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Evaluation of Citrus Cultivars for tolerance to Citrus Tristeza Virus (CTV), *Aphis gossypii* and their Management by limiting vector Population †

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Abstract: Citrus tristeza virus (CTV) is one of the most destructive diseases of citrus and is major cause of reduction in citrus yield. CTV epidemics have caused the death of millions of citrus trees globally. Present study was aimed to evaluate citrus cultivars against CTV and its vector (aphid) population. Highest infection and vector population were recorded in Mangal Singh whereas the lowest in early fruiter (20%). Early fruiter has a maximum level of tolerance against Citrus tristeza virus. CTV is replicated in phloem cells of the plants and transmitted by aphid specie *Aphis gossypii*. Thus, maximum vector population mirrors to highest infection. Chemical Plant nutrients i.e., micro-mix (Zn, Fe, Cu, Mn), NPK, Zinc and insecticide (Lufenoron) were used for limiting the most challenging CTV and *A.gossyii.* Lufenuron caused maximum disease inhibition followed by plant nutrients Zinc, NPK and Micro mix respectively. However, *Aphis gossypi* population was decreased at great extent with Leufenoron. The results indicate that early fruiter has lowest percent disease index and its vector population. Moreover, lufenuron is best solution for controlling vector population and disease inhibition.

Keywords: Citrus; Epidemics; Nutrients; CTV; *Aphis gossypii*; Lufenoron

1. Introduction

Citrus tristeza virus (CTV), a member of the genus Closterovirus, represents one of the intricate viruses with an overwhelmingly complex biology. Moreover, the characterization of CTV has also been performed on molecular basis [1]. Citrus tristeza virus is the most challenging virus due to efficient (vector) transmission system and lack of resistant cultivars. CTV causes stem pitting in different citrus cultivars and leads to significant losses in fruit quality and quantity worldwide. It spreads all over the world through aphid vector and exchange of infected budwood [2]. *Toxoptera citricida* and *Aphis gossypi* are the most efficient and important vectors of CTV in citrus growing countries [3] while in Pakistan two aphid species *A.gossypii* and *A. spiraecola* are mainly responsible for disease transmission

Symptom phenology of CTV is based on virus strains. Mild isolates of CTV don't cause decline on sour orange root stock while virulent strains causes stem pitting in main trunk [4], when favorable environment conditions prevails, plant becomes dry and dead [5]. Much success for controlling losses of Citrus tristeza virus has been obtained by using cross protection and transgenic plants in different citrus producing countries i.e. South Africa, Australia and Brazil [6]. CTV is controlled by limiting vectors (aphid) population. Biological control involves the use of natural enemies and it showed significant results against aphid population *P. longispinus* sp. can be completely controlled by biological

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method [7]. Use of cross protection and transgenic plants against CTV is laborious and takes a long time. Thus, present study was designed for the determination of resistant source against CTV.

2. Methods

Present study was carried out at research area, Department of Plant Pathology, University of Agriculture, Faisalabad Pakistan (31.4278° N, 73.0758° E). Two individual experiments were carried out by following Randomized Complete Block Design (RCBD) and RCBD with factorial arrangement. In first experiment fourteen citrus cultivars were planted by following R×R and P×P distance of 90cm. In second experiment a highly susceptible cultivar "Mangal Singh" was planted by following the same planting geometry. All cultural and agronomic practices were followed to keep field healthy.

Cultivars were screened by following scale describes in Table 1. In second experiment nutrients i.e. NPK, Zinc, Micromix (Mn, Fe) and chemical Lufenuron were evaluated at three different concentration (3, 5, 7 g/liter of water) against aphid population and CTV on a highly susceptible cultivar "Mangal Singh"

Percent disease index was measured by following equation.

$$\text{Percent Disease Index}(\%) = \frac{\text{Total number of numerical ratings}}{\text{Number of observation}} \times \frac{100}{\text{Maximum disease rating}}$$

3. Results and discussion

Results of first experiment (Table 2) revealed that there was not even a single cultivar that showed the immune or resistant response against CTV. Early fruiter showed moderately resistant response with minimum Percent Disease Index (20%) and aphid population. Early fruiter expressed moderately resistant response which can be used by researcher for incorporating resistant genes in advanced lines of citrus with good horticultural attributes. Results of current study are in line with the work of Broadbent, *et al*., [7] who also evaluated citrus cultivars towards CTV and concluded that use of resistance source is the only way for the management of CTV.

