



T.R.

NIĞDE ÖMER HALISDEMİR UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

DEPARTMENT OF PLANT PRODUCTION AND TECHNOLOGIES

GREENHOUSE EVALUATION OF EGGPLANT CULTIVARS FOR TOMATO
SPOTTED WILT VIRUS (TSWV) DISEASE

AFZAL ALAM

January 2021

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Master Thesis

Supervisor

Assoc. Prof. Dr. Eminur ELÇİ

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The study entitled “**Greenhouse Evaluation of Eggplant Cultivars for Tomato Spotted Wilt Virus (TSWV) Disease**” and presented by Afzal ALAM under the supervision of Assoc. Prof. Dr. Eminur ELÇİ has been accepted 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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THESIS CERTIFICATION

It is certified that I have written this thesis by myself. I further confirmed that all information included in this thesis is scientific and is in accordance with the university rules and regulations. Any materials that I have used from external sources as well as help received and all sources used in finalizing this research work and preparing this thesis, all have been acknowledged in the thesis.

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Afzal ALAM

SUMMARY

GREENHOUSE EVALUATION OF EGGPLANT CULTIVARS FOR TOMATO SPOTTED WILT VIRUS (TSWV) DISEASE

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The objective of this study was to evaluate the effect of TSWV on different five eggplant (*Solanum melongena* L.) cultivars (Kemer, Aydin Siyahi, Halep Karasi, Topan and Silindirik 153-74) under greenhouse condition. Eggplant cultivars were mechanically inoculated three times with varying days interval (7, 14 and 21 days) and after 28 days of last inoculation, collected samples were tested by ELISA method. According to DAS-ELISA, the highest infection rate was observed in Kemer (58%) followed by Topan (52%) whereas, the lowest infection rate was noticed in Silindirik 38%. Infection of TSWV caused significant ($p \leq 0.05$) reduction in fruit number (32.99-59.34%), fruit length (17.12-49.76%), fruit diameter (12.44-38.30%), fruit weight (31.31-67.70%), flesh thickness (18.11-46.05%), total soluble solid (16.83-40.69%), fruit color, fruit firmness (4.88-29.25%) and yield (50.22-84.22%) in inoculated plants. According to the results obtained the cultivar Silindirik (153-74) performed better against TSWV among all the cultivars, after Silindirik the performance of Halep Karasi was good among the selected cultivars. Whereas, the performance of the Kemer was poor making it sensitive to TSWV.

Keywords: Eggplant, ELISA, tomato spotted wilt virus, yield, quality

ÖZET

SERA KOŞULLARINDA PATLICAN ÇEŞİTLERİNİN DOMATES LEKELİ SOLGUNLUK VIRÜSÜ (TSWV) HASTALIĞINA KARŞI REAKSIYONUNUN DEĞERLENDİRMESİ

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Bu çalışmanın amacı, sera koşullarında beş farklı patlıcan çeşidi (Kemer, Aydın Siyahi, Halep Karasi, Topan ve Silindirik 153-74) üzerinde TSWV'nin etkilerinin değerlendirilmesidir. Patlıcan çeşitleri, farklı gün aralıklarıyla (7, 14 ve 21 gün) üç kez mekanik olarak inokule edilmiş ve ELISA yöntemi ile test edilmiştir. DAS-ELISA'ya göre en yüksek enfeksiyon oranı Kemer'de (%58), ardından Topan'da (%52) görülürken, en düşük enfeksiyon oranı (%38) Silindirik'te görülmüştür. İnokule edilmiş bitkilerde TSWV enfeksiyonu, meyve sayısında (%32.99-59.34), meyve uzunluğunda (%17.12-49.76), meyve çapında (%12.44-38.30), meyve ağırlığında (%31.31-67.70), meyve eti kalınlığında (%18.11-46.05), toplam çözünebilir katı madde oranı (%16.83-40.69), meyve rengi, meyve sertliği (%4.88-29.25) ve verim (%50.22-84.22) parametrelerinde önemli ($p \leq 0.05$) azalmalara neden olmuştur. Elde edilen sonuçlara göre, test edilen çeşitler arasında Silindirik (153-74) ve Halep Karasi çeşitlerinin dayanıklılık açısından TSWV'ye karşı daha iyi performans gösterdiği, Kemer çeşidinin ise daha hassas reaksiyon gösterdiği tespit edilmiştir.

Anahtar Sözcükler: Patlıcan, ELISA, Domates lekeli solgunluk virüsü, Verim, Kalite

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SYMBOLS AND ABBREVIATIONS

Symbols	Descriptions
%	Percentage
µl	Microliter
g	Gram
Kg	Kilogram
mm	Milimeter
cm	Centimeter

Abbreviations	Description
TSWV	Tomato Spotted Wilt Virus
TMV	Tobacco Mosaic Virus
ELISA	Enzyme linked Immunosorbent Assay
FAOSTAT	Food and Agriculture Organization Statistical Databases (United Nations)
ToMV	Tomato Mosaic Virus
CMV	Cucumber Mosaic Virus
TYLCV	Tomato Yellow Leaf Curl Virus
ToRSV	Tomato Ring Spot Virus
PVY	Potato Virus Y
RT-PCR	Reverse Transcription Polymerase Chain Reaction
DAS	Double Antibody Sandwich Assay
ArLV	Artichoke Latent Virus
NC	North-Carolina
DTBIA	Direct Tissue Blot Immunoassay

CHAPTER I

INTRODUCTION

Eggplant (*Solanum melongena* L.) belongs to the family Solanaceae having mostly famous and widely consumed vegetables such as potato, tomato, and pepper. Aubergine, brinjal and eggplant are the common names used for *S. melongena*. Globally, the eggplant is cultivated in all regions including the subtropical, tropical and temperate region (Sihachakr et al., 1994). *S. melongena* is adopted for fresh consumption as well as it is cooked in different ways (Bhasker and Ramesh Kumar, 2015). It can be fried, grilled, baked, boiled, pickled, dried, sauté, microwaved, mashed and also its leaves are cooked as a green vegetable (Sekara et al., 2007).

Firstly, the eggplant cultivation is reported from India, which is known as the center of its origin and diversity whereas, China is regarded as the secondary center of eggplant's diversification which is reported by (Karihaloo and Gottlieb, 1995). In 1300 AD its cultivation started in all over the Europe (D'Anna and Sabatino, 2013). In 16th century the eggplant was considered as a food item. Secondly, its primary cultivation started around the Mediterranean region in the west which was the secondarily domestication of eggplant to these regions including Turkey, Persia and Syria (Daunay et al., 2001; Kucuk 2003; Tumbilen 2007). Many indigenous cultivars are grown in Turkey which was developed by the breeders found in almost all the regions (Balkaya and Karaagac 2005).

Worldwide eggplant was harvested from the land of 1.86 million hectares (ha) with the total production of 52.3 million tons, Figure 1.1. Asia is the core of eggplant production. China grew on 0.80 million hectares with total production of 34.13 million tons and India 0.73 million Ha with production 12.82 million tons in 2018. Furthermore, this crop was widely cultivated in Egypt 0.04 million ha land with production of 1.40 million tons (FAOSTAT 2018), (Figure 1.2).

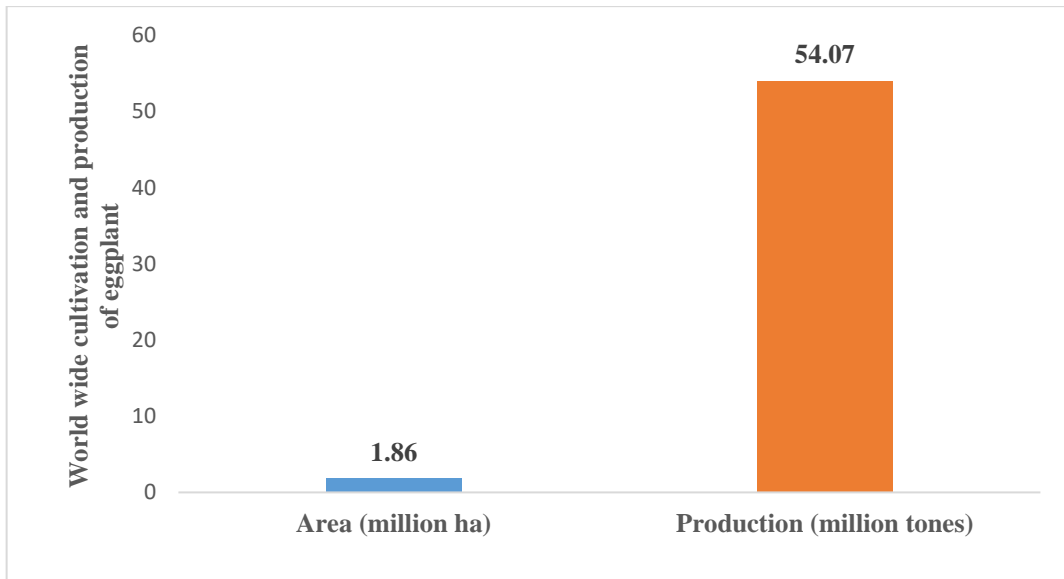


Figure 1.1. Worldwide cultivation and production of eggplant

Turkey is famous for its widely cultivated vegetables; eggplant is one of them which was introduced by traders during 16th century. Turkey grew 0.02 million ha with total production of 0.83 million tons of eggplant in the last decade which put the country in 4th place among the five biggest growers in region (FAOSTAT 2018).

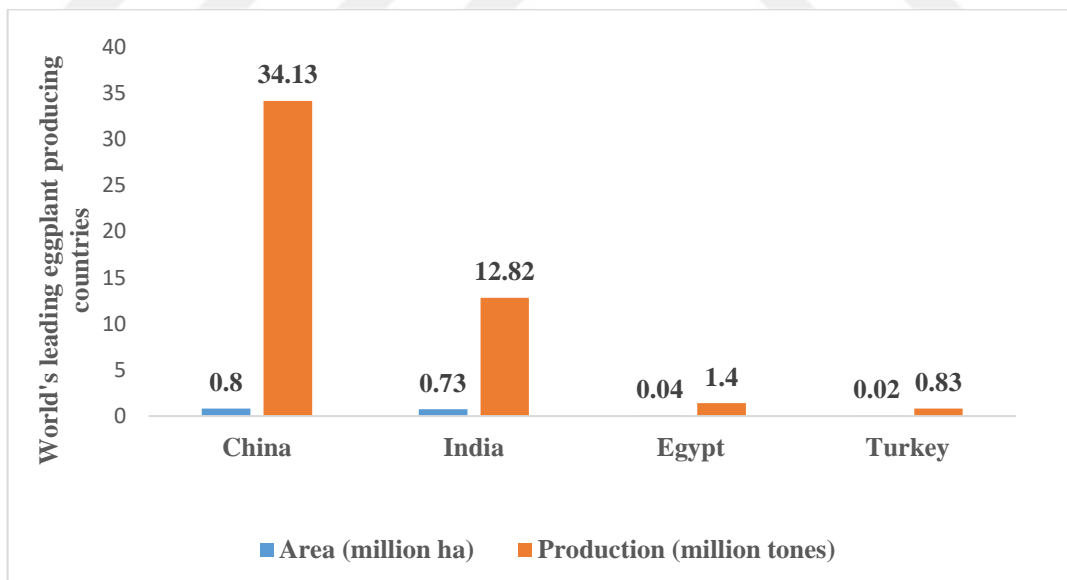


Figure 1.2. World's leading eggplant producing countries

Vegetables intake helps in good health because each year 2.7 million people die due to improper intake of vegetable diet that contain proteins, carbohydrates and fibers (Dias, 2011). Eggplant is considered as a rich source of minerals and as a low-calorie fruit.

Among the top ten vegetables, the eggplant plays a vital role having high phenolic content which enhance the radical absorbing capacity (Cao et al., 1996, Caguiat and Hautea, 2014). Eggplant contain desirable nutrients i.e. carbohydrates, sugar, dietary fiber, fat and proteins which are necessary for repair of damaged cells or tissues, provide protection and contribute in body growth. Eggplant also have a set of different vitamins (Vit. A, Vit. B complex, Vit E and K), minerals (Ca, Mg, Fe, P and K), Phytochemicals (for scavenging activity) and antioxidants (Noda et al., 2000, Whitaker and Stommel. 2003).

