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Disinfectant Efficacy Trial on Tomato Plants Infected with ToBRFV (Tomato Brown Rugose Fruit Virus)

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Abstract

In this study, experiments were conducted to determine the effectiveness of disinfectants against ToBRFV under controlled and field conditions. A total of 184 plants was involved this study using the Samyeli F1 variety from Anamas company with the random blocks trial design. Tomatoplants which are known hosts of ToBRFV were planted according to a randomized block experimental design consisting of one infected plant in each block. To ensure that control group plants were infected with ToBRFV; the tomatoes were mechanically inoculated (three times) using the soft sponge pad method when they reached the true two-leaf stage. In the evaluation of the effectiveness of disinfectants in farmer conditions against ToBRFV; disinfectants with active substances such as 0.5-1% HCl, 11.2% Hydrogen peroxide and disinfectant containing silver (300cc/100lt) were found to be effective. It has been observed that these disinfectants, which are applied with hand sprayers, have a preventive effect on the spread of the virus in an uncontaminated greenhouse after single application in a week. At the end of the experiment, the plants in the greenhouse were subjected to molecular tests. The bands seen in the gel electrophoresis show that the disinfectants are not effective on the related virus agent and the plants are 100% infected with ToBRFV. As a result, it was observed that disinfectants that were effective under controlled conditions delayed the disease epidemic in the greenhouse. However, the disinfectants was not effective in the prevention of ToBRFV infection in studies conducted under field conditions. In addition to the early diagnosis of the presence of virus diseases and the removal of diseased plants from the greenhouse, routine hygiene procedures in the greenhouse are also very important for the control of this virus. For this reason, disinfectant studies to prevent ToBRFV from infecting tomato production areas should be considered.

Keywords

Disinfectants, ToBRFV, Tomatoes, Virüs infection

1. Introduction

Significant loss in tomato production areas due diseases and pests are reported every year. In addition to fungal and bacterial diseases in tomatoes, many viral factors also cause significant economic damage. Tomato brown rugose fruit virus (ToBRFV), which is in the genus Tobamovirus, is a viral disease with high virulence that has recently emerged in greenhouses and production areas and. ToBRFV first appeared in Israel in 2014. Its first occurrence was reported from Jordan in 2016. The disease agent was detected 7 months after this report in Israel's Ramat Negev Region. Studies show

that the disease spreads in various tomato-growing areas of the country within a year [1].

Once ToBRFV infection is recorded in a greenhouse, it can spread be spread through mechanical means. This virus significantly reduces crop production and the market value of tomatoes and peppers, as these crops are the main hosts of this diease. ToBRFV causes chlorosis, mosaic (mild or severe), mottling and narrowing of the leaf blades on the leaves. However, the symptoms of ToBRFV on tomato plants may vary according to the variety and time of contamination. Coarsening of the leaves, browning of the fruit calyx is seen in the early period of infection. In infected tomatoes, the fruits show yellow or brown spots and the wrinkled fruit becomes unmarketable. Fruits may show various forms of deformation and irregular ripening.

Since new isolates of Tobamoviruses infecting tomatoes have been identified, the effectiveness of Tm-2^2 resistance against this virus infection is debated. Tomato mottle mosaic virus (ToMMV), which can overcome the Tm-2^2 resistance gene, has been reported in Mexico and against ToBRFV from Jordan [2]. The absence of a resistant variety and the absence of chemical control highlight the studies on routine hygiene procedures in the greenhouse in the fight against this virus.

2. Materials and Methods

In the 2021 sprng season,the study was conducted in a greenhouse which belonged to Anamas Seed Company in Antalya.

2.1 Material

The isolate 'ToBRFV-Ant-Tom: MT107885' which were the main inoculum materials for mechanical inoculation stagewas obtained from Akdeniz University Faculty of Agriculture Plant Protection Laboratory.

2.2 Method

Disinfectants (Hydrochloric acid (HCl), hydrogen peroxide (H_2O_2), silver-containing) that are frequently used in seed and nursery production facilities and recommended disinfectants in previous studies were selected.

2.2.1 Research plot design

160 tomato plants were used in this study together with a random block experimental design.The experimental design of the research consisted of 4 blocks in each plot. Each plot had ten plants. Disease-free plants were planted in the trial area on November 1, 2021.

Figure 1. Research plot design.

2.2.2 Mechanic inoculation of ToBRFV

Mechanic inoculation was performed on each plant in each block.1gram of infected leaf was mixed with 10ml of 0.02M phosphate buffer (pH:7) and 0.1% 2-mercaptoethanol was added and grounded. Inoculation was performed on the plants durng the two-leaf stage of tomatoes using the softsponge pad method as previously descibed by Fidan and Koç (2021) [3].

Seven days after the initial inoculation,the second inoculation was performed. In total, inoculation was repeated thrice. The purpose of the repeated inoculation processes was to ensure effective and quick infection.

Figure 2. Mechanic inoculation.

2.2.3 Disinfectants application

In this study,hydrochloric acid, hydrogen peroxide disinfectants and a silver-containing chemical obtained from a private company was tested as to whether they could offer tomato plants protection against ToBRFV. The optimum doses of these disinfectants were determined based on the recommendations of the manufacturer. During the study and an upper and a lower dose of these doses were applied. 0.1%, 0.5%, 1% for HCl; 10%, 11.2% and 12% for hydrogen peroxide; For disinfectant containing silver, lower dose: 2mg/L, target dose: 3mg/L, upper dose: 4mg/L was applied.

The effectiveness of disinfectants was measured in farmer conditions while there was a source of infection in the environment, where disinfection was carried out once a week until the fruits came to the third cluster. Different hand sprayers were used for each disinfectant. Starting from the lowest dose of each disinfectant, disinfectants were applied to the plants by spraying from the top in determined amounts into 2 liters of water.

2.2.4 Molecular analysis

DNA isolