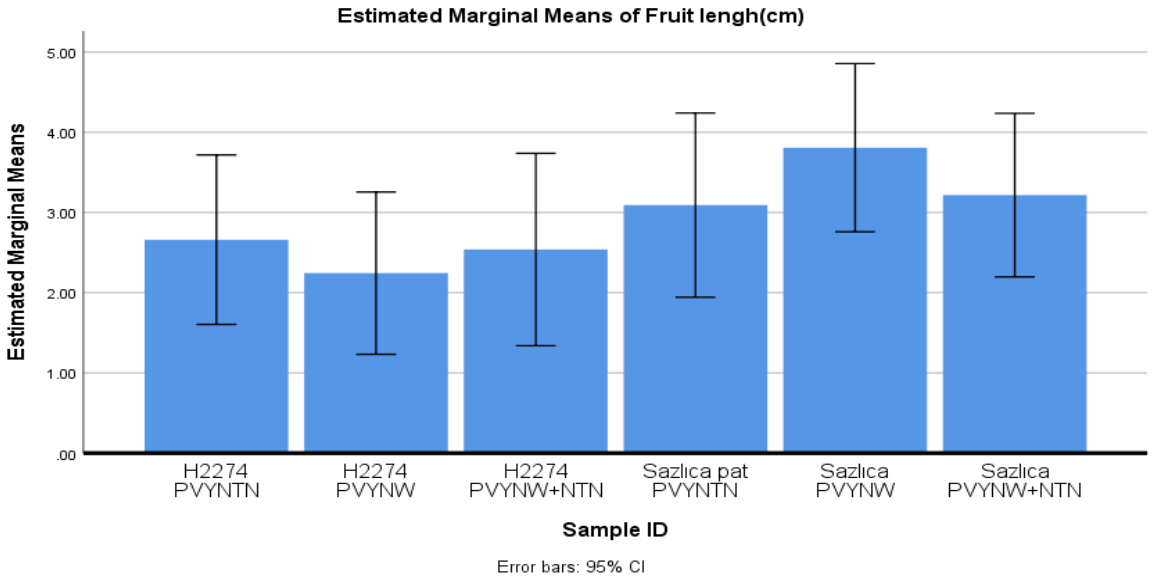

Figure 4.8. Average fruit Length of 14 days inoculations

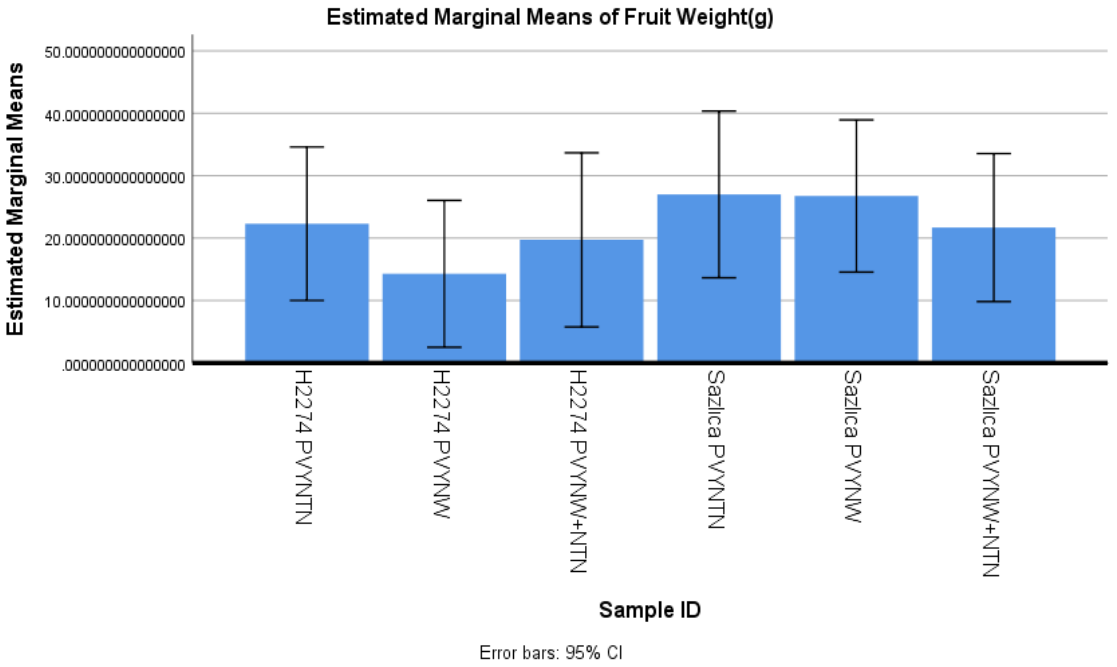

Figure 4.9. Average fruit weight of 14 days inoculations

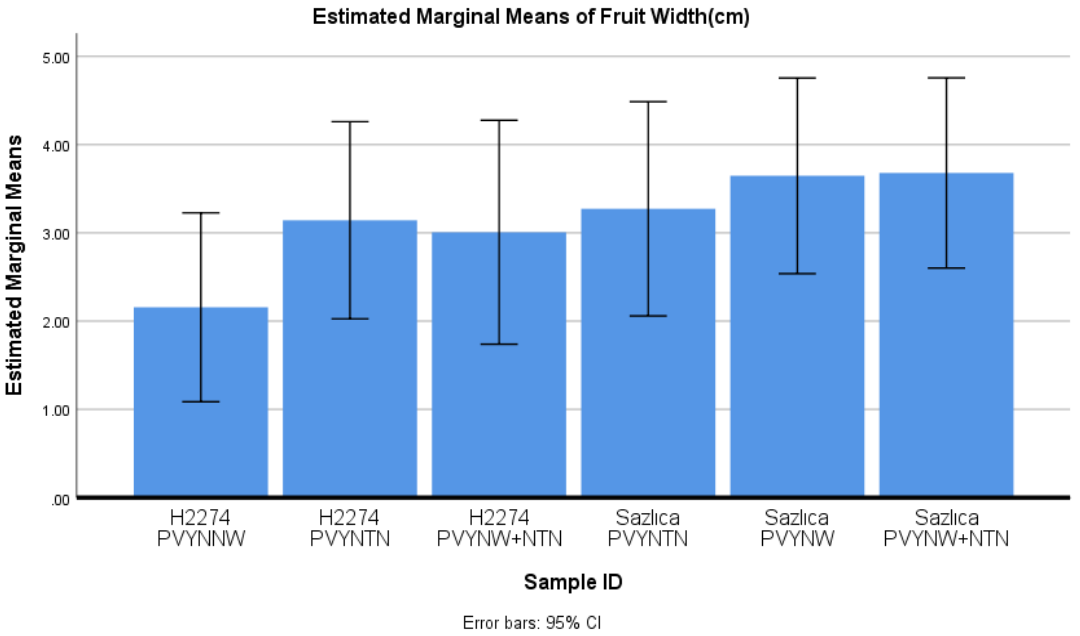

Figure 4.10. Average fruit width of 14 days inoculations

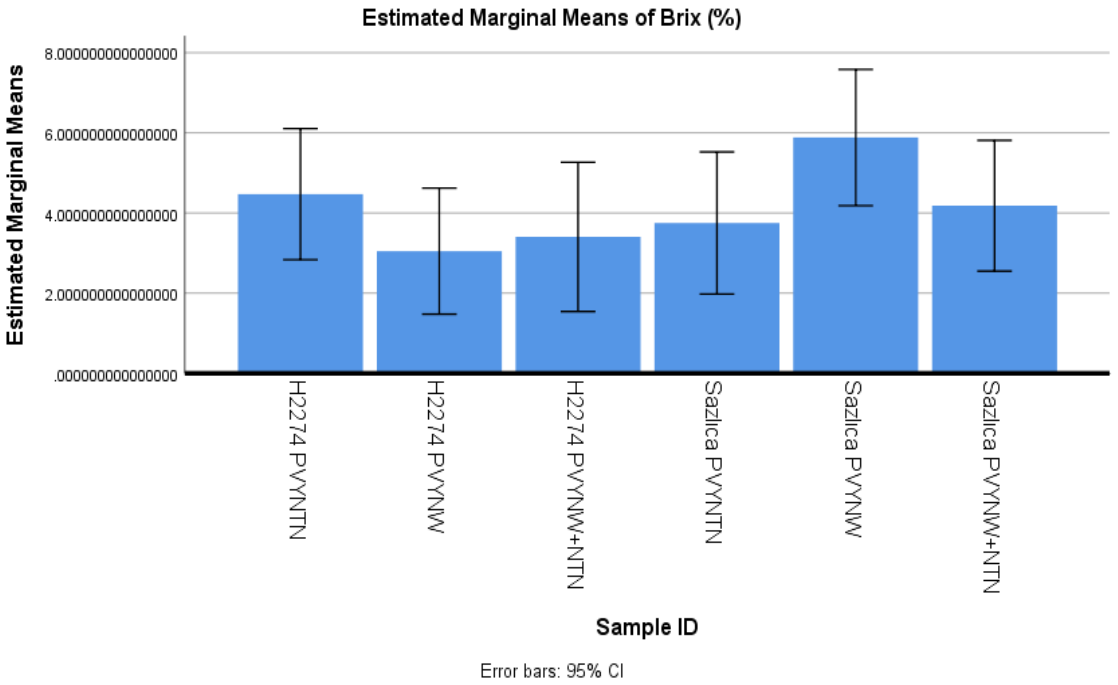

Figure 4.11: Average fruit brix of 14 days inoculations

Table 4.4. Fruit quality parameters results of twenty-one days inoculated plants (the average of infected plants along with control plants).

Tomato variety/ virus strain	Fruit length (cm)	Fruit weight (g)	Fruit width (cm)	Brix (%)
H2274 PVY^{NW}	3.6250	21.4512	3.750	4.500
H2274 PVY^{NW+NTN}	1.8333	12.0133	1.500	1.730
H2274 PVY^{NTN}	1.1667	6.11833	1.333	1.830
Sazlıca PVY^{NTN}	3.6667	20.1600	4.167	4.260
Sazlıca PVY^{NW}	1.5000	13.7066	1.667	1.900
Sazlıca PVY^{NW+NTN}	4.1667	38.3900	4.500	6.160
H2274 Control	4.1000	48.2000	4.300	6.600
Sazlıca Control	4.8000	47.3100	4.100	6.500

For twenty-one days inoculated plants, the highest H2274 fruit length is obtained by H2274 PVY^{NW+NTN} and PVY^{NW} with the same number of 5.5 cm while the highest length of Sazlıca is recorded for Sazlıca PVY^{NW} with an average of 4.5 cm. The

maximum length of H2274 negative control is 5 cm whereas Sazlıca negative control is for 6 cm. For the fruit width of twenty-one days inoculated plants, the highest H2274 fruit width is reached by PVY^{NW} with the number of 5 cm and the highest Sazlıca fruit width is obtained for PVY^{NW+NTN} with the number of 5.5 cm. The maximum H2274 and Sazlıca negative control of fruit width is similar with the number of 5 cm. For the fruit weight of twenty-one days inoculated plants, the highest fruit weight of Sazlıca is obtained by Sazlıca PVY^{NW+NTN} with the number of 41.31 g/plant whereas the maximum fruit weight of H2274 is achieved by H2274 PVY^{NW} with the number of 38.59 g/plant. The maximum fruit weight of Sazlıca negative control is 56.32 g/plant whereas the H2274 is 60.32 g/plant. In the fruit sugar soluble content (brix) of twenty-one days inoculated plants, H2274 PVY^{NW+NTN} has the maximum fruit brix of 5.9% whereas the maximum sugar soluble content of Sazlıca is obtained by Sazlıca PVY^{NW+NTN} with the number of 6.2%. The maximum fruit brix of H2274 negative control is recorded for 7% whereas Sazlıca maximum negative control is also 7%.

Post hoc, Duncan analysis showed significant differences between control and infected twenty-one days inoculated plants and non-significant effect in the fruit length (Sazlıca and H2274; P=0.071), fruit weight (Sazlıca and H2274; P=0.109), width (Sazlıca and H2274; P=0.089) and total soluble solid content (brix) which became (Sazlıca and H2274;P=0.085) between varieties, respectively.

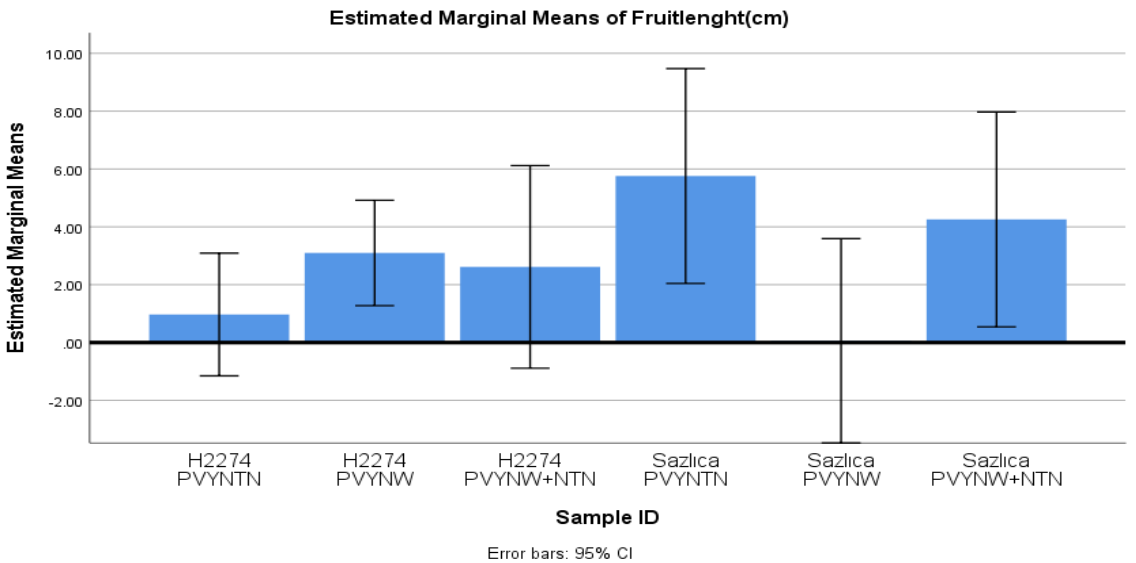

Figure 4.12. Average fruit length of 21 days inoculations

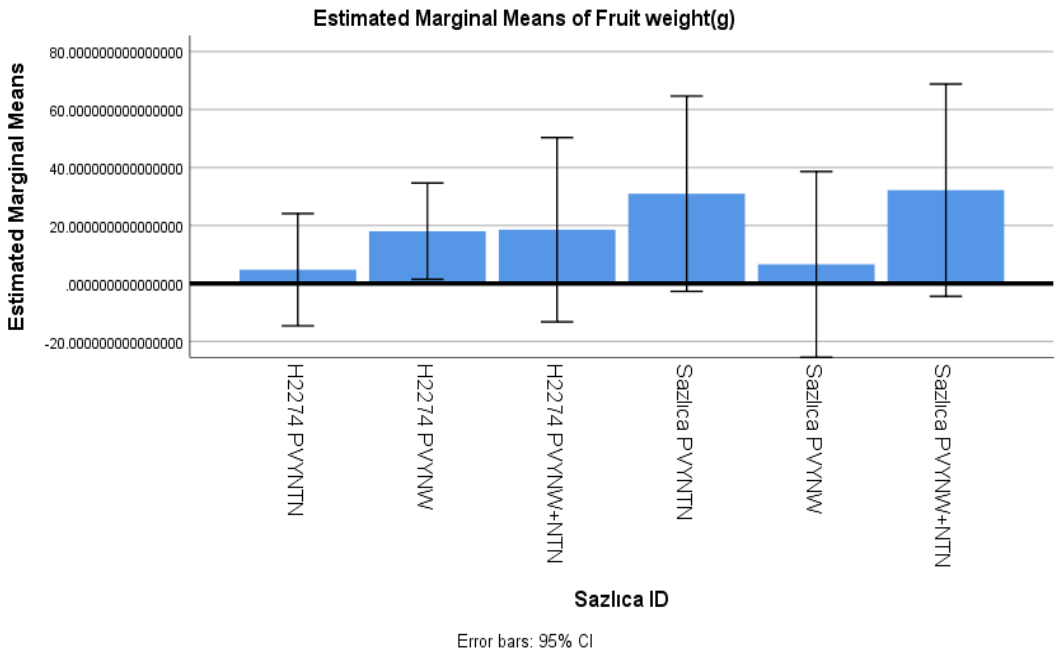

Figure 4.13. Average fruit weight of 21 days inoculations

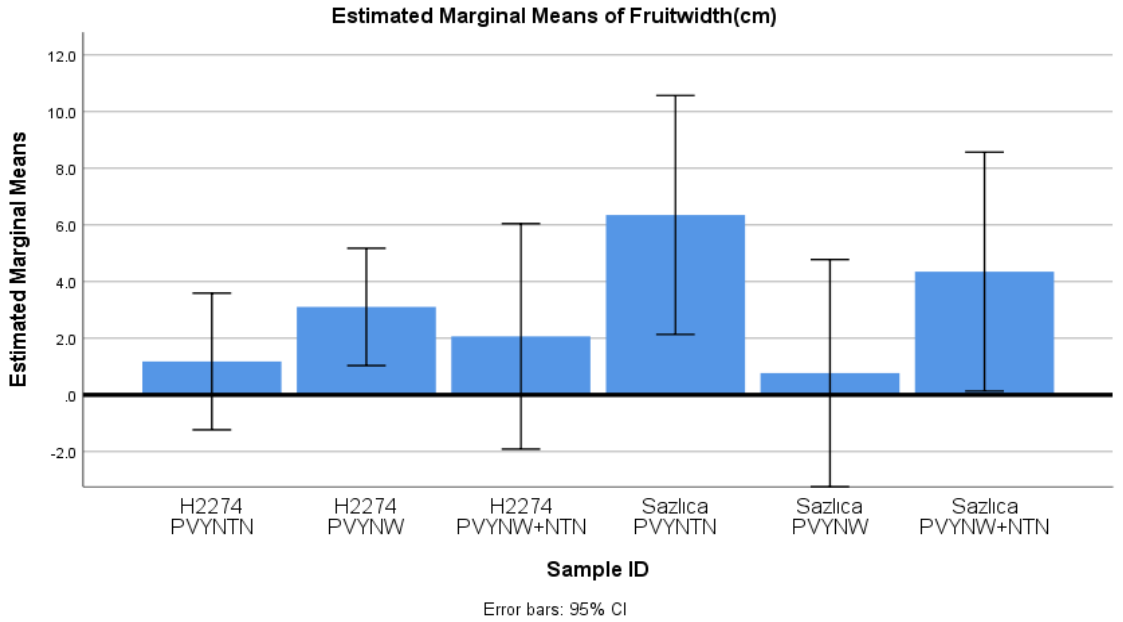

Figure 4.14. Average fruit width of 21 days inoculations

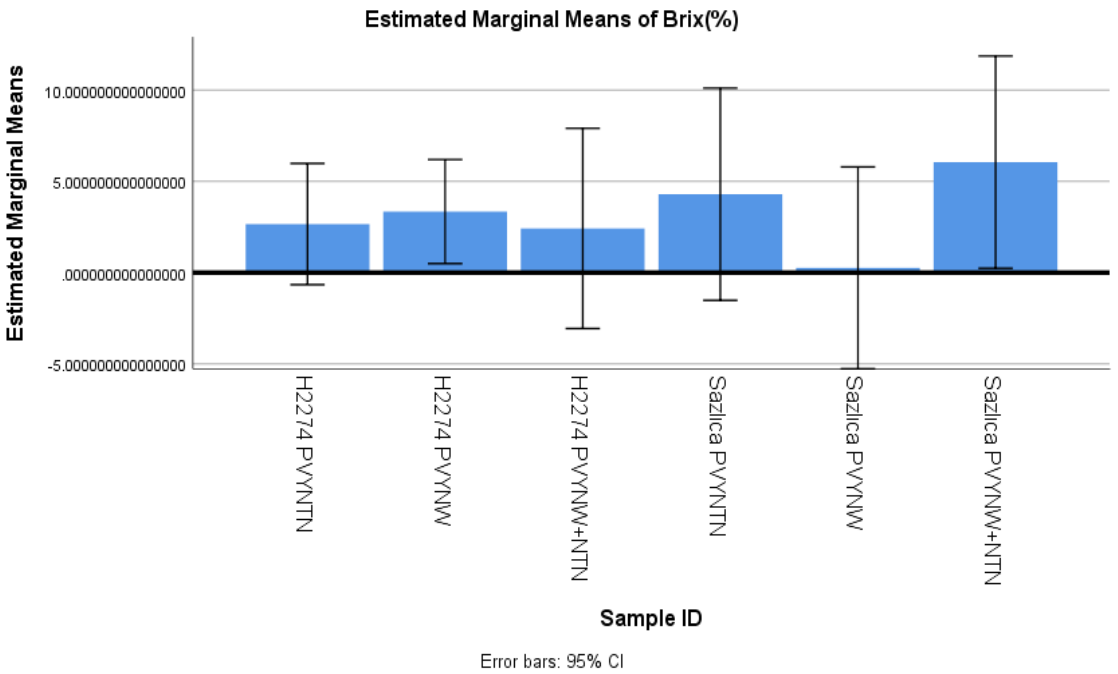

Figure 4.15. Average fruit brix of 21 days inoculations

CHAPTER V

DISCUSSION

PVY is the most economically significant disease problem in tomato plants in many places of the world. This virus is responsible for decrease in yield and quality and causes serious diseases in cultivated hosts, such as tomato, potato, tobacco, and pepper. A total of 379 tomato plant from local genotype "Sazlıca" and PVY susceptible H2274 commercial tomato varieties were mechanically inoculated with PVY strains in different times (7 days, 14 days, 21 days and 28 days). After 6-8 weeks, samples were collected from inoculated plants along with 50 survey samples and tested by DAS-ELISA. The positive result was variable based on the replications, varieties and effect of PVY infection. Based on the survey studies in Sazlıca region, some virus specific symptoms were observed on tomato plants and the results indicate PVY incidence in that region. Due to the high amount of potato and other *Solanecous* plant production, PVY could be transmitted to Sazlıca tomato genotypes. It shows the importance of PVY management in Sazlıca tomatoes.

For evaluation the reaction of PVY strains on Sazlıca tomatoes, mechanical inoculations were done under greenhouse conditions. In the varieties (Sazlıca and H2274), although have showed a close positive result, H2274 is found to be more susceptible to PVY infection than to Sazlıca variety. In seven days inoculated plant, H2274 has shown 25 positive samples out of 39 samples whereas Sazlıca have indicated 20 positive samples out of 33. In fourteen days inoculated planted, Sazlıca was obtained 22 positive results out of 45 samples while H2274 has shown 21 positive samples out of 45 samples. In twenty-one days inoculated plants, H2274 has shown 16 positive samples out of 45 samples whereas Sazlıca has displayed 7 samples out of 45 samples. In twenty-eight days inoculated plants, Sazlıca has only 1 positive sample out of 45 samples and H2274 has not indicated any positive sample in all samples.

Among the PVY strains, PVY^{NW} has shown the highest positive reaction in the varieties and the replications except PVY^{NW+NTN} in seven days inoculated plants which showed the highest infection rate in seven days inoculated plants. The infection of PVY^{NW} in Sazlıca seven days is (73%) compared PVY^{NTN} (64%) and PVY^{NW+NTN} (45%) while H2274 seven days inoculated plants, PVY^{NW+NTN} (85%) has shown the highest infection rate compared with PVY^{NW} (64%) and PVY^{NTN} (38%). In Sazlıca fourteen days inoculated plants; PVY^{NW} is the highest percentage which is (53%) whereas PVY^{NTN} and PVY^{NW+NTN} have reached a similar percentage which is (47%). On the other hand, H2274 fourteen days inoculated plants, PVY^{NW} has the maximum infection rate which is (53%) compared with PVY^{NW} (47%) and PVY^{NTN} (40%). In Sazlıca twenty-one days inoculated plants, PVY^{NW} has the maximum infection rate which is (20%) whereas the two other strains (PVY^{NTN} and PVY^{NW+NTN}) have similar infection rate (13%). No any symptoms were observed on fruits for all replications. It can be concluded that PVY^{NW} strain has an importance on PVY management of Sazlıca tomatoes.