Eggplants has a number of medicinal properties and it is potentially used in pharmaceutical products. Moreover, it is said to be the father of modern medicine because it has ayurvedic medicinal properties (Gürbüz et al., 2018; Sekara et al, 2007). The eggplant fruit provide natural food supplements which helps in the human development and also helps in the health care of pregnant and nursing mothers. The Aubergine is recommended for anemic patients with low iron content in their bodies (Asiedu-Addo, 2014). It helps in the blood circulation by clearing the damaged radicals and relaxing blood vessels resulting in prevention of cardiovascular diseases. Brinjal fruits has also some antioxidants which can lower the risks of brain tumor and keep the mind healthy by maintaining the brain cells strong. It is also used for the curing of certain diseases like asthma, dysentery, Alzheimer's, high blood pressure, diabetes, dermatic and stomach cancer (Fraikue B. F., 2016).

There are certain biotic and abiotic factors that affect the yield of eggplant crop. The abiotic factors include extreme changes in temperature, water shortage, rainfall and soil humidity which effect the plant during the vegetative period resulting in lower yield (Adamczewska-Sowińska et al., 2016). The biotic factors include some insect pests and pathogens. Some major insect pests effecting the growth and yield of the brinjal include whiteflies, aphids, variegated grasshoppers, cotton strainers, fruit and shoot borers (Mochiah et al., 2011). Pathogens that develops certain diseases can cause serious economic loss by yield reduction. Different diseases are developed by bacteria, phytoplasmas, fungi and viruses either in roots or shoots region of eggplant (Tsitsigiannis et al., 2008).

Eggplant is very important, several kinds of disease which affect the productivity of brinjal (Bacterial, fungal and viral) among the viral disease which effect on eggplant are Tobacco mosaic virus (TMV), Tomato mosaic virus (ToMV), Cucumber mosaic virus (CMV) and Tomato spotted wilt virus (TSWV). Tomato yellow leaf curl virus (TYLCV) is also affecting tomato and pepper but brinjal consider resistant to this virus (Czosnek et al. 1993).

TSWV (tomato spotted wilt virus) belongs to the genus *Tospovirus* of the family *Bunyaviridae*. TSWV was reported in 1915 (Best, 1968). There are a lot of plant families including *Solanaceae*, *Asteraceae*, *Brassicaceae*, *Bromiliaceae* and *Leguminaceae* which infect by TSWV (Momol and Pernezny 2006). This virus has single stranded DNA (Tripartite) which consists of large, medium and small RNA fragments. Large RNA codes negatively for RNA dependent RNA polymerase. Non-structural proteins and glycoproteins are encoded by medium RNA while small RNA encodes for nucleocapsid protein (Whitfield et al. 2005; Pappu et al. 2009). TSWV virion is 80-120 nm in diameter and is quasi-spherical, bounded by double membrane (German et al., 1992). TSWV has the capability to be transmitted in various ways with the help of different thrips species including *Frankliniella occidentalis*, *F. schultzei*, *F. Intonosa*, *F. bispiosa*, *F. fusca*, *Thrips setosus*, and *T. tabaci* and it is not known to be seed transmitted (Tsuda, 1999, Mound, 1996). Mechanical inoculation of TSWV causes more infection than insect vector thrips as reported by Nascimento et al. (2006) after evaluating the response of three peanut breeding lines to TSWV by mechanical and thrips inoculation. The effect of TSWV on plants varies with type of plant, time and duration of infection, environmental surroundings; normally wilting, ringspots and stunting of leaves, necrosis and chlorosis can be seen on infected plants (Holguin Pena and Rueda Puente 2007).

Among the viruses TSWV can cause systemic infection which lead yield loss in many important crops by producing un-marketable plants, flower and fruit (Ramkat et al. 2006). This virus destroys all flowering crops and is currently causing the most severe disease of these host plants in the US as well as in the world's temperate and subtropical regions (Pfleger et al. 1989). Severe TSWV-related yield losses have been reported in potato, pepper, tomato, tobacco, peanut and in some ornamental crops (Culbreath et al. 2006, Diffie et al. 2006). TSWV was first detected with an infection rate of 80-100

(Azeri, 1981) percent in tobacco plants in the Canakkale region and in eggplants, tomatoes, peppers, squash and lettuce, as well as buttercup plants from various parts of Turkey were then recorded (Azeri, 1994; Turhan and Korkmaz, 2006).

Recently the occurrence of TSWV and thrips are problematic in vegetable growing areas of Turkey, also in other parts of the world. The occurrence of TSWV and its distribution causes sever losses in many parts of Turkey. Due to these losses, the current study was conducted to evaluate the effect of TSWV on yield and quality parameters of eggplant. The result of this study will be instrumental in affecting the means used to control the incidences of diseases in regards to yield and quality of eggplants in this region and to improve its production.



CHAPTER II

REVIEW OF LITERATURE

Tomato spotted wilt virus belongs to the family Bunyaviridae is valuable horticultural and agronomic plants virus, affecting around 900 different plants specie include monocotyledonous (8) and dicotyledonous (90) families (Cho et al., 1986). In Turkey TSWV was first reported in 1979 Çanakkale Province (Azeri, 1981). It causes reduction in yield in Aegean region of Turkey, including crops are pepper, tomato, lettuce and eggplant (Fidan, 1995; Ozdemir et al., 2007). It has been proven that the effective control of TSWV is difficult because of their wide host and vector (thrips), but the researchers are struggling to find the solution for this issue and improve the production of vegetables which is affecting by TSWV.

2.1 World Distribution of TSWV

Moriones et al., (1998) conducted experiment over tomato crops which are naturally infested by TSWV, the infected plants grown-up in the north-eastern area of Spain from May-September. For finding the infection of TSWV, the plants were labelled and tested by DAS-ELISA. Plants that showed signs at 24, 38, or 45 days after transferring produced significantly lesser amount of tomato as compared to those who developed signs at 60, 67, and 74 days after shifting. These plants which infected later disclose alike patterns of production with high yields among July and August, when fruits were collected. Production component like yield of mature fruits per plant, number of fruits per plant and fruit weight increased. However, the marketable value of fruit was extremely reduced by TSWV due to irregular growth of plants and ripening.

Diaz-Perez et al., (2003) designed a disease management strategy to determine the relation among fruit yield and plant growth with the time interval for TSWV symptoms appearance. He used different colored mulches for two susceptible varieties namely Sun Chaser and Florida-91 and one resistant variety BHN-444. The varieties and mulches showed its effect on time for appearance of TSWV symptoms. An increase was noticed with time in vegetative top fresh weight, number of fruits and total yield in the plants

free from TSWV disease. As the time gap increased in the transplanting and TSWV appearance the marketable tomato yield increased.

Abad et al., (2005) designed a research to study TSWV in potato, samples were collected at the base of symptoms from affected plants. He collected tuber, stem and leaf samples in North-Carollina (NC), for the confirmation of TSWV he used RT-PCR, bio-assay and DAS-ELISA methods. All the samples were tested and showed positive results. *Nicotiana benthamiana* kind of tobacco plant were inoculated from the samples and the result was confirmed by RT-PCR and ELISA. According to a phylogenetic investigation of expected nucleocapsid protein 3 isolates of TSWV obtained from potato in NC and eleven isolates were got in Georgia from tomato pepper and peanut, NC implied that these isolates are interconnected. For testing the vector transmission 1 isolate was chosen from NC potato and virus transferred to potato by *Frankliniella fusca* (tobacco thrips), but no result was found from effected potato to new sprouting tubers and leaves. In North America it was first certification of the occurrence and characterization of TSWV in field cultivated potato.

Ramkat et al., (2006) conducted a research study on the mechanical inoculation of TSWV and its effect on yield in different varieties of tomato crop namely, Roma, Money maker, Riogrande, Cal J and Marglobe. Riogrande and Marglobe varieties were less effected while the Cal J was severely affected. The total yield is highly affected by mechanical inoculation in different varieties ranging from 27-60%. The virus inoculation affected the marketable yield significantly in all varieties except Roma, which was resistant to its disease and can be used in Integrated Pest Management strategy for minimizing TSWV disease.

Sharman and Persley (2006) revealed that different varieties of capsicum has a *Tsw* resistance gene which can develop symptoms of TSWV in almost 100% of greenhouse grown crops. Samples responded in ELISA using TSWV antibodies. Virus isolates caused severe systemic symptoms from infected plants instead of hypersensitive reaction in the capsicum cultivars having *Tsw* resistance gene. Virulent isolates toward the *Tsw* gene had a very close molecular and biological properties to normal TSWV isolates including a hypersensitive reaction in the cultivars of *Sw-5* tomatoes. In

Virginia during a study *Tsw* virulent isolates were found in both of tolerant and susceptible varieties of tomato and pepper.

Farooq and Akanda (2007) studied the occurrence of Tomato Wilt Virus infecting eight different tomato varieties, namely Manik (BARI-T1), Ratan (BARI-T2), Apurba (BARI-T3, BARI-T7), Shila (BARI-T8), Lalima (BARI-T9), BINA-T1 and BINA-T2. The symptoms of TSWV were observed on some plants and others were not affected throughout the life stages in all varieties. The lowest occurrence of TSWV was observed for the variety BINA-T2 while the highest was observed for BARI-T2. The occurrence of the virus was at peak during the mid-stages, whereas, it was lower in the early stages of infection. Variety BINA-T2 was less affected and performed better compared to other varieties.

Hajiabadi et al., (2009) design a research to study TSWV in tomato fruits and leaves, the samples were collected from different provinces of Iran. For the confirmation of TSWV DAS-ELISA test and biological-assay was performed. Tomato plant after mechanical inoculation shown symptoms like necrotic brown ring spot on leaves and also yellow ring spots on fruits, other plants like *Vigna unguiculate*, *Petunia hybrida* and *Nicotiana rustica* shown symptoms like ring-spot, wilting and stem necrosis. He also tested 300 samples of tomato which were collected from different areas in North Khorasan and Khorasane-razavi, only 12 plants showed positive response in ELISA test. He also collected 320 samples from Shirvan, Bojnourd and Neishabour only 25 samples were infested by TSWV.

Abu-Shirbi et al., (2012) studied the occurrence of viruses in tomato field in Jordan. He collected 1647 samples of tomato plants from different areas of Jordan (Central Jordan Valley, Southern Jordan Valley and Northern Jordan Valley) and tested by DAS-ELISA. According to test results 39.6% of the samples affected by more than one virus. The finding proved occurrence of Potato virus Y (PVY), TSWV, Tomato aspermy virus (TAV), TYLCV, Tomato ring spot virus (ToRSV), Tobacco rattle virus (TRV) and Tobacco etch virus (TEV). During the study he stated that TSWV infected 17.7% of collected samples followed by PVY and ToRSV with (8.7%,7.8%) infection rate. However, the rest of viruses is not noticed in collected samples of tomato.

Kulshrestha et al., (2013) in current studies TSWV its presence and internal molecular structural biology was enhanced belong to specie Tospovirus according to the classification and a packed virus which leads obliteration growth and economical losses worldwide. It boosts growth in insect cell and transmitted by many species of thrips. *Frankliniella occidentalis* is imminent vector followed transmission of the virus maintained propagative way. TSWV is a negatively strand RNA virus and there are many factors effecting the subsequent transmission through insects from both vector and virus likewise. Composition and coding of TSWV from different protein helps in virus protection, vector transmission, movement of cellular level and recent replication in RNA silencing activity. The current review aimed on radical compositional structure, genome orientation, molecular grounds of transmission and recent advances in TSWV investigation.

Salamon et al., (2016) stated that TSWV arose significant pathogen of pepper and observed in open-field in last 20 years in Hungary. During the study he observed symptoms in Hungarian TSWV-tolerant variety (White cecei pepper). Fruit melanotic ringspot (FMRS) showing strains were also isolated for the first time which were resistant to TSWV in pepper fruit. Several macroscopic symptoms were developed by the TSWV infection and there are also about seven other Tospovirus which can cause similar symptoms. A gene from *Capsicum. chinense* has been isolated which can be successfully used in developing resistant vegetables. Though, there is no TSWV resistant spice peppers variety in Hungry. In the laboratory, a *Tsw* gene is inserted into spice pepper by crossing a homozygous resistant male with (" Kocséri ", " PalF4 " line) a high valued susceptible line. For dihaploids parent generation, homozygous (BC1) and heterozygous (F1 & F2) resistant spice pepper were used, linked with a *Tsw* gene by using SCAR markers. Recently, in hybrid testing programs and agronomic evaluation the ca. DH lines are used.

Karavina and Gubba (2017) conducted a study in Zimbabwe to confine topographical distribution, phylogeny, and host-range of TSWV. He also stated that TSWV causes more than 1 billion dollars losses yearly in green house and open field crops. During the survey, samples were collected from 18 districts, 50% of virus was noticed of districts surveyed. For the identification of TSWV, RT-PCR and DAS-ELISA follow by phylogenetic and sequencing analysis. Furthermore, some earlier described hosts, for the first

time TSWV was noticed in *Gyposphila elegans*, *Cucurbita moschata*, *Cucumis sativus* and *Cucurbita pepo*. The molecular investigation showed that TSWV isolate were extremely identical 99.02% and similar 93.99% at amino-acid and nucleotide arrangement. When Zimbabwean TSWV isolates compared rest of the biosphere, were firmly connected to isolate of New Zealand, Montenegro, Serbia and Italy. By providing protocols and procedures, the bases of future research regarding TSWV is offered through this study. Effective control measures against TSWV can be regulated by the information that this study provides.

Gupta et al., (2018) reported several biotic and abiotic factors that limits the productivity of tomato crops. One of these are, the interaction of tomato spotted wilt virus (TSWV) and its vector which effects on tomato yield by causing disease. Thrips are small insects which transmits this virus. Some strategies are applied in past decades to realize the association among TSWV and its transmitting insect pest. Few methods were used to identify the proteins or genes that are involved in the TSWV and thrips interaction. There are also a few genes (*Sw-7*, *Sw-6*, *Sw-5*, *Sw-4*, *Sw-3*, *Sw-2* and *Sw-1a,1b*) which can cause the resistance in plants and a protein against this lethal virus.

Lazaro et al., (2019) stated that conventional agriculture has injurious effects on human and environment and it is increasing with the passage of time. As a result, more sustainable and alternative systems for better production was adopted. Globally, the organic agriculture is quickly adopted strategy to meet the need of food for world population and to manage the pests and disease problems. Though, there are no studies present on the organic management of plant viruses. In Eastern Spain a survey on TSWV, CMV and Tomato mosaic virus (ToMV) in tomato crops was performed to check the virus's management under conventional and organic agriculture. Samples were collected from 40 different small farms in which ToMV showed highest existence, CMV showed in few plants where none of the plants were affected by TSWV. It was estimated that the plants were affected by one of the three viruses separately. It was concluded from the study survey that the infection risks by viruses is higher in conventional and lower in organic farms.

2.2 Occurance of TSWV in Turkey

Turhan and Korkmaz (2006) designed a research to find and study the occurrence of TSWV in tomato crops grown in field in Turkey (Çanakkale Province). Hence a survey was managed in the tomato open field and samples were collected at the base of symptoms and tested by ELISA to confirm. During the survey 200 samples were collected from 99.2-hectare area of tomato field and tested by ELISA, showed positive result and also tested by Direct Tissue Blot Immunoassay (DTBIA) for comparison. Corresponding to ELISA test result, 9 samples showing positive response and also tested these samples by DTBIA method and positive result was found. Infected samples were also tested by inoculation to indicator plant. DTBIA method was effectively operated and pink reddish color on nitrocellulose membrane was detected in affected samples under binocular (10X magnification).

Ozdemir et al., (2007) conducted a study on tomato grown in greenhouse and on weeds to verify the occurrence of TSWV in Turkey (Denizli Province). The plants which showed samples like TSWV were collected (71 leaf and 7 fruit samples) and tested by ELISA method. According to the test, 43 leaf samples were infected by TSWV with (60.6%) infection rate and also these samples were used for inoculation on indicator plants including (*Nicotiana tabaccum cvs*, *N. rustica L* and *Datura stramonium L*). After inoculation symptoms like local lesion, rings on leaves, chlorotic and necrotic spots were noticed. The ELISA test also shown positive response for fruit samples, results showed that fruit samples were also affected by TSWV. The effect of TSWV was also found in weeds in this region for the confirmation, 10 species of weeds belonging to different families were tested and positive result was noticed for 7 species of weed.

Kamberoglu et al., (2009) discovered that the eggplants are showing some symptoms similar to the incidence of TSWV in Turkey, in the regions of Eastern Mediterranean region. Plants were mottled, stunted, deformed and necrotic leaves including ringspots on the fruit. DAS-ELISA was used to identify the occurrence of TSWV. RT-PCR was used with two primers (Virus specific) revealed the presence of TSWV in the infected plants and the infected leaves showed the range from 1.6 while the healthy plants were recorded below than 0.160. The expected (276bp) amplicons were studied in 1%

agarose gel. Presence of TSWV was identified in 72 tested samples and there was no incidence of virus on the healthy plants.

Sevik and Arli-Sokmen (2012) conducted a research to find the influence of TSWV on production and marketable value of tomato crops. This research was carried out in the Agricultural Faculty of Ondokuz Mayıs University in Turkey (Samsun province). According to the investigation TSWV reduced 95.5% marketable value and 42.1% yield. The infection of TSWV significantly caused reduction in fruit number (20.18%), weight (26.61%), length (11.93%) and width (10.94%) of tomato fruit. In the current study it was suggested that 0.9-million-dollar loss occurs in the Samsun region of Turkey due to TSWV infection in tomato crops.

Arli-Sokmen and Mehmet Ali Sevik (2013) studied the spatial and temporal distribution of TSWV during 10 weeks in 2004. After putting the source plants in the field, the infecting plants detection was started in week 4 which were increased till 7th week. At the end of experiment, 147 plants were recorded to be infected. Population of thrips were also monitored in the experimental area in which thrips specie *tabaci* (Lindemann) was found more frequent (84.9%). A significant correlation was noticed among the thrips and infected plants. The majority of infected plants were at the distance of 21-30 m from internal virus source, whereas the nearer plants were not too much effected. Significantly higher number of infected plants were infected at the distance of 21-25 m. When time progressed, there was no gradual rise in the distances of TSWV-infected plants within 0-30m. The virus tends to be carried by vectors via primary infections and spread within 30 m radius, but in the case of a small internal source of virus, minimal secondary infections are likely to occur after this point.

Bozdogan and Kamberoglu (2015) designed a study to screen the distribution and occurrence of TSWV in pepper, lettuce and tomato plants growing glass-house in Turkey (Antalya province). Samples were collected from different areas of Antalya province, including the Center (Camkoy, Altinova, Aksu and Kursunlu villages), Çakallık villages (Serik) and Beykonak, Mavikent villages (Kumluca). Total number of collected samples were 596 (345 from pepper, 58 lettuce and 193 tomato). DA-SELISA test were used for the confirmation of viruses. TSWV was noticed in 526 out of 596 samples, 81% from 316 pepper, 93% in 54 lettuce and 88% from 156 tomato plants. For

biological characterization 3 isolates were chosen from pepper, lettuce and tomato having the code (K1, A1 and S1). Symptoms were detected in 7-10 by using A1 isolate (lettuce) in indicator plant, while for S1 and K1 isolates the symptoms were noticed after 20-24 days of inoculation. Furthermore, these isolates were also confirmed by using RT-PCR.

Sevik and Arli-Sökmen (2016) stated that viral disease is main factor which can cause destruction in vegetable growing area in Turkey (Samsun region). For this purpose, survey was conducted to determine the prevalence of viruses in plants belonging to two different families *Cucurbitaceae* and *Solanaceae*. From different 5 locations total 985 samples were collected and analyzed for TSWV, Impatiens necrotic spot virus, Watermelon silver mottle virus and Tomato chlorotic spot virus. DAS-ELISA method was used to determine the virus, only pepper and tomato shown positive result for TSWV while the rest of viruses were not detected in collected samples. According to the test results, the prevalence of TSWV was 9.91% in 2011 and 8.09% in 2012 correspondingly.

Kiliç et al., (2017) stated that the virus not only effect the open field crop, also affecting greenhouse growing crops. Due to this reason the experiment was conducted in the Western Mediterranean region of Turkey, where he observed the loss caused in tomato crops by viruses in the greenhouse. Three different viruses, including Pepino mosaic virus (PepMV), TSWV and TYLCV examined for this intention. From six location 186 samples of infected tomato leaf were collected and confirmed by using DAS-ELISA method, and result showed that 118 samples were affected by more than one virus. TSWV infected 53 plants of tomato with an infection rate (28.48%), 43 and 22 plants were affected by TYLCV and PepMV with (23.12% and 11.83%) infection rate. Positive samples were also tested by mechanical inoculation.

Dikova et al., 2017 tested the essential lavender and fennel oils to inhibit TSWV in pepper plants. DAS-ELISA and artificial inoculation of test (indicator) plants were used to evaluate the deformed and spotted fruits obtained from supermarkets during the summer months. Results revealed that the deformities were caused by TSWV which is an important viral pepper pathogen. Lavender and fennel essential oils were used as a potential source for controlling TSWV, diluted in the concentrations of 10000 ppm,

5000 ppm, 3000 ppm, 2000 ppm and 1000 ppm. The oils were mixed with the TSWV inoculum and in each variant pepper plants of the variety Sofiyska kapiya have been inoculated. The results show that fennel oils have no inhibitory effect whereas, lavender oils have inhibiting effect on TSWV *in vivo* trails.

Çulal-Kiliç et al., (2017) conducted experiment to study the occurrence of TSWV in pepper producing regions of Turkey (Denizli, Isparta and Burdur). In survey 448 of TSWV infected leaf was collected from different places of pepper growing areas. DAS-ELISA method was used for the existence of TSWV. All the infected samples were tested by ELISA and result revealed that 61 samples out of 448 were affected by TSWV (13.61%). For mechanical inoculation positive samples were used and symptoms were also noticed on indicator plant after inoculation. Molecular investigation was tested by using RT-PCR method total 86 samples (25 negative and 61 positive) were tested and positive result was found in 63 samples. Immunocapture reverse transcriptase polymerase chain reaction (IC-RT-PCR) method were also used and seventy (70) samples showed result mean affected by TSWV.

Güneş and Mustafa (2018) did a study the molecular characterization of infection causing TSWV and CMV. They collected the samples from pepper open fields from various parts of Antalya. They used DAS-ELISA and found out that among the total 148 samples, 53 were infected by TSWV and 11 were infected by CMV. The genomes of these viruses were amplified by RT-PCR using specific primers. Nucleotide resemblance for nucleocapsid protein gene, glycoprotein gene and RNA genes (RNA dependent) was ranging between 92-98% for TSWV and 89-96% for CMV. TSWV showed up to 97% similarity with *TSW* resistance breaking isolates; collected from *Capsicum* plants from various regions of Turkey, Spain and Italy. They concluded that resistance for *TSW* is controlled by S segment but alterations in amino acid sequences cause breakdown of TSWV resistance.

Ozturk and Baloglu (2019) examined the viruses in pepper plants, samples which showed like virus symptoms were collected from different regions of Turkey (Mersin, Adana, Hatay and Kahramanmaras). These samples were tested by DAS-ELISA and RT-PCR method. According to investigation numerous virus was found in pepper plants like Alfalfa mosaic virus (AMV), Pepper mild mottle virus (PMMoV), Potato Y virus

(PVY), Cucumber mosaic virus (CMV), TSWV, TEV, Tomato mosaic virus (ToMV) and Tobacco mosaic virus (TMV). Totally 1225 samples were analyzed and 728 showed positive result which was infected by one or more than one virus. CMV was the leading one with 33.68% infection rate, followed by TEV with a 22.26% infection rate. The infection ratio of TSWV was 4.66%, PVY 19.64%, PMMoV (1.52%), PepMoV (3.3%), while the infection rate of AMV and TMV is 1.08%.

Öncü and Yeşil (2019) described that the most convenient way of virus control is development of resistant cultivars and use them in the field. A gene “*Sw-5*” is responsible of the virus resistance (TSWV) and many seed companies around the globe have incorporated it into the cultivars. While the mutations in this virus genome can cause the cultivar to become again susceptible. Different studies from the past have showed this pattern of broken resistance. Now researchers are in a race to identify other sources of resistance against TSWV. As a result of this pursuit, a new gene “*Sw-7*” has been identified as a potential source of resistance; that can be used in variety development programs especially against TSWV.

Fidan and Koç (2019) explained that Artichoke is important agricultural product of Northern Cyprus because of its export potential. The used DAS-ELISA to examine infections (belonging to groups of Nepovirus, Cheravirus, Fabavirus, Ilarvirus, Cucumovirus, Tombusvirus, Tobamovirus, Tobravirus, Potyvirus, Carlavirus, Potexvirus, Crinivirus, Tospovirus, Anulavirus, in the families of Rhabdoviridae and Bromoviridae respectively) which can affect Artichoke production. Among the collected samples 33 samples were found positive for TSWV and ArLV (Artichoke latent virus) in ELISA and RT-PCR tests. In RT-PCR test 485bp region of ArLV and 276bp of TSWV were collected on agarose gel (2%). While other viruses were not identified in the selected samples. It was found out that the disease incidence increases by the transmission of aphids and thrips vectors.

CHAPTER III

MATERIALS AND METHODS

3.1 Experimental Site Description

This research work was carried out in the greenhouse of the Faculty of Agricultural Science and Technologies at Niğde Ömer Halisdemir University during 2019-2020. In Niğde the warm season lasts for 4 months (June-September) and average temperature are high 29°C/85F and low 24°C/75F. while the cold season lasts for 3.4 months (November 28 to March 9) and average temperature are high 8°C/47F and low 1.6°C/35F. The city is located at 37.97 latitude and 34.68 longitude and 1243 m above the sea level. This study was conducted in greenhouse because of control condition and the temperature inside the greenhouse is high as compared to outside.

3.2 Materials

3.2.1 Plant materials

For evaluating the effects of TSWV on eggplants, five different cultivars were used. Eggplant commonly known as Patlıcan in Turkey are highly consumed vegetable. During this study five different cultivars of eggplant (*Solanum melongena* L.) having names Topan, Kemer, Halep Karasi, Aydin Siyahi and Silindirik 153-74 were used. These cultivars were selected based on their common usage in the region. The seeds were obtained from private breeding companies.

3.2.2 Seed trays and pots

During the study both seed trays and pots were used. Each seed tray contains 32 holes and for each cultivar 3 seed trays were used. First the seed were sown in trays for germination. The total number of seeds sown from each cultivar was 96. Later the pots were used for transplanting for each cultivar 25 pots were used.

3.2.3 Virus isolates

TSWV inoculum sources (tomato plant) were obtained from Mr. Ali Ferhan MORCA (T.R. Ministry of Agriculture and Forestry, Ankara Directorate of Agricultural Quarantine).

3.3 Experimental Design

To evaluate the effects of TSWV infection on different eggplant cultivars, seeds were sown in plastic seed trays under greenhouse conditions and after germination of seeds, seedling were transplanted to pots. From each cultivar 25 plants were used for inoculation and 25 plants were used as a control. After seven days of transplanting the plants were mechanically inoculated three times with 7 days, 14 days and 21 days of interval with virus. Each cultivar contains 50 plants, 25 inoculated and 25 non inoculated (control).

3.4 Biological Assay

3.4.1 Plants transplanting

For mechanical inoculation the seeds were sown in seed trays and waited when they reach to optimum size and get ready for transplanting, figure (3.1). 250 number of pots were filled with soil and turf. For each cultivar 50 pots were used. One day before transplanting the seedlings were irrigated and next day transplanted to pots. The pots were arranged and tagged accordingly with their specification in order to easily identify. Irrigation to the plants was carried out on daily basis.



Figure 3.1. Seedlings before transplanting and after transplanting

3.4.2 Mechanical inoculation

Mechanical virus inoculation method was used for inoculation eggplants. For eggplant virus inoculation the following steps were followed. First inoculation buffer having pH: 7.4, containing 0.199 g/l KH_2PO_4 , 1.14 g/l Na_2HPO_4 and 0.1% Na_2SO_3 and 1% PVP-40 was prepared. After that infected plants samples were grinded by mortar and pestle to get leaf extract for inoculation and mix these extracts with inoculation buffer. After transplanting, plants were inoculated with (7, 14 and 21) days intervals. One day before the inoculation the plants were irrigated well and putted the plants in dark. Next day early in the morning the eggplants were inoculated because in the morning the stomata opening and absorption rate is high as compared to evening. Before starting the inoculation, carborundum was sprinkled on the surface of leaves to cause abrasion of the cells and the virus preparation was rubbed over the surface of eggplants leaf by using cotton-swab with making small wound by paper pin to achieve infection because virus particles cannot penetrate an intact leaf surface, with its cuticle and cell walls acting as a major barriers. figure (3.2). Tap water was also spray on the surface of leaves after 2-5 mints of inoculation. After 10-15 days of virus inoculation, plants show a symptoms of TSWV and for testing of the virus the leaf from inoculated plants were collected and tested by ELISA method.



Figure 3.2. TSWV inoculated eggplants

3.5 DAS-ELISA

DAS-ELISA method was used to detect TSWV in eggplant according to Clark and Adams (1977) and instructions of the antisera's manufacturer (Bioreba AG, Switzerland) for the monoclonal antisera of TSWV. For ELISA, leaves from inoculated plants were collected, each leaf represents the whole plant. After that 1000 μ l of extraction buffer were added for getting leaf extraction and grinded by mortar and pestal and then extraction was transferred to 2 ml tubes (figure 3.3). The first step in ELISA is coating which were done by diluting 10 μ l of IgG in 10 ml of coating buffer and 100 μ l of solution were added to each well. The plate was covered tightly with plastic and putted in incubator at 30 °C for 4 hours, then plate was removed after four hours and washed three times with washing buffer. After washing and drying the plate, leaf extraction (juice) of 100 μ l were added to each well by using 100 μ l pipet and also positive, negative and extraction buffer were added to first five well. After putting the extraction into the wells, the plate was covered tightly and putted at 4°C overnight. Next day in morning time plate was washed again three times with washing buffer. Conjugate

buffer was prepared by diluting 1 ml of conjugate buffer in 10 ml of distal or sterile water and then 10 µl of enzyme conjugate in 10 ml of conjugate buffer were diluted and 100 µl solution were added to each well. Plate was covered and puted in humid box and incubated at 30 °C for five hours. After removing the plate from incubator washed again 3 times and drying, then dissolved Para-nitrophenyl-phosphate (pNPP) 10 mg in 10 ml of substrate buffer and 100 µl of substrate buffer solution were added to each well. The plate was covered and incubated at room temperature (22-25 °C) at dark place. After 90 minutes the ELISA plate was read by Biotek el.800 ELISA reader.



Figure 3.3. Grinding leaf samples for TSWV detection

3.6 Calculation of Infection Rate of TSWV (%)

The following formula was used for calculation of TSWV infection rate in eggplants to find how much eggplants were infected.

$$\text{TSWV (\%)} = \frac{Z1}{Z2} \times 100 \quad (3.1)$$

Z1= Positive samples of eggplant tested by ELISA

Z2= Total samples of eggplants tested by ELISA

3.7 Yield and Quality Parameters of Eggplant

Fruits of inoculated and control plants were harvested as they mature. All fruit morphological traits related to quality were evaluated for 10 ripened fruits per cultivar. The ripen fruits from infected and control plants were harvested by hand and putted in plastic bags and the following parameters were analyzed for ripen fruits. Fruit number (FN) were counted and tagged accordingly. The fruit length (FL) was measured by using measuring tape and data were recorded in centimeter (cm), whereas the fruit diameter (width) was measured by using vernier caliper and data was recorded in millimeter (mm). For fruit fresh (FW) and dry matter, the digital scale was used for fresh weight the ripen fruits were weighted immediately after harvesting and for dry matter the samples were kept on room temperature to lose moisture and then oven dried for 48 hours, data was recorded in percent. The fruit color (FC) was determined by using chromometer and fruit firmness (FF) was measured by using penetrometer. For finding the total soluble solid (TSS) (Brix) the refractometer was used and for flesh thickness of fruits (FT) vernier caliper were used. Yield per plant was measured by using digital scale (Fig 3.4).

3.8 Data Analysis

Analysis of Variance (ANOVA) statistical tests was performed using the statistix 8.1. Duncan multiple comparison test was used to compare the differences between the averages which were statistically significant according to the variance analysis results.



Figure 3.4. Measuring fruit length by using measuring tap (a), weighting individual fruit weight by using digital scale (b), measuring flesh thickness by using vernier caliper (c) measuring tss (brix) by using a. krüss optronic gmbh, ar-2008 (d) finding fruit firmness by using penetrometer (e), checking color of fruits by using chroma meter (f) fruits were dried in oven for calculating dry matter (g), measuring fruit diameter with vernier caliper (h) and calculating yield per plant with digital scale (i)

CHAPTER IV

RESULTS

Results obtained from this study were mainly divided into three sections. The first section includes mechanical inoculation, second was DAS-ELISA analysis and third one was analysis of yield and quality traits of local Turkish eggplant cultivars.

4.1 Disease Symptoms

For inoculation, the isolate of TSWV were used three times at different days interval and disease symptoms started to appear after second inoculation in some plants whereas, majority of plants showed symptoms after the third inoculation. The virus source were inoculated in young leaves of the plants which showed symptoms after the second inoculation. The cultivar Silindrik 153-74 showed bronzing of the leaves (a). Symptoms observed for the remaining cultivars, Aydin Siyahi showed ringspot (d). Kemer cultivar showed necroses (f), whereas, the necrotic ringspot was observed for Halep Karasi (c, e, h, and i) and concentric ringspot was observed for Topan cultivars (b and g) on inoculated plants as shown in Figure 4.1. The symptoms of TSWV varied from cultivar to cultivar in host plants. The symptoms were observed only on the leaves while no symptoms were noticed. On fruits and stem. Samples were collected from the inoculated plants randomly and tested by DAS-ELISA for the confirmation of TSWV infection.

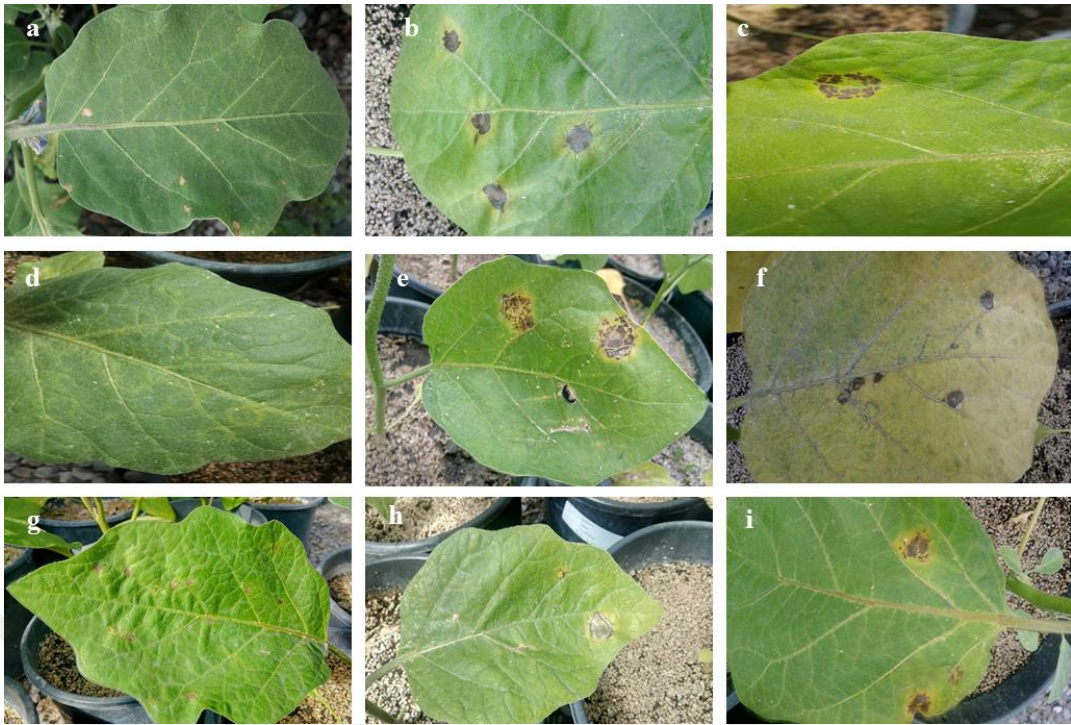


Figure 4.1. Eggplants leaf showing symptoms of tswv, bronzing (a), concentric ringspot (b and g), ringspot (d), necrosis (f) and necrotic ringspot (c, e, h, and i)

4.2 DAS-ELISA

Leaf samples were randomly selected after four weeks of last inoculation from all cultivars of eggplant and were tested by DAS-ELISA method. The ELISA results were observed visually and dark yellow color in ELISA plate indicated positive result as shown in Figure 4.2. The highest positive ELISA result was detected for Kemer cultivar 58% followed by Topan 52%, whereas the lowest positive result was observed from Silindrik 153-74 cultivar 38%. The results of positive samples are summarized in following Table 4.1.

