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INDUCTION OF RESISTANCE IN TOMATO PLANTS AGAINST TOMATO LEAF CURL VIRUS BY USING BIOCHAR AND SEED PRIMING

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ABSTRACT

The experiment was conducted to check the effects of biochar and different priming agents on whitefly transmitted tomato leaf curl virus. Biochar was applied to potting mixture at 1%, 2% and 3% concentration. In order to check the effects of priming, tomato seedlings were primed with NaCl, glycerine and distilled water at three concentrations. After biochar application 22.43% disease severity was recorded that was significantly lower than control (40.12%). In graft inoculated plants, disease severity was in 15.32% while in control 50.23% disease severity was recorded. From primed seedling NaCl and glycerine shows good results then distilled water. In case of biochar, disease reduction was more at higher concentration (3%) than other two concentrations. Priming gave better results in case of whitefly inoculation.

Keywords: Defence, Resistance, Biochar, Priming, Virus and Tomato.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) belongs to nightshade family and its cultivation started in South American region (Knapp, 2000). Tomato provides balanced and energetic dietary components as it contains various mineral nutrients, vitamins and lycopene (Glick *et al.*, 2009). Apart from second most consumable vegetable, it is used in many industrial products and also has share a considerable share in export (Nagaraju *et al.*, 2002). The quality and quantity of tomato produce is badly affected when it is challenged by different disease causing agents (Mari *et al.*, 2007). Tomato crop is heavily devastated by leaf curl disease caused by tomato leaf curl virus (TLCV) (Polston and Anderson, 1997). This disease is prevalent

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all over the world (Valverde *et al.*, 2001). Under epidemic conditions it may cause 80-100% yield losses (Moriones *et al.*, 2017). Tomato leaf curl virus (TLCV) is a DNA virus from the genus Begomovirus and the family Geminiviridae (Gutierrez, 1999). TLCV causes the most destructive disease of tomato, and it can be found in tropical and subtropical regions causing severe economic losses (Moriones and Navas-Castillo, 2000). TLCV virus is transmitted by an insect vector, the whitefly (*Bemisia tabaci*), commonly known as the silver leaf whitefly or the sweet potato whitefly that comes from family Aleyrodidae and order Hemiptera (Ghanim *et al.*, 1998). The primary host for TLCV is the tomato plant, and other plant hosts where TLCV infection has been found include eggplant, potato, tobacco, beans, and peppers (Anfoka *et al.*, 2009). Diseased plants become stunted with upward and downward foliage curling and show chlorotic symptoms (Papayiannis *et al.*, 2011). Due to the rapid spread of TLCV in the last few decades, there is an increased focus in research trying to understand

and manage this damaging pathogen (Lapidot and Polston, 2006). Some interesting findings include virus being sexually transmitted from infected males to non-infected females (vice versa), and an evidence that TLCV is transovarially transmitted to offspring for two generations (Melzer *et al.*, 2010). In the past, different management options including management of insect vectors, removal of weed hosts and adjustment in sowing dates were used against tomato leaf curl virus disease (Polston and Lapidot, 2007). This work was conducted to find out the non-conventional ways for the management of tomato leaf curl virus instead of using insecticides and plant extracts against whitefly. Biochar and priming could trigger the plant mechanisms for maintaining the incoming stress and fending off the pathogen. Effect of biochar and different seedling priming agents was evaluated against TLCV disease severity.

MATERIALS AND METHODS

Field survey and sample collection: Tomato field in the research area Department of Horticulture, University College of Agriculture, University of Sargodha, Sargodha (Pakistan) was surveyed. Tomato leaf curl virus disease was assessed in the aforementioned tomato field (72.740°N and Longitude 32.6861°E). Diseased leaves and stems were detached from the symptomatic plants to perform biological assays in the greenhouse. Whitefly was collected from the diseased plants by using aspirator.

Experimental plan and Design: Experiment was conducted under greenhouse conditions using CRD (Completely Randomized Design). The purpose was to evaluate the efficacy of biochar and priming against the tomato leaf curl virus disease. Pots were filled with soil, sand and farm yard manure (FYM) in equal proportions (50:50). Each treatment was replicated three times. Tomato variety Pakit was transplanted in pots. Recommended agronomic practices were followed.

