INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
15–023/2016/18–5–1061–1066
DOI: 10.17957/IJAB/15.0208
http://www.fspublishers.org

Full Length Article

Epidemiological Studies of Tomato Leaf Curl Virus Disease Based upon Environmental Variables

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Abstract

Tomato leaf curl virus (TLCV) is an emerging problem in tomato growing areas of Pakistan. Relationship of TLCV disease and environmental conditions were studied on five tomato cultivars. Temperature (maximum and minimum) and relative humidity significantly contributed in the development of TLCV disease during two years (2012 and 2013). The TLCV disease incidence increased with rise in maximum and minimum temperature and decreased with the increase in relative humidity. The contribution of maximum temperature was explained by linear regression which showed 83 to 91% variability in the disease incidence and minimum temperature explained 75 to 85% variability. Relative humidity exerted 78 to 85% contribution in the disease development. The linear regression could not explain the contribution of rainfall and wind speed in disease development. The characterization of favorable environmental conditions for TLCV disease development would contribute towards accurate prediction and timely management of the disease.

Keywords: Epidemiology; TLCVD; Environment; Tomato; Disease

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is the second most important vegetable crop by production after potato. Tomatoes contribute to a healthy diet by providing rich amount of minerals, essential amino acids, sugars and dietary fibers etc. It comprises of abundant vitamin B, C, iron and phosphorus (Glick *et al*., 2009).

The inferior quality of seed, lack of advanced production technology, poor management practices and the impact of pests and diseases contribute to low tomato yield (Varela, 1995). Tomatoes are generally attacked by different groups of plant pathogens. Among them, viral diseases are more serious than fungal, bacterial or nematodes infestations because viral diseases are difficult to control (Verlaan *et al*., 2013). Tomato is host for several viruses and attacked by tobacco mosaic virus (TMV), tobacco streak virus (TSV), tomato leaf curl virus (TLCV), tomato chlorosis virus (ToCV) and cucumber mosaic virus (CMV) etc. (Hanssen *et al*., 2010).

Among the viral diseases, tomato leaf curl virus (TLCV) has emerged as the most important disease (Chakraborty, 2008) and belongs to the Geminiviridae family that has a circular, single-stranded DNA genome virus with two incomplete icosahedral geminate particles (Pandey *et al*., 2009). It is transmitted by whitefly (*Bemisia tabaci* Genn.) in a persistent and circulative manner (Uchibori *et al*., 2013).

As plant diseases have major economic and environmental concerns, it is necessary to describe their dynamics for sustainable management strategies (Medina *et al*., 2009; Majeed *et al*., 2014). Environmental factors play a vital role in the development of the disease in epidemic form by influencing pathogen and vector population (Khan and Khan, 2000). An epidemic is the progress of disease in time and space. Each epidemic has a structure whose temporal dynamics and spatial patterns are jointly determined by the pathosystem characteristics and environmental conditions (Maanen and Xu, 2003). Environment is an integral component of disease development without the knowledge of which disease management is very difficult (Ghent *et al*., 2013). The characterization of favorable environmental conditions for disease development facilitates farmers and researchers for timely management of the disease (Moral *et al*., 2012). To eliminate unnecessary use of pesticides, precise knowledge of the risk of an epidemic at field level is essential (Dani, 2013).

Due to the heavy losses caused by TLCVD, it is necessary to record the disease incidence with respect to environmental conditions. The main objective of the study was to identify favorable environmental conditions under which the virus causes severe crop losses. The relationship of environmental conditions with TLCVD would provide a base line for developing a simple and reliable disease prediction system to help the farmer to take up plant protection measures more precisely and economically.

To cite this paper: Zeshan, M.A., M.A. Khan, S. Ali, M. Arshad and N. Ahmed, 2016. Epidemiological studies of tomato leaf curl virus disease based upon environmental variables. *Int. J. Agric. Biol.*, 18: 1061–1066

Materials and Methods

Experimental Material

Five susceptible and highly susceptible tomato cultivars i.e. Big Beef, Caldera, Sitara-TS-101, 014276 and Salma were sown in research area Department of Plant Pathology, University of Agriculture Faisalabad, Pakistan. All these genotypes were obtained from Ayub Agricultural Research Institute (AARI), Faisalabad. Each test entry was planted in a row of 3 m length with 30 cm plant to plant and 70 cm row to row distance in augmented design. One row of the most susceptible check (Fanto) was repeated after every two entries in the experiment as a spreader of the virus. These cultivars were sown in spring seasons 2012 and 2013.

