

TOMATO LEAF CURL VIRUS DISEASE (TLCVD) AND ITS RESISTANCE MANAGEMENT PRACTICES

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Tomato (*Lycopersi comesculentum*) is an important vegetable crop of Pakistan which is affected by various biotic and abiotic diseases. There are many diseases that infect tomato among which *Tomato leaf curl virus* (TLCV) is the major disease of tomato crop all over the world. TLCV spreads by *Bemisiatabaci* under natural conditions in persistent-circulative manner. The environmental factors and the population of *Bemisiatabaci* considerably increase the spread of disease. The purpose of this study is to identify the source of resistance by screening tomato germplasm against TLCV under natural field conditions and determination of favorable environmental factors (maximum temperature, minimum temperature, relative humidity and rain fall) on disease development. Management of disease and its vector through insecticides tested.

Keywords: Tomato, chemicals, *Tomato leaf curl virus*, resistance, disease, management.

INTRODUCTION

Tomato (*Lycopersicon esculentum*) belongs to Solanaceae family is an important short duration vegetable crop attacked by many pathogenic diseases. TLCVD is most important which damage the crop very badly and affect the fruit quality and quantity badly. This disease is due to a complex of viruses which are transmitted through the whitefly. To manage the disease control of its vector is very important which is done by using different approaches. Management of TLCVD and *B. tabaci* through plants extracts, nutrients and insecticides are useful. Climatic conditions helps in development of disease as well as in management of disease. The management of this disease is very important due to the importance of this crop. First time tomato was cultivated in Andes and imported to Europe in 16th century (Paduchuri *et al.*, 2010). Tomato export was negligible until 2000 from Pakistan and it has increased since 2007. These days main focus of Pakistani exporters is to export tomato to Middle East and Afghanistan (Tahir *et al.*, 2012). Worldwide production of tomato is about 161.79 million tones with total yield 53681.2 kg/ha. In Pakistan, total production was 560,000 tones and total yield was recorded 101818.9 (kg/ha) with 4803.680 thousand hectares (FAOSTAT, 2014-15). Different studies have shown that consumption of tomatoes reduces the risk of cancer, digestive tract, lungs and stomach disorders. Tomatoes also provide protective effects against mouth, pharynx and prostate cancer (Helyes and Lugasi, 2006). Tomato contains important health promoting compounds, which play a role in energy balancing, weight management and in reducing the risk of cancer (Dorais *et al.*, 2008). The area under tomato cultivation is high, but the production of

tomato is low, this attributed is to the potential loss in yield due to number of various diseases. Tomato leaf curl disease (TLCD) is an important viral disease which affects tomato crop and causes severe economic losses throughout the world. TLCD was first time found in Middle East during 1930 to 1950 and now it is distributed worldwide (Lefevre *et al.*, 2010).

Tomatoes are very important due to short duration crop contributing a healthy diet by providing rich amount of minerals, essential amino acids, sugars, fibers etc. (Glick *et al.*, 2009). A number of biotic and abiotic factors affect the quantity and quality of tomato fruit. Among the pathogenic diseases the viral diseases are most important. Tomato leaf curl virus is a serious threat to this crop. Basically it is a complex of viruses. This complex of viruses in Pakistan, India and Australia region is known as TLCV (tomato leaf curl virus) and in Israel and Europe region called as TYLCV (tomato yellow leaf curl virus (Pandey *et al.*, 2009). TYLCV was reported firstly in Israel region in 1920 which appear in epidemic form in 1960 (Glick *et al.*, 2009). TLCV belongs to the genus *Begomovirus* (Xie *et al.*, 2013). *Begomoviruses* have icosahedral single-stranded DNA (ssDNA) genomes and are transmitted by the sweet potato whitefly (*Bemisiatabaci*) in persistent-circulative manner (Ghanim *et al.*, 2014). TLCD symptoms on infected plants include stunting, wrinkling of leaves and yellowing between the veins, leaf margins curl upward giving cup-shape appearance and flower drop occurs before fruit set (Melzeret *et al.*, 2009). Chemicals play significant role in reducing the vector populations by reducing the number of individuals which acquire and transmit the virus which ultimately decrease the disease incidence (Castle *et al.*, 2009). Different insecticides are being used for

controlling insect pests on different crops that include acetamiprid, nitanpyram, imidacloprid, thiacloprid, clothianidin, dinotefuran and thiamethoxam are commonly used insecticides specifically for the sucking pests (Ishaaya, 2001). Plant extracts are also used these days extensively because they are eco-friendly and safe for humans.