Preparation of priming solutions: Seedlings were primed with sodium chloride (NaCl), glycerine and distilled water before transplanting. For priming with 0.2M NaCl, 5.85g of salt was dissolved in 1 liter distilled water. Glycerine solution was prepared by taking 50 ml of 5% glycerine and dissolving in 1000ml of distilled water. Seedlings were dipped in the solutions for 1 hour (Tufe, 2016).

Application of Biochar: Twenty pots were reserved for Biochar experiment. Biochar was prepared with the

help of maize clipart without grains. This was prepared by heating them in a tin for 2 hours (Yeboah *et al.*, 2016). Biochar was applied in 15 pots by 1-3% of total mixture in the pot.

Biological assays: Ten pots were used for biological assays, out of which five were transplanted with tomato and five with indicator plants. Suspected viruliferous whiteflies collected from the infected fields were released on to the potted plants covered with polythene sheet. Similarly, leaf patch and cleft grafting techniques were also used for the confirmation of virus. Symptoms were observed on the plants after one week. After the successful completion of biological assays, viruliferous whiteflies were used to inoculate the primed and biochar amended plants.

DATA RECORDING AND STATISTICAL ANALYSIS

Disease severity data were recorded at weekly intervals as follows. Data was subjected to ANOVA and means were compared by LSD test. Analysis was done through Statix 8.1 software.

$$\text{Disease serverity (\%)} = \frac{\text{No. of diseased leaves}}{\text{Total No. of leaves}} \times 100$$

(Bhyan *et al.*, 2007).

RESULTS

Biochar and three priming agents were tested against TLCV disease severity under controlled conditions. All the treatments were used at three concentrations (1%, 2% and 3%). Significant reduction in disease severity was recorded in tomato plants treated biochar and priming agents (distilled water, NaCl and Glycerol).

Biochar amendment with potting mixture was the most effective at 3% followed by 2 and 1% respectively as compared to control in both graft and whitefly inoculation (Figure 1, 2). However, better results were recorded in graft inoculated tomato plants. Mixing of biochar at highest concentration (3%) showed minimum disease severity.

Primed plants with all three agents retained the TLCV infection and showed significant decrease in disease severity as compared to non-primed tomato plants. Priming with distilled water gave better reduction in whitefly inoculated plants than grafted ones as compared to control (Figure 3). All three concentrations of NaCl performed better against TLCV disease severity in graft and whitefly inoculated plants when compared with untreated check (Figure 4).

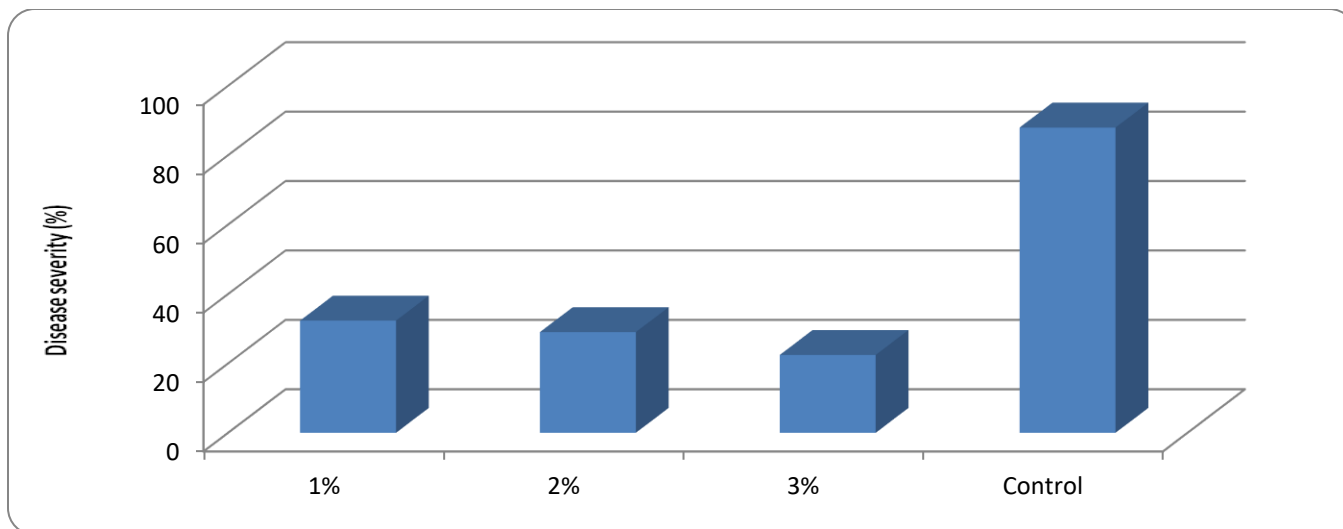


Figure 1. TLCV disease severity in whitefly inoculated tomato plants treated with biochar in potting mixture.

