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EFFECT OF ENVIRONMENTAL FACTORS ON BIOCHEMICAL PROPERTIES OF TOMATO LEAF CURL VIRUS INFECTED LEAVES OF TOMATO

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ABSTRACT

Tomato is a commodity of great economic importance in the world. One of the destructive viral diseases known as Tomato leaf curl virus belongs to *Begomovirus* has negatively impacted on tomato production in world. Tomato leaf curl virus widely distributed in the tomato crop and transmitted through whitefly in nature. Virus duplicates inside the host and altered various biochemical and physiological process. In this project, it was observed that the photosynthesis rate had positive correlation while lycopene and starch content (max temp) were negatively correlated in relation to environmental variables when compared with healthy plants. In case of phenolic compounds, it was increased as temperature increased and vice versa. There is no effect of relative humidity on chlorophyll contents in infected plants.

Keywords: Temperature, vector, importance, phenolic compounds.

INTRODUCTION

Tomato is one of the most extensively grown vegetable across the world having potential health benefits. Lycopene present in tomato is one of the unique phytochemicals which provides protection against oxidative damage that lead towards slowing down the ageing process in plant (Agarwal and Rao, 2000). Due to perennial growth on a large scale; tomato is susceptible to various biotic and abiotic

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factors which adversely affect the plant growth, crop yield, different biochemical changes and physiological processes in plants (Hasegawa *et al.*, 2000; Tajul, 2011). Viruses are serious threat among biotic factors which causes substantial yield losses both qualitatively and quantitatively. Almost seven viral diseases have been reported in Pakistan (Mughal, 1985). Among these viruses tomato leaf curl virus belongs to genus *Begomovirus*, single-stranded-DNA (Gottlieb *et al.*, 2010; Hanssen *et al.*, 2010) is most destructive virus that causes heavy yield losses up to 30-40% (Tariq, 1999). Tomato leaf curl virus infected tomatoes both in field conditions and greenhouses (Green, 1994). It is naturally transmitted through different species of whitefly and mechanically as well.

The most obvious and characteristic symptom is distortion and upward curling of the leaves (Melzer, 2010). Wobbes (2004) described that biochemical changes due to viral diseases affect the normal physiological functions and directly involved in photosynthesis inhibition that may also be due to suppression of genes. Hemida (2005) observed the chlorophyll degradation in infected plants after the attack of TLCV. Epidemiological factors play a very significant role in development and spread of the disease (Mubeen *et al.*, 2017). Although this disease has been reported in Pakistan but very little work and inadequate information is available on TLCV in Pakistan. Therefore, this experiment was designed to observe the impact of tomato leaf curl virus on physiological functions of tomato plants in relation to various environmental factors.

MATERIALS AND METHODS

Plant materials: The samples were collected from infected field and healthy samples from the previous trial (Arooj *et al.*, 2017). Healthy and diseased leaves of tomato were collected for the estimation of chlorophyll, photosynthetic rate, starch, lycopene and phenolic contents in relation to environmental factors.

Estimation of Chlorophyll: Leaves (infected and healthy) were collected and washed with sterilized water. Chlorophyll contents in TLCV were compared using chlorophyll meter (SPAD-502 Minolta, Japan).

Estimation of Photosynthetic rate: Change in photosynthetic activity of leaves due to TLCV in both infected and healthy leaves was compared through portable photosynthesis apparatus (IRGA).

Estimation of Lycopene: Lycopene contents were determined through the method purposed by Ranganna (1979). The tomato pulp from infected and healthy samples was extracted in acetone and absorbance was recorded in a spectrophotometer (503 nm) by using petroleum ether as blank.

Estimation of total soluble phenolics: The plant material was extracted by dipping in absolute methanol for one week. The methanol extract was determined through rotary evaporator. The phenolic contents were examined through folin ciocalteau phenol reagent method according to the description of (Chaovanalikit and Wrolstad, 2004; Iftikhar *et al.*, 2011).

Estimation of Starch: Accumulations of starch contents in plant leaves and roots play an important role of virus

infections on host plant. Therefore, starch contents were observed by Iodo-starch test (Iftikhar *et al.*, 2015) for quick indexing used in citrus for both virus and virus-like diseases. Tomato leaf curl virus infected leaves and healthy leaves were immersed in iodine solution for about 1.5-2 minutes followed by washing with distilled water. The edges of leaf samples at advancing margins were examined for dark staining by using magnifying glass.

Correlation of Environmental Factors: Environmental factors were recorded fortnightly from In-service Agriculture Institute Sargodha. Factors included maximum and minimum temperature and relative humidity.

STATISTICAL ANALYSIS

The data was correlated in relation to various environmental factors by using "R" software.

RESULTS

Correlation of physiological parameters with environmental factors: Physiological parameters viz; chlorophyll contents, lycopene determination, photosynthesis rate, starch contents and total soluble phenolic contents were observed and correlated with the environmental factors.

Correlation of chlorophyll contents with environmental factors: Chlorophyll contents of leaves (healthy and infected) were compared. Chlorophyll contents in virus infected leaves decreased significantly as compared to healthy leaves. Maximum temperature has negative correlation with healthy and infected leaves. No changes in chlorophyll contents of infected leaves have been observed in minimum temperature (Figure 1A) whereas it showed negative correlation with healthy leaves (Figure 1B). Chlorophyll contents of infected leaves showed a positive correlation with relative humidity. Chlorophyll were directly proportional to an increase in relative humidity but no significant effect was observed in plants infected with TLCV (Figure 1B).

Relationship of photosynthesis rate with environmental factors: Photosynthetic rate was measured with the help of IRGA. Photosynthesis rate increased as temperature increased in case of infected plants. At minimum temperature photosynthesis rate also increased in infected plants whereas it decreased in healthy plants and same in the case of relative humidity for both infected and healthy plants (Figure 2).

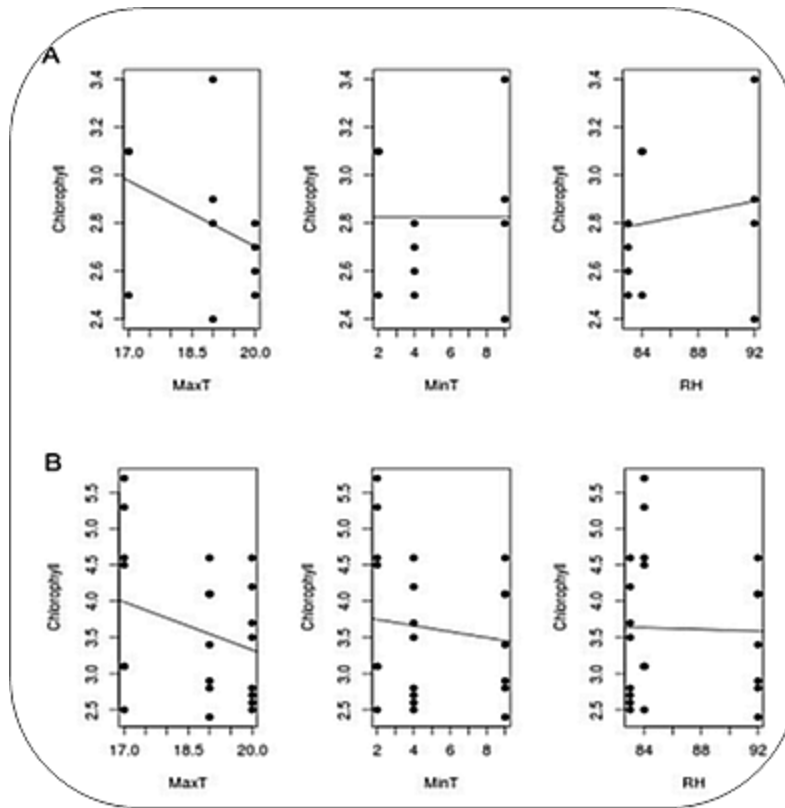


Figure 1. Chlorophyll contents of infected leaves (A) and healthy leaves (B)

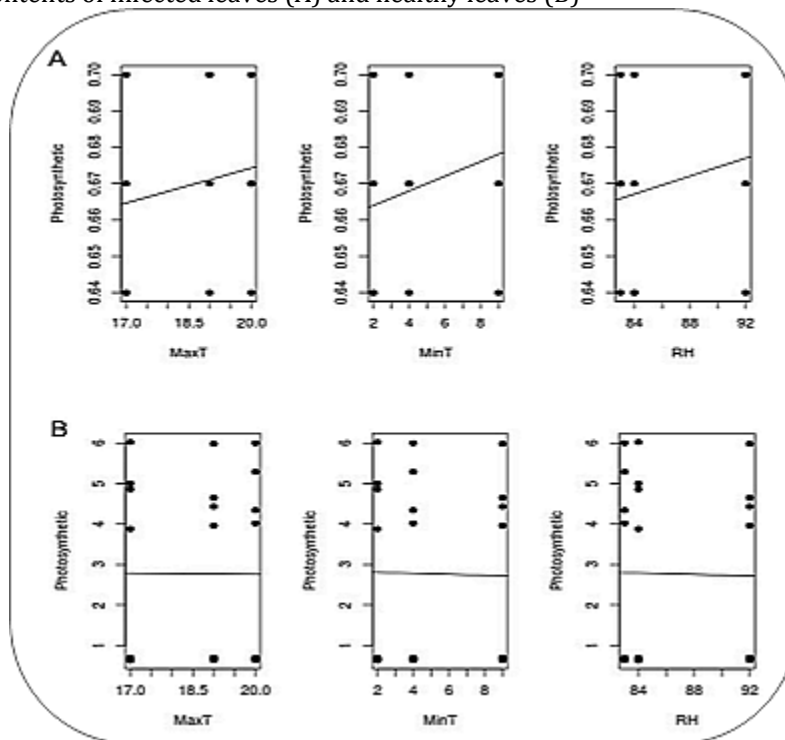


Figure 2. Photosynthetic rate of infected leaves (A) and healthy leaves (B)

Relationship of lycopene with environmental factors: Lycopene contents showed a negative correlation as its rate decreased at all environmental

parameters (max temp, minimum temp, relative humidity) for both infected as well as healthy plants (Figure 3).

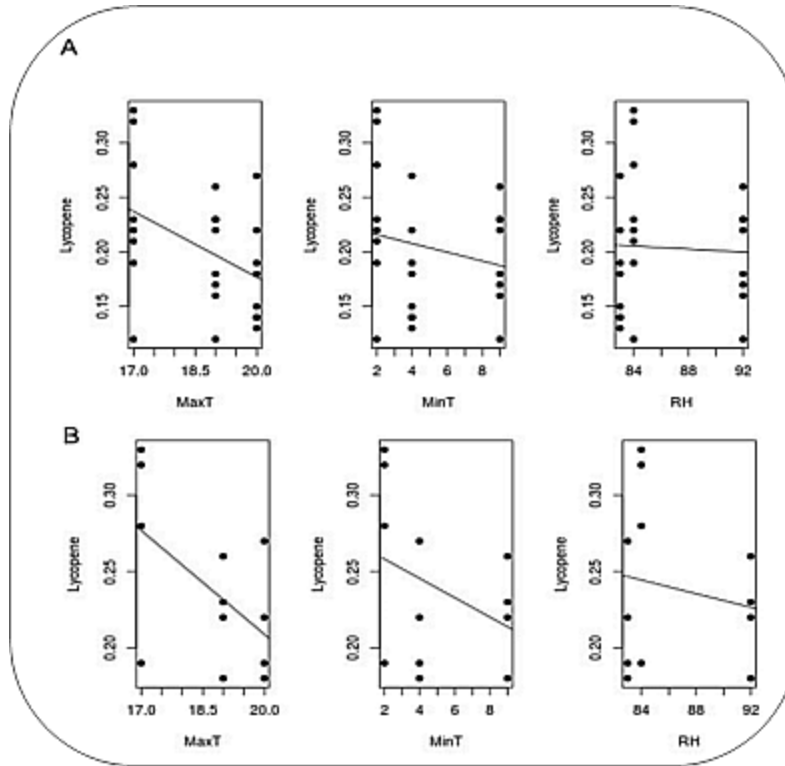


Figure 3. Lycopene contents of infected leaves (A) and healthy leaves (B)

Correlation of phenolic with environmental factors: Phenolic contents of leaves viz; healthy and infected were calculated and it was obvious from investigation that phenolic compound at maximum

temperature in case of infected plants increased whereas for both other factors it is negatively correlated for both infected and healthy plants (Figure 4).

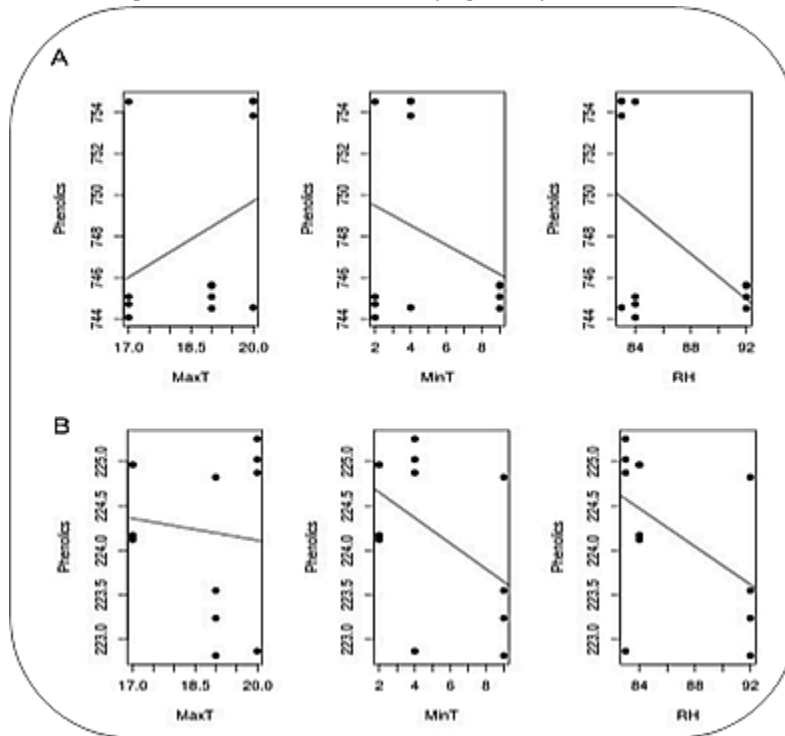


Figure 4. Phenolic contents of infected leaves (A) and healthy leaves (B)

Starch content: Healthy leaves showed no color while infected leaves showed dark grey to black stains after Table 1. Starch contents of healthy and infected leaves

immersed in iodine and reactions were observed negative and positive respectively (Table. 1 and Figure 5).

Healthy Sample	Reaction	Infected Sample	Reaction
1	-VE	1	+VE
2	-VE	2	+VE
3	-VE	3	+VE
4	-VE	4	+VE
5	-VE	5	+VE
6	-VE	6	+VE
7	-VE	7	+VE
8	-VE	8	+VE
9	-VE	9	+VE
10	-VE	10	+VE

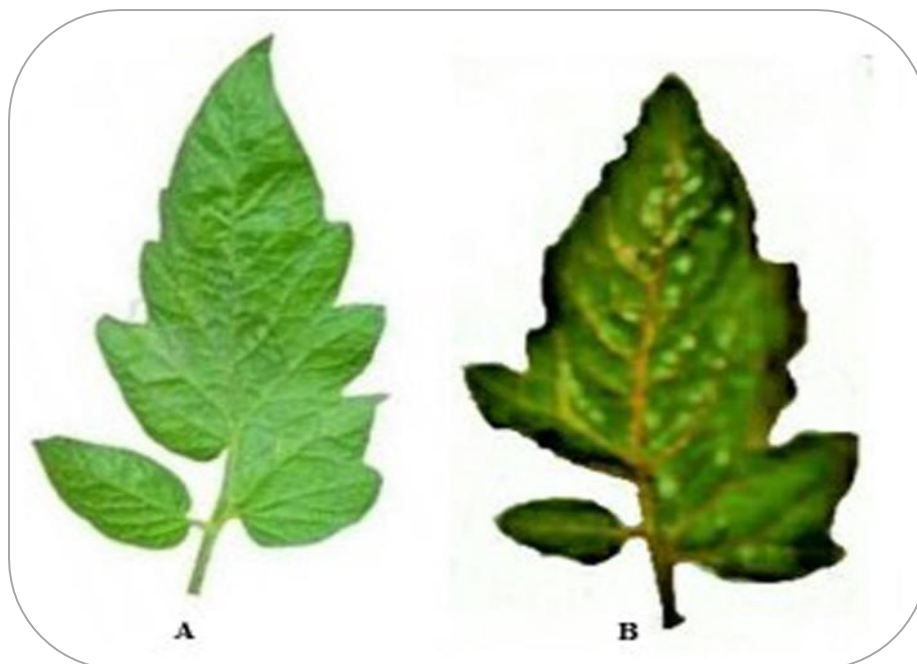


Figure 5. Healthy tomato leaf (A) and infected tomato leaf (B).