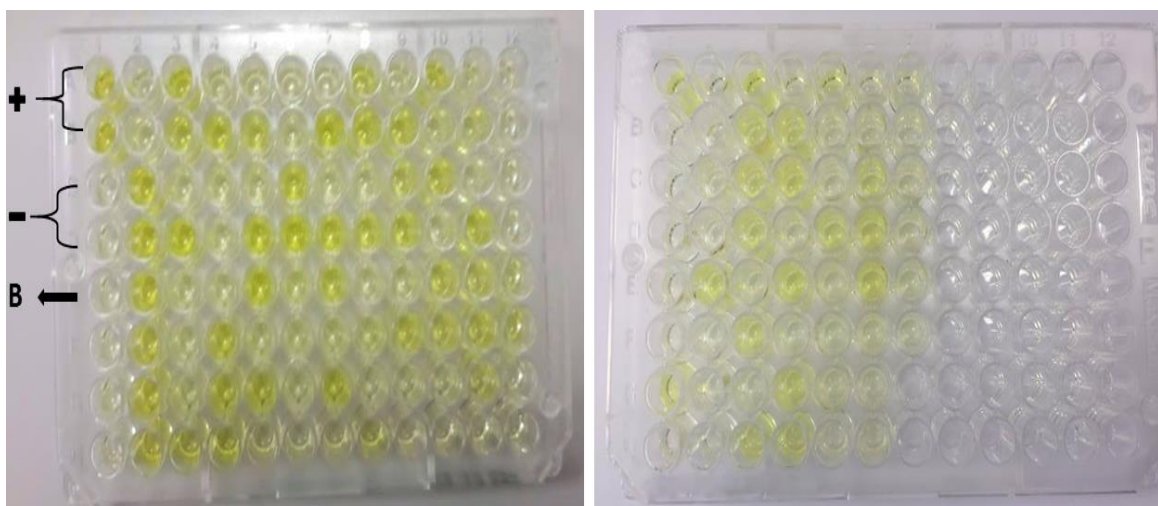


Figure 4.2. ELISA plate results, positive control (+), negative control (-) and extraction buffer (B), yellow color indicating positive reaction and colorless wells indicates negative reaction

Table 4.1. Details of ELISA positive results and infection rate in eggplant cultivars

Cultivars	Number of inoculated plants	Number of total tested samples	Number of infected samples	Virus	Infection rate (%)
Silindirik	25	18	7	TSWV	38%
Kemer	25	17	10	TSWV	58%
Aydin Siyahi	25	19	9	TSWV	47%
Halep Karasi	25	18	8	TSWV	44%
Topan	25	19	10	TSWV	52%

4.3 Yield and Fruit Quality Results

For evaluating the effect of TSWV on different eggplant cultivars, fruits were harvested and the number of fruits per plant, fruit length, fruit diameter (mm), fruit fresh weight (g), fruit color, total soluble solid (TSS), flesh thickness of fruits (mm), firmness of fruits (Kg), total yield (g per plant) and dry matter (%) were examined.

Table 4.2. Effect of TSWV on fruit number, fruit length and fruit diameter of different cultivars of eggplant

Cultivars	Fruit Number		Percent Reduction over control	Fruit Length		Percent Reduction over control	Fruit Diameter		Percent Reduction over control
	Inoculated	Control		Inoculated	Control		Inoculated	Control	
Silindirik	5.26c	7.85a	32.99	12.59c	15.19a	17.12	40.96c	46.97b	12.80
Kemer	2.89f	6.47b	55.33	7.32e	14.57ab	49.76	21.31f	34.54d	38.30
Aydin Siyahi	1.79g	3.85de	53.50	8.7e	14.88ab	41.53	29.58e	38.28c	22.73
Halep Karasi	1.90g	3.49ef	45.55	7.89e	13.54bc	41.73	28.65e	32.72d	12.44
Topan	1.87g	4.60cd	59.34	5.6f	10.36d	45.95	38.93c	52.76a	26.21
LSD 0.05	0.84			1.52			2.73		

Means value in columns with different letters are significantly different at $p \leq 0.05$ using LSD Test

4.3.1 Fruit number

In the current research experiment analysis of variance for fruit number of eggplant cultivars exhibited highly significant ($p \leq 0.05$) variation among all the inoculated and control plants. Among the control condition the highest number of fruit was recorded from Silindirik 153-74 cultivar (7.85) which was statistically different from the remaining treatments followed by control plants of Kemer (6.47). Whereas, among the inoculated cultivars the highest fruit number was recorded for Silindirik 153-74 (5.26) and lowest number of fruits were recorded for cultivars Aydin Siyahi (1.79), Topan (1.87) and Halep Karasi (1.90) which were statistically non-significant from each other. It was recorded that inoculation of virus reduced the fruit number significantly among the inoculated plants. Percent reduction over control was calculated for each cultivar because each cultivar is different from other. Highest percent of reduction in fruit number over control in inoculated plants was recorded for Topan 59.34%, followed by Kemer 55.33% whereas the lowest percentage of reduction 32.99% was recorded for Silindirik 153-74 cultivar (Table.4.2), (Figure.4.3).

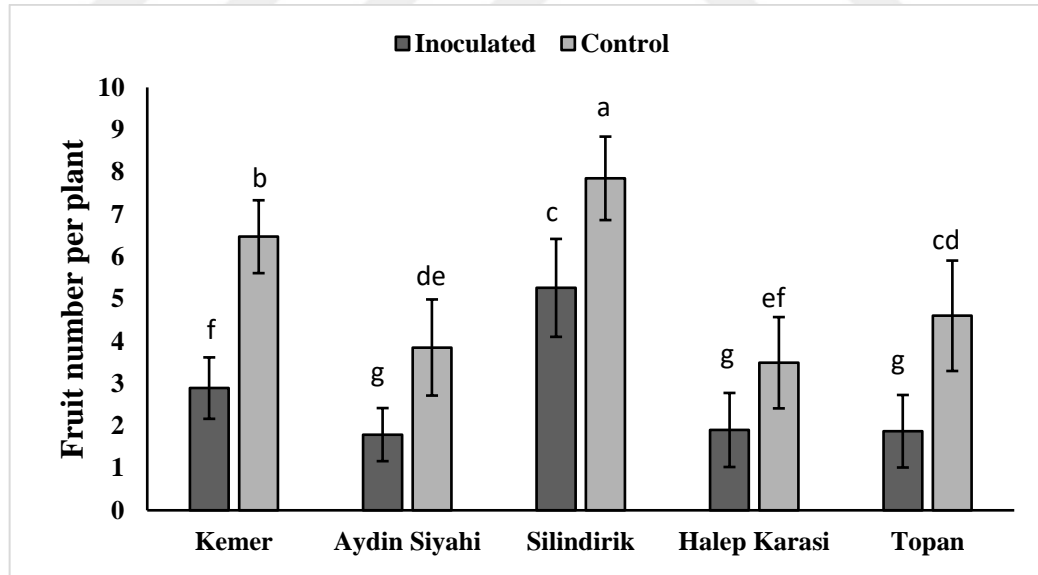


Figure 4.3. Number of fruits per plant of eggplant cultivars effected by TSWV and control plants. Bar shows mean value, vertical bar represents standard deviation, alphabets sharing same letter are statistically non-significant ($p \geq 0.05$) whereas different letters shows significant ($p \leq 0.05$) difference among cultivars

4.3.2. Fruit length

Fruit length of the eggplant cultivars was significantly ($p \leq 0.05$) affected by mechanical inoculation of TSWV. The inoculated plants comparatively showed lower fruit length while the control showed higher fruit length. The highest fruit length among inoculated plants was recorded for cultivar Silindirik 153-74 (12.59 cm). The lowest fruit length (5.6 cm) was recorded from the cultivar Topan inoculated plants which showed reduction by 45.95% compared to its control plants fruit length (10.36 cm). Highest mean fruit length (15.19 cm) was recorded from Silindirik 153-74 cultivar which was statistically at par with the fruit length of Aydın Siyahi (14.88 cm) and Kemer (14.57 cm) followed by Halep Karasi control plants with the mean fruit length of (13.54 cm). Among the eggplant cultivars Kemer resulted in highest percent of reduction in fruit length (49.76), followed by Topan (45.95) and Aydın Siyahi (41.53). Lowest percent decrease in fruit length (17.12) was recorded from Silindirik 153-74 cultivar. (Table.4.2), (Figure.4.4).

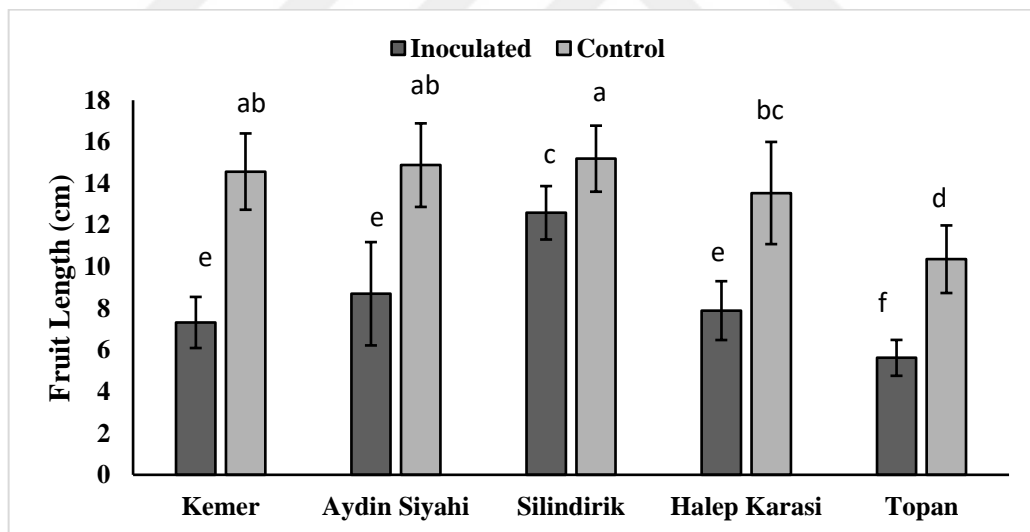


Figure 4.4. Fruit length of eggplant cultivars effected by TSWV and control plants. Bar shows mean value, vertical bar represents standard deviation, alphabets sharing same letter are statistically non-significant ($p \geq 0.05$) whereas different letters shows significant ($p \leq 0.05$) difference among cultivars

4.3.3 Fruit diameter

Mechanical inoculation of TSWV significantly ($p \leq 0.05$) reduced the fruit diameter in inoculated plants compared to control plants. Highest fruit diameter (52.76 mm) was recorded for Topan because of round shape followed by Silindirik 153-74 cultivar (46.97 mm) and Aydin Siyahi (38.28) control plants. Whereas, among the inoculated plants the highest fruit diameter was recorded for Silindirik 153-74 (40.96 mm) and lowest fruit diameter was recorded from Kemer (21.31 mm) followed by Halep Karasi and Aydin Siyahi with the mean diameter of (28.65 mm) and (29.58 mm). The highest percent reduction of fruit diameter was noted in Kemer cultivar 38.30% followed by Topan 26.21%. Silindirik 153-74 and Halep Karasi cultivars resulted with the lowest percent fruit diameter reduction of 12.80% and 12.44%. (Table.4.2). (Figure.4.5).

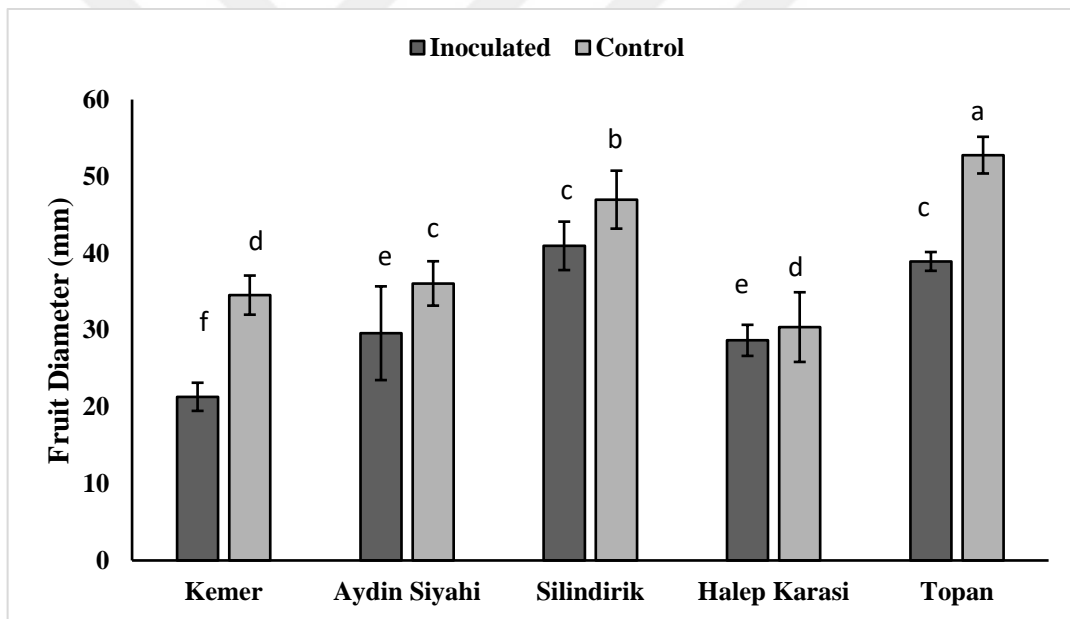


Figure 4.5. Fruit diameter of eggplant cultivars effected by TSWV and control plants. Bar shows mean value, vertical bar represents standard deviation, alphabets sharing same letter are statistically non-significant ($p \geq 0.05$) whereas different letters shows significant ($p \leq 0.05$) difference among cultivars