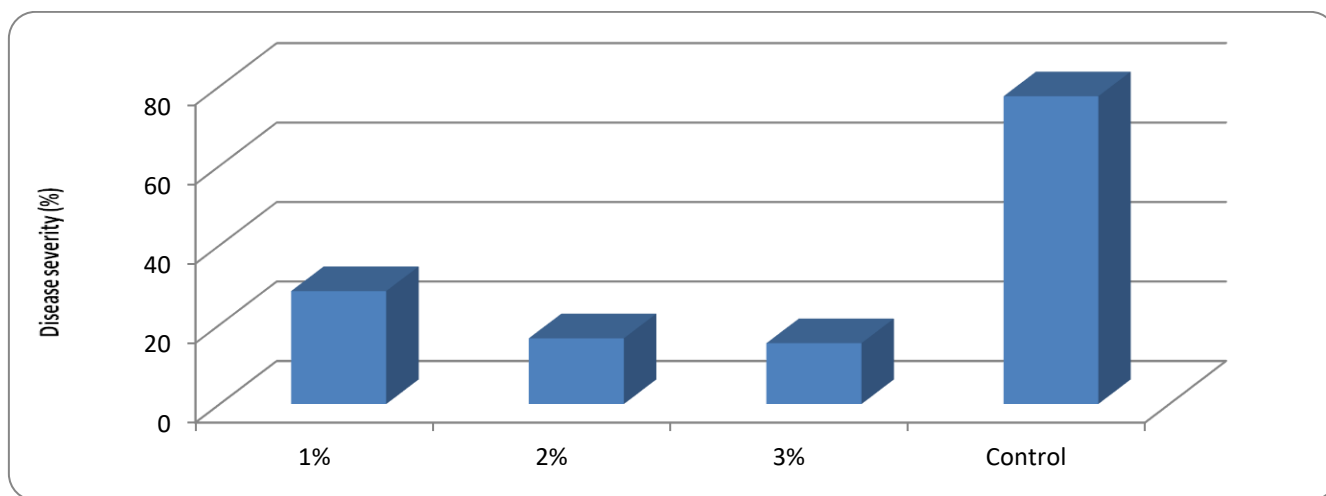


Figure 2. TLCV disease severity in grafted tomato plants treated with biochar in potting mixture.