In H2274 twenty-one days inoculated plants, the PVY^{NW} is also has the highest infection rate which is (47%) compared with PVY^{NTN} (40%) and PVY^{NW+NTN} (20%). In the last inoculated plants, which are twenty-eight days inoculated plants, Sazlıca only has shown one positive sample and this is belonged by PVY^{NW} strain while the other strains did not indicate any positive reaction.

For plant ages, the age of the plant is an important factor that takes part the susceptibility of the plants to the virus. As the age of the plant was bigger the effects of the virus was lower and the plants that inoculated after seven days were the most susceptible ones to virus when compared to other plants inoculated after fourteen days and twenty-one days, whereas the plant inoculated after twenty-eight days were affected by other factors and did not infected by the virus except one sample. The total samples that were infected by the PVY strains were 45 out of 72 samples and this shows how the plant age is significant for the virus, as expected.

The most previous studies focused on the resistance of plant to the virus and the virus vector. The symptoms of PVY infection differ with varieties, PVY strain, plant age and environmental conditions. A biological assay resulted that cv. Agria is more susceptible to PVY^{N-Wi} than to PVY^{NTN}, whereas cv. Charlotte is susceptible to both strains. The biological (inoculation) assay also displayed that the expression of symptoms on varieties is strain-dependent. These shows stress the main role of the resistance profile of varieties to explain the balance of the PVY strains in potato crops (Dupuis et al., 2018). The occurrence of PVY on potato plants were investigated and samples were collected from main tomato growing fields in west-bank Palestine by utilizing serological, biological and molecular methods. In DAS-ELISA method, the occurrence of PVY virus was identified at an average of 15.29% and also confirmed by RT-PCR analysis and bioassay test (Khudiesh, 2016). Bostan and Haliloğlu (2004) studied the distribution and percentage of PVY strains (PVY^{N}, PVY^{O}, PVY^{C}) along with other viruses such as PLRV and PVS and seed tubers were used for sowing materials in the significant potato producing provinces in Turkey. The symptoms induced by single or mixed infection were observed under field conditions. At first, virus-specific polyclonal antibodies were used to analyze a total of 880 leaf samples and almost 83 samples were detected the presence of PVY infection of the first result were re-analyzed by utilizing PVY^{O}, PVY^{N}, PVY^{C}-virus-specific monoclonal antibodies. The ELISA result showed seed potato tubers utilized for the planting materials were infected with the percentage of PVY (17.7%), PLRV (14.2%), PVS (4.6%) and PVX (11.8%). Ibaba (2009), found that all PVY isolates infecting tomato and pepper as a positive for the normal strains of PVY^{O} both ELISA and RT-PCR whereas PVY isolates infecting potato have more heterogeneous and consisted of PVY^{N}, PVY^{NTN} and $PVY^{N\ Wilga}$ strains and some cases mixed infection shown. However, our research had several limitations which are fruit quality parameters and have showed a nonsignificant in statistical analyzing. In our findings, the PVY susceptible commercial H2274 tomato variety is more susceptible to PVY^{NW} than to PVY^{NTN} and PVY^{NW+NTN}. It is reported that infection rate was higher for plants inoculated at pre-flowering relative to those inoculated at the post-flowering and the replication different for mechanical inoculation, the interaction of strain and genotype was not statistically significant (Shrestha et al., 2014). Mature-plant resistance can also inhibit PVY^{N} infections but plants need to be physiologically old at the time of highest infection pressure in the late season. Therefore, the use of chatted seed linked

with planting as early as possible, and early haulm destruction, could, together, be a helpful part of an approach to manage PVY^{N} (Weidemann, 1988). The current study also showed us that the infection rate was higher to the plants inoculated seven days when compared to the plant inoculated fourteen days and twenty-one days and this indicates how plant age is important at the time of virus infection.