Table 4.3. Effect of TSWV on fruit weight, flesh thickness and total soluble solid (TSS) of different cultivars of eggplant

Cultivars	Fruit Weight		Percent Reduction over control	Flesh Thickness		Percent Reduction over control	TSS (Brix)		Percent Reduction over control
	Inoculated	Control		Inoculated	Control		Inoculated	Control	
Silindirik	116.43c	169.49a	31.31	8.00b	9.77a	18.11	5.09b	6.12a	16.83
Kemer	39.45e	122.12c	67.70	4.10ef	7.60bc	46.05	3.06e	5.16b	40.69
Aydin Siyahi	56.54e	127.74c	55.74	4.65e	6.80cd	31.61	3.88d	5.51ab	29.58
Halep Karasi	53.65e	98.81d	45.70	4.37ef	6.29d	30.52	4.24cd	5.45ab	22.20
Topan	51.78e	145.53b	64.42	3.80f	6.43d	40.90	3.10e	4.91bc	36.86
LSD 0.05	17.49			0.83			0.70		

Means value in columns with different letters are significantly different at $p \leq 0.05$ using LSD Test

4.3.4 Fruit weight

TSWV significantly ($p \leq 0.05$) reduced the individual fruit weight of inoculated eggplants compared to control plants. Among the inoculated plants the lowest fruit weight (39.45 g) was recorded for Kemer cultivar which was statistically at par with mean value of fruit weight Topan (51.78 g), Halep Karasi (53.65 g) and Aydin Siyahi (56.54 g) and highest fruit weight was recorded from Silindirik 153-74 cultivar (116.43 g). whereas, the highest mean value of fruit weight was recorded from Silindirik 153-74 cultivar (169.49 g) control (non-inoculated) plants followed by Topan (145.53 g), Aydin Siyahi (127.74 g), Kemer (122.12 g) and Halep Karasi (98.81 g). The highest percent reduction of fruit weight was calculated for Kemer 67.70% followed by Topan 64.42%, Aydin Siyahi 55.74% and Halep Karasi 45.70%. The lowest percent reduction was recorded from Silindirik cultivar 31.31%. (Table.4.3). (Figure.4.6).

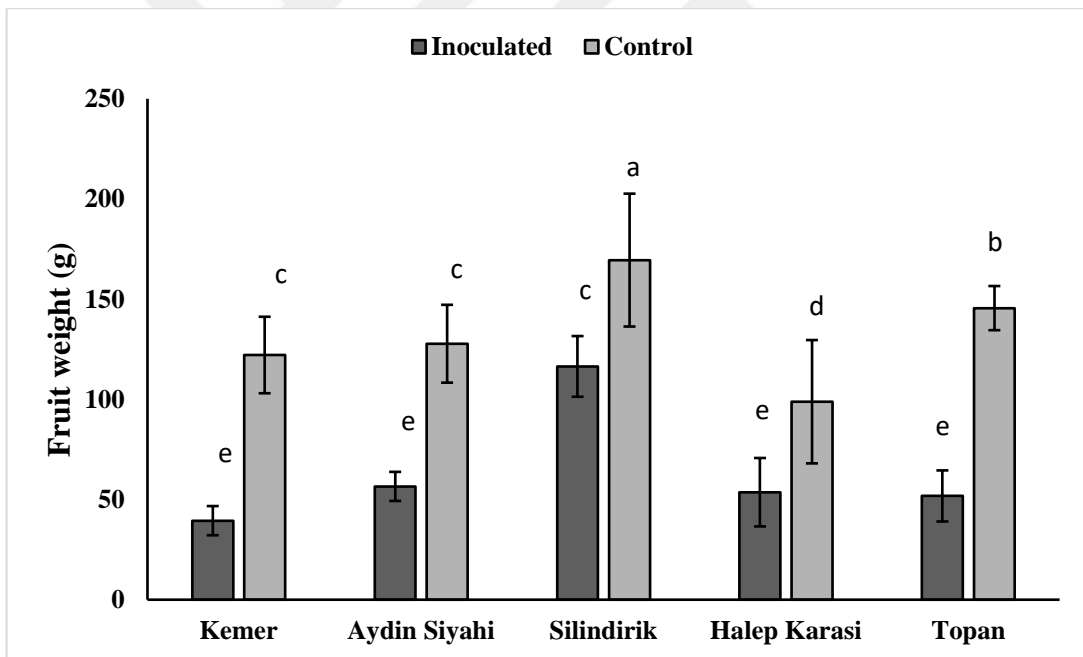


Figure 4.6. Fruit weight of eggplant cultivars effected by TSWV and control plants. Bar shows mean value, vertical bar represents standard deviation, alphabets sharing same letter are statistically non-significant ($p \geq 0.05$) whereas different letters shows significant ($p \leq 0.05$) difference among cultivars

4.3.5 Flesh thickness

Flesh thickness of eggplant cultivars was significantly ($p \leq 0.05$) reduced by TSWV. The inoculated plants comparatively showed lower flesh thickness whereas for the control plants higher flesh thickness was recorded. The lowest mean value of flesh thickness was recorded for Topan (3.80 mm) inoculated cultivar which is statistically at par with mean value of Kemer (4.10 mm), Halep Karasi (4.37 mm) and Aydin Siyahi (4.65 mm) whereas the Silindirik 153-74 cultivar (8.00 mm) was statistically different from the remaining of the cultivars. The highest mean value for flesh thickness was recorded for cultivar Silindirik 153-74 (9.77 mm) control plants followed by Kemer (7.60 mm) and Aydin Siyahi (6.80 mm). The highest percent reduction was calculated for Kemer 46.05%, followed by Topan 40.90%, Aydin Siyahi 31.61% and Halep Karasi 30.52%. Whereas, the lowest percent reduction was calculated from Silindirik 153-74 cultivar 18.11%. Table (4.3), (Figure.4.7).

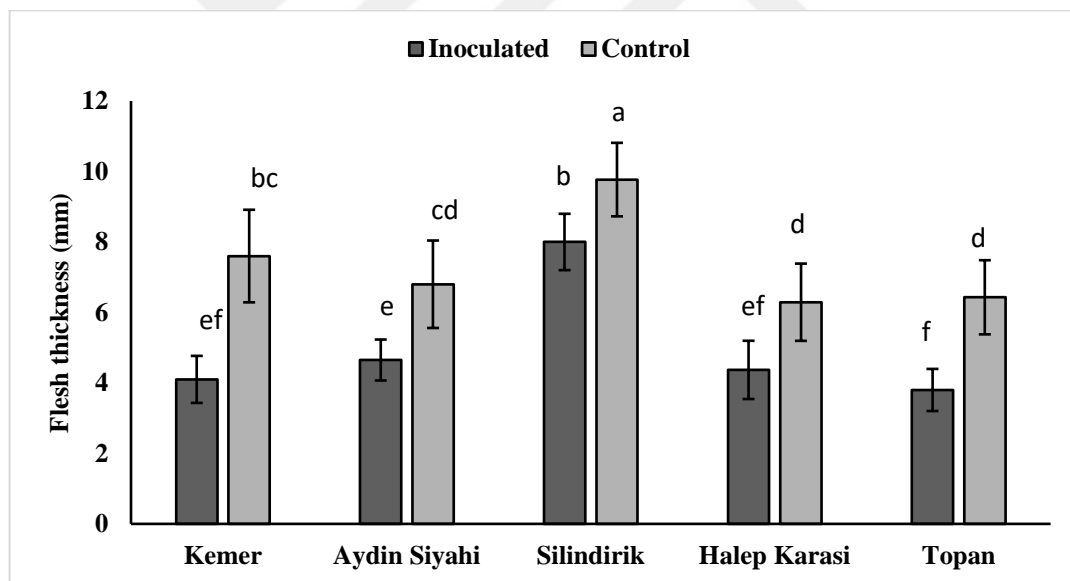


Figure 4.7. Flesh thickness of eggplant cultivars effected by TSWV and control plants. Bar shows mean value, vertical bar represents standard deviation, alphabets sharing same letter are statistically non-significant ($p \geq 0.05$) whereas different letters shows significant ($p \leq 0.05$) difference among cultivars

4.3.6 Total soluble solids (Brix)

TSWV significantly ($p \leq 0.05$) reduced the TSS content in inoculated eggplant cultivars compared to controls. Among the inoculated cultivars the lowest mean value of brix was recorded for Kemer (3.06%) and Topan (3.10%) followed by Aydin Siyahi (3.88%) and Halep Karasi (4.24%) and highest brix was recorded for Silindirik 153-74 (5.09%). Whereas, the highest mean value for brix was recorded from Silindirik 153-74 cultivar (6.12%) under control condition, followed by Aydin Siyahi (5.51%), Halep Karasi (5.45%), Kemer (5.16%) and Topan (4.91%) which is statistically with at par. The highest percent reduction for brix was recorded in Kemer 40.69% and Topan 36.86% cultivars. Whereas, the lowest percent reduction was recorded from Silindirik 153-74 cultivar 16.83%, Table (4.3), (Figure.4.8).

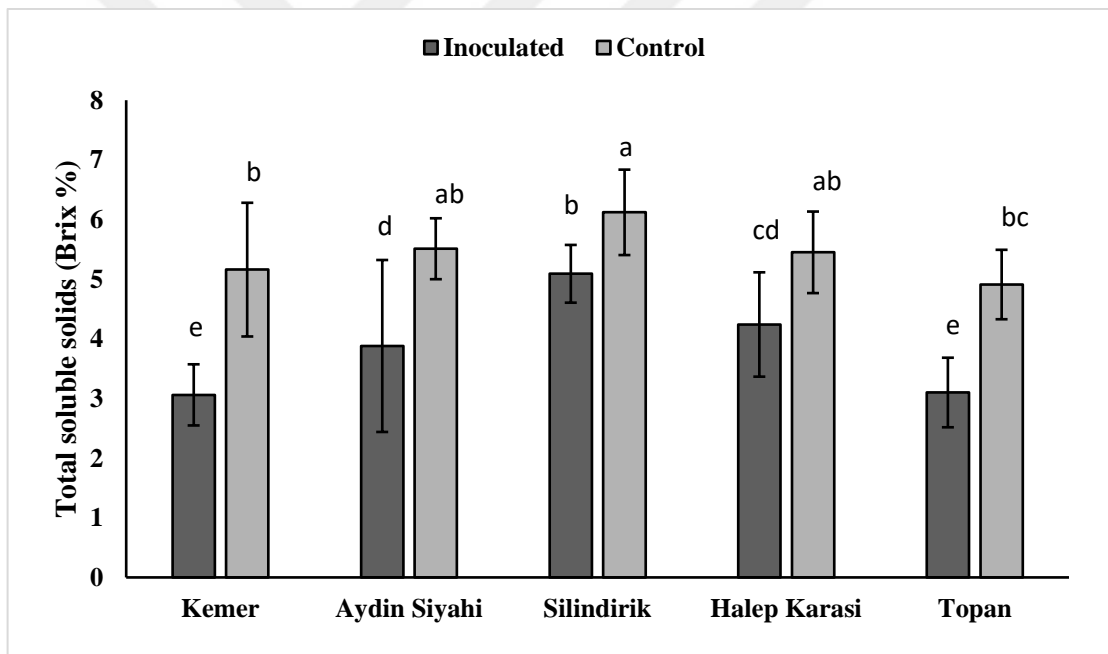


Figure 4.8. Total soluble solids of eggplant cultivars effected by TSWV and control plants. Bar shows mean value, vertical bar represents standard deviation, alphabets sharing same letter are statistically non-significant ($p \geq 0.05$) whereas different letters shows significant ($p \leq 0.05$) difference among cultivars

Table 4.4. Effect of TSWV on fruit color of different cultivars of eggplant

Cultivars	Fruit Color					
	Inoculated			Control		
	L	a	b	L	a	b
Silindirik	34.03d	2.94	5.09	34.47cd	3.33	5.31
Kemer	35.20cd	4.80	5.35	35.00cd	3.88	5.35
Aydin Siyahi	38.29b	4.55	4.97	36.00c	3.76	5.98
Halep Karasi	35.11cd	3.29	5.81	38.95b	5.90	6.77
Topan	51.23a	10.75	3.22	38.91b	10.58	2.88
LSD 0.05	1.80					

Means value in columns with different letters are significantly different at $p \leq 0.05$ using LSD Test

4.3.7 Fruit color

Fruit color of eggplant was examined for five different cultivars under inoculated and control (non-inoculated) conditions. Chroma meter describes the color distribution in eggplant cultivars in three dimensions “L”, “a” and “b”. The dimension “L” was considered since it explains the alteration of color from dark/black to white (light), commonly observed in fruit skin of eggplant. The cultivar Topan showed light fruit skin color (51.23) under inoculated conditions in comparison to dark fruit skin color (38.91) depicted under control conditions. Same trend was observed for Aydin Siyahi, which showed a significantly different ($p \leq 0.05$) light color (38.29) under inoculated condition as compared to its control (36.00). Contrarily, inoculated eggplant of Halep Karasi exhibited dark fruit color (35.11) as compared to the light color (38.95) displayed under non-inoculated conditions. The eggplant cultivars Kemer and Silindirik 153-74 cultivar did not show significant difference ($p \geq 0.05$) in fruit skin color under inoculated and non-inoculated conditions. Table (4.4), (Figure 4.9).

