Impact of Environmental Factors on Tomato Leaf Curl Virus and Its Management through Plant Extracts

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Management of TLCV with plant extracts

In the present study the correlation between TLCV incidence on different lines/varieties and the environmental factors (temperature, relative humidity and rainfall) was conducted. Furthermore, the whitefly population was also correlated with the environmental factors. It was revealed that there exists a strong positive and significant interaction in case of maximum and minimum temperature (8ºC-32ºC) and TLCV incidence but negative and significant interaction was observed in case of relative humidity and rainfall. The whitefly population showed a positive significant interaction with maximum and minimum temperatures prevailing. While whitefly population decreases with an increase in relative humidity and rainfall. It was also studied that the plant extracts (Neem, Bitter melon and Itsit) play a vital role in the management of TLCV in tomato plants. Where it was observed that the Plant extracts of *Azadirachta indica* (Neem), *Momordica charantia* (Bitter melon) and *Trianthema monogyna* linn. (Itsit) at 5% concentration was effective in reducing the disease incidence. However, Neem extract at 5% concentration showed better results as compared to control and other plant extract i.e., Bitter melon and Itsit, in controlling the whitefly population and disease incidence of TLCV.
Keywords: Tomato leaf curl virus, environmental factors, whitefly, plant extracts

Introduction

Tomato (*Lycopersicon esculentum* L.) Karst is one of the most important vegetable after potato, by the virtue of its high nutritive value. Virus diseases are considered one of the most important problems affecting tomato production in many countries and act as a strong limiting factor (Daniela et al. 2009; Murad et al. 2009; Hanseen et al. 2010). More than 20 viruses are known to infect tomato around the world and losses up to 20-90 % by different viruses have been reported (Hameed 1995). Heavy losses were observed due to the lack of antiviral products; control strategies rely on genetic resistance or hygienic measures, or on eradication of diseased crops to control such diseases (Hanseen et al. 2010). At least seven viral diseases are known to be present in Pakistan; tomato leaf curl virus (TLCV), Tomato mosaic virus (ToMV), potato virus X (PVX), Cucumber mosaic virus (CMV), tomato yellow top virus (TYTV), tomato spotted wilt virus (TSWV) and tomato ring spot virus (TRSV) (Mughal 1985).

Tomato leaf curl (TLCV) is one of the most devastating viral diseases of cultivated tomato in tropical and subtropical regions worldwide, and losses of up to 100% are frequent. In many regions, TLCV is the main limiting factor in tomato production (Moriones and Navas-Castillo 2000). Tomato leaf curl virus is the most important constraint for tomato production in Pakistan causing 30-40% yield losses (Tariq 1999). Most of the tomato varieties are susceptible to tomato leaf curl virus. TLCV is transmitted by the whitefly *Bemisia tabaci* and fails to infect plants when inoculated mechanically (Akad et al. 2004; Bosco et al. 2004). The symptoms of TLCV on infected plants include upward or down ward curling of leaves, smalling of leaves, vein thickening, and stunting of the whole plant (Ridgen et al. 1993). An average incidence of 29.79% of TLCV was recorded on tomato leaves (Khan 1997). It affects all the stages and cause 40–90% loss depending on stage of crop and time of infection (Dasgupta et al. 2003; Ansari et al. 2007)Environmental factors play an important role in the development and spread of disease. Good insight into the biology and epidemiology of these viruses is critical for the development of suitable control strategies (Hanseen et al. 2010).

Accordingly the objectives of present study were to determine correlation between environmental factors and TLCV incidence and also the effect of environmental factors on whitefly population. Secondly, an ecofriendly management of its vector (whitefly) and TLCV through different plant extracts.

Materials and methods

Disease incidence of TLCV on tomato

Four lines of tomato (VRI-5, VRI-11, VRI-15 and VRI-20) were taken from the vegetables research institute, AARI, Faisalabad, Pakistan. These were sown in the research area of Department of Plant Pathology, University of Agriculture, Faisalabad. The plant to plant distance was maintained 30 cm and row to row distance was

maintained 70 cm. The disease incidence was determined by using the following formula.

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

Disease severity of TLCV and whitefly population data

The data of disease severity was taken using disease severity scale reported by Banerjee and Kalloo (1987). Conformation of tomato leaf curl virus was done through transmission of virus from infected to healthy plants by side grafting method and transmission of virus through its vector i.e., whitefly. The data of whitefly population was recorded early in the morning. Three plants from each plot were selected at random and populations of whitefly were recorded from upper, middle and lower leaves per plant.

Correlation of environmental factors with TLCV and whitefly

The data of different environmental factors (Maximum temperature, minimum temperature, relative humidity and rainfall) during the experimental period was obtained from the Department of Crop Physiology, University of Agriculture, Faisalabad. The weekly averages of these parameters were calculated and correlated with disease severity and whitefly population. The data was analyzed statistically (Steel et al. 1997)

Management of whitefly and TLCV with plant extract

For the management of tomato lines/varieties against TLCV, seedlings of three varieties (Money maker, VRI-20, VRI-19) were planted in pots. The experiment was carried out in completely randomized design (CRD) with three replications. The three treatments were randomized on varieties and replications. The data of whitefly population was recorded early in the morning 24 hrs before spray and then 48 hrs after spray. Three plants from each pot were selected at random and populations of whitefly were recorded from upper, middle and lower leaves per plant. Extract at 5% concentration of *Azadirachta indica* (Neem), *Momordica charantia* (Bitter melon) and *Trianthema monogyna* linn. (Itsit) were prepared in the laboratory. Extracts were prepared by following method reported by Hilji et al. (2003) with slight modification as grinding leaves of these three plants in equal quantity of water in a grinding machine. The juice was filtered through muslin cloth in three separate jars one for each extract and the jars were labeled. 50 ml extract was mixed in distilled water to make volume up to 1000 ml to make 5% plant extract solution. These extract sprayed on tomato varieties to manage the disease and control whitefly population. A control was kept on which only tap water was sprayed.

Results and discussion

Four tomato lines were sown in the field (VRI-5, VRI-11, VRI-15 and VRI-20). The data of disease severity, disease incidence and whitefly population was recorded from these lines. Two Lines (VRI-5 and VRI-15) showed mild resistance against leaf curl virus. The line VRI-11 appeared to be moderately susceptible, whereas the line VRI-20 appeared to be highly susceptible to TLCV. The conformation of tomato leaf curl virus was done through transmission of virus from infected to healthy plants by side grafting method and transmission of virus through its vector i.e., whitefly.

Correlation of environmental factor with TLCV

The correlation of maximum temperature with diseases severity of TLCV was found to be positive in all four lines. There was increasing trend in TLCV disease development with maximum air temperature 20ºC-32ºC and it was explained by linear regression as indicated by r values 0.89, 0.84, 0.87 and 0.82 for the four lines (Fig.1). Similarly, in Fig. 2. it was clear that with the increase in minimum temperature from 11ºC -17ºC. The diseases severity also increased as explained by r values 0.70, 0.94, 0.91 and 0.96. The relative humidity was negatively significant with disease severity as indicated by r values -0.71, -0.26, -0.33 and -0.22 (Fig. 3). The rainfall was also negatively correlated with diseases severity of TLCV (Fig. 4).

Relationship of maximum and minimum temperature, relative humidity and rainfall with whitefly population

The relationship of environmental parameters with whitefly population on all the varieties was significant. There was positive correlation of temperature either maximum or minimum with whitefly population and explained by linear regression as indicated by r values 0.73, 0.85, 0.83, 0.86 and 0.81, 0.94, 0.83, 0.85 for maximum and minimum temperature, respectively (Fig. 5 & 6). The correlation of whitefly population with relative humidity was negatively significant on all the varieties as indicated by r values -0.74, -0.70, -0.88 and -0.86 (Fig. 7). The rainfall was also negatively correlated with whitefly population i.e., the increase in rainfall causes decrease in whitefly population as explained by r values -0.18, -0.25, -0.48 and -0.49 (Fig. 8). These results were in accordance with the findings of Hassan et al. (1993) who studied the epidemiology of tomato viruses. He found

that the initial and primary source of tomato virus infection was attributed to vector whitefly, aphid and different species of leaf hoppers.

Management of TLCV on tomato through plant extract

Statistical analysis had revealed significant results between different plant extracts and varieties. The individual effects of all the plant extracts were significant, while the effect of varieties and the interaction between varieties and extracts was non-significant. The effect of time of application of plant extracts on disease severity was significant and the two way interaction between extracts and time of application of plant extracts was non-significant. The two way interaction between varieties and time of application of plant extracts was significant and the three way interaction of time of application of plant extracts, plant extracts and varieties was also non significant (Table 1).

The diseases severity of TLCV was calculated on the basis of disease rating scale on Money maker, VRI-19 and VRI-20. Neem extract had lower mean value (1.85) suggested that this plant extract was more effective than Bitter melon and Itsit having mean value of 2.53 and 3.48, respectively as compared to control having mean value 4.500. This extract showed more significant results after 2nd fortnight of March (Table 2) and more significance response had given by Money maker after extract application having low mean value of 3.05 as compared to other varieties VRI-20 and VRI-19 having mean value of 3.09 and 3.12, respectively (Table 3). In the three way interaction of varieties, plant extract and time of application of plant extract , it indicated that using neem plant extract disease severity were 1.66, 1.00 and 1.67 on money maker, VRI-20 and VRI-19 respectively. Similarly, by Bitter melon extract at 5% concentration disease severity were 2.50, 2.16 and 2.00 on Money maker, VRI-19 and VRI-20 by the application of itsit extract at 5% concentration the diseases severity were 3.66, 3.33 and 3.00 on Money maker, VRI-19 and VRI-20. In case of control, maximum disease severity was 4.66, 4.33 and 4.16 on Money maker, VRI-19 and VRI-20. From the results, it was concluded that the disease severity by TLCV was higher in untreated control followed by It sit, Bitter melon and Neem. The most effective plant extract against TLCV on tomato was Neem extract (Table 4).

Effect of plant extracts on whitefly population

The results showed that the individual effect of all the plant extracts, varieties and dates was significant on whitefly population. The interaction of varieties and dates was also highly significant but the interaction of extracts and varieties was not significant (Table 5). Average number of whitefly per plant was significantly higher in untreated control having mean value 4.40 as compared to others where plant extract Neem, Bitter melon and Itsit applied having mean values 1.79, 2.66 and 3.29 respectively on Money maker, VRI-20 and VRI-19. The most effective treatment was Neem extract followed by Bitter melon extract and itsit extract in the order of effectiveness (Table 6). The best response are given by Money maker having mean value 2.50 as compared to VRI-20 and VRI-19 after 3rd application of plant extracts on whitefly population (Table 7).

Interaction between time of application of plant extracts, plant extracts and varieties showed significant results. Among the plant extracts and the time period the last date of application of extracts showed better results in controlling the disease and whitefly population. It indicated that Neem extract at 5% concentration the average whitefly population per plant were 1.00, 2.16 and 2.33 on Money maker, VRI-19 and VRI-20, respectively. Similarly, by Bitter melon extract at 5% concentration whitefly population were 2.67, 2.83 and 3.00 on Money maker, VRI-19 and VRI-20. By the application of itsit extract at 5% concentration the whitefly population were 3.33, 3.33 and 3.00 on Money maker, VRI-19 and VRI-20. In control, maximum whitefly population was 4.00, 4.33 and 4.00 on Money maker, VRI-19 and VRI-20 (Table 8).

The results in respect of whitefly population are also in accordance with the results of Rao et al. (1990), Nandihalli et al. (1991) and Aslam (2000). According to them, whitefly population was significantly reduced by using botanical insecticides, especially Neem based products, like Neemark, Neem guard and Neem oil. Dimetry et al. (1996) the bioactivity of different formulations of Neem seed extracts against the whitefly *Bemisia tabaci* (Genn.) were assayed in semi field trials during 1992. Also, the different treatments reduced the population density of the adult whiteflies compared with the control.

Conclusion

In the present study there was a significant effect of environmental parameters (temperature, relative humidity and rainfall) on TLCV severity and whitefly population. Correlation of temperature with disease severity of TLCV on all the varieties was positive showing that disease increases with the increase in temperature. The correlation of relative humidity with disease severity of TLCV was negative showing that disease increased with the decrease in relative humidity. Rainfall was also negatively correlated with TLCV severity on tomato lines. Whitefly population increased with the increase in temperature either maximum or minimum. While the effect of relative humidity and rainfall was negative. Whitefly population increased with the decrease in relative humidity and rainfall. These finding can be used to develop a disease forecasting model to manage disease economically.

The plant extracts applied on tomato proved to be helpful in reducing the disease. The most effective plant extract against TLCV on tomato was Neem extract at 5% concentration. Among the plant extracts and the time period the last date of application of extracts showed better results in controlling the disease and whitefly population. These results will help in environment friendly management of the TLCV and whitefly.

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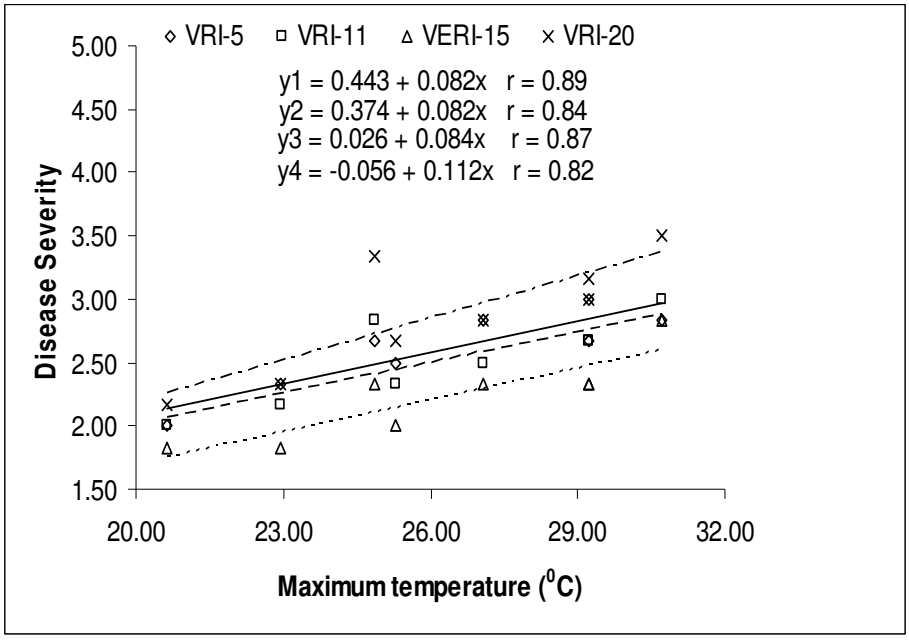

Fig. 1. Relationship of maximum temperature with disease severity of TLCV.

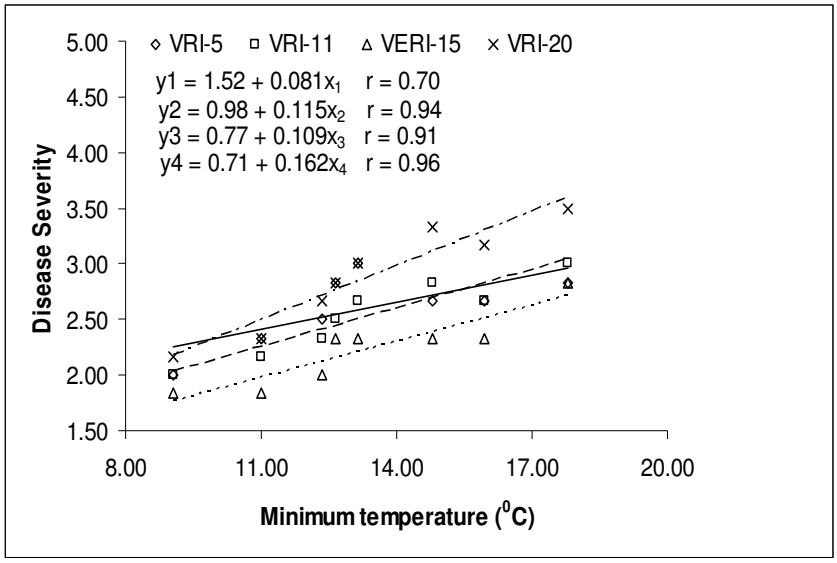

Fig. 2. Relationship of minimum temperature with disease severity of TLCV.

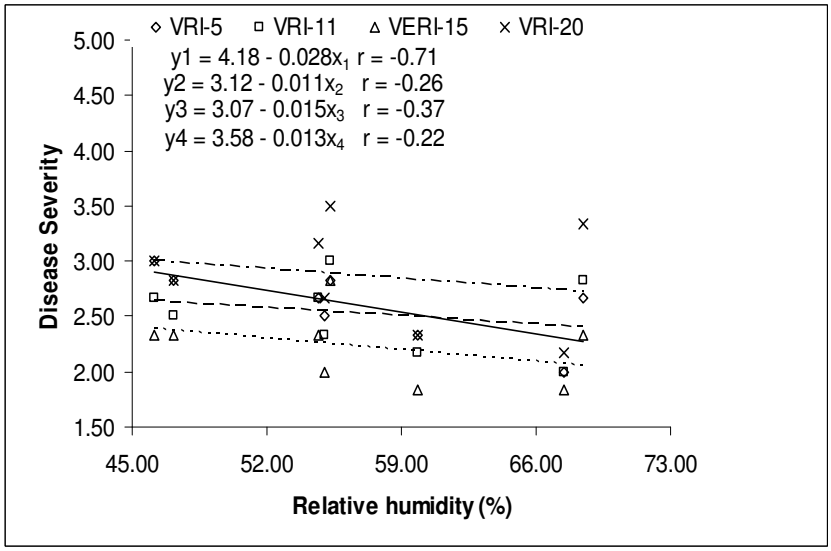

Fig. 3. Relationship of relative humidity with disease severity of TLCV.

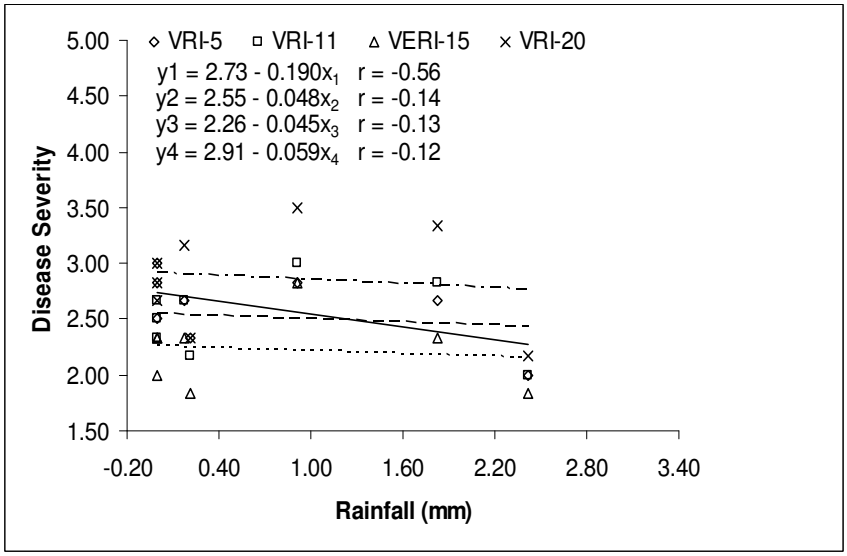

Fig. 4. Relationship of rainfall with disease severity of TLCV.

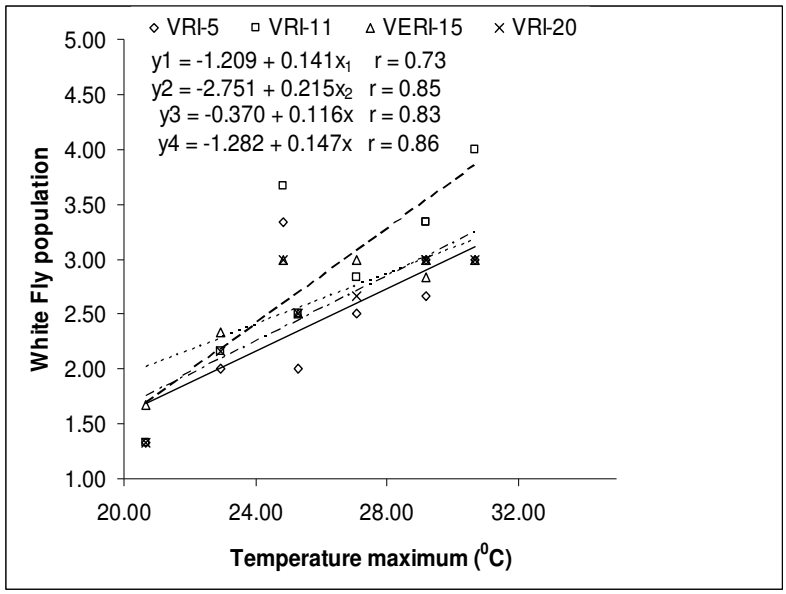

Fig. 5. Correlation of maximum temperature (ºC) with whitefly population on tomato plants.

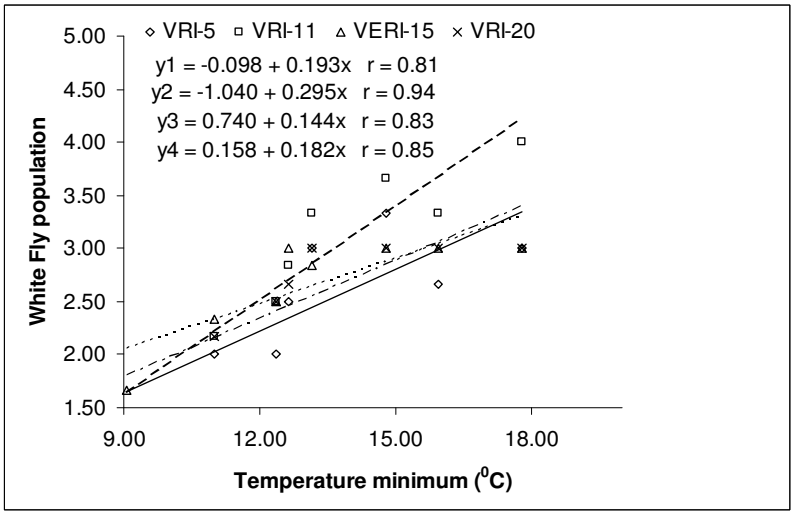

Fig. 6. Correlation of minimum temperature (ºC) with whitefly population on tomato plants.