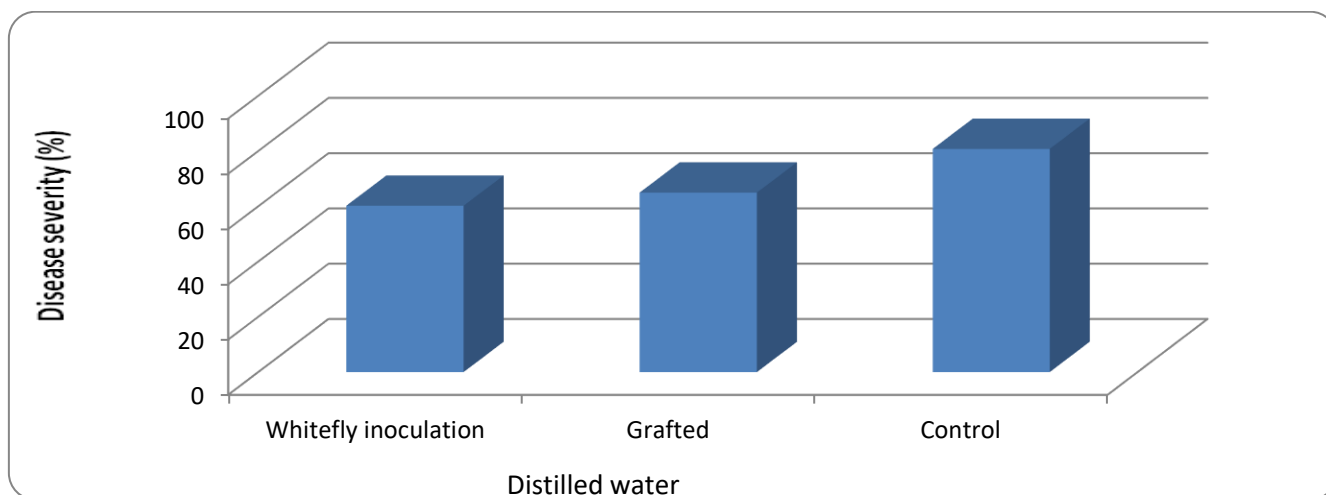


Figure 3. Effect of distilled water priming on TLCV disease severity in whitefly and grafted inoculated plants.

NaCl was the most effective in reducing the disease at 3% concentration while maximum disease was observed in plants primed with 1% concentration followed by non-primed. The overall disease reduction was higher in whitefly inoculated plants than grafted ones in NaCl priming.

Among two sets of tomato plants that were stressed with two inoculation methods, glycerol reduced the disease upto 36 and 40%, respectively in whitefly and graft inoculation. Disease severity was the lowest in

plants where glycerol was applied in 3% concentration while it was the highest in 1% concentration after control.

As a whole high dose of biochar and priming agents were effective in reducing the disease severity. Biochar gave better results in graft inoculated plants whereas all priming agents performed better in whitefly inoculated tomato plants. Among priming agents, minimum disease severity was exhibited by glycerol followed by NaCl and distilled water.

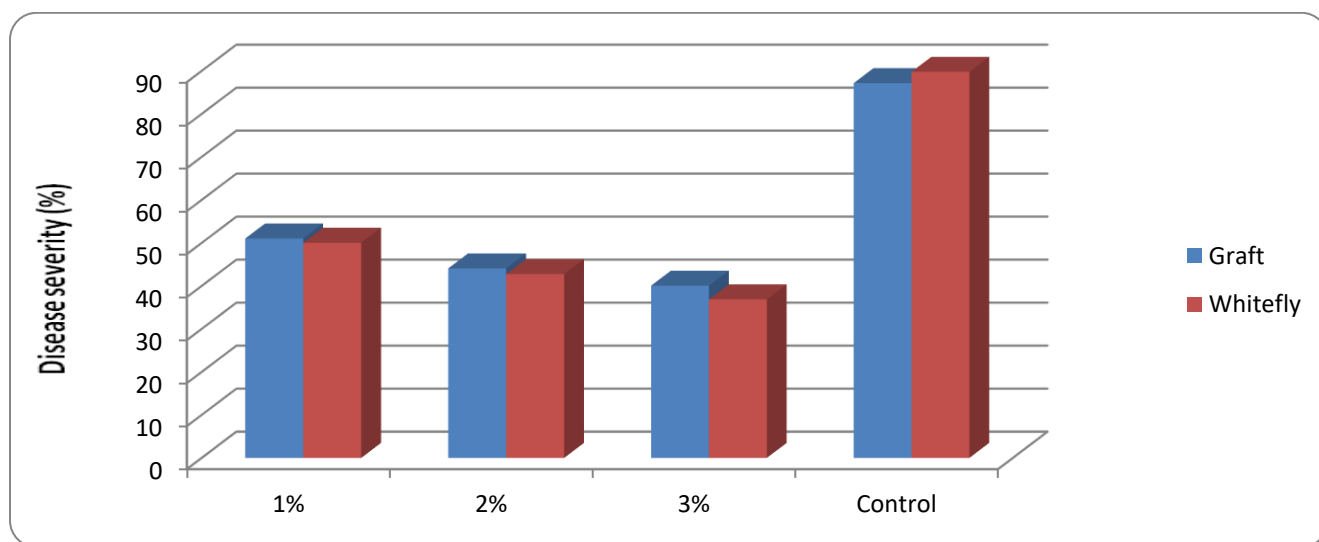


Figure 4. Effect of NaCl priming on TLCV disease severity in whitefly and grafted inoculated plants.