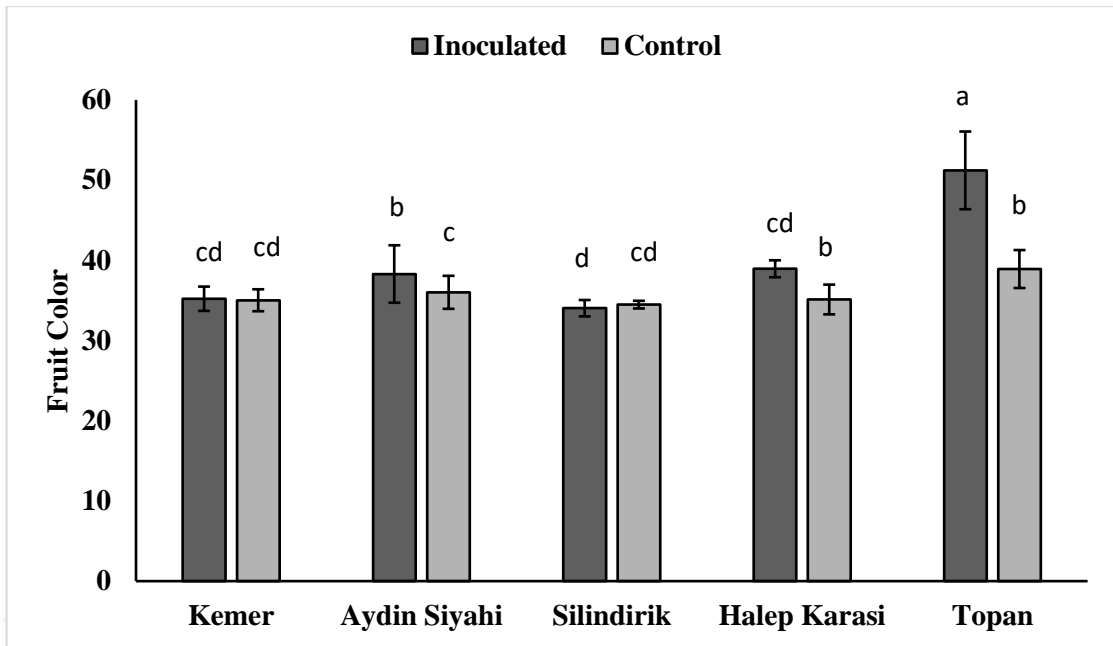


Figure 4.9. Fruit color of eggplant cultivars effected by TSWV and control plants. Bar shows mean value, vertical bar represents standard deviation, alphabets sharing same letter are statistically non-significant ($p \geq 0.05$) whereas different letters shows significant ($p \leq 0.05$) difference among cultivars

Table 4.5. Effect of TSWV on fruit firmness, dry matter and yield of different cultivars of eggplant

Cultivars	Fruit Firmness		Percent Reduction over control	Dry Matter		Percent Reduction over control	Yield (g/plant)		Percent Reduction over control
	Inoculated	Control		Inoculated	Control		Inoculated	Control	
Silindirik	5.06ab	5.32a	4.88	10.27	10.13	NS	650.1bc	1310.1a	49.61
Kemer	2.77e	3.90d	28.97	10.05	10.24	NS	124.2e	770.6b	83.88
Aydin Siyahi	3.85d	4.56bc	15.57	10.52	10.40	NS	106.5e	541.4c	80.32
Halep Karasi	4.25cd	4.60bc	7.60	10.36	9.60	NS	113.1e	364.0d	68.92
Topan	3.24e	4.58bc	29.25	10.57	10.50	NS	101.7e	644.5bc	84.22
LSD 0.05	0.56			NS			136.45		

Means value in columns with different letters are significantly different at $p \leq 0.05$ using LSD Test

4.3.8 Fruit firmness

Fruit firmness was significantly ($p \leq 0.05$) affected by TSWV. Inoculated eggplant cultivars showed lower fruit firmness compared to control plants. Among the inoculated cultivars the highest fruit firmness was recorded for Silindirik 153-74 (5.06) and the lowest mean value for fruit firmness was recorded for cultivar Kemer (2.77) and Topan (3.24) followed by Aydın Siyahi and Halep Karasi (3.85), (4.25). For control cultivars, the highest fruit firmness was recorded from Silindirik 153-74 cultivar (5.32) followed by Halep Karasi (4.60), Topan (4.58), Aydın Siyahi (4.56) and which is statistically non-significant from each other. The maximum percent reduction over control was calculated for Topan 29.25% and Kemer 28.97%, whereas the lowest percent reduction was calculated from Silindirik 153-74 cultivar 4.88%. Table (4.5), (Figure.4.10).

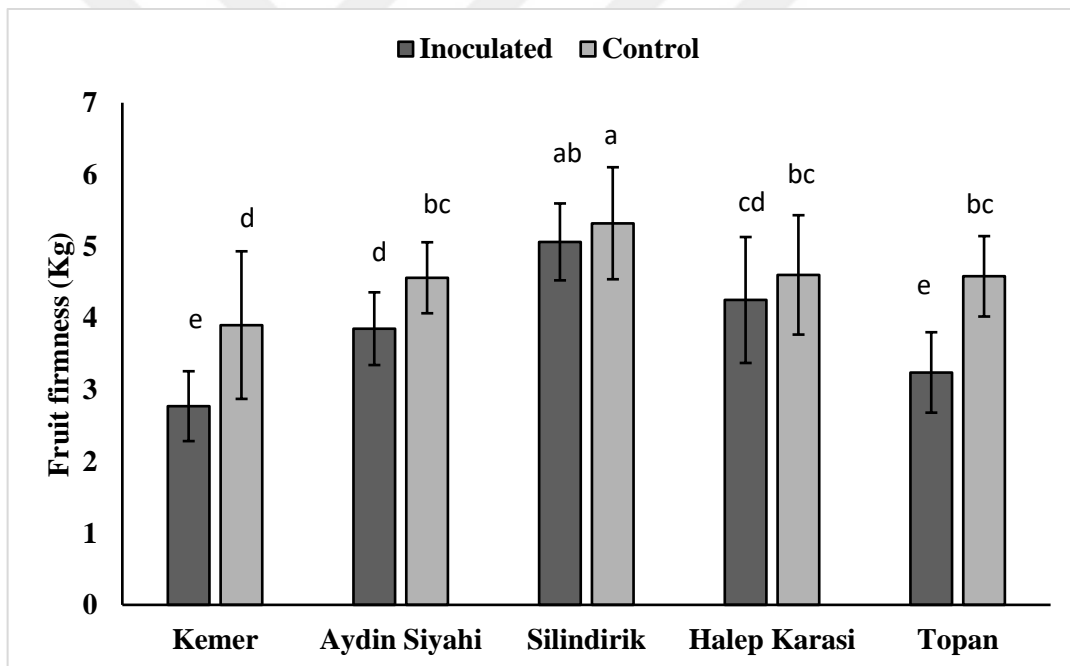


Figure 4.10. Fruit firmness of eggplant cultivars effected by TSWV and control plants. Bar shows mean value, vertical bar represents standard deviation, alphabets sharing same letter are statistically non-significant ($p \geq 0.05$) whereas different letters shows significant ($p \leq 0.05$) difference among cultivars

4.3.9 Dry Matter content

Results regarding dry matter content, showed non-significant ($p > 0.05$) difference among the cultivars with the inoculation of TSWV as well as among control plants. The

cultivar Kemer showed dry matter content of 10.05% in inoculated one which was also statistically similar to its control (10.24%). Similarly, for the cultivar Aydin Siyahi (10.52%), Silindirik 153-74 cultivar (10.27%), Halep Karasi (10.36%) and Topan (10.57%) dry matter content was statistically similar to their control plants. (Table.4.5), (Figure.4.11).

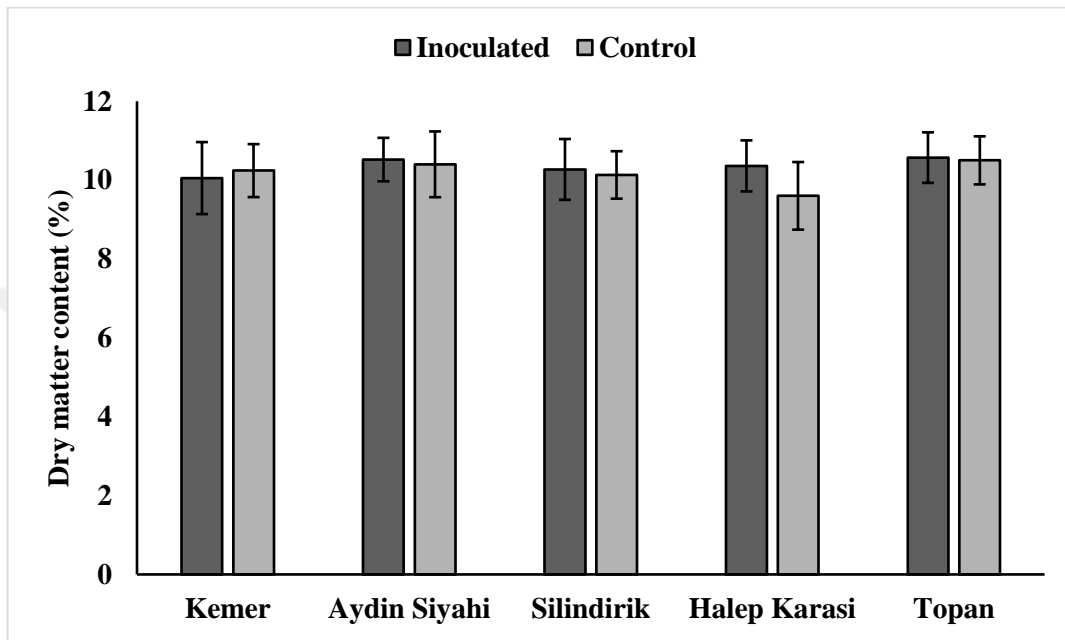


Figure 4.11. Dry matter contents of eggplant cultivars effected by TSWV and control plants. Bar shows mean value, vertical bar represents standard deviation, statistically it was non-significant ($p \geq 0.05$)

4.3.10 Yield (g/plant)

TSWV infection cause reduction in fruit yield. The fruit yield of inoculated eggplant cultivars was significantly ($p \leq 0.05$) lower than control. The lowest mean value for yield was recorded for cultivar Topan (101.7 g/plant) which is statistically similar to the remaining treatment of eggplant cultivars Aydin Siyahi (106.5 g/plant), Halep Karasi (113.1g/plant) and Kemer (124.2 g/plant). Whereas, Silindirik cultivar (660.1 g/plant) perform best in inoculated condition. The highest yield per plant was showed by Silindirik 153-74 cultivar (1310.1 g/plant) in control condition followed by Kemer (770.6 g/plant), Topan (644.5 g/plant), Aydin Siyahi (541.4 g/plant) and Halep Karasi (364.0 g/plant). The highest percent reduction over control was calculated for cultivar

Topan 84.22% and Kemer 83.88%. Whereas for yield the lowest reduction was calculated for Silindirik 153-74 cultivar 49.61%. (Table 4.5), (Figure.4.12).