In Pakistan, most of tomato varieties being grown are susceptible to TLCV. Environmental factors play an important role in the TLCV disease spread and vector population. Knowledge of environmental factors which influence disease development is required to manage the disease. Control of insect whitefly with different chemicals and plant extracts is helpful to reduce the disease incidence.

MATERIALS AND METHODS

Growing of tomato seedlings in green house was be an easy, cost effective and healthy method. Tomato plants were raised in earthen pots (12.50 cm × 12.50 cm). Seeds were covered lightly with potting compost then pots were placed in a warm (up to 27 °C) and dark place. When the roots were come out from the base of pot plants were transferred to the larger pot. Plants were ready for transplanting outside. After 7 days of sowing the seeds and potting plants were kept indoors for pot experiment for the confirmation of virus through graft transmission and vector transmission.

Infected section of tomato plant was collected from the field. A slanting cut of about 2cm long on the stock and 0.2cm deep was mad so that the exposed area on scion was matching the cut area on the stock. The wedge shaped end of the section was inserted into the cut on the stock and both tied immediately by a polythene ribbon over the union. The grafted seedlings were maintained in the greenhouse (25 ± 5°C, 72.4% R.H) for 2 months. Symptoms development was recorded for disease confirmation (Kashina *et al.*, 2007).

For the transmission of TLCV though whitefly, plants was grown under insect free environment. A viruliferous adult whiteflies was collected with the help of Aspirator and Sweep method and these were transferred on virus infected plants in wooden cage for 40 -60 minutes and was allowed a latent period of 24 hours. Whiteflies were collected and transferred on the test plants by means of an Aspirator and these were allowed an inoculation feeding period of 1 hour. After inoculation whiteflies were killed with insecticide and symptoms development was recorded for 2, 3, and 4 weeks (Kashina *et al.*, 2007).

The experiment was conducted in three replications in RBCD. After the transplantation of seedling in field their response was checked against TLCV. Observation were noted on weekly basis and data of disease incidence was recorded with the help of following formula

$$\text{Disease incidence} = \frac{\text{No.of infected plants}}{\text{Total no.of plants}} \times 100$$

Five varieties Nagina, Morgal, Rio grande, Baby red and Thorgal were used for epidemiological studies. The experiment was conducted in three replications in RBCD. After the transplantation of seedling in field their response was checked against TLCV. Observation were noted on weekly basis and these were correlated with environmental parameters (maximum temperature, minimum temperature, rainfall and relative humidity).The data of different environment conditions (maximum temperature, minimum temperature, rainfall and relative humidity) during the experimental period was collected from the Website of University of Agriculture, Faisalabad (www.uaf.edu.pk). It was used to identify the effect of climatic factors on the development of TLCV and its vector and fluctuations in their response against environmental changes (Muqit *et al.*, 2006).

The data of whitefly feeding on tomato plants was recorded early in the morning at this time whitefly was in active and was not moving as rapidly as during day time. For this purpose three plants from each plot were selected at random and population of whitefly was recorded from upper, middle and lower leaves per plant, then mean was taken to calculate the total whitefly population per plant. For the management of TLCV it is necessary to control its vector. Tomato varieties were sown in three sub-plots with row length 6 meters, row to row spacing 65 cm and plant to plant distance 35 cm. The experiment was conducted in RCBD design with three replications of each treatment. The data on whitefly population was recorded early in the morning 24 hours before spray and then 48 hours after the spray. Three plants from each plot were selected at random and population of whitefly was recorded from upper, middle and lower leaves per plant. The insecticides were applied randomly for management of whitefly vector. Data regarding whitefly population and disease incidence was recorded two days before the spray of chemical and on seven days interval after each spray.

The data obtained will be analyzed statistically and means were compared by using least significant difference test (LSD). Influence of environmental conditions on TLCVD by correlations. Most favorable conditions for disease development were evaluated by regression analysis.

