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MANAGEMENT OF TOMATO LEAF CURL VIRUS AND ITS *BEMISIA TABACI* VECTOR IN RELATION TO PREVAILING ENVIRONMENTAL CONDITIONS OF FAISALABAD

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A B S T R A C T

Tomato (*Lycopersicon esculentum*) production is severely affected by the tomato leaf curl virus (TLCV) and its vector populations under favourable environmental conditions. This disease and its vector *Bemisia tabaci* are effectively controlled by the plant extracts and many chemicals. Therefore, current study was designed in the Research Area of Plant Pathology Research Institute, Ayub Agriculture Research Institute (AARI) Faisalabad following Randomized Complete Block Design (RCBD) during crop seasons 2018-2019. The data for disease incidence were recorded based on disease rating scale proposed by Khalid (2013) and subjected to analysis of variance at a 5% level of significance. Correlation and regression analysis were performed, to determine the relationship between environmental factors and disease incidence. Only two varieties i.e. Naqeeb and Morgal indicated a resistant response against disease development. All epidemiological variables showed a positive association with the disease incidence and *Bemisia tabaci* populations except relative humidity. Maximum disease incidence and whitefly population were observed at the highest temperature (34-41ºC), the lowest temperature (22-24ºC), rainfall (1.4-4.5 mm) and wind speed (3.1-5.8 km/h) whereas, this populations decreased by increasing relative humidity from 15-25%. Among chemicals and plant extracts, acetamiprid and neem proved to be the most effective for the management of disease incidence and whitefly populations, respectively.

Keywords: Disease incidence, meteorological variables, chemicals, tomato.

INTRODUCTION

Tomato (*Lycopersicon esculentum*) belongs to the family Solanaceae is the most cultivated vegetable crop after potato (Knapp and Peralta, 2016). In Pakistan, it is cultivated on 52.3 thousand hectares area and 4.6 million hectares all over the world (FAO, 2018). Its production is influenced by several destructive diseases of nematodes, viruses, bacteria and fungi. Among all these diseases, TLCV is the most devastating viral disease affecting both the quality and quantity of crops (Ramos *et al.,* 2019).

Disease symptoms include the curling of leaves, yellowing, flower abortion, the irregular shape of the fruit, stunted growth, reduction in leaflet area, puckering, crinkling and growth reduction (Kumar *et al.,* 2012). Tomato leaf curl virus (TLCV) is spread from infected to healthy plants through *B. tabaci* which belongs to the family Aleyrodidae and order Hemiptera (Guo *et al.,* 2019). Single whitefly has five minutes acquisition period to acquire the virus from diseased host and transfer it to healthy plants after successful inoculation of 4 to 8 hours. The whitefly has an 8 to 24 hours latent period. The TLCV can be also transferred from diseases to healthy host through grafting (Pandey *et al.,* 2009).

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Weather parameters have important role in the epidemic of TLCV disease incidence and whitefly populations. The TLCV severity increased with an increase in wind speed and temperature whereas, decreased by increasing the humidity and rainfall (Zeshan *et al.,* 2015). The 25-30ºC temperature is considered optimum for the buildup and rapid replication of the whitefly (Khan *et al.,* 2006). The disease predictive models are helpful for site selection, disease prediction in future and the implementation of management practices to protect crop from diseases (Naerstad *et al.,* 2007).

In the country, most of the tomato genotypes are vulnerable to TCLV infection. Therefore, it is important to sort out some suitable and efficient solutions for the effective management of TLCV disease and its vector population. Several cultural techniques such as destruction of residual crop roots, sanitation and avoidance, alternation with non-hosts, and host plant resistance are being used but the application of chemicals and plant extracts gave the best results against TLCV and its vector control (Dhaliwal *et. al.,* 2020). Moreover, to reduce huge yield losses caused by TLCV disease as well as whitefly, the cultivation of resistant genotypes is a prerequisite because it is a simple, efficient, cheap and easy to use method (Achari *et. al.,* 2019). Hence, keeping in view all above mention facts the aim of the present study were (1) to evaluate tomato germplasm in relation to epidemiological factors and (2) the management of TLCV and its vector population through chemicals and plant extracts.

MATERIALS AND METHODS

Establishment of tomato nursery: Seeds of five tomato varieties namely; Nagina, Baby Red, Nemador, Morgal and Naqeeb were obtained from the Vegetable Research Institute, Ayub Agriculture Research Institute (AARI) Faisalabad. The soil with the ratio of 1:1:1 of sand, farmyard manure and clay loam was prepared. The seeds of different varieties were sown in the pots filled with sterilized soil and allowed to germinate for 30 days under controlled conditions. The evaluation of tomato varieties experiment was conducted in the Research Area of Plant Pathology Research Institute, Ayub Agriculture Research Institute Faisalabad during September 2017-2018.

Screening of tomato varieties against TLCV: The 35-45 days old seedlings with 6-7 leaves were transplanted on ridges under natural field conditions by using a Randomized Complete Block Design (RCBD). The Plant–plant, row–row and path–distance was kept at 30, 75 and 60 cm, respectively. All agronomic practices were performed to keep the crop in good condition. The disease occurred naturally and no artificial inoculation was performed.

Growing Tomato Seedlings in Green House: The seeds of three tomato varieties namely Nagina, Nemador and Baby Red were sown on September 27, 2018, in the earthen pots (12.50 Cm ×12.50 Cm) consisting 1:1:1 ratio of farmyard manure, soil and sand. The seeds were covered with compost and placed in the dark and warm (up to 28°C) conditions in a greenhouse. After the emergence of seedlings in 8-12 days, plants were transferred to larger pots under insect free environments for the confirmation of the virus through vector and graft transmission.

Confirmation of TLCV disease through vector and graft transmission: For the vector transmission of TLCV, adults of aviruliferous whiteflies were collected and transferred them in the wooden cage for one hour to acquire the virus. Then whiteflies were placed in the separate wooden cage for one day (24 hours) to complete their latent period. After acquiring the virus, whiteflies were released on the four isolated test plants from each of two genotypes viz. Nagina and Baby Red with the help of Aspirator. The vectors were allowed to complete their inoculation feeding periods of one hour. After inoculation, these were killed by the application of insecticides. The plants were kept in controlled conditions to examine the TLCV disease symptoms. The symptoms appeared after 2, 3, and 4 weeks of vector transmission (Kashina *et al*., 2007).

For the confirmation of the transmission of TLCV by grafting, 8 healthy plants from each of two genotypes such as Nagina and Baby Red were selected to grow in the pots. The scions of TLCV infected plants were obtained from the research area and surface sterilized with distilled water. A wedge-shaped cut of one centimeter on the scion and a slanting cut on the stock were given with the help of scissor so that scion could be inserted easily into the stock. After insertion, these were tied immediately with the polythene ribbon over the union. These plants were kept under greenhouse conditions at 25 ± 5°C and 75% relative humidity for 2 months. The symptoms were recorded for disease assessment.

Data Recording: The data for TLCV disease was recorded on weekly basis by using disease rating scale proposed by Khalid (2013) (Table 1**).** The data of vector populations were recorded in the early morning because during this time whiteflies were inactive and does not move as rapidly as during day time. To record its populations, 3 plants were selected randomly and whiteflies numbers were counted from the lower, upper, and middle leaves on each plant and then mean was taken to determine its total populations on each plant.

Table 1. Evaluation of tomato varieties by using disease rating scale

Sr. No.	Description	Disease incidence (%)	Response
1	No viral symptoms	0-9	Highly resistant
2	Transient and very mild symptoms (up to 25% of leaves curl)	10-20	Resistant
3	Mild disease symptoms and chlorosis on the leaves with no serious effects on the yield and growth	21-35	Moderately Resistant (MR)
4	Intermediate prominent chlorosis with leaves curling and reduction in fruit size	51-65	Susceptible (S)
5	Severe chlorosis leaves curling and rolling with less growth of plants and high reduction in fruits and yield	66-75	Highly susceptible (HS)

Study of epidemiological variables: The data of different weather parameters such as temperature (minimum & maximum), winsd speed, relative humidity and rainfall during the experimental period were collected from the Meteorological Research Station of Ayub Agriculture Research Institute Faisalabad. The weekly mean of these variables were determined and correlated with the TLCV disease and *B. tabaci* populations. The critical ranges conducive for TLCV and its vector populations were also recorded on all five varieties through regression analysis.

Management of TLCV disease incidence and its vector populations: For the management of TLCV and *B. tabaci* two susceptible tomato genotypes such as Baby Red and Nagina were sown in the Research Area of Plant Pathology Research Institute, Ayub Agriculture Research Institute during October 7, 2019, in the Randomized Complete Block Design (RCBD) with six treatments and 3 replications. Two chemicals acetamiprid (T_1) and diafenthiuron (T_2) and 3 plant extracts viz. neem (T_3), garlic (T_4) and onion (T_5) were sprayed with their recommended dose for the control of vector populations (Table 2).The six treatment (T_6) applied was distilled water as a control. The data of TLCV disease and its vector were recorded after every five days intervals. The plant extracts and chemicals were applied in different dates in the month of April and May. The data for TLCV disease incidence and whitefly population was recorded by adopting methods as described earlier.

Table 2. Name of insecticides and plant extracts used for the control of *B. tabaci* populations and TLCV

Common Name	Active ingredient	Manufacturer	Recommended Concentration
Acelan (T_1)	Acetamaprid	FMC	125ml/acre
Polo (T_2)	Diafenthiuron	Syngenta	200ml/acre

Common Name	Botanical Name	Part Used	Recommended Concentration
Neem (T_3)	*Azairachta indica*	Leaves	5ml/L
Garlic (T_4)	*Allium sativum*	Blub	5ml/L
Onion (T_5)	*Allium cepa*	Blub	5ml/L
Control (Distilled water) (T_6)	-	-	-

STATISTICAL ANALYSIS

The data recorded of TLCV disease and its vector populations were subjected to ANOVA, regression and correlation analysis by using the software Minitab ver.17**.** Fisher's Least Significant Difference (LSD) test was used for statistical comparison among treatments (Ali *et al.,* 2017).

RESULTS

Evaluation of tomato varieties against TLCV disease under field conditions: Results showed that Naqeeb, Morgal and Nemador indicated resistant, moderately resistant and moderately susceptible response against tomato leaf curl virus disease incidence, respectively. The genotypes Baby Red and Nagina demonstrated susceptible and highly susceptible response to TLCV disease incidence, respectively. None of the variety showed highly resistant and immune response against TLCV disease incidence (Table 3).

Table 3. Response of tomato varieties against TLCV disease incidence

Sr. No.	Variety Name	Response
1	Naqeeb	Resistant
2	Morgal	Moderately Resistant
3	Nemador	Moderately Susceptible
4	Baby Red	Susceptible
5	Nagina	Highly Susceptible

Confirmation of TLCV disease through vector and graft transmission: It was observed that in vector transmission, whiteflies that feed on the diseased plants transfer more efficiently TLCV disease (60%) as compared to the healthy plants where only 20% plants were infected (Table 4). In graft transmission, 25 and 75 disease incidence was recorded from healthy and disease plants, respectively (Table 5).

Table 4. Vector transmission of TLCV disease

Treatments	Total number of insect feed	Total number of plants used	Disease plants/observed plants	Disease incidence (%)
Healthy plants	9	4	¼	25
Disease plants	9	4	¾	75

Table 5. Graft transmission of TLCV disease

Treatments	Total number of plants grafted	Diseased plants/observed plants	Disease incidence (%)
From healthy plants	8	2/8	25
From Disease plants	8	7/8	87.5

Correlation of meteorological variables with whitefly population and TLCV disease incidence: Three meteorological variables such as temperature (minimum & maximum) and humidity exhibited significant association with the TLCV disease and vector populations. A positive correlation was observed between the temperature (maximum & minimum) and whitefly population and TLCV disease incidence whereas, relative humidity demonstrated negative association with variables. The epidemiological variables namely wind speed and rainfall showed non-significant relationship with the whitefly populations and TLCV disease incidence (Table 6 & 7).