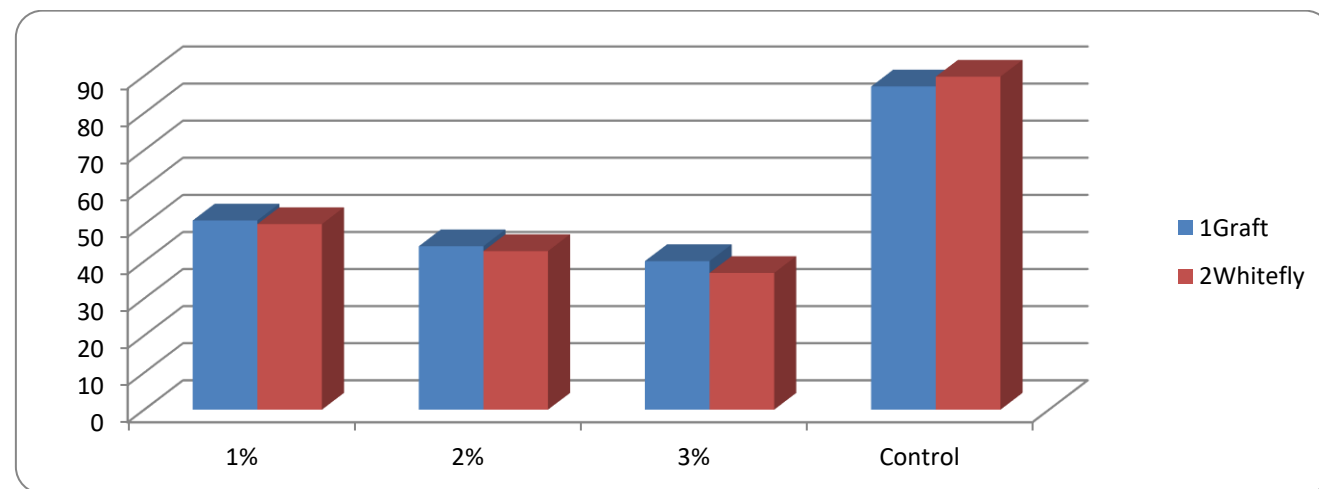


Figure 5. Effect of Glycerine priming on TLCV disease severity in whitefly and grafted inoculated plants.

DISCUSSIONS

Tomato plants treated with different quantities of biochar significantly reduced the disease severity. The results of this experiment are in close conformity with

the results of Elad, (2010) who concluded that biochar could reduce the disease severity upto 68.65%. Application of biochar changes soil physical properties that enhance the systemic acquired resistance in plants

(Elad *et al.*, 2011). Due to improvement in physical conditions of soil, the disease triangle may be affected (Whitman *et al.*, 2016). Biochar increases nutrients availability to plants (Silber *et al.*, 2010) that affect the plant growth and strengthen the plant biochemical mechanisms (Graber *et al.*, 2013). Nutrients enhance the accumulation of inhibitory substances by altering morphology, histology and functions of the tissues (Datnoff *et al.*, 2007). Calcium affects the plant defense by strengthening cell wall, cell membrane and middle lamella (Bateman and Basham, 1976). Biochar also releases hormone like compounds that are absorbed by the plants and trigger the existing biochemical mechanisms (Graber *et al.*, 2010).

Priming induces the disease resistance in plants at cell level and encounters the incoming stress (Ahn *et al.*, 2007). Seedling or seed treatment with different agents may affect the systemic acquired resistance mechanisms. Defense in treated plants activates quickly upon pathogenic attack (Conrath, 2009). Defense activation speeds up bursting of reactive oxygen species and regulating the genes (Slaughter *et al.*, 2012). Priming increases, the receiving of pathogenic signals and stimulating the plant defense signals (Conrath *et al.*, 2006). Molecular characterization of primed plants revealed the increased mitogen activated protein kinases (Conrath, 2011). Primed plants might develop stability against pathogenic exposure and are ready to fend off the physiological interruption by it (Frost *et al.*, 2008). However, the application of biochar was effective than primed seeds and from primed seeds NaCl and glycerol showed good results than distilled water. Glycerol increases the production of pathogenesis related proteins and reactive oxygen species in plants (Zhang *et al.*, 2015). Signals that activated the defense were stimulated by glycerol (Mandal *et al.*, 2012). The result of primed seeds showed close conformity with the result of (Ashraf, 2007) whose study concluded that primed seed control 62% of the disease.

On the basis of aforementioned facts it can be concluded that both biochar and priming might be the best source of defense activation in tomato plants against tomato leaf curl virus. These two strategies would also be helpful in reducing the environmental pollution caused by injudicious use of pesticides.

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