DISCUSSION

Tomato leaf curl virus is the most destructive disease resulted in severe crop losses across the world and prime limiting aspect in production of tomato in Pakistan which approximately caused 30-40% yield losses (Tariq, 1999). Tomato leaf curl virus spread from one place to other via a vector called White fly (*Bemesia tabacii*) and cause huge destruction both qualitatively and quantitatively (Sánchez-Campos *et al.*, 2013). Keeping in view the spread and severity of TLCV disease, the present study was planned to investigate different physiological parameters in relation to various environmental factors.

Physiological parameters and metabolic activities of the host plant considerably influenced by the attack of the

disease. The biochemical changes caused due to morphological variations and the degree of yield loss can be observed by apparent symptoms caused by infected plant (Levy and Marco, 1982). Khalil *et al.*, (2014) reported that virus infected tomato plants caused considerable decrease in total of soluble sugar; insoluble sugar and carbohydrate contents in stem and plant leaves respectively as compared with control. Tomato yellow leaf curl virus causes definite changes in physiological functions of virus infected plants resulted in reduction of the main components of the plant viz; Plant growth, photosynthetic pigments, total carbohydrate contents and Mg⁺⁺ ions.

Viruses are directly accountable for degradation of chlorophyll contents in virus infected plants. Bertamini

et al., (2002) and Hemida (2005) observed almost similar outcomes with our findings that chlorophyll contents of virus infected leaves were found to be reduced at maximum temperature. Our experimental results are also comparable with Singh *et al.*, (2014) who investigated that occurrence of TLCV has a significant effect on chlorophyll concentration in infected host plant which exhibit very close association among susceptible and resistant genotypes. Chlorophyll concentration reduced in infected virus plants at high temperature has been studied many scientists which might be due to chlorosis which are more frequently occurred in the infected leaves. Our results were also in line with the findings of Marco (1975) who examined that interveinal chlorosis is the one of the most obvious symptoms expressed by virus infected host plants.

Contrary to results of Bertamini *et al.*, (2004) who confirmed the photosynthetic level in virus infected tomato plants generally reduced as compared to healthy plants whereas in our study we observed that Photosynthetic rate has been increased after the attack of virus as compared to healthy plants which might be due to different environmental factors and geographical distribution of the viruses across the world. The lycopene contents reduced in both viruses infected as well as in healthy fruits. Our results were in accordance with Paranita *et al.*, (2012) who investigated that lycopene contents of virus infected fruits were reducing after attack while Akanda *et al.*, (1998) reported that the level at which chlorophyll contents, β carotene and lycopene contents reduced significantly depends upon virus infected tomato plants. The max. temperature showed positive association while mini. temperature and relative humidity showed negative correlation with phenolic contents in virus infected tomato leaves. The similar findings have been reported by several researchers (Kumar *et al.*, 2010; Raj *et al.*, 2005; Huang and Backhouse, 2005).

CONCLUSION AND RECOMMENDATION

Chlorophyll contents showed a declining trend in virus infected leaves. Maximum temperature had no significant impact on healthy and infected leaves, whereas minimum temperature has only impact on healthy leaves. Relative humidity increased the Chlorophyll contents of infected leaves. Maximum temperature increased the photosynthetic rate of infected plants. At minimum temperature photosynthetic rate was increased in infected plants

whereas it little bit decreases in healthy plants and same in case of relative humidity for both infected and healthy plants. Lycopene contents (infected and healthy Plants) decreased if environmental factors increased. Maximum temperature increased the phenolic compound in infected plants whereas both other factors (minimum temperature & relative humidity) decreased phenolic compound in both infected and healthy plants. Healthy leaves showed no color while infected leaves showed dark grey to black stains after immersed in iodine and reactions were observed as negative and positive. Therefore, it is recommended study of environmental factors in relation to physiological functions of plants in future will not only be helpful in determining the response against plant diseases but also for monitoring the progression of disease.

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