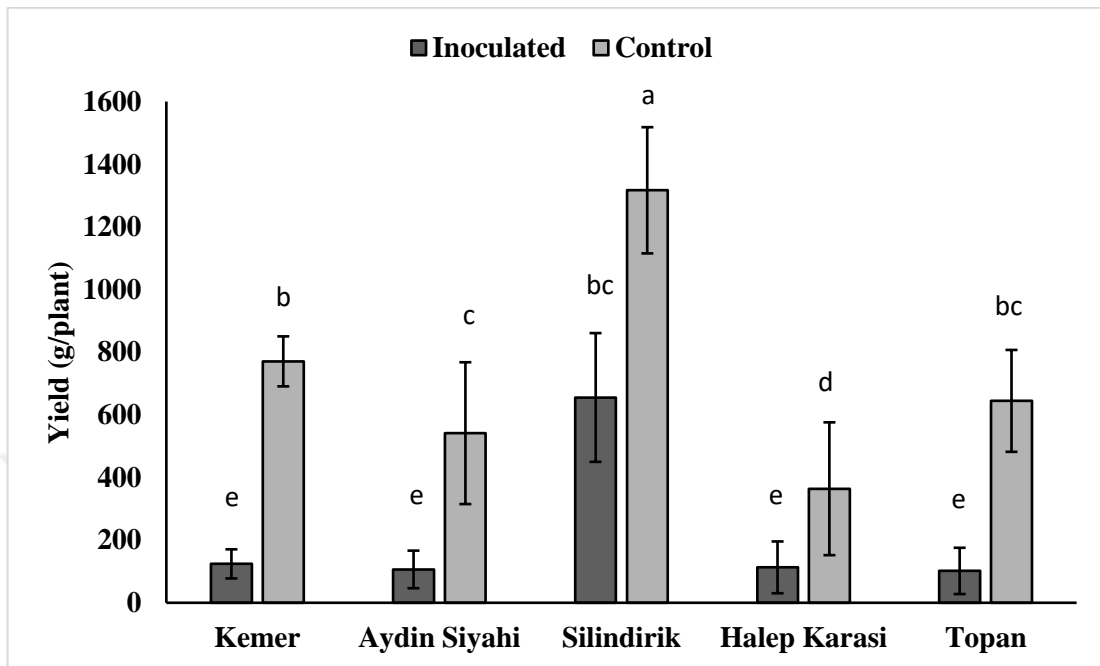


Figure 4.12. Yield of eggplant cultivars effected by TSWV and control plants. Bar shows mean value, vertical bar represents standard deviation, alphabets sharing same letter are statistically non-significant ($p \geq 0.05$) whereas different letters shows significant ($p \leq 0.05$) difference among cultivars

CHAPTER V

DISCUSSION

Objective of the current study was to evaluate the effect of TSWV on different cultivars of eggplant in the greenhouse of the Faculty of Agricultural Science and Technologies at Niğde Ömer Halisdemir University during 2019-2020. TSWV was mechanically inoculated three times on five different eggplant cultivars. The first inoculation was done after seven days of seedlings transplantation, second time TSWV was inoculated after two weeks of first inoculation whereas, third time the TSWV was inoculated after three weeks of second inoculation. Characteristic symptoms including ringspots, necrosis, necrotic ringspot and concentric ringspot started to appear after 10 to 14 days of inoculations. Similarly, Ramkat et al., (2006) observed the TSWV symptoms like ringspot, leaf yellowing and necrotic lesions in tomato plants after 14 days of inoculation and also stated that disease severity based on symptoms varies among the cultivars. In eggplants, Kamberoglu et al. (2009) also found symptoms like deformed leaves, necrotic, molted and stunted plants. The infection rate of TSWV in this experiment was noted which was highest in cultivar Kemer (58%) followed by Topan (52%), Aydin Siyahi (47%), Halep Karasi (44%) whereas, lowest infection rate was noticed in Silindirik 153-74 cultivar (38%). Similar level of infection ranging from 41% to 68% and also the same symptoms were reported by Farooq and Akanda, (2007) in different cultivars of tomato. Mechanical inoculation cause more infection because of applying high concentrations of inoculum source. Mechanical inoculation of TSWV causes more infection than insect vector thrips as reported by Nascimento et al. (2006) after evaluating the response of three peanut breeding lines to TSWV by mechanical and thrips inoculation. The fruit number was significantly affected by TSWV in inoculated plants compared to its control. The lowest fruit number was recorded for Aydin Siyahi inoculated plants and the highest number was recorded from Silindirik 153-74 cultivar. The reduction percentage in number of fruits per plant ranged from 32.99 to 59.34% in all eggplant cultivars. Farooq and Akanda, (2007) stated that TSWV has a significant effect in reduction of fruit number ranging from 50 to 72%. TSWV causes reduction in fruit number reported by Sevik and Sokmen (2012) who found a decrease of 20.18%, compared with the production of fruits by healthy plants. This study was further supported by the earlier report of Ramkat et al. (2006) who reported

reduction of 90% in fruits by the infection of TSWV. The fruit length of control plants was significantly higher than the inoculated plants. The reduction percentage of fruit length was 17.12 to 49.76% in all the eggplant cultivars. Our results are in accordance to the findings of Farooq and Akanda (2007) who reported the impact of TSWV on reduction of fruit length from 11 to 68%. Similar decrease in fruit length of 11.93% was also reported by Sevik and Sokmen, (2012) during a study of estimating the effect of TSWV infection in some yield components. Reduction in potato tuber length was also noticed by El-Shazly et al. (2017) for Potato Virus Y infection along with nutritional yield parameters. These studies showed that the infection of viruses can reduce the fruit length significantly.

In the current study the fruit diameter was also significantly affected by mechanical inoculation of TSWV causing a reduction of 12.80 to 38.30% which is in accordance with the findings of Sevik and Sokmen (2012), who reported a reduction of 10.94% fruit diameter in Tomato plant. Likewise, the reduction rate of diameter was 12.1 to 22.2% caused by TEV in pepper population reported by Ozturk et al. (2019). Reduction in diameter was also reported by El-Shazly et al. (2017) in potato tubers by Potato Virus Y. Fruit weight was significantly affected by TSWV resulting in percent reduction of 31.31 to 67.70%. The highest mean single fruit weight of 169.49 g was measured for Silindirik cultivar whereas, Kemer inoculated resulted in lowest i.e. 39.45 g. The response of cultivars was different regarding fresh weight reduction against TSWV that might be due to their natural resistance level. Ramkat et al. (2006) also stated that viral diseases reduce yield, TSWV disease is being one of them. They noticed a significant decrease in fruit weight ranging from 27% to 60% depending on the response of varieties to TSWV infection. In another study Díaz-Pérez et al (2007) reported that TSWV affected the fresh fruit weight as well as the vegetative plant weight. Kim et al. (2004) noticed that TSWV caused weight and quality loss in paprika fruit.

TSWV has a significant effect on fruit flesh thickness in the current study causing a reduction of 18.11 to 46.05%. The reduction rate varies among the cultivars due to several factors. Fruit size and shape also could be a reason for difference in flesh thickness. Different studies reported that TSWV has effect on fruit size, length and diameter which means that if these factors got effect, it could result in reduced flesh thickness. Though, Ozturk et al. (2019) determined that there were losses in fruit wall

thickness after getting infected by TEV. Owing to the results it was recorded that the infection of TSWV has reduced the total soluble solid (Brix) of fruit. The reduction rate was in the range of 16.83 to 40.69 percent in different cultivars. According to the findings of El-Shazly et al. (2017) viral infection reduces the percent starch and total soluble sugar content of fruit. It could be due to reduction in water supply, photosynthesis rate or disorder in assimilation of nutrient contents in plants. Our results are also supported by the study of Perez-Benlloch et al. (2001) who reported the decrease of Solids soluble content in pepino (*Solanum muricatum* L.) by Tomato mosaic virus.

The results of current study indicated that TSWV has an effect on fruit color of eggplant. The cultivar Topan and Aydin Siyahi showed light color under inoculated conditions. The changes in fruit color could also be due to different varieties. The fruits from the infected plants indicated light color compared to healthy fruits. Similar kind of results were also obtained by Farook and Akanda, (2007) for color response in tomato crop against TSWV. Our results are also comparable to the study of Swift, (2006) who reported the color variation of fruits due to TSWV infection. Whereas, the findings of Ozturk et al. (2019) are in contrast to our results who reported a non-significant difference among the healthy and infected plants of pepper effected by TEV. Fruit firmness traits attributes describes fruit texture and are vital in determining final fruit quality. In the current study, TSWV has influence on fruit firmness in a highly significant way. The firmness of vegetables is affected by different traits such as biochemical constituents, cellular organelles, cell wall composition and water content or turgor. Thus, TSWV affecting any of these traits could modify firmness and could lead to bring changes in fruit quality. Examination of the dry matter (DM) content of eggplant fruits in the inoculated and control plants demonstrated that the difference between the cultivars were not statistically significant. Ozturk et al. (2019) observed that, TEV has non-significant effect on the dry matter content. Dry matter yield could be variety-influenced but mosaic virus disease infection had no effect on dry matter yield (Elegba et al. 2013).

Eggplant yield is generally associated with healthy vegetative growth of plants throughout the growing season. Results of this study revealed that TSWV extremely disrupts normal plant growth. An increase was observed in disease symptoms and there was an increasing reduction in yield parameters during eggplant development. Plant

infection with TSWV typically resulted in significantly reduced fruit yield per plant. Results indicated that the eggplant cultivars had different tolerance level as percent reduction in yield varies among the cultivars. Most infected cultivar was Topan and Kemer with highest percent fruit yield reduction of 84.22% and 83.88 while, the lowest infected one was Silindirik 153-74 cultivar with 49.61% fruit yield reduction. These results are in agreement with the study of Ramkat et al. (2006) who reported the fruit yield loss of 37-90% due to TSWV in different tomato varieties. Farooq et al. (2017) found that under different tolerant conditions, TSWV resulted in 44.10 - 55.68% reduction in fruit yield. Similarly, Díaz-Pérez et al., (2003) determined that prior to harvesting TSWV reduces the crop yield by 2.1% to 2.3% for each day. Previous reports showed that viruses cause different diseases in Solanaceae family, among them TEV was the very common and destructive virus which reduced the Karaisali pepper yield by 77.5% (Ozturk et al. 2019). The effect of TSWV on yield varied with variety and stage of inoculation. Rapando et al. (2009) also found that TSWV disease caused 57% and 32% crop yield reduction in two different cultivars of cucumber. Most fruits formed on the infected plants by TSWV exhibited abnormal coloration and the marketable yield of tomatoes was drastically reduced due to the abnormal ripening (Moriones et al. 1998).

Using the relations of inoculated to that of control plants, the cultivar Silindirik 153-74 can be rendered tolerant to TSWV. It had the lowest infection rate and lowest reduction in both yield and quality traits. Whereas, the cultivar Kemer and Topan can be rendered sensitive. It had the highest infection rate and highest reduction in yield and quality traits. Information presented here clearly illustrated that TSWV had devastating effects on quality and yield parameters of eggplants. Therefore, strategies to avoid TSWV infection and control measures before crop losses should be implemented.

CHAPTER VI

CONCLUSION

Eggplant is highly consumed vegetables such as potato, tomato, and pepper. It has numerous medicinal properties and it is potentially used in pharmaceutical products. Turkey produced 0.83 million tons of eggplant in the last decade which hold the country at 4th position. Among the viruses' TSWV can cause yield loss in several important vegetable crops by producing un-marketable fruit. Current study was devised to elaborate the effects of TSWV on five selected eggplant cultivars (Kemer, Aydin Siyahi, Halep Karasi, Topan and Silindirik 153-74) under greenhouse conditions, for the screening of cultivars on the basis of their performance against TSWV. According to DAS-ELISA, the cultivar Kemer showed the highest infection rate followed by the cultivar Topan and Aydin Siyahi whereas, the lowest infection rate was recorded for Silindirik 153-74 cultivar. Infection of TSWV significantly reduced the yield and quality traits of eggplant such as fruit number, fruit length, fruit diameter, fruit weight, flesh thickness, total soluble solid, fruit color, fruit firmness and yield in inoculated plants as compared to their respective control plants. For fruit number the highest reduction was recorded from Topan cultivar whereas, lowest was recorded for Silindirik 153-74. For fruit length, fruit diameter, fruit weight, flesh thickness and TSS the highest percent reduction was recorded for Kemer and lowest was recorded for Silindirik 153-74. Change in fruit color was observed for Topan and Aydin siyahi. For fruit firmness and yield highest reduction was observed for Topan and Kemer and lowest reduction was observed for Silindirik 153-74. The results showed that among the five cultivars, the performance of Kemer and Topan was poor which suggested that these cultivars are highly sensitive to TSWV. Whereas, the performance Silindirik 153-74 was good which suggested that this cultivar is tolerant to TSWV. This study will enable plant scientists to understand effects of TSWV on eggplant cultivars. Results of the study will be helpful for plant breeders as well for the development of sustainable virus resistant cultivars by screening/selection of the tolerant cultivars to cope TSWV.

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