RESULTS AND DISCUSSION

Chemical control method is the major method for the management of insect pests but it become less effective due to resistance develop in insect pest against the insecticides (Siebert *et al.*, 2012). *B. tabaci* adults, nymphs and eggs are found resistant against the chemicals so plant extracts are used. As the chemical control of insect pest is costly so the plant extracts are more effective than the chemicals. Foliar spray of Neem (azadairachtin) and neem plus can kill the eggs, nymphs and adults of *B. tabaci*. Ethanolic and aqueous extracts of *Annona squamosal*, *Carlowrightiamyriantha*, *Trichilliarborea*, *Azadirachtaindica* and *Acalyphagaumeri*

are effective against *B. tabaci* population. Neem oil, garlic and eucalyptus extracts give significant result against this disease (Khan *et al.*, 2013). I used ten varieties of tomato for this experiment. These varieties are Nagina, Naqeeb, Rio Granda, Morgal, Thorgal, Baby Red, SBS_292, VRI-575, Nemador and GSL-198 as shown in Table 1. In this experiment used three replications in RBCD. I was recorded the data about disease incidence and white fly population on the weekly bases. The results are described below. One variety naqeeb was found to be resistant against the TLCV while one line VRI-575 was found to be moderately resistant. Thorgal, Rio Grande and Morgal showed response as moderately susceptible for this disease. Highly susceptible lines were SBS-292, Baby Red, GSL-198 and Nemador.

Table 1: Reaction of tomato lines against TLCV disease.

Rating	Varieties	Disease incidence (%)	Level of resistance
1.	Naqeeb	10-20	Resistant(R)
2.	VRI-575	21-35	Moderately resistant(MR)
3.	Thorgal, Rio Grande & Morgal	36-50	Moderately susceptible(MS)
4.	SBS-292, Baby RED, GSL-198 and Nemador	51-65	Susceptible(S)
5.	Nagina	66-75	High susceptible(HS)

Confirmation of TLCVD through grafting: TLCVD affected plant show a number of symptoms which are reduction in leaf area as well as leaf size, stunted growth, puckering of leaf, upward curling, vein clearing, abnormal shoot proliferation, deformation of leaflets and reduction in yield quantity as well as quality. Tomato is the primary host of this disease. Some other plants are also acts as its host are bean (*Phaseolus vulgaris*), lisianthus (*Eustoma grandiflourm*) and petunia (*Petunia hybrid*) are also host plants of TYLCV. Some weeds including *Cleome viscosa* (Capparidaceae) as well as *Carton lobatus* (Euphorbiaceae) are also susceptible against TYLCV but no disease symptoms are produce (Kurata *et al.*, 2016). For the confirmation of transmission of tomato leaf curl virus through grafting experiment was performed which shows following results shown in Table 2.

Table 2: Graft transmission of TLCV.

Graft transm. Sr. No	Lines	No. of plants with symptoms/ no. of plants grafting	Disease incidence %	levels
1	SBS-292	5/8	62	S
2	Baby Red	5/8	62	S
3	GSL-198	5/8	62	S
4	Nagina	6/8	62	S

Four varieties SBS-292, Baby Red, GSL-198 and Nagina used for the grafting experiment in the greenhouse condition as shown in Table 2. The diseased scions of same thickness

were grafted on tomato stock in green house. It was taken care for that scions and stock were of same varieties. Taken eight plants in each variety were grafted with TLCV diseased scions. These plants kept in greenhouse and recorded data after 7,14 and 21 days. Recorded data showed that all varieties were susceptible to TLCV. SBS-292, Baby Red and GSL-198 showed the disease incidence in the range between 51-65%. Due to disease rating scale was used in screening experiment which showed the all varieties were susceptible for TLCV. In all these varieties showed the 62% disease incidence (5 plants out of 8 plants in each variety). Nagina showed disease incidence more than 75% (6 plants out of 8 plants). The confirmation of results supported the results of literature.

Confirmation of tomato leaf curl virus disease through insect vector: The chemical control method is easy and most commonly used approach against the insect pest. A number of insecticides are used. Among them imidacloprid, acetamiprid, nitenpyram, thiamethoxam and diafenthiuron give significant result against aphids, whiteflies and other insect pests (Bacci *et al.*, 2007). The comparative efficacy of these insecticides show significant result against this disease. These chemicals are very effective against the *B. tabaci* and TLCVD (Zeshan *et al.*, 2015). White fly was used in the experiment for the confirmation of vector in TLCV. Results obtained are shown in Table 3.

Table 3: Vector Transmission of TLCV.

Treatments	No. of insects feeding on plants	No. of plants	Observation	Disease incidence
Insect feed on healthy plant	8	6	4/8	50
Insect feed on diseased plants	8	6	6/8	75

Taken the eggs of whitefly which belong to aviruliferous whiteflies. After hatching the nymphs were reared on the suitable host. Adult aviruliferous whiteflies transferred TLCV infected plants in wooden cage for 40-60 minutes for acquisition and allowed a latent period of 24 hours. After acquisition whitefly were collected and transferred on plants for the inoculation. Whitefly allow for feeding plants for 1 hour. Whitefly allows to transmit TLCV in 16 healthy plants as shown in Table 3. The data showed that those whitefly which were allowed to feed on TLCV diseased plant before disseminating them on healthy plants were more efficient in virus transmission and caused 75% disease incidence of TLCV. While the whiteflies which were fed on healthy plants it showed less than 50% disease incidence. This experiment confirmed that whitefly transferred the TLCV from infected plants to healthy plants.

These results proved that insect *B. tabaci* transferred the disease TLCV in healthy plants under natural field conditions. So the management strategies applied in field conditions

which control the transmission of disease of TLCV in healthy plants.

Relationship of maximum temperature, minimum temperature, relative humidity, rainfall and wind speed with TLCV: Plant health play an important role the management of a disease. Nutrients play an important role in plant health which enable the plants to fights against the chewing type insects. Therefore, the nutrients (N, P, K, Zn, and B) are very important in crop health and these nutrients were evaluated. The application of micro and macro elements can affect the relationship between the insect pest and plants. Studied shows that Zinc improve the defense system of plants. Plants with higher amount of N, P, K, and B give significant result against the disease. Some of these nutrients act as cofactor in enzyme activation (Maathuis *et al.*, 2009). The effect of meteorological parameters with TLCV disease incidence was highly significant on all varieties. The relation between disease and temperature showed positively results because disease incidence increases with the increase in temperature while when temperature decrease then disease also decreases. Whereas relative humidity shows the negative correlation in TLCV. It showed in liner regression explained by r value.

Disease incidence % were easily observed when growth occurring in tomato crops. The relationship of maximum temperature, minimum temperature and wind speed were showed significant results as shown in Table 4. While rainfall and humidity were non-significant with TLCV disease incidence on all varieties. All the varieties showed the highly significant correlation in maximum temperature and TLCV disease. When increase the temperature 25-40°C it also increase the disease incidence in all verities. It has positively correlation in temperature and disease incidence because temperature increases as it increase the disease rate in plants Table 4.

The resistant tomato varieties are only growing in economical way of managing tomato leaf curl virus. For this purpose used ten tomato varieties (Nagina, Naqeeb, Rio grande, Morgal, Thorgal, Baby red, SBS-292, VRI-575, Nemador and GSL-198) was evaluated on the bases of resistance and susceptibility in field conditions and find the disease incidence. Naqeeb was a resistant variety against TLCV when it sown in field while VRI-575 was found as moderately resistant. Some varieties shows the moderately susceptible these varieties are Thorgal, Rio grande and Morgal whereas other four varieties as SBS-292, Baby red, GSL-198 and

Nemador were susceptible while Nagina was highly susceptible to TLCV.

These results were matched with the Ragupathi and Narayanaswamy (2000) who performed experiment on 160 germplasm against TLCV during summer season 1994 in tamilnadu, Coimbatore and india. They found 2 varieties *Lycopersiconglaboratum* and *Lycopersiconhirsutum* which shows the resistance under natural conditions. *Lycopersiconglaboratum* and *Lycopersiconhirsutum* and H-24 were artificially inoculated with TLCV in green house. These results showed that only 2 varieties were resistance against TLCV under natural condition. Pilowsky and Cohn (2000) screened 25 *lycopersicon* sown in green house to find out the resistance source of germplasma against white fly in TYLCV.

Tomato yellow leaf curl virus was detected in 7 to 9 accession in *lycopersi comperuvianum* in all 5 accession *lycopersiconchilense* shows the maximum level of resistance while CIAS 27 show the moderately resistance against TYLCV, other 7 accessions show the highly susceptibility against TYLCV. The results of screening experiment also similar with the findings of Nainar and Pappiash (2000) who reported the results under field condition 2 accessions such as LE.118 and IIHR.1942 did not show any infection TLCV up to 75 days after transplanting. These varieties show that six were susceptible, 7 were moderately susceptible, five more moderately resistance and 2 were resistance.

