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SOME BIOCHEMICAL CHANGES IN TRISTEZA INFECTED CITRUS TREES IN PAKISTAN

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ABSTRACT
Fifty samples from each citrus variety viz. Sweet orange, Kinnow mandarin and grapefruit infected with citrus tristeza virus were analyzed for Phenolics, Chlorophyll and Starch contents followed by ELISA. Starch contents and total chlorophyll among CTV-infected samples were with mean values of 2.96µg/ml and 13.19µg/ml, respectively which were found lower than non-infected plants (5.63µg/ml, and 14.79µg/ml, respectively). However, phenolic compounds were same in healthy and infected citrus samples. Chlorophyll-A and starch contents showed a significant lowering trend in the infected citrus samples, with mean values of 8.07µg/ml and 3.89µg/ml, respectively. Among citrus varieties Chlorophyll-A, B and total chlorophyll contents were significantly lower in sweet orange with mean values of 7.91, 3.96 and 11.83µg/ml, respectively whereas, starch contents were significantly lower in grapefruit (3.79µg/ml).

KEYWORDS: CTV, Chlorophyll-A and B, total chlorophyll, starch and phenolics

INTRODUCTION

Tristeza disease in citrus, caused by *Citrus tristeza closterovirus* (CTV) is one of the most serious, destructive and wide spread diseases, damaging citrus orchards all over the world. It is a ssRNA virus with flexuous rod shaped particles measuring group, approximately 12 x 2,000 nm in size. CTV consists of a number of strains which ranges from mild to severe stem pitting isolates (Moreno et al., 2008; Schneider, 1959). All species, cultivars and hybrids of citrus are variably attacked by CTV, regardless of rootstock inducing mild or masking conditions to severe symptoms. In fact, "Tristeza" is a disease mainly infects sweet orange (*Citrus sinensis*) and other citrus varieties when grafted on sour orange (C. aurantium) rootstock (Anonymous 2004; Bar-Joseph and Lee, 1989).

Several biochemical changes can occur the reflection of virus infection including; the change in rate of nitrogen fractions, carbohydrates, sugars, phenolics, alkaloids, growth regulators and nucleic acid (Verma, 2003). The disease also interrupts the normal pathways of photosynthetic process, water and nutritional transport. Effective blockage of the photosynthetic pathway causes the necrosis and death of conductive tissue. The roots of trees use the stored starch when it is not available from leaves, which ultimately leads to death (Futch and Brlansky, 2005). Discoloration of leaves due to the loss of chlorophyll, wilting due to abnormal transportation of water and nutrients and ultimately die back is the result of CTV infection (Lee and Bar-Joseph, 2000; Roberts et al., 2001). Phenolic compounds play an important role in defense mechanism against the disease development, in a wide range of pathogens. Plants can produce these compounds instead of antibodies, and are considered as secondary metabolites (Bennett and Wallsgrove, 1994).

CTV has already been reported in Pakistan during the limited preliminary survey of citrus orchards (Anwar and Mirza, 1992; Catara *et al.*, 1988; Iftikhar *et al.*, 2009). Though advanced protocol has been developed for biochemical study, but literature on biochemical study of CTV infected plants is scarce like production of phenolic compounds and estimation of chlorophyll and starch contents. This study was therefore an attempt with the objective to analyze the changes in biochemical factors of CTV infected plants.

MATERIALS AND METHODS:

Sample Collection

Citrus samples of three varieties; Sweet orange, Kinnow mandarin and grapefruit were collected from orchards of major growing areas of the Punjab and Khyber Pakhton khowa (former N.W.F.P), Pakistan, during a survey at random (Iftikhar et al., 2009).

Biochemical Methods

On the basis of ELISA, 50 samples from each variety were selected randomly for the following biochemical analysis.

a) Total soluble Phenolics: Soluble Phenolics from infected and healthy leaves of citrus trees were determined as described by Julkunen-Titto (1985). Forty to fifty mg of leaves were ground in 80% acetone in a pestle and mortar and placed in a water bath at 50 °C for 1 hour. Supernatant was collected in a microfuge tubes after centrifuge for 10 minutes at 10,000 rpm at 4°C. 100 µl aliquot was diluted with 2ml distilled water in a test tube. 1 ml of Folin-

ciocalteus phenol reagent was added and shaken vigorously. The volume was made up to 10 ml by adding 5 ml of 20% Na_2CO_3 and vortex vigorously for 5-10 seconds. Absorbance was measured at 750 nm after 20 minutes in a Spectrophotometer (model U2020) by setting zero against 80% acetone. A standard curve was prepared from 20, 40, 60, 80, and 100µg of Galic Acid (prepared from 100 µg/ ml stock).

b) Determination of Chlorophyll Contents: a sample of 0.5g of fresh leaves were ground in 80% acetone were ground and filtered through filter paper (Whatman 40) to remove debris. The absorbance for each sample was measured at 645nm, 663nm and 652 nm for chlorophyll a, b and total chlorophyll, respectively. The chlorophyll contents were calculated using the following formula described by Arnon (1949).

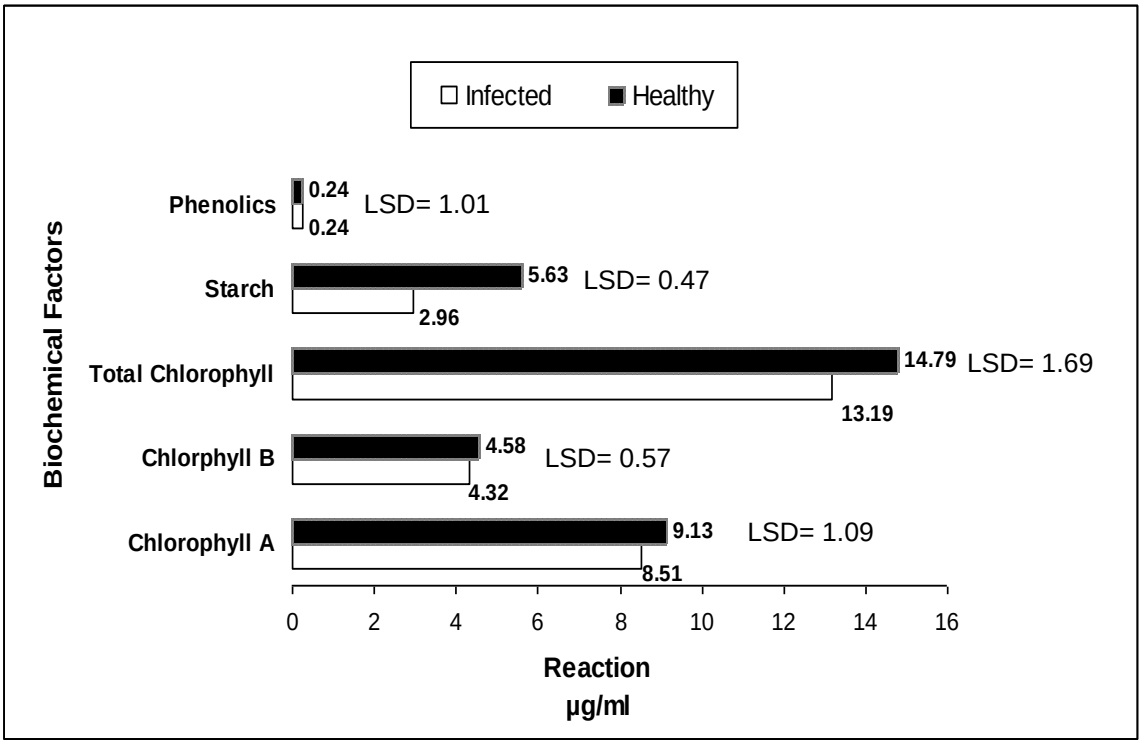
c) Determination of starch contents in root hairs: Root samples were collected and subjected to process for the determination of starch content as described by Hedge and Hofreiter (1962) and Thimmaiah (2004) through a rapid and convenient anthrone reagent method. One gram roots along with root hairs was ground in 5ml 80% ethanol and centrifuged at 10,000 rpm for 10 minutes at 4°C. The pellet was taken and again centrifuged after washing with ethanol. The supernatant was discarded and 5ml water was added to the pellet following with 6.5 ml perchloric acid, after drying. Material was extracted by shaking and again it was centrifuged at 10,000 rpm and 4°C for 10 minutes. The supernatant was collected and again 6.5 ml perchloric acid was added to collect the supernatant. Supernatant, after centrifuging twice was pooled and made up to volume 1ml, by adding 0.1-0.2 ml distilled water. Four ml anthrone reagent was added and the material was cooled after heating at 80 °C for 8 minutes. Absorbance was measured at 630nm using an ELISA reader (EXL- Bio-Tek 800).

Statistical analysis: Means values of the processed citrus samples were calculated and compared through LSD test at P = 0.05% (Steel and Torrie, 1997).

RESULTS

The biochemical factors studied such as; chlorophyll, phenolics and starch contents were found lower in infected samples as compared to healthy. Starch contents were found to be significantly low in infected samples with mean value of 2.96µg/ml as compared to healthy samples with mean value of 5.63µg/ml. Although chlorophyll A, B and total cholorphyll were low in CTV infected samples as compared to healthy one but differences were not significant. Levels of phenolics were same in healthy and infected samples (Fig. 1).

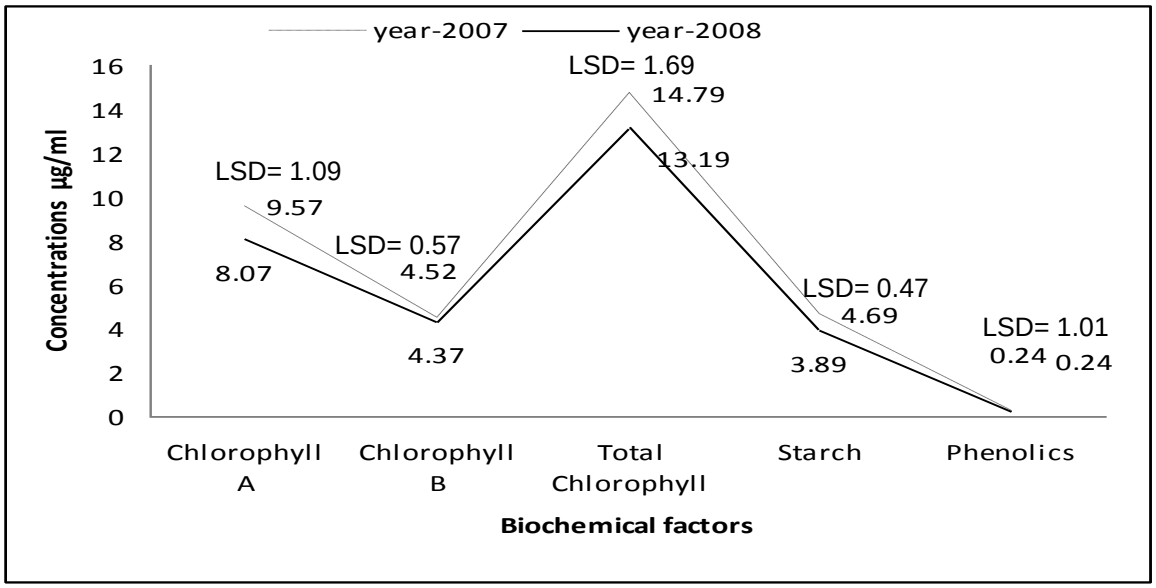
FIGURE 1. Comparison of biochemical factors in CTV infected and healthy samples



The concentration of chlorophyll, phenolics and starch contents were low in 2007 as compared to the previous year. It was found that only chlorophyll-A and starch contents were significantly low with the mean value of 8.07µg/ml and 3.89 µg/ml, respectively during 2007 as compared to 2006 (9.57 µg/ml and 4.69 µg/ml respectively). Total chlorophyll contents were also considerably low (13.19µg/ml) in year 2008 as compared