Data of second experiment in table 3 revealed that among plant nutrients/insecticide, Lufenuron caused maximum CTV disease inhibition with minimum (20.12%) percent disease index. Among concentration, maximum suppression of disease was observed when all these nutrients/insecticide were applied at 7 g^{-1}liter of water, followed by 5 g^{-1}liter of water while minimum suppression was recorded concentration of 3 g^{-1}liter of water as it showed maximum percent disease index.

Aphis gossypii is the major vector of CTV transmission and the application of insecticides is the primary pest management strategy to control aphid population many effective insecticides are available [8]. Frequent applications of chemicals (insecticides) may accelerate the development of aphid resistance; strategies to reduce aphid resistance should be implemented by chemicals rotation and using nonchemical strategies [9].

Application of chemicals (Thiamethoxam) lowers aphid pressure by increasing aphid mortality and delaying their colonization [10]. Among four chemicals lufenuron showed significant results by showing minimum vector population. Outcomes of contemporary study are supported by the work of Kerns and Stewart [11] that used carbofuran, Acephate and acephate against aphid population. Current study is also in agreement with the work of Franco *et al.,* [12] that application of chemicals is the best way to control citrus mealy bug and aphid population. Results of present study are supported by the finding of Barnier *et al*., [13] that use of insecticides for the suppression of *A.gossypii* also controls CTV.

Table 1. Disease data was recorded by following visual observations and rating scale. According to this scale: .

Sr.	Description	Score	Reaction
1	Disease symptoms are not present	0	Immune
2	Few spots present on the tip, covers less than 10% leaf area	1	Resistant
3	Purplish brown patches, covering less than 20% leaf area	2	Moderately resistant
4	Patches along with paler outer region, covering up to 40% leaf area	3	Moderately susceptible
5	Long lines are present covering up to 75% leaf area	4	Susceptible
6	Complete leave dried or its breakdown occur from stalk	5	Highly susceptible

Table 2. Evaluation of Citrus cultivars against Citrus Tristeza Virus disease under field conditions.

Sr.	Cultivars	Percent Disease Index (PDI)	Aphid Population (per plant)	Score	Reactions
1	Early Fruiter	20 k	49 i	2	MR
2	Sweet Lemon	33 j	83 h	3	MS
3	Mayer Lemon	33.06 j	85 h	3	MS
4	Saccri	39.33 i	93 gh	3	MS
5	Malta	40 h	101 fgh	3	MS
6	Zarica XI	41.33 h	106 fgh	4	S
7	Jafa	41.50 h	116 efg	4	S
8	Kinnow	46.16 g	120 efg	4	S
9	Grape Fruit	52.90 f	129 def	4	S
10	Feultral's lemon	56.83 e	142 cde	4	S
11	Mitha	66.53 d	157 bcd	5	HS
12	Red blood	69.33 c	165 bc	5	HS
13	China Lemon	80 b	175 ab	5	HS
14	Mangal Singh	85.90 a	203 a	5	HS
	LSD	**1.3026**	**29.93**		

*Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test (P≤0.05).

Table 3. Percent Disease Index of CTV affected by different nutrients/chemical at their different concentration.

Concentration	Concentration			
Treatments	**3 g l^{-1}**	**5 g l^{-1}**	**7 g l^{-1}**	**Mean**
NPK	28.90 e	26.77 f	24.50 g	**26.72 C**
Zinc	22.80 h	21.63 hi	18.83 j	**21.09 D**
Micro-Mix	46.60 b	42.53 e	39.60 d	**42.91 B**
Leuran	21.93 hi	20.80 i	17.63 j	**20.12 D**
Control	85.80 a	85.80 a	85.80 a	**85.90 A**
Mean	**41.23 A**	**39.55 B**	**37.27 C**	

LSD at (p≤0.05) for Treatments = 0.746, Concentration= 0.578 and Treatments ×Concentration= 1.291.

4. Conclusion

Present investigations were conducted to find the source of resistance against Citrus tristeza virus (CTV) in citrus cultivars. Results revealed that early fruiter has the maximum tolerance against CTV and exhibits minimum vector population. Moreover, leufenoron application significantly limits *A.gossypii* polulation and disease incidence.

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