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Proceeding Paper

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 affecting citrus and is a major cause of reductions in citrus yield. CTV epidemics have caused the death of millions of citrus trees globally. The present study aims to evaluate citrus cultivars against CTV and its vector (aphid) population. The highest levels of infection and vector population were recorded in Mangal Singh, whereas the lowest were found in the early fruiter (20%). Early fruiter had a maximum level of tolerance against Citrus tristeza virus. CTV is replicated in the phloem cells of plants and is transmitted by the aphid specie *Aphis gossypii*. Thus, the maximum vector population mirrors the highest infection. Chemical plant nutrients, including micro-mix (Zn, Fe, Cu, Mn), NPK, zinc, and the insecticide Lufenoron, were used to limit the impact of CTV and *A.gossyii.* Lufenuron caused maximum disease inhibition, followed by the plant nutrients zinc, NPK, and micro-mix, respectively. However, Lufenoron significantly decreased the population of *Aphis gossypi.* The results indicate that the early fruiter has the lowest percent disease index and vector population. Moreover, Lufenuron is the best solution for controlling vector population and disease inhibition.

Keywords: citrus; epidemics; nutrients; CTV; *Aphis gossypii*; lufenoron

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1. Introduction

Citrus tristeza virus (CTV), a member of the genus Closterovirus, represents one of the most intricate viruses with an overwhelmingly complex biology. Moreover, the characterization of CTV has been performed on a molecular basis [1]. Citrus tristeza virus is the most challenging due to its efficient vector transmission system and the 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 vectors and the 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, the two aphid species *A. gossypii* and *A. spiraecola* are mainly responsible for disease transmission.

The symptom phenology of CTV is based on virus strains. Mild isolates of CTV do not cause decline on sour orange rootstock, while virulent strains cause stem pitting in the main trunk [4]. When favorable environmental conditions prevail, plants can become dry and dead [5]. Much of the success in controlling Citrus tristeza virus losses has been obtained by using cross-protection and transgenic plants in citrus-producing countries such as South Africa, Australia, and Brazil [6]. CTV is controlled by limiting its vector (aphid) population. Biological control involves the use of natural enemies, and has shown significant results against aphid populations, with *P. longispinus* sp. being completely controlled by biological methods [7]. The use of cross-protection and transgenic plants against CTV is laborious

and takes a long time. Thus, the present study is designed to determine resistant sources against CTV.

2. Methods

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

Cultivars were screened by following the scale described in Table 1. In the second experiment, nutrients (NPK, zinc, micro-mix (Mn, Fe)) and the chemical Lufenuron were evaluated at three different concentration (3, 5, 7 g^{-1} L of water) against aphid populations and CTV on the highly susceptible cultivar "Mangal Singh".

Table 1. Disease data were recorded by following visual observations and a rating scale, as seen.

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, covers less than 20% leaf area	2	Moderately resistant
4	Patches along paler outer region, covers up to 40% leaf area	3	Moderately susceptible
5	Long lines are present, covers up to 75% leaf area	4	Susceptible
6	Leaves completely dried, or its breakdown occurs from the stalk	5	Highly susceptible

The percent disease index was measured using the 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 from the first experiment (Table 2) revealed that none of the cultivars showed an immune or resistant response against CTV. However, early fruiter showed moderately resistant response with a minimal Percent Disease Index (20%) and low aphid population. The moderately resistant response expressed in early fruiter could be used by researcher to incorporate resistant genes into advanced lines of citrus with good horticultural attributes. The results of the current study are in line with the work of Broadbent et al., [7] who also evaluated citrus cultivars towards CTV, and concluded that the use of resistance sources was the only way for effective management of CTV.

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

Aphis gossypii is the major vector for CTV transmission, and the application of insecticide is the primary pest management strategy for controlling aphid populations [8]. The frequent application of chemicals (insecticides) may accelerate the development of aphid resistance. Therefore, strategies such as chemical rotation and nonchemical approaches should be implemented to reduce aphid resistance [9].

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 ($p \leq 0.05$), lower case alphabet shows the means difference among PDI and aphid population.

Table 3. Percent Disease Index of CTV affected by different nutrients/chemicals at different concentrations.

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	

Lower case alphabet shows the means difference among PDI and aphid population. LSD at ($p \leq 0.05$) for Treatments = 0.746, Concentration = 0.578, and Treatments × Concentration = 1.291. NPK = Mixture of Nitrogen, Phosphorus and Potassium.