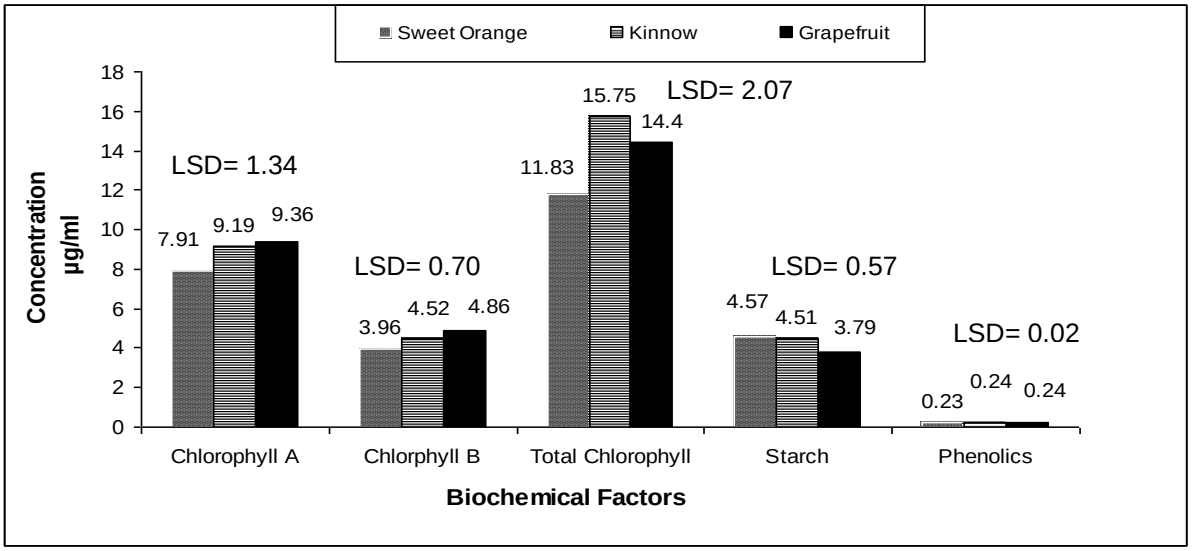
to 2007 with mean value of 14.79µg/ml. Phenolic compounds did not exhibit any change (Fig. 2).

FIGURE 2. Concentration of phenolics, chlorophyll and starch contents in CTV infected samples during 2007-2008



Citrus varieties reacted differently towards CTV infection in relation to biochemical factors such as chlorophyll-A, chlorophyll-B, total chlorophyll, starch and phenolics. In sweet orange chlorophyll-A, B and total chlorophyll was significantly low (7.91µg/ml, 3.96µg/ml and 11.83µg/ml, respectively) as compared to kinnow mandarin and grapefruit. Overall Starch contents were significantly low in grapefruit (3.79 µg/ml), followed by kinnow and sweet orange, which were at par with each other. All varieties reacted towards phenolics non-significantly (Fig 3).

FIGURE 3. Biochemical changes in citrus cultivar after CTV infection



DISCUSSION

CTV is a phloem- limited pathogen; its infection is likely to disturb the nutritional activity of the plant which leads to the death of tree due to necrosis of phloem tissue at the bud union. Decrease in photosynthesis in the virus infected plants is usual (Bos, 1999). Our results are in accordance with the study of biochemical changes in virus infected plants, reported by many scientists (Anonymous, 2004; Brown *et al.*, 1988; Futch and Brlansky, 2005). Virus infection clearly alters the normal pathways of photosynthesis, respiration, transpiration and affects accumulation or depletion of starch and sugars in differentparts of plants. Virus infection develops chlorotic lesion due to loss of chlorophyll and producing the yellowing state in leaves (Verma, 2003). Therefore, capacity of plants for production of chrolophyll and transfer of starch contents in infected plants can be decreased in virus infected plants.

Although phenolic compounds produced in other cases of viral infections provide an encouraging response for the disease development and the resistance. However phenolic compounds did not reveal any real change in their level which may be attributed to the rootstock used being

tolerant to CTV and the environmental factors that influence the production of phenolic compounds. It is hard to make the bench mark for the characterization of CTV only as phenolics, chlorophyll and starch contents are generally influenced by the virus infection. These changes are also dependent upon the host and virus relationship. CTV infection causes reduction in root system resulting in less water supply and minerals which ultimately leads to wilting, chlorosis and dieback symptoms. These symptoms can be avoided by introducing decline tolerant citrus cultivar against the strains of CTV (Lee and Bar-Joseph, 2000). It was concluded that CTV infected samples showed lower concentration of chlorophyll-A, B, total chlorophyll and starch contents as compared to healthy samples, in general. It can also be safely concluded that these biochemical activities may be hindered due to disturbance in the biochemical pathways of the infected citrus samples. However, more work is required to be done on biochemical activity in the CTV infected samples to differentiate and monitor the disease on a biochemical basis.

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