In the fruit quality parameters, among the three replications (seven days, fourteen days and twenty-one days), varieties and strains, the highest fruit length is achieved by Sazlıca PVY^{NW+NTN} which is recorded for 4.1667 cm in twenty-one days inoculated plants. The highest fruit weight is reached by Sazlıca PVY^{NW+NTN} which is recorded for 38.2983 g/plant in 21 days inoculated plants whereas the minimum fruit is obtained by seven days inoculated plants. On fruit width, the maximum fruit width among the replications, tomato varieties and strains are obtained by Sazlıca PVY^{NW+NTN} with the maximum average of 4.500 cm in twenty-one days inoculated plants. For fruit total soluble solid content (brix), Sazlıca PVY^{NW+NTN} has the maximum average of 6.16% in twenty-one days inoculated plants when compared with other replications. After seven days inoculated plants yielded smaller and produced less tomato compared to 14 days and 21 days plants. In general fruit brix, the fourteen days inoculated plants obtained the maximum fruit brix with the number of 6.8 % (Sazlıca) and 8.1 %(H2274) when compared the seven days and twenty-one days inoculated plants. On the other hand, control plant all of them are closer and have shown a higher percentage when compared with infected plants. Only one infection was observed on 28 days inoculated plants even for repetitions. The findings also showed us that the survey samples have infections and at least one positive sample was found in each field and this indicates how PVY exists in Sazlıca town area. The twenty-eight days inoculated plants also had not produced any infections due to age and physiological conditions of plants. The mechanical inoculation methods were more effective than virus vector transmissions because of the virus was directly transmitted to plants through leaves with high concentrations. This study is a base for future study of the effect of PVY strains at different growth stages on yield and fruit quality of local genotype in Turkey particularly Sazlıca town/Niğde region. This research is also helpful for knowing the effect of PVY strains on tomato fruit, infection

time for plant and are recommended to make a further research in the future and expended to a larger area.

CHAPTER VI

CONCLUSION

Tomato is one of the most important vegetable grown worldwide and is now the fourth most saleable fresh-market vegetable after potatoes, lettuce, and onions. Turkey ranks as the 4th biggest producers of tomatoes around the world. Tomato is the most important vegetable after potato; this plant creates an outstanding source of health-advancing compounds owing to balanced mixture of minerals and antioxidants. Infection of PVY in different strains and growth ages of tomato hindrances the total quality-based production of tomato. As a result of extreme nature and huge losses, PVY is ranked at 5th position in term of worldwide economic damages. In the current study almost 379 samples of both Sazlıca and H2274 varieties were mechanically inoculated with PVY to evaluate the effect of PVY strains on tomato plants in different replications. Symptomatic samples from different fields on Sazlıca town were collected. Both inoculated plants and surveyed samples were screened by DAS-ELISA to confirm the infection (for inoculated plants) and incidence (for surveyed plants) of PVY. The survey samples collected from Sazlıca town showed 5 % of positive samples and from each field at least one infected sample was found by DAS-ELISA test. For the evaluation of PVY strains, the results showed that PVY^{NW} strain has the greatest number of positive samples when compared with the strains PVY^{NTN} and PVY^{NW+NTN}. Between the varieties, H2274 obtained the maximum PVY infected samples. For the fruit quality parameters, the greatest fruit weight is recorded for Sazlıca PVY^{NW+NTN} of after twenty-one days inoculated plants whereas the highest fruit length is a reached by Sazlıca PVY^{NW+NTN} of after twenty-one days inoculated plants. On the other hand, the maximum fruit width is obtained by Sazlıca PVY^{NW+NTN} of after 21 days inoculated plants and finally the maximum fruit total soluble solid content (brix) is achieved by Sazlıca PVY^{NW+NTN} of after 21 days inoculated plants. The current study has importance for knowing the effects of PVY strains on local tomato genotype Sazlıca and this research can be used as starting point for effect of PVY strains on tomato at different growth stages. The farmers in Sazlıca town are recommended to remove host plants to minimize or eliminate virus inoculum

sources and also, they should take precaution to aphids which are the most dangerous vector of PVY in early stages of tomato plantlets to decrease the PVY incidence.

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