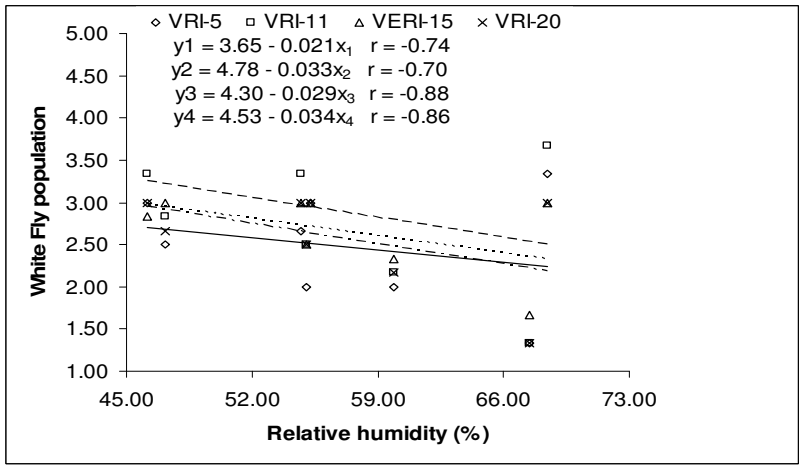

Fig. 7. Correlation of relative humidity (%) with whitefly population on tomato plants.

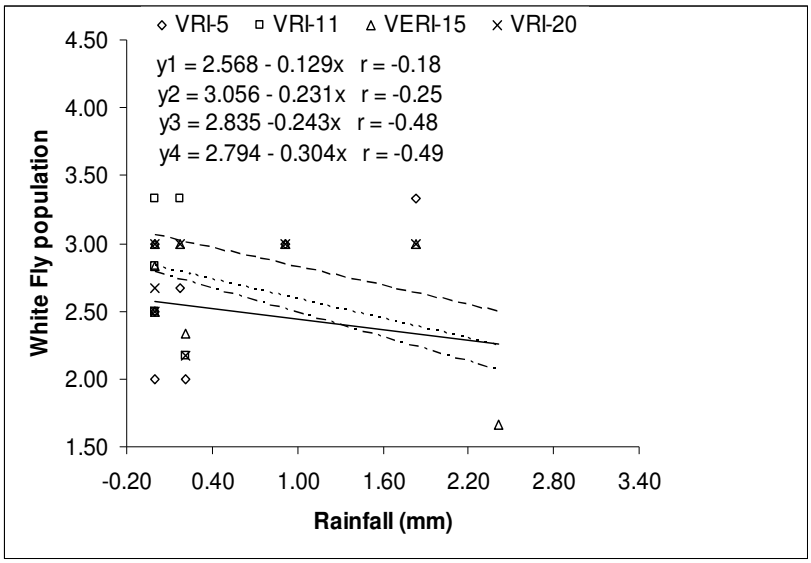

Fig. 8. Correlation of rainfall (mm) with whitefly population on tomato plants.

Table 1. Analysis of variance of TLCV Disease Severity on tomato including the two way interaction between varieties and time of application of plant extracts and the three way interaction of time of application of plant extracts, plant extracts and varieties .

Source of variation	DF	SS	MS	F-value
Replications	2	1.060	0.530	3.3919
Extracts (E)	3	107.463	35.821	229.2058**
Varieties (V)	2	0.088	0.044	0.2814NS
Interaction (E x V)	6	0.523	0.087	0.5579NS
Dates (D)	2	7.032	3.516	22.4989**
Interaction (E x D)	6	1.579	0.263	1.6836NS
Interaction(V x D)	4	2.704	0.676	4.3250**
Interaction(E x V x D)	12	1.685	0.140	0.8986NS
Error	70	10.940	0.156	
Total	107	133.074		

**= Highly significant NS= Non significant

Table 2. Comparison of mean values of different plant extracts and time of application of plant extracts on disease severity of TLCV on tomato.

		Time of Application of plant extracts			Means
		01-03-2009	15-03-2009	30-03-2009	
Extracts	Neem	1.889	2.222	1.444	1.852 d
	Bitter melon	2.444	2.944	2.222	2.537 c
	It Sit	3.167	3.944	3.333	3.481 b
	Control	4.444	4.667	4.389	4.500 a
Means		2.986 b	3.444 a	2.847 b	

LSD (E) = 0.2144
LSD (D) = 0.1857

Table 3. Comparison of mean values of time of application of plant extracts, interaction of time of application of plant extracts and varieties (on disease severity of TLCV

		Varieties		
		Money maker	VRI-20	VRI-19
Time of Application of plant extracts	01-03-2009	2.792 cd	3.042 bc	3.125 b
	15-03-2009	3.250 ab	3.542 a	3.542 a
	30-03-2009	3.125 b	2.708 d	2.708 d
Means		3.056	3.097	3.125

LSD (V x D) = 0.3216

Table 4. Comparison of mean values of interaction of extracts, varieties and time of application of plant extract on disease severity of TLCV.

