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Characterization of epidemiological factors for the whitefly (*Bemisia tabaci* Genn.) population and tomato leaf curl virus disease (TLCVD) incidence on tomato genotypes in Faisalabad, Pakistan

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Abstract
Relationship of epidemiological factors among whitefly (*Bemisia tabaci* Genn.) population and tomato leaf curl virus disease (TLCVD) incidence was studied on six tomato varieties. Epidemiological factors have significant role for the development of whitefly and TLCVD incidence during 2016. Temperature (maximum and minimum) had positively significant relationship for whitefly population and disease incidence. With increase in maximum temperature whitefly population showed 77-99% variability while with minimum temperature it was 66-99% variability. TLCVD incidence showed 84-96% variability with minimum temperature while 86-96% with maximum temperature. Relative humidity had negatively significant relationship with whitefly population and disease incidence. With increase in relative humidity whitefly population and TLCVD incidence decreased. Relative humidity showed 75-96% and 83-93% variability in case of whitefly population and disease incidence respectively. Wind speed and rainfall have very poor relationship with vector population and disease incidence.

Keywords: Characterization, epidemiological factors, TLCVD incidence, significant

1. Introduction
Tomato (Lycopersicon *esculentum* Mill.) is most important crop grown worldwide after potato [2]. Present globally tomato production is about 150 million tones cultivated at 4.6 million hectares. In Pakistan it is cultivated on area of 52.3 thousand hectares with 529.6 thousand tones production [11]. Poor management practices, lack of information and advanced technology, lower class of seed and many fungal, bacterial and viral diseases contribute to the lower tomato production in Pakistan [28].

In Pakistan tomato crop is attacked by many viral diseases such as Tomato Mosaic virus (ToMV), Tomato Yellow Top Virus (TYTV), Potato Virus x (PVX), Cucumber Mosaic Virus (CMV), Tomato Spotted Wilt Virus (TSWV), Tomato Ring Spot Virus (TRSV) and Tomato Leaf curl Virus (TLCV) [14]. Among these viral diseases Tomato Leaf Curl virus Disease (TLCVD) is most devastating disease [14]. TLCV is a complex of virus species including tomato yellow leaf curl virus (TYLCV), tomato tallow leaf curl Malaga virus (TYLCMalV), tomato yellow leaf curl Mali virus (TYLCMV), tomato yellow leaf curl Sardinia virus (TYLCSV) and tomato yellow leaf curl Axarquia virus (TYLCAxV), all producing same symptoms [4]. On the basis of the world worldwide survey, protein and DNA comparison, TYLCV is grouped into three groups stipulating viruses from, Indian/Australian/Far Eastern region, African region/Middle Eastern/Mediterranean and American regions [8].

In the many countries of the old world as well as the in Southeast and East Asia, viruses associated with leaf curl diseases are termed as TLCV or TYLCV [27]. Whitefly- transmitted viruses infecting tomato plants are known as TLCV and have been identified in Indo-Pak and Australia. TYLCV in sub- Saharan Africa, India and Southeast occurs are different strain from the Mediterranean viruses, which are known as Israeli, western Mediterranean, eastern Mediterranean and Sardinia strains [1, 10, 16, 9].

TLCV isolates from the Northern India having two genomic components whereas the isolates from the Taiwan, Southern India and Australia having single genomic component (DNA-A) [15]. The Viral complex in the Australia and India is named as TLCV instead of TYLCV because the viral complex in this region is bipartite whereas TYLCV is monopartite [15].

Tomato yellow leaf curl virus disease (TYLCVD) like symptoms were first time observed in late 1920s in the Israel (Jordan valley) and in 1960s it turned into epidemics [5, 13]. TLCV belong to the genus begomovirus and family Geminiviridae which is transmitted by Whitefly [29]. These viruses have circular *ss*-DNA of about 2.8 kb twinned quasi-isometric geminate virions [6].

Epidemiological factors play significant role for the whitefly population density and TLCVD incidence. Temperature is most important for the growth and development of whitefly and disease incidence [11]. As the temperature increase the vector population and disease incidence increases but rainfall and relative humidity has inverse relation with whitefly population and disease incidence [29].

The main objective of this study was to study the characterization of epidemiological factors for the development of whitefly population and TLCVD incidence on different tomato varieties in Faisalabad district Pakistan.

2. Materials and Methods

This study was carried out in the research area Department of Plant pathology, University of Agriculture, Faisalabad, Pakistan during the months of March to May 2016 to find out the effect of epidemiological factors on whitefly population density and TLCVD incidence on tomato genotypes. Six tomato varieties (Roma, Gola, Moneymaker, Baby Red, Nemador and Nagina) were collected from the Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. These varieties were sown under augmented design during the last week of March 2016. Each variety had three replication with 30cm plat to plant distance and 75 cm row to row distance. A row of Fanto variety (spreader) was sown after every three rows of each line/ verity to ensure the viral inoculum in field. Data of disease incidence was recorded on weekly basis. Percentage of disease incidence was calculated by using following formula

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

To accesses the resistance against disease, a revised Ssekyewa, 2006 scale (disease rating scale) was used.

2.1. Data collection

Data of whitefly population was recorded from the disease screening nursery sown in March 2016 on weekly basis. From each replication of variety three plants were selected randomly and whitefly was counted at upper, middle and lower leaves. The data of epidemiological factors such minimum temperature, maximum temperature, wind speed, rain fall and relative humidity was collected from last week of April 2016 to the end of the May 2016. This epidemiological data was obtained from the meteorological station run by University of Agriculture Faisalabad, Pakistan.

2.2. Data Analysis

The data of this research trail was analysed by using two different software *i.e* Statistics 8.1 and IBM SPSS statistics 22. The data of whitefly population and epidemiological factors was analysed by using Pearson's correlation coefficient. Comparison between whitefly population and epidemiological factors and analysis of variance (ANOVA) were find out by least significant difference test (LSD at $P<0.05$). Impact of epidemiological factors (minimum, maximum temperature, wind speed, rainfall and relative humidity) on whitefly population and TLCVD incidence was access by the correlation analysis [26]. Epidemiological factors showed significant response with whitefly population and TLCVD incidence were graphically plotted and critical ranges of epidemiological factors favourable for whitefly population and TLCVD incidence were determined.

2.3. Pathogenicity Test of Virus

Vector transmission and graft inoculation methods were used for the pathogenicity tests of the virus

2.3.1. Vector Transmission Test

Under greenhouse ten plants of each three tomato varieties (Baby Red, Nemador and Nagina) were sown in earthen pots and were kept in insect free cage to avoid from the insect vector. Nine plants out of these of each variety were inoculated through vector transmission techniques [18]. For the comparison of the results one plant of each verity was kept as control. Twenty Aviruliferous whiteflies were feed on the TLCVD infected plants for 2 days to give them acquisition feeding period. After acquisition feeding period these whiteflies were collected through aspirator and then transferred to the healthy tomato plants grown in earthen pots at 2nd leaf stage for 2 days. After inoculation feeding period of 2 days these plants were sprayed with Imidacloprid (insecticide) to kill the whiteflies. After 7 days of inoculation symptoms were observed.

2.3.2. Graft Transmission Test

Six tomato varieties were sown in the pots under greenhouse condition for the pathogenicity test through graft transmission. Ten plants of each variety were sown and were placed in good condition. All the agronomic practices were performed to maximize the output and best results. For the grafting TLCVD diseased plants were collected from the field. A slanting cut was made on the scion (stem of infected plant) of 0.2cm deep and 2cm long and wedge grafting method was adopted as suggested by [30]. Grafted portion of scion and stalk was wrapped with polythene bags. Non-grafted plants were as control.

2.3.3. Statistical Analysis

To analyse the data two statistical software *i.e* IBM SPSS statistics 22 and Statistics 8.1. The data of epidemiological factors and whitefly population was statistically analysed through Pearson's correlation coefficient. The compression of epidemiological factors among whitefly population, TLCVD incidence and ANOVA (Analysis of variance) were made by least significant difference test (LSD at $P<0.05$). Impact of epidemiological factors (minimum, maximum temperature, wind speed, rainfall and relative humidity) for the development of whitefly population and TLCVD incidence was find out by correlation analysis [26]. The epidemiological factors showed significant relationship with whitefly population and TLCVD incidence were graphically plotted.

3. Results

3.1. Effect of Epidemiological Factors on Whitefly and TLCVD Incidence

The data of whitefly population was recorded on weekly basis from the upper middle and lower leaves of randomly selected plants of each variety. The data of environmental parameters (minimum temperature, maximum temperature, rainfall, relative humidity and wind speed) was recorded up-to six

weeks from 20 April to 31 May, 2016. As the temperature (minimum and maximum temperature) increases the population of whitefly increases but in case of rainfall and relative humidity it decreases. In the case of TLCVD incidence same effect of epidemiological factors was found. Temperature was directly proportional to the disease incidence, as the temperature increase the disease incidence is also increases. With increase in relative humidity TLCVD incidence decreases. The wind speed and rainfall shows non-significant results with the TLCVD incidence.

3.2. Correlation of Epidemiological Factors with Whitefly Population and TLCVD Incidence.

Temperature played important role for the development of whitefly population on all six verities i.e., Roma, Gola, Moneymaker, Nemador, Baby Red and Nagina during 2016 as predictive by the probability at 5% level of significance and the values of correlation of coefficient. The overall correlation of relative humidity was negatively significant wit whitefly population on all six varieties. Wind speed and rainfall had non-significant relationship with whitefly population on all varieties (Table 1). Maximum whitefly average was found on variety Nagina. In case TLCVD incidence temperature has positively correlated with disease incidence while relative humidity was negatively correlated with disease incidence (Table 1). Same results were shown by the wind speed and rainfall as in case of whitefly. The disease incidence was higher at variety Nagin.

Table 1: Correlation of Epidemiological Factors with Whitefly Population and disease incidence

Variety	Max Temp	Min. Temp	RH	RF	WS
Correlation of Epidemiological Factors with Whitefly Population					
Roma	0.4410* 0.02677	0.7938* 0.0163	-0.6075* 0.1158	0.2928 0.0441	0.4929 0.0214
Gola	0.3150* 0.0577	0.8388* 0.0236	-0.0175* 0.2158	0.8238 0.0541	0.8526 0.0149
Moneymaker	0.8644* 0.0019	0.7938* 0.0363	-0.0157 * 0.0058	0.8928 0.0941	0.8295 0.0142
Nemador	0.0410* 0.0277	0.2983* 0.0163	-0.6275 * 0.0158	0.2923 0.0341	0.8729 0.0144
Baby Red	0.3110* 0.0277	0.8838* 0.0362	-0.6570* 0.0058	0.2282 0.0743	0.3459 0.0249
Nagina	0.3410* 0.0077	0.7389* 0.0063	-0.0175 * 0.0058	0.1938 0.0421	0.8459 0.0134
Correlation of Epidemiological Factors with disease incidence					
Roma	0.0410* 0.0267	0.8833* 0.0063	0.0276* 0.0098	-0.8138 0.0414	-0.8853 0.0149
Gola	0.0404* 0.04717	0.7811* 0.0036	0.0229* 0.0812	-0.8198 0.0143	-0.1789 0.1986
Moneymaker	0.5347* 0.0000	0.8899* 0.0131	0.1367 * 0.0010	-0.5675 0.0491	-0.6619 0.2189
Nemador	0.3176* 0.0213	0.3899* 0.0031	-0.6686 * 0.0076	0.1314 0.0129	-0.6129 0.4118
Baby Red	0.2121* 0.0172	0.6612* 0.0162	-0.678* 0.0000	0.9876 0.03769	-0.7742 0.1761
Nagina	0.5872* 0.0000	0.0298* 0.0032	-0.187* 0.0008	-0.6129 0.04761	-0.7792 0.3967

Upper values indicate Pearson's correlation coefficient and lower value indicates the probability value at 5%.
* = Significant when P-value < 0.05
**= Highly Significant when P-value < 0.01
NS= Non Significant when P-value > 0.05

3.3: Characterization of Epidemiological Factors Favourable for the Development of Whitefly Population and TLCVD Incidence

The epidemiological factors favourable for the development of whitefly population. All the environmental parameters subsidised toward the development of whitefly population. There was a positive significant correlation between temperature and whitefly population. The relationship between relative humidity and whitefly population was negatively significant. There was very poor relationship of whitefly with wind speed and rainfall. As the temperature increases the population of whitefly increases. The minimum temperature was 20-35 ^{0}C from the month of April to May 2016 and significantly correlated with whitefly population. The population of whitefly increases with increase in minimum temperature. As one degree increased in minimum temperature, the population of whitefly increased by 0.18, 0.58, 0.35, 2.72, 0.28, and 0.44 percent. Same in case of maximum temperature as one degree increases the population of whitefly increased by 0.06, 0.39, 0.2, 0.21, 0.18, and 0.27 percent (Fig. 1). Wind speed and rainfall showed very poor relationship with whitefly population. Relative humidity was negatively correlated with whitefly population. As one degree increased in relative humidity the population of whitefly was decreased by 0.03, 0.08, 0.02, 3.05, 0.08 and 0.09 percent on all the six tomato varieties Roma, Gola, Moneymaker, Nemador, Baby Red and Nagina. The maximum whitefly was found on Nagina verity as compared to all other varieties (Fig. 1).

In case of TLCVD incidence, the epidemiological factors favourable for the development of TLCVD incidence on six tomato varieties (Roma, Gola, Moneymaker, Nemador, Baby red and Nagina) were characterized during 2016. The relationship among minimum temperature, maximum temperature and TLCVD incidence was found positively significant. As one degree increased in minimum temperature, the TLCVD incidence increased by 1.83, 2.92, 4.87, 6.97, 7.08 and 8.49 percent. Same in case of maximum temperature as one degree increases the TLCVD incidence increased by 0.41, 0.58, 0.96, 1.39, 1.59, and 1.42 percent (Fig. 1). Wind

speed and rainfall showed very poor relationship with the development of TLCVD incidence. Relative humidity was negatively correlated with TLCVD incidence. As one degree increased in relative humidity the disease incidence was increased by 0.55, 0.92, 1.28, 1.75, 1.94 and 2.57 percent on all the six tomato varieties Roma, Gola, Moneymaker, Nemador, Baby Red and Nagina. The maximum disease incidence was found on Nagina verity as compared to other varieties.

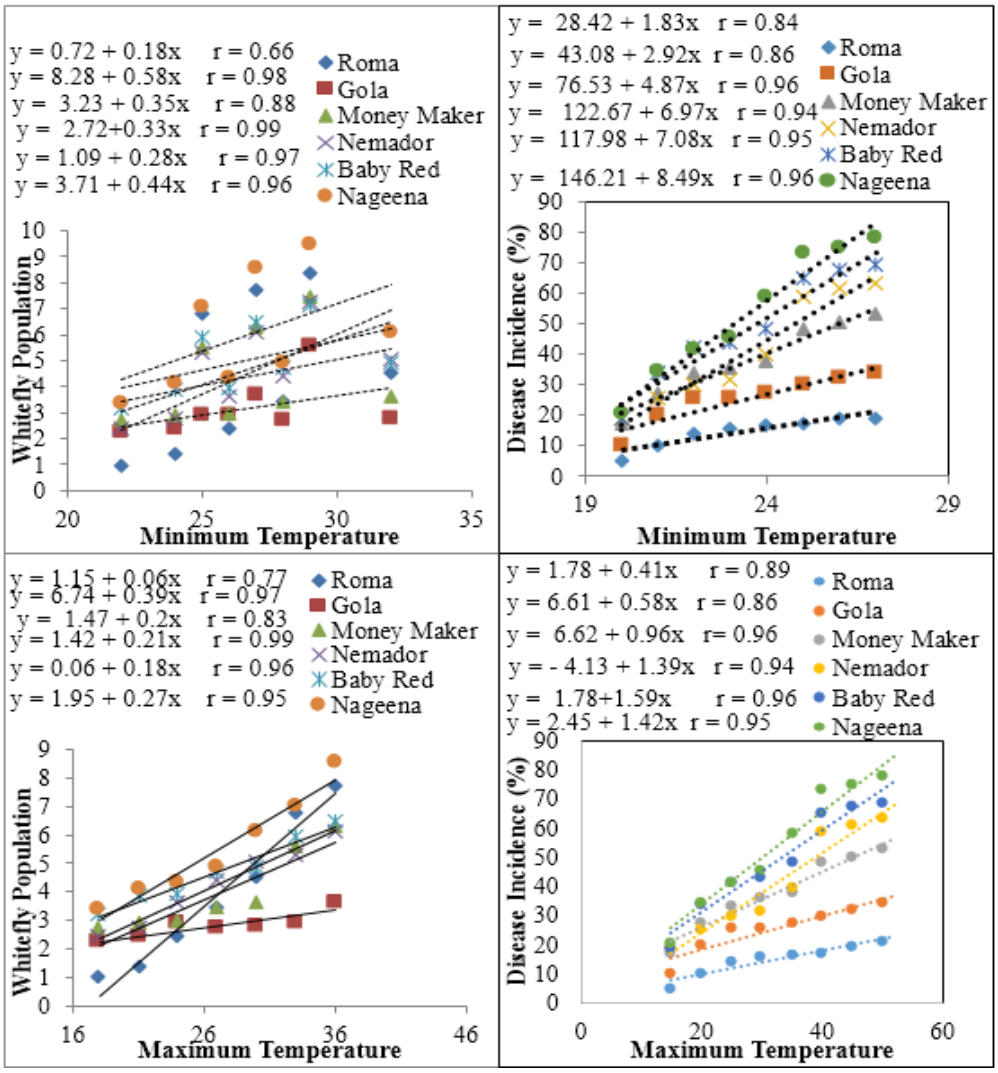

Fig 1: Relationship of temperature (Max & Min) with whitefly population and disease incidence