Data Recording and Pathogenicity Tests

Disease symptoms appeared in the first week of May and spread of TLCVD in the experimental plot was recorded at seven days intervals till maximum infection (last week of June) was achieved. Plants showing clear symptoms such as curling, yellowing, downward cupping and reduction in size of the leaves (Zhang *et al*., 2008) were counted and percent incidence was calculated. Assessments of disease incidence (%) in the field were backed up by graft inoculation as described by Kashina *et al*. (2007) and whitefly mediated inoculation as suggested by Lapidot *et al.* (1997) in the glass house. The disease incidence (D.I) was calculated by using following formula (Allen *et al*., 1983):

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

Whitefly Mediated Inoculation

For the confirmation of virus, seedlings of all the above mention cultivars were transplanted in pots under greenhouse conditions. All the pots were covered with muslin cloth. Suspected viruliferous whiteflies were collected from the infected plants grown in the field with an aspirator. These whiteflies were released on the three weeks old tomato plants covered with muslin cloth and given an inoculation access period of 2 days. After 10–12 d symptoms were recorded.

Statistical Analysis

The data of different environmental factors such as temperature (maximum and minimum), relative humidity (%), rainfall (mm) and wind speed (km/h) during the growth period of the crop was acquired from the website www.uaf.edu.pk.

The data were statistically analyzed using Pearson's correlation coefficient for disease incidence (%) and environmental factors. Effects of environmental variables (maximum and minimum temperatures, relative humidity, rainfall and wind speed) on disease incidence were determined by correlation analysis (Steel and Torri, 1997). The purpose of correlation was to find out the most appropriate environmental variable for disease development. The data were analyzed using two statistical software packages i.e. Statistix 8.1, IBM SPSS statistics 22. Analysis of variance (ANOVA) and comparison between disease incidence and environmental conditions were made through least significant difference test (LSD at P<0.05). Environmental variables exhibited significant relationship with disease incidence was graphically plotted and critical ranges of environmental variables conducive for TLCVD development were determined.

Results

Analysis of Variance of TLCVD Incidence during Two Years (2012 and 2013)

During both the years (2012 and 2013), the individual effects of variety, week and year were observed highly significant for TLCVD incidence. The two way interaction of variety with week and week with year was also found highly significant. The two way interaction of variety with year was seen non-significant while three way interaction of variety, week and year was significant (Table 1). This showed that the variation of disease incidence was not high with respect to varieties and years.

Overall Correlation of Weekly Environmental Conditions with TLCVD Incidence during Two Years (2012 and 2013)

Overall correlation of environmental conditions with TLCVD incidence was significant during both the years except rainfall and wind velocity (Table 2). The relationship of maximum and minimum temperature was significantly positive with TLCVD incidence on all five varieties. There was significantly negative relationship seen between relative humidity and TLCVD incidence. Rainfall and wind speed were found non-significant in overall correlation with TLCVD incidence.

Year Wise Correlation of Weekly Environmental Conditions with TLCVD Incidence during Two Years (2012 and 2013)

A highly significant correlation was observed between environmental parameters i.e., maximum and minimum temperature, relative humidity, rainfall and TLCVD incidence during both the years while non-significant correlation was observed between wind speed ($r < 0.20$ and $r < 0.22$) and TLCVD incidence during both the years (Table 3).

Table 1: Analysis of variance for TLCVD incidence during two years (2012 and 2013)

SOV	DF	SS	MS	F-value	P-value
Replication	2	0.002	0.0012		
Variety (V)	4	14.908	3.727	138.4	0.001**
Week (W)	5	290.866	58.173	2160.21	0.001**
Year (Y)	1	7.938	7.938	294.77	0.001**
V×W	20	1.964	0.098	3.65	0.001*
V×Y	4	0.162	0.041	1.5	0.21NS
W×Y	5	21.384	4.276	158.82	0.001**
V×W×Y	20	1.026	0.051	1.9	0.018*
Error	118	3.178	0.026		
Total	179	341.428			

*Significant at $P<0.05$;** Highly Significant at $P<0.05$; NS Non-Significant

Table 2: Correlation of weekly environmental conditions with TLCVD incidence during 2012 and 2013

Varieties	Maximum temperature (°)	Minimum temperature (°)	Relative humidity (%)	Rainfall (mm)	Wind speed (km/h)
Salma	0.786**	0.743**	-0.814**	0.511 NS	0.275 NS
	0.002	0.006	0.001	0.090	0.388
014276	0.785**	0.739**	-0.788**	0.510 NS	0.292 NS
	0.002	0.006	0.002	0.090	0.357
Sitara-	0.772**	0.735**	-0.763**	0.510 NS	0.298 NS
TS101	0.003	0.006	0.004	0.090	0.348
Caldera	0.795**	0.743**	-0.809**	0.511 NS	0.266 NS
	0.002	0.006	0.001	0.090	0.404
Big Beef	0.799**	0.745**	-0.816**	0.511 NS	0.245 NS
	0.002	0.005	0.001	0.089	0.442

**Significant at $P=0.05$; NS= Non-significant

Table 3: Year wise correlation of weekly environmental conditions with TLCVD incidence during 2012 and 2013

Environmental variables	2012	2013
Maximum temperature (°C)	0.927**	0.849**
	0.000	0.000
Minimum temperature (°C)	0.847**	0.842**
	0.000	0.000
Relative humidity (%)	-0.895**	-0.945**
	0.000	0.000
Rainfall (mm)	0.649**	0.624**
	0.000	0.000
Wind speed (km/h)	0.197 NS	0.215 NS
	0.063	0.41

*Significant at $P=0.05$; NS= Non-significant

Characterization of Environmental Conditions Conducive for the Development of Disease Incidence on Five Varieties during Two Years (2012 and 2013)

The environmental conditions conducive for TLCV disease development were characterized on five tomato varieties i.e., Big Beef, Caldera, Sitara-TS-101, 014276 and Salma. All the environmental variables significantly contributed in disease development. Significantly positive relationship was found between temperature (maximum and minimum) and TLCV disease incidence (Fig. 1 and Fig. 2). The relationship between relative humidity and TLCVD incidence was significantly negative (Fig. 3). The relationship of TLCV disease incidence was very poor with rainfall and wind velocity (Fig. 4 and Fig. 5). The TLCV disease incidence increased with increase in maximum temperature from 36 to 45^{0}C and linear regression model explained 79 to 85% variability in the disease development (Fig. 1). Correlation of maximum temperature with TLCV disease incidence was found highly significant in variety Salma and it contributed 85% towards disease development. The minimum temperature, ranged from 25 to 32^{o}C significantly correlated with TLCV disease incidence during both the years (Fig. 2). The correlation of minimum temperature was best explained by linear regression model as indicated by higher r values (95%). The minimum temperature explained 84 to 95% of the variability in TLCVD development. The minimum temperature contributed 95% towards disease development in case of line 014276.

Relative humidity had significantly negative influence on TLCV disease incidence and linear regression model explained 78 to 87% variability in disease development as the relative humidity increased, the disease incidence was decreased (Fig. 3). The maximum influence of relative humidity was observed in case of Big Beef where it contributed 87% towards disease development. Rainfall had not significant influence on TLCV disease incidence and polynomial regression explained 47 to 54% of the variability in disease development (Fig. 4). A linear relationship was not found in case of rainfall with TLCVD development as indicated by very low r values (54%). The rainfall explained 54% variability in disease development in case of Sitara-TS-101.The wind speed had non-significant effect in the TLCVD development and its contribution was negligible (Fig. 5). The linear model indicated very low r values. The wind speed exerted maximum influence of about 34% in disease development in case of Sitara-TS-101.

Discussion

Variations in environmental conditions can affect the virus disease epidemic components in different ways, including altering host morphology, physiology and resistance to vectors or viruses, life cycles of vector and pathogen, abundance, reservoirs and inoculum (Jones, 2014).

In the present study, TLCVD incidence was increased with the increase in temperature while decreased with increase in relative humidity and not supported by the previous studies where the relative humidity was directly proportional to the disease incidence (Rai *et al*., 2001).

Temperature influence disease resistance to viruses and also affects host-pathogen interactions at different temperature ranges (Garrett *et al*., 2006). The activities of different resistance genes inhibited at high temperatures due to which disease incidence increased (de Jong *et al*., 2002).

The effect of rainfall on TLCVD incidence was not significant, because of low rainfall during the experimental period. High rainfall resulted in increased relative humidity

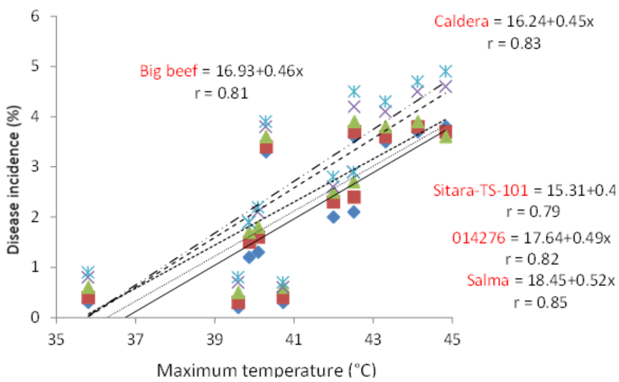

Fig. 1: Relationship of maximum temperature with TLCVD incidence on five tomato varieties i.e. y_1=Big Beef, y_2=Caldera, y_3=Sitara-TS-101, y_4=014276 and y_5=Salma during two years (2012 and 2013)

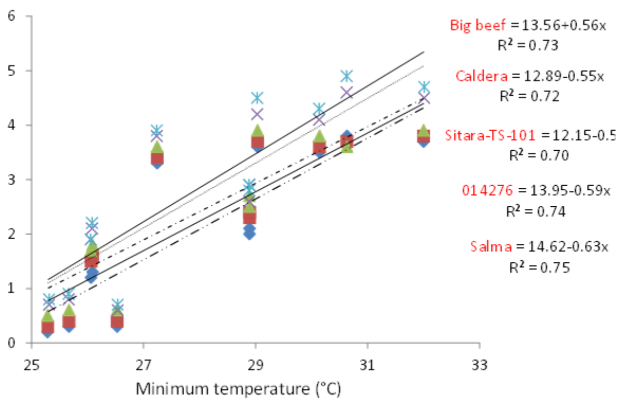

Fig. 2: Relationship of minimum temperature with TLCVD incidence on five tomato varieties i.e. y_1=Big Beef, y_2=Caldera, y_3=Sitara-TS-101, y_4=014276 and y_5=Salma during two years (2012 and 2013)

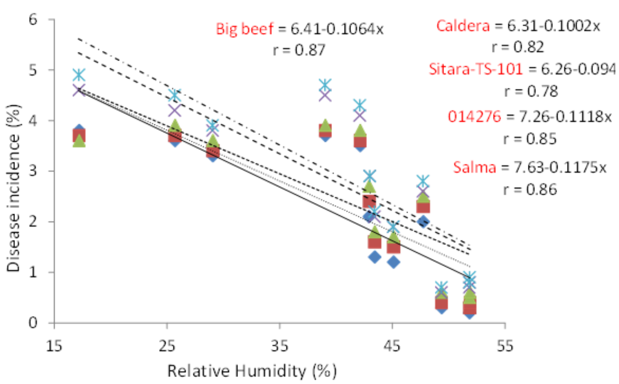

Fig. 3: Relationship of relative humidity with TLCVD incidence on five tomato varieties i.e. y_1=Big Beef, y_2=Caldera, y_3=Sitara-TS-101, y_4=014276 and y_5=Salma during two years (2012 and 2013)

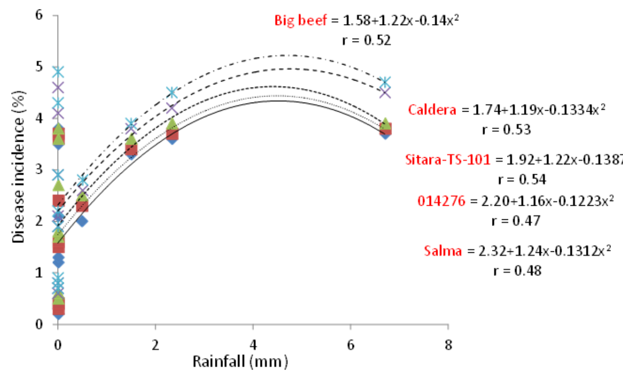

Fig. 4: Relationship of rainfall with TLCVD incidence on five tomato varieties i.e. y_1=Big Beef, y_2=Caldera, y_3=Sitara-TS-101, y_4=014276 and y_5=Salma during two years (2012 and 2013)

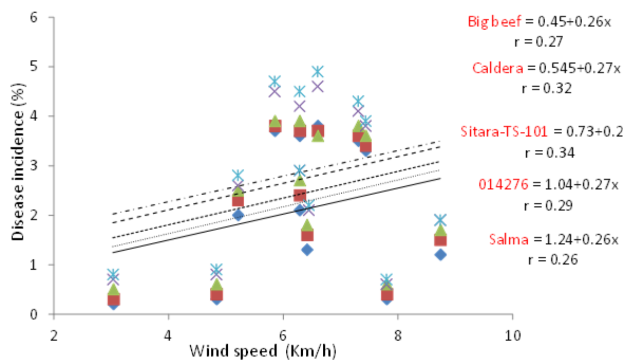

Fig. 5: Relationship of wind speed with TLCVD incidence on five tomato varieties i.e. y_1=Big Beef, y_2=Caldera, y_3=Sitara-TS-101, y_4=014276 and y_5=Salma during two years (2012 and 2013)

due to which activity of whitefly decreased and consequently TLCVD incidence minimized. During the months of low rainfall and high temperature disease incidence increased (Sastry *et al.*, 1978). During hot period, a linear relationship was observed between temperature and disease incidence as symptomatology depends on the temperature and the time of infection (Polizzi and Asero, 1993). These findings could be further strengthened as the seasonal pattern of disease incidence and severity determined in Mediterranean and Middle Eastern countries indicated that disease incidence was highest and symptoms were most severe during the hot and dry summer months but negligible during the cold and rainy winter months (Makkouk and Laterrot, 1983; Ioannou and lordanou, 1985). Disease incidence was high in hot weather despite of low vector population that may be the result of host vulnerability under very high temperature conditions (Yassin, 1983).

Epidemiological studies of insect transmitted viral diseases revealed that sowing time significantly affects the disease. The incidence, severity and rate of spread of the disease were maximum during summer, while minimum during winter. Temperature not only affects the activities of the vector but also virus availability for the vector in plants. Usually, viral diseases appear in more severe form at higher temperatures because virus requires an optimum temperature for its multiplication and symptom development. When temperature varied above or below the optimum temperature, the viral disease incidence also affected (Glasa *et al*., 2003).

The correlation of maximum temperature and relative humidity was negative with CLCuVD (Beniwal *et al.,* 2006). The influence of air temperatures, rainfall and relative humidity on whitefly and MYMV severity was significant when analyzed through stepwise regression (Khan *et al.*, 2006).

In the present study, there was a non-significant relationship between disease incidence and wind speed. Outcomes of the current study were supported by the work of Yassin (1975). In another study, Ali *et al*. (2005) found that minimum temperature and relative humidity had significant correlation with OYVMV disease severity while wind speed showed non-significant relationship.

Conclusion

Five susceptible and highly susceptible cultivars were sown for accurate characterization of favorable environmental variables with TLCV disease development because these categories (susceptible and highly susceptible) are more predisposed to disease as compared to other categories (resistant, moderately resistant and moderately susceptible). As the disease incidence increased with increase in maximum and minimum temperature, in order to avoid from TLCV disease, the dates of crop sowing should be adjusted accordingly. This strategy will reduce the cost of curative sprays.

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(Received 09 January 2015; Accepted 02 July 2016)