Similarly screening experiment results were resembled with the results of Marthi *et al.*, 2003, who screened Different and domesticated tomato varieties against two viral strains such as tomato leaf curl virus from Bangalore isolated 4 India and tomato yellow leaf curl virus Isreal and find out resistant resource of germplasm. They performed their experiment under Field condition and green house in India at the University of Agriculture sciences and screened 34 different tomato genotype for the resistance of TLCV these are susceptible of TYLCV. Tomato plants were inoculated with TLCV through vectors in all inoculated tomato plants produce symptoms but in some plants of lines 902 and 910 virus was not identified by hybridization. The tomato genotype which were susceptible to TLCV through *B.tabaci* facilitated inoculation were also found susceptible to the virus under field conditions but there were great difference between various genotype of tomato in crop yield, symptoms severity, disease spread and disease incidence. They tested sixteen tomato genotypes for the resistance to TYLCV is at the Hebrew University of Jerusalem, rehovet, which were

Table 4: overall correlation of metrological parameters with whitefly population and TLCV disease incidence%.

Treatments	Maximum temp	Minimum temp	Relative humidity	Rainfall	Wind speed
TLCV disease incidence %	0.600**	0.647**	-0.652 ^{NS}	-0.079 ^{NS}	0.508**
p-value	0.000	0.000	0.562	0.518	0.000
Whitefly population	0.599**	0.647**	-0.651-	-0.067 ^{NS}	0.492**
p-value	0.000	0.000	0.512	0.583	0.012

resistance or tolerant to the TLCV in India. They resulted that *Lycopersiconhirsutum* L. A1777 and PI 390659 were the best source of resistance to both viral strains and lines 902 and 910, those were resistant to TYLCV was only tolerant to TLCV and *lycopersiconperuvianum* CMV Sel. INRA resistance to TLCV was only tolerant to TYLCV.

The results of confirmation of TLCV through grafting and vector resembled with the results of the other researchers. The grafting experiment showed that tomato leaf curl virus transmitted through grafting. But there was difference in disease incidence in all the four varieties (SBS-292, Baby red, GSL-198 and Nagina). Maximum disease incidence was recorded in Nagina while SBS-292, Baby red, GSL-198 gave less TLCV disease incidence. So it was confirmed that TLCV can be transmitted through grafting. Similar findings were found were reported by Hafiz, (1986) who said that virus is retained when vector molts and neither multiplies in vector or transmitted directly transmitted directly transmitted to progeny. The vector transmission TLCV proved that insect vector (*B.tabaci*) plays a major role in the transmission of TLCV under natural field condition. These findings were similar to the result of (Rashid *et al.*, 2008) who studied virus vector relationship in TYLCV during 2000-2001 at Bangladesh Agriculture Institute and horticulture research Center Gazipur.

There are 16 different viruses have been recorded in Bangladesh on tomato crop among these viruses TYLCV most devastating one, even a single whitefly can transmit it within 30 minutes. They resulted that young seedlings of 20 days were found to be extremely susceptible to the virus. Likewise the results of vector transmission of TLCV were in accordance with the result of Kashina *et al.*, 2007 who worked on insect vector transmission at Tanzania and they made intensive study on the transmission properties of viruses such as acquisition feeding period, inoculation feeding period, virus persistence in the vector, seed transmission and mechanical inoculation of tomato yellow leaf curl virus. They resulted that TYLCV is transmitted only through vector whitefly in persistent manner but it is not transmitted through mechanically and seed of tomato.

Conclusion: Tomato is cultivated in Pakistan and India also in other countries of Asia as well as throughout the world due to its high nutritional value and short duration crop. A number of biotic and abiotic factors affect the quantity and quality of tomato crop. Among the viral diseases TLCVD is very important which is a serious threat to tomato crop. To manage this disease it is very important to control its vector first. For this purpose a number of approaches are used which includes the management through plant extracts, through the nutrients and through the insecticides. Cultural practices, removal of host plant, vector control and crop rotation are also useful approaches against the disease.

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