Applications of chemicals (Thiamethoxam) can lower aphid pressure by increasing mortality and delaying colonization [10]. Among the four chemicals, Lufenuron showed significant results in minimizing the vector population. Outcomes of contemporary studies are supported by the work of Kerns and Stewart [11], who used carbofuran and acephate against aphid populations. The current study is also in agreement with the work of Franco et al., [12] highlighting that the application of chemicals is the best way to control citrus mealy bug and aphids. The results of the present study are supported by the findings of Barnier et al., [13] emphasizing the use of insecticides in the suppression of *A. gossypii*, which also controls CTV.

4. Conclusions

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 a minimum vector population. Moreover, Lufenuron application significantly limits the *A. gossypii* population and disease incidence.

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References

1. Davino, S.; Willemsen, A.; Panno, S.; Davino, M.; Catara, A.; Elena, S.F.; Rubio, L. Emergence and phylodynamics of Citrus tristeza virus in Sicily, Italy. *PLoS ONE* **2013**, *8*, e66700. [CrossRef] [PubMed]
2. Cambra, M.; Gorris, M.T.; Marroquın, C.; Román, M.P.; Olmos, A.; Martınez, M.; de Mendoza, A.H.; López, A.; Navarro, L. Incidence and epidemiology of Citrus tristeza virus in the Valencian Community of Spain. *Virus Res.* **2000**, *71*, 85–95. [CrossRef] [PubMed]
3. Marroquín, C.; Olmos, A.; Gorris, M.T.; Bertolini, E.; Martınez, M.C.; Carbonell, E.A.; de Mendoza, A.H.; Cambra, M. Estimation of the number of aphids carrying Citrus tristeza virus that visit adult citrus trees. *Virus Res.* **2004**, *100*, 101–108. [CrossRef] [PubMed]
4. Yokomi, R.K.; Selvaraj, V.; Maheshwari, Y.; Saponari, M.; Giampetruzzi, A.; Chiumenti, M.; Hajeri, S. Identification and characterization of Citrus tristeza virus isolates breaking resistance in trifoliate orange in California. *Phytopathology* **2017**, *107*, 901–908. [CrossRef] [PubMed]
5. Moreno, P.; Garnsey, S.M. Citrus Tristeza diseases—A worldwide perspective. In *Citrus Tristeza Virus Complex and Tristeza Diseases*; Karasev, A.V., Hilf, M.E., Eds.; American Phytopathological Society: Saint Paul, MN, USA, 2010; pp. 247–260.
6. Da Graca, J.V.; Van vuren, S.P. Managing Citrus tristeza virus losses using cross protection. In *Citrus Tristeza Virus Complex and Tristeza Diseases*; Karasev, A.V., Hilf, M.E., Eds.; American Phytopathological Society: Saint Paul, MN, USA, 2010.
7. Gross, S.; Blumberg, D.; Drishpon, Y.; Mendel, Z. The spherical mealybug, Nipaecoccusviridis: New data on its distribution, damage, natural enemies and control. *Phytoparasitica* **2001**, *29*, 62.
8. Hodgson, E.W.; VanNostrand, G.; O'Neal, M.E. *Yellow Book: Report of Insecticide Evaluation for Soybean Aphid*; Department of Entomology, Iowa State University: Ames, IA, USA, 2010.
9. O'Neal, M.E.; Johnson, K.D. Insect Pests of Soybean and Their Management. In *The Soybean: Botany, Production and Uses*; Singh, G., Ed.; CABI: Cambridge, MA, USA, 2010; pp. 300–324.
10. McCornack, B.P.; Ragsdale, D.W. Efficacy of thiamethoxam to suppress soybean aphid populations in Minnesota soybean. *Crop Manag.* **2006**, *5*, 1–8. [CrossRef]
11. Kerns, D.L.; Stewart, S.D. Sublethal effects of insecticides on the intrinsic rate of increase of cotton aphid. *Entomol. Exp. Appl.* **2000**, *94*, 41–49. [CrossRef]
12. Franco, J.C.; Suma, P.; da Silva, E.B.; Blumberg, D.; Mendel, Z. Management strategies of mealybug pests of citrus in Mediterranean countries. *Phytoparasitica* **2004**, *32*, 507. [CrossRef]
13. Barnier, J.; Grafton-Cardwell, B.; Polek, M. Citrus tristeza virus (CTV): Then and now. *Citrograph* **2010**, *1*, 16–23.