		Varieties								
		Money maker			VRI-20			VRI-19		
		Time of Application of plant extracts								
		D1*	D2**	D3**	D1	D2	D3	D1	D2	D3
Extracts	Neem	1.667	2.000	1.667	2.000	2.500	1.000	2.000	2.167	1.667
	Bitter melon	2.333	3.000	2.500	2.500	3.000	2.167	2.500	2.833	2.000
	It Sit	2.833	3.500	3.667	3.333	4.000	3.333	3.333	4.333	3.000
	Control	4.333	4.500	4.667	4.333	4.667	4.333	4.667	4.833	4.167

*D1-01.03.2009 **D2-15.03.2009 ***D3-30.03.2009

Table: 5 Anova of dependent variable whitefly with the interaction of extracts , varieties and interaction of varieties and dates .

Source of variation	DF	SS	MS	F-value
Replications	2	0.264	0.132	0.7983
Extracts (E)	3	97.785	32.595	197.2129**
Varieties (V)	2	1.389	0.694	4.2017*
Interaction (E x V)	6	1.778	0.296	1.7927NS
Dates (D)	2	3.764	1.882	11.3866**
Interaction (E x D)	6	1.125	0.188	1.1345NS
Interaction (V x D)	4	2.472	0.618	3.7395**
Interaction(ExVxD)	12	3.917	0.326	1.9748*
Error	70	11.569	0.165	
Total	107	124.063		

*= Significant** Highly significant Ns =non significant

Table 6. Comparison of mean values of plant extract and varieties of tomato and their effect on whitefly population

		Varieties			Means
		Money maker	VRI-20	VRI-19	
Extracts	Neem	1.556	1.833	2.000	1.796 d
	Bitter melon	2.389	2.833	2.778	2.667 c
	It Sit	3.333	3.389	3.167	3.296 b
	Control	4.333	4.667	4.222	4.407 a
Means		2.903 b	3.181 a	3.042 ab	

LSD (E) = 0.2205
LSD (V) = 0.1910

Table 7. Comparison of mean values of time of application of plant extracts, interaction of time of application of plant extracts and varieties on whitefly population

		Varieties			Means
		Money maker	VRI-20	VRI-19	
Time of application of plant extracts	01-03-2009	2.875 bc	3.083 ab	2.750 cd	2.903 b
	15-03-2009	3.333 a	3.292 a	3.292 a	3.306 a
	30-03-2009	2.500 d	3.167 ab	3.083 ab	2.917 b

LSD (D) = 0.1910
LSD (V x D) = 0.3307

Table 8. Comparison of mean values of interaction of extracts, varieties and time of application of plant extracts on whitefly population.

		Varieties								
		Money maker			VRI-20			VRI-19		
		Dates			Dates			Dates		
		D_1	D_2	D_3	D_1	D_2	D_3	D_1	D_2	D_3
Extracts	Neem	2.000 hij	1.667 ijk	1.000 k	1.333 jk	2.000 hij	2.167 ghi	1.667 ijk	2.0 hij	2.33 fghi
	Bitter melon	2.500 fgh	2.67 efgh	2.000 hij	2.67 efgh	3.000 def	2.83 defg	2.333 fghi	3.0 def	3.00 def
	It Sit	3.000 def	4.000 bc	3.000 def	3.333cde	3.500 cd	3.333 cde	3.000 def	3.50 cd	3.000 def
	Control	4.000 bc	5.000 a	4.000 bc	5.000 a	4.667 ab	4.333 ab	4.000 bc	4.67 ab	4.000 bc

LSD (E x V x D) = 0.6615
D1-01.03.2009
D2-15.03.2009
D3-30.03.2009

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