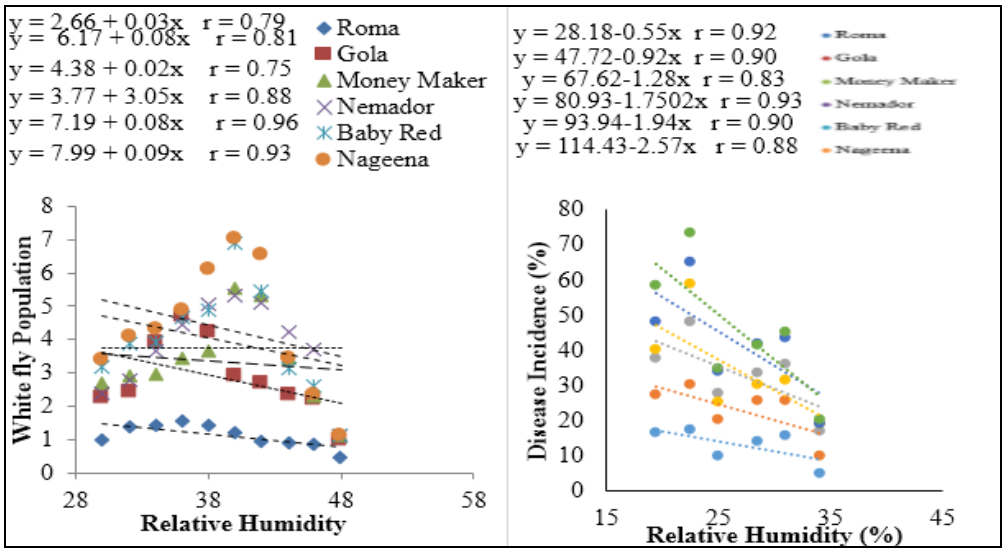

Fig 2: Relationship of relative humidity with whitefly population and disease incidence

4. Discussion

The population of whitefly decreased with increase in relative humidity and increased with increase in temperature. In the semi-arid regions of India the maximum temperature was significantly correlated with whitefly population [13]. Due to increase in temperature the activities of whitefly is enhanced. The mean growth time (days) from egg to adult was 37 at 20 ^{0}C and 20 at 25-30 ^{0}C. The temperature ranges between 25-30 ^{0}C was conducive for the development of whitefly [9]. Sowing and transplanting time is affected the whitefly invasion. Whitefly attacks on tomato during the months of April-November and with high infestation in the months of the August-October whereas tomato crop planted in February is rarely infested with whitefly. In April tomato is severely attacked by the *B. tabici* during the flowering and fruiting stage that causes 40% crop losses [22]. In India environmental factors of TLCVD incidence were studied, result reveled that disease incidence was increased with high temperature and relative humidity. Maximum TLCV infection was observed at 79.73% relative humidity and 25 ^{0}C temperature [21]. As the minimum temperature increase the population of whitefly increases and decrease as the relative humidity increases [3]. During the month of March, inoculative whitefly started to appear and reached to maximum value 7% in July and August in the Tobas district whereas in Jenin district the population of inoculative whitefly was recorded 6% in August [24]. There was negative correlation between wind direction and TLCV incidence in Sudan for five different tomato growing seasons. During 7-10 weeks after planting there was the maximum rate of spread of TLCV in the early stages of growth [27]. In Israel the epidemics of TYLCV was continually recorded in months with mean maximum temperature of 30 ^{0}C and mean relative humidity (RH) less than 60% [19]. Tomato crop planted during the months of July-November are subjected to low temperature, high relative humidity and high rainfall, subsequent low vector population, low TLCD incidence and better yield of crop whereas the crop planted during the months of December-May are exposed to high temperature, low rain fall and low relative humidity which leads to high population of whitefly and high TLCD incidence that causes low yield [23]. In Pakistan during the year of 2010 and 2012, minimum temperature 25-37 ^{0}C, maximum temperature 35-40^0 C and relative humidity 17-51% were the acute environmental range for the *B. tabici* population and TLCVD. These environmental conditions played significant role for the increase of TLCVD and vector population during these two years. There was significant correlation of environmental conditions and whitefly population [29].

5. Conclusion

It is concluded from this experiment that epidemiological factors are conducive for the development of whitefly population and TLCVD incidence. With one unit increase in epidemiological factors disturbs the whitefly population and TLCVD incidence. With increase in temperature (minimum and maximum) cause for the development of whitefly population and disease incidence. Similarly within increase in relative humidity decreases the whitefly density and TLCVD incidence. While wind speed and rainfall have very poor relationship with whitefly population and disease incidence.

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