Table 6. Correlation of meteorological variables with white fly population

Sr. No.	Variety Name	Maxi. T. (˚c)	Mini. T. (˚c)	Rainfall (mm)	Wind speed (km/h)	Relative Humidity (%)
1	Naqeeb	0.983**	0.997**	0.433	0.660	-0.930*
		0.008	0.001	0.283	0.170	0.035
2	Morgal	0.963*	0.970*	0.252	0.555	-0.952*
		0.019	0.015	0.374	0.223	0.024
3	Nemador	0.937*	0.955*	0.143	0.318	-0.946*
		0.031	0.023	0.428	0.341	0.027
4	Baby Red	0.989**	0.975*	0.292	0.373	-0.994**
		0.006	0.013	0.354	0.314	0.003
5	Nagina	0.983**	0.991**	0.209	0.496	-0.807
		0.008	0.004	0.395	0.252	0.097

Upper values indicate Pearson's correlation coefficients; Lower values indicate probability of significance level at P = 0.05; * Significant, ** Highly Significant

Table 7. Correlation of meteorological variables with tomato leaf curl virus disease incidence

Sr. No.	Variety Name	Maxi. T. (˚c)	Mini. T. (˚c)	Rainfall (mm)	Wind speed (km/h)	Relative Humidity (%)
1	Naqeeb	0.902*	0.884	0.722	0.849	-0.769
		0.049	0.058	0.139	0.076	0.116
2	Morgal	0.897*	0.866*	0.921*	0.649	-0.969*
		0.042	0.003	0.039	0.175	0.015
3	Nemador	0.885*	0.955*	0.364	0.431	-0.859*
		0.037	0.023	0.318	0.284	0.031
4	Baby Red	0.947*	0.630*	0.815	0.461	-0.994**
		0.026	0.045	0.093	0.270	0.003
5	Nagina	0.904*	0.888*	0.896*	0.273	-0.930*
		0.048	0.046	0.042	0.363	0.035

Upper values indicate Pearson's correlation coefficients; Lower values indicate probability of significance level at P = 0.05; * Significant, ** Highly Significant

Characterization of meteorological variables conducive for white fly population and TLCV disease incidence: All five tomato varieties viz. were Naqeeb, Morgal, Nemador, Baby Red and Nagina were employed to regression analysis to determine the critical ranges of meteorological variables conducive for whitefly population and TLCV disease incidence. A significant relationship was determined among maximum temperature, whitefly population and disease incidence. It was noted that with every one unit increase in maximum temperature from 34-41°C whitefly population (Figure 1) and disease incidence (Figure 2) also increased on all five varieties as indicated by their correlation coefficient (r) values. The impact of minimum temperature on TLCV disease and its vector populations was recorded significant positive. The maximum whitefly population and disease incidence were noted at 22-24ºC minimum temperature on all varieties. This association was best described by the linear regression model as indicated by the 0.80, 0.88, 0.73, 0.78 and 081 r values of whitefly population and minimum temperature (Figure 3) and 0.89, 0.87, 0.71, 0.87 and 0.94 r values of disease incidence and minimum temperature (Figure 4). Wind speed and rainfall indicated a weak relationship with whitefly population and TLCV disease incidence. However, highest vector populations and disease incidence was noted at 3.1-5.8 km/h wind speed and 1.4-4.5 mm rainfall (Figure 5-8), respectively. A negative linear relationship was observed among relative humidity, whitefly population and disease incidence indicating with every one unit increase in relative humidity from 15-25% whitefly population and disease incidence decreased (Figure 9-10).

Management of TLCV disease and its vector populations: The individual impact of all applied treatments was significant (P ≤ 0.05) on vector populations (Table 8) and TLCV disease incidence (Table 9). It was observed that among chemicals and plant extracts, acetamiprid and neem proved the most effective to control whitefly population (1.05) (Figure 11) and TLCV disease incidence (Figure 12) followed by diafenthiuron, neem, garlic and onion as compared to the control.

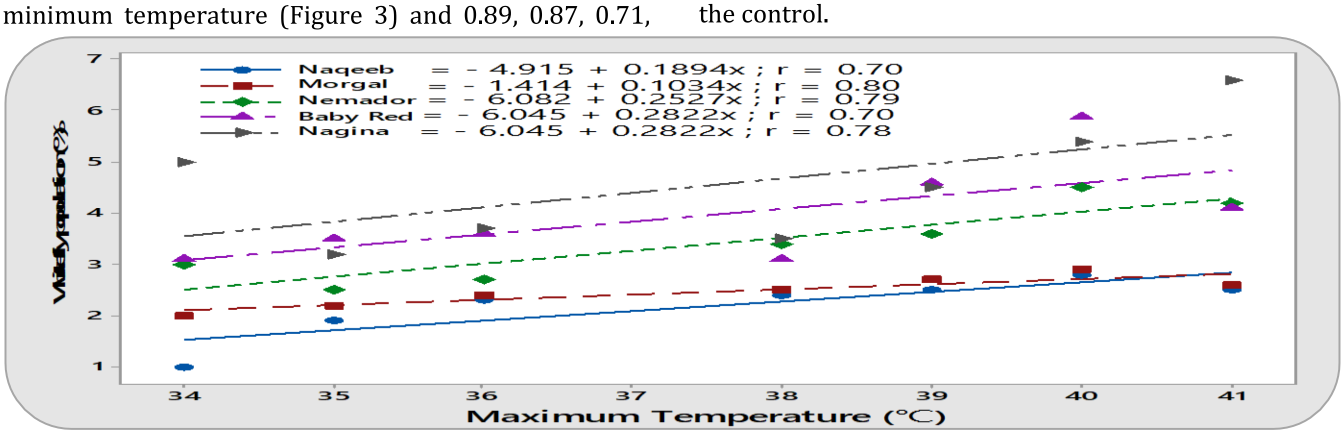

Figure 1. Relationship between maximum temperature and whitefly population on five tomato varieties

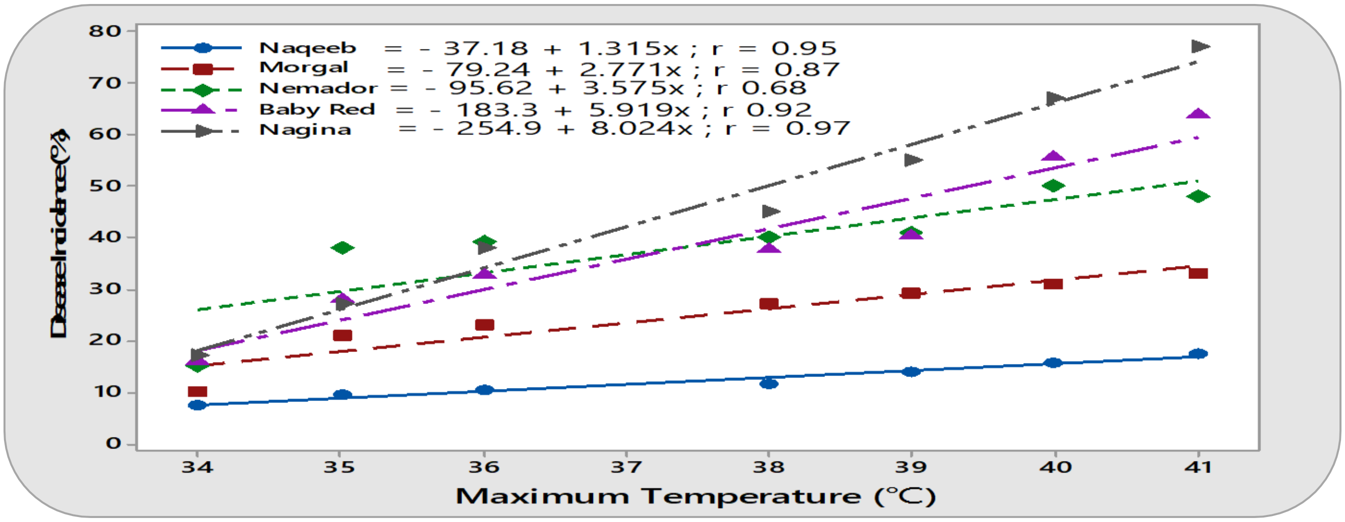

Figure 2. Relationship between maximum temperature and disease incidence on five tomato varieties

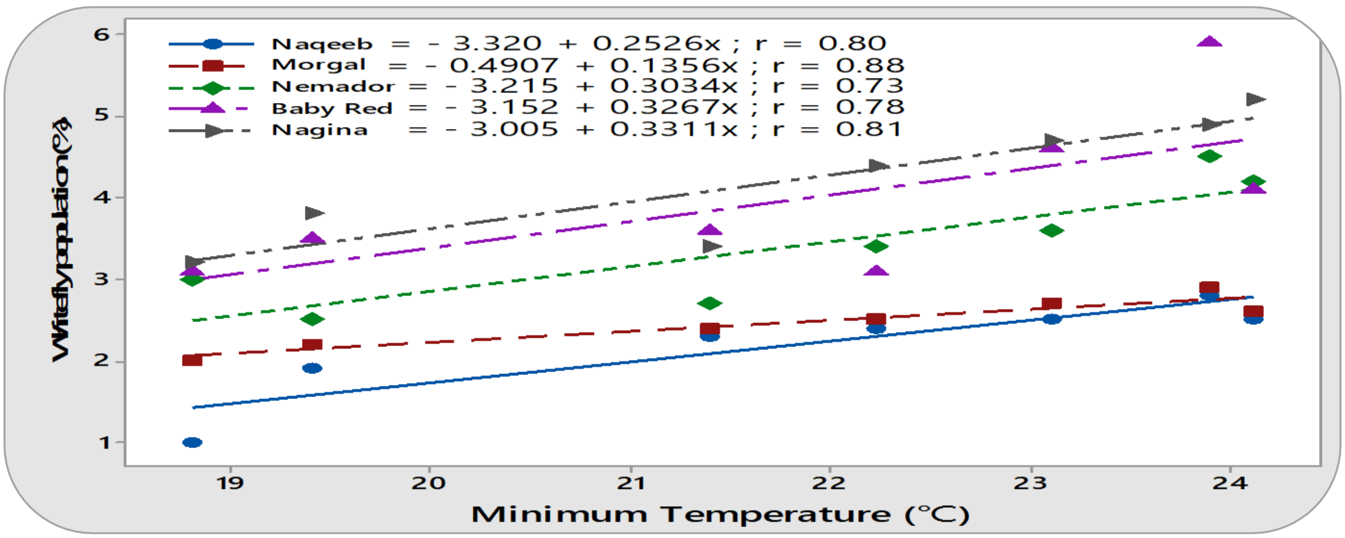

Figure 3. Relationship between minimum temperature and whitefly population on five tomato varieties

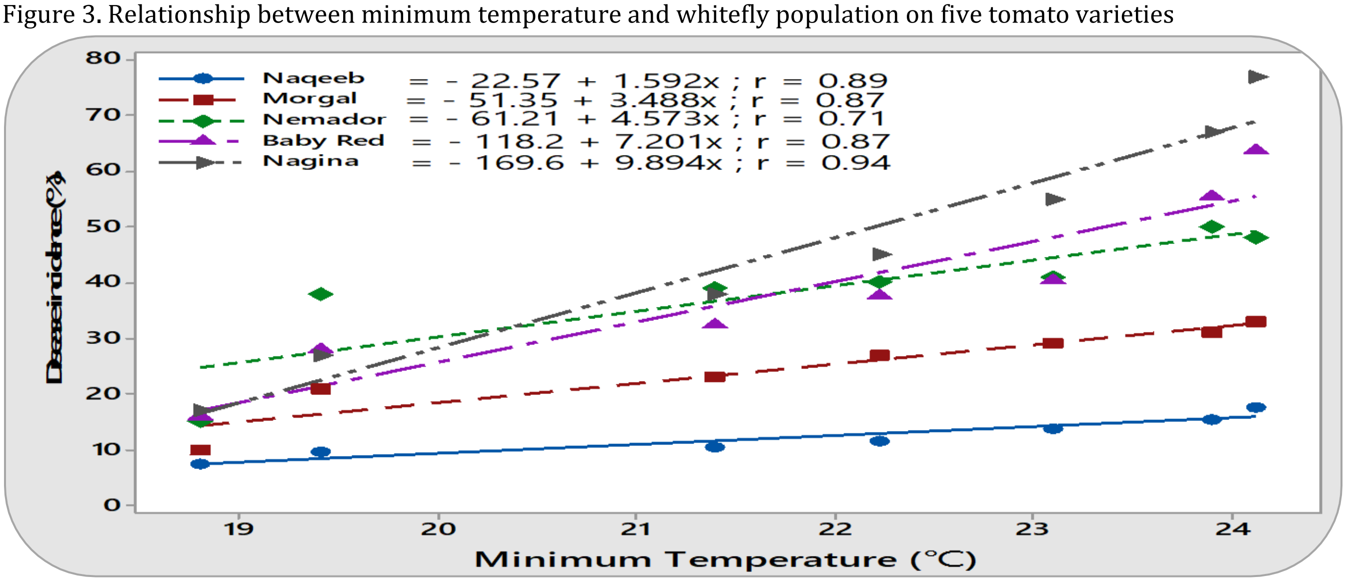

Figure 4. Relationship between minimum temperature and disease incidence on five tomato varieties

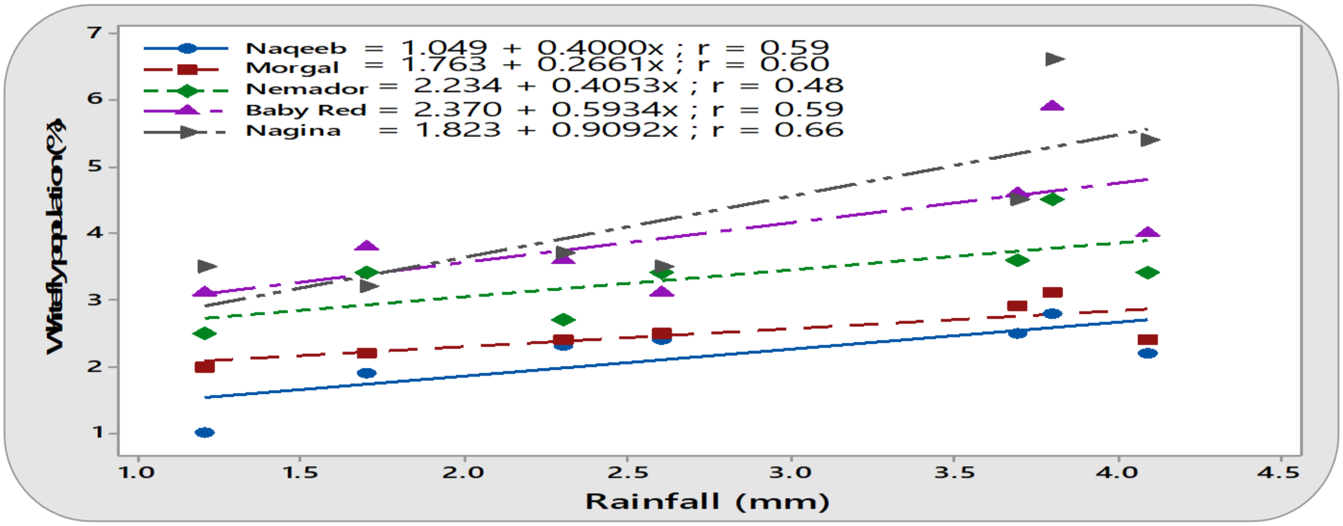

Figure 5. Relationship between rainfall and whitefly population on five tomato varieties

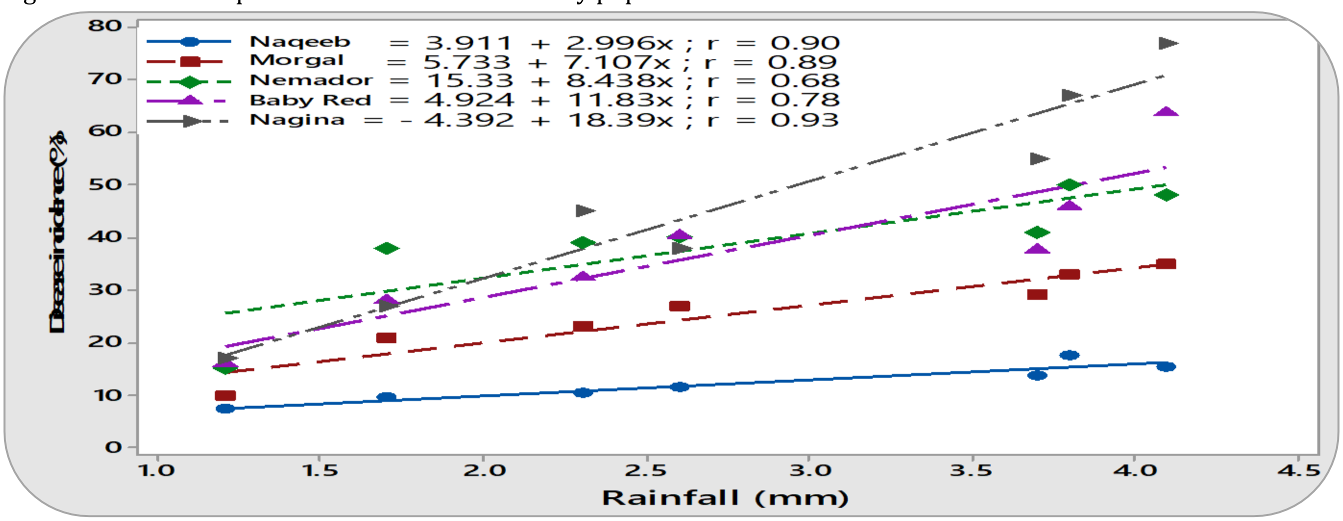

Figure 6. Relationship between rainfall and disease incidence on five tomato varieties

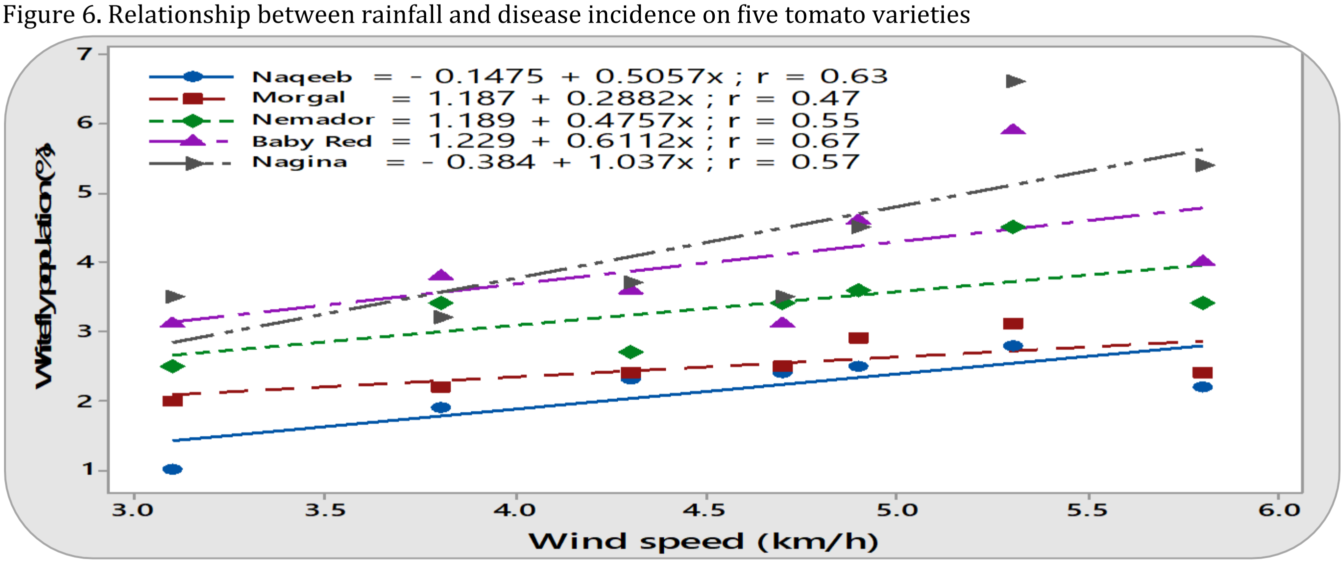

Figure 7. Relationship between wind speed and whitefly population on five tomato varieties viz. Naqeeb, Morgal, Nemador, Baby Red and Nagina

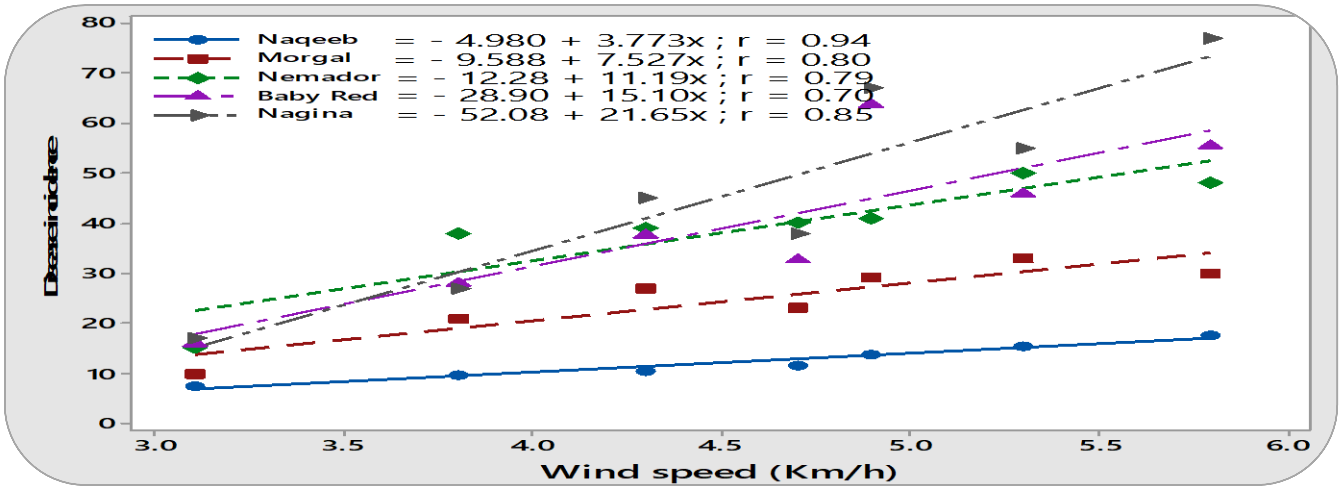

Figure 8. Relationship between wind speed and disease incidence on five tomato varieties viz. Naqeeb, Morgal, Nemador, Baby Red and Nagina

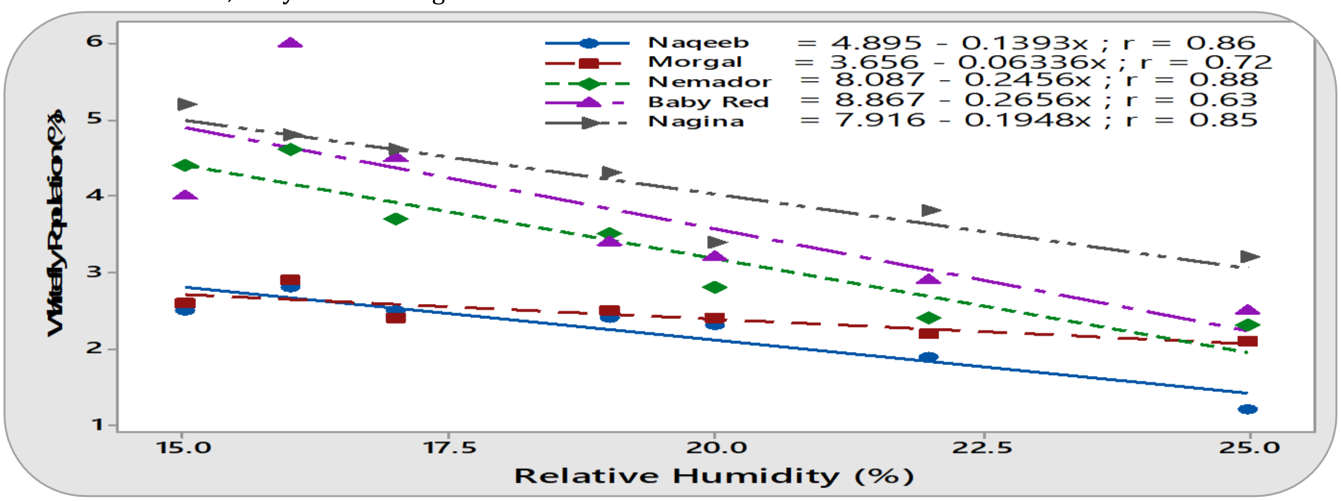

Figure 9. Relationship between relative humidity and disease incidence on five tomato varieties viz. Naqeeb, Morgal, Nemador, Baby Red and Nagina

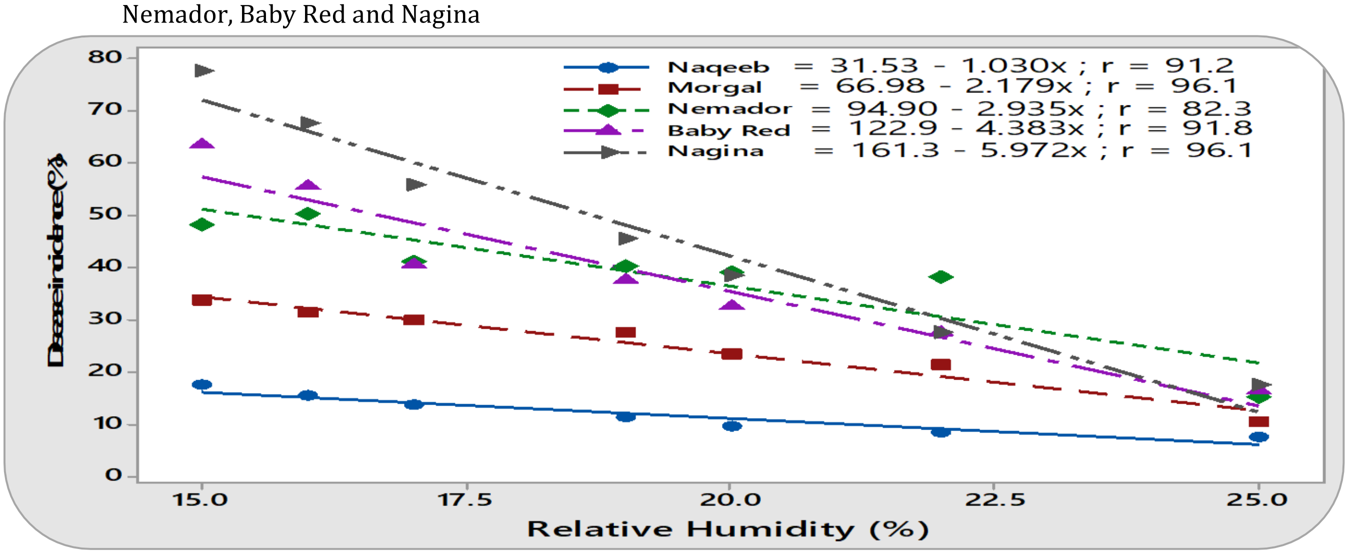

Figure 10. Relationship between relative humidity and disease incidence on five tomato varieties viz. Naqeeb, Morgal, Nemador, Baby Red and Nagina

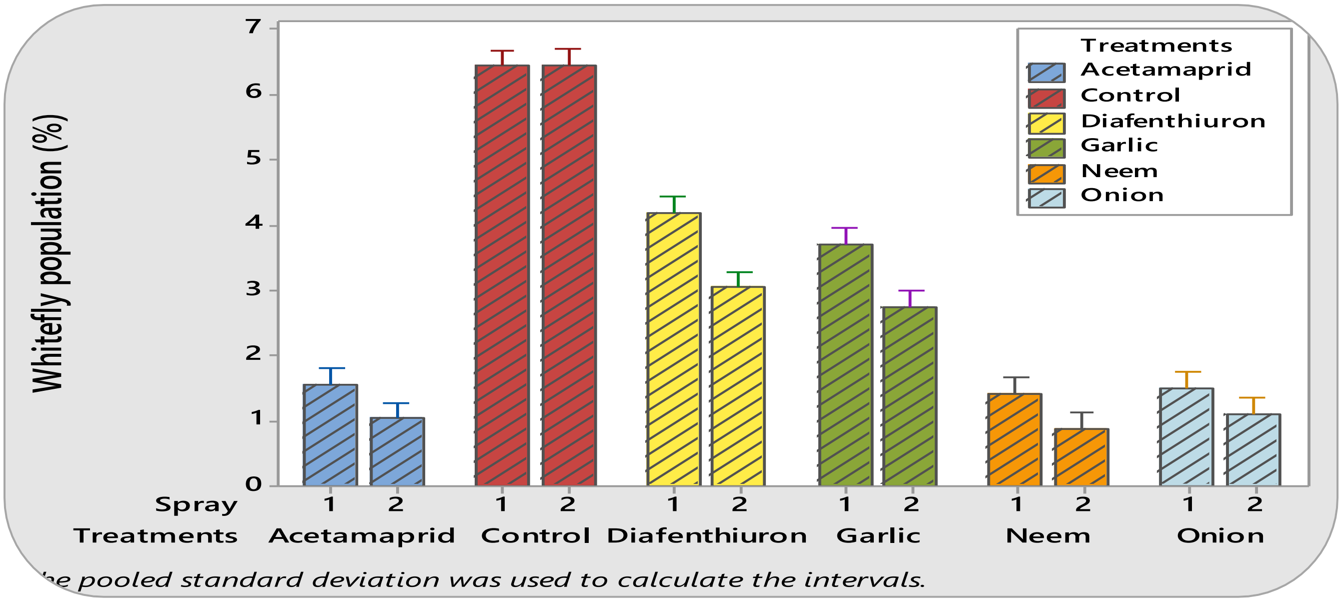

Figure 11. Effects of different chemicals and plant extracts with number of sprayed on whitefly population

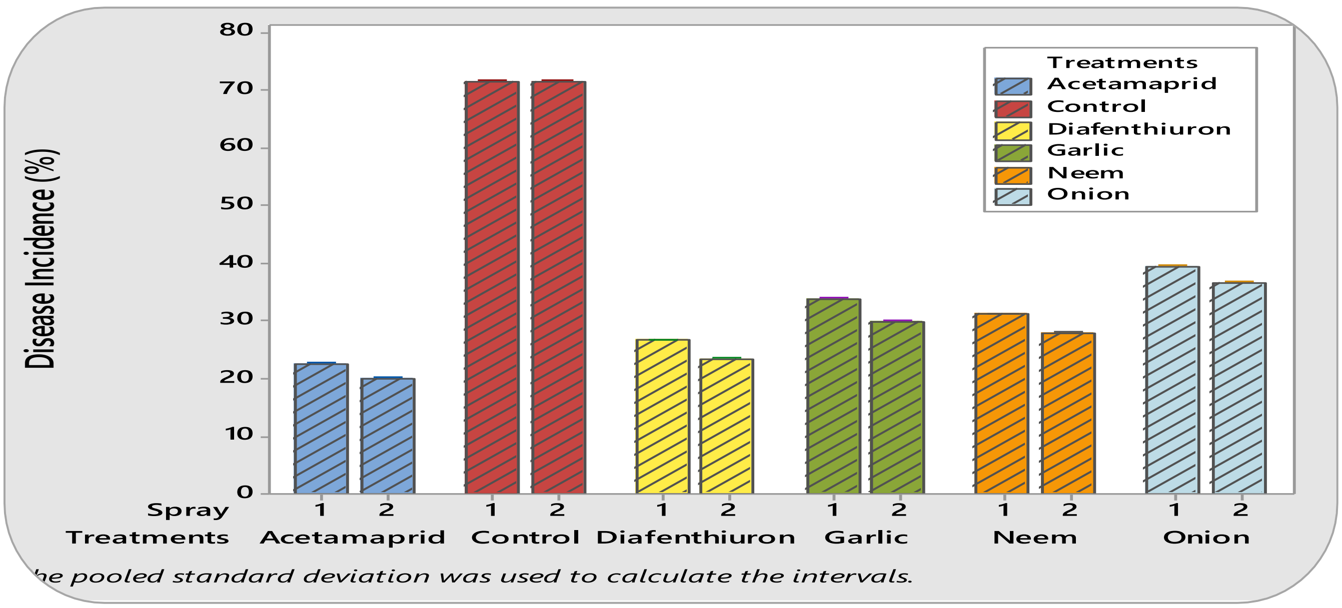

Figure 12. Effects of different chemicals and plant extracts with number of sprayed on tomato leaf curl virus (TLCV) disease incidence

Table 8. Analysis of variance indicating the effect of chemicals and plant extracts on whitefly populations

Source	DF	SS	MS	F-Value	P-Value
Variety (V)	1	0.081	0.0807	2.72[NS]	0.106
Treatment (T)	5	256.476	51.2951	1726.54*	0.000
Spray (S)	1	6.355	6.3546	213.89*	0.000
V*T	5	80.218	16.0436	540.01*	0.000
V*S	1	0.160	0.1596	5.37*	0.025
T*S	5	2.592	0.5185	17.45*	0.000
V*T*S	5	1.239	0.2478	8.34*	0.000
Error	48	48	1.426		
Total	71	71	348.546		

Table 9. Analysis of variance indicating the effect of chemicals and plant extracts on tomato leaf curl virus (TCLV) disease incidence

Source	DF	SS	MS	F-Value	P-Value
Variety (V)	1	2.5	2.46	44.85*	0.000
Treatment (T)	5	19998.4	3999.68	72798.70*	0.000
Spray (S)	1	124.1	124.14	2259.42*	0.000
V*T	5	1.5	0.29	5.28*	0.000
V*S	1	0.0	0.02	0.38*	0.001
T*S	5	27.9	5.58	101.52^{NS}	0.543
V*T*S	5	0.9	0.18	3.31*	0.000
Error	48	2.6	0.05		
Total	71	20157.9			

DISCUSSION

In Pakistan, most of the tomato genotypes are vulnerable to TLCV. Cultivation of resistant varieties is prerequisite for the control of viral maladies. Screening is considered an effective tool by which we can evaluate the tomato germplasm against pathogens and identify a resistant source to reduce the level of disease incidence. The results obtained from screening experiments were in line with the investigation of Zeshan *et al.* (2015) who evaluated 27 tomato lines/varieties for the source of resistance against TLCV disease. Eight lines were found resistant, 6 moderately resistant, 4 moderately susceptible and 9 indicated susceptible to highly susceptible response. Similarly, Hussain, (2016) evaluated ten tomato lines/varieties and showed that one genotype Naqeeb was found to be resistant and VRI-575 moderately resistant to tomato leaf curl virus incidence. Three varieties Morgal, Rio Grande and Thorgal showed susceptible and four varieties Nemado, GSL-198, Baby Red and SBS-292 demonstrated susceptible response against disease development. The grafting experiment showed that TLCV successfully transmitted through grafting in all tomato varieties with a strong difference in disease incidence. Zeshan *et al.,* 2015 also exhibited that, all other attempts to transfer TLCV through nematodes, soil, seed and sap have been failed. The vector transmission of TLCV played a significant role in virus transmission under field conditions. It was observed that among all sixteen plant viruses reported in the Bangladesh TLCV is the most dangerous and its vector *B. tabaci* transfer it from diseased to healthy host within 30 minutes (Rashid *et al.,* 2008). Moreover, TLCV transmitted only through vector *B. tabaci* persistently rather than mechanically or seed of tomato (Kashina *et al.,* 2007).

The epidemiological parameters significantly influence the establishment of pathogens on any crop. Consequently, understanding the role of these parameters with TLCV disease incidence is the important factor for the timely management of the disease. Tomato leaf curl virus disease incidence and whitefly population are significantly influenced by the epidemiological variables. The association of weather parameters with the TLCV and its vector populations were in line with the findings of Zeshan *et al.* (2015) who reported the similar correlation of these variables with the TLCV incidence and whitefly populations. The maximum percentage of TLCV incidence and *B. tabaci* populations were recorded at a higher temperature and lower rainfall and relative humidity. The egg and nymph stages of *B. tabaci* were rapidly developed at maximum temperature ranging from 25-30℃. The rainfall had negative impacts on the whitefly population indicating whitefly population decline after rainfall incident (Aregbesola *et al.,* 2019). Wind speed and wind direction affect the travel of *B. tabaci* to longer distance they have ability to maintain their flights for more than two hours (Isaacs *et al.,* 1999; Ludwig *et al.* 2019). Harshita *et al.* (2019) and Khan, (2019) showed that relative humidity negatively influences the vector populations because with an increase in relative humidity its populations decrease.

Considering the management of disease incidence and whitefly population both the insecticides (acetamiprid, and diafenthiuron) and plant extracts (neem, garlic and onion) proved most effective to control tomato leaf curl virus and its vector. The application of acetamiprid is the most effective strategy to control TLCV and its vector populations in vegetables (Palumbo *et al.,* 2001). The application of insecticides is direct, rapid and easy method to reduce the disease incidence and *B. tabaci* population but it has some environmental hazards and leads to development of resistance in the host plants (Hussain, 2016). The antimicrobial plant extracts significantly reduced the feeding behavior and growth of whiteflies populations (Sabokkhiz *et al.,* 2019). Populations of whitefly, jassid, and thrips were significantly reduced with the application of neem extracts (Khattak *et al.,* 2006). Choudary (2016) demonstrated that neem extract is the most effective against TLCV and whitefly population as compared garlic and onion extracts.

CONCLUSION

It was concluded that environmental conditions of Faisalabad exhibited a significant relationship with tomato leaf curl virus and its vector population except relative humidity. Among chemicals and plant extracts, acetamiprid and neem proved most effective to control TLCV and its vector populations.

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Yasir Ali	:	Prepared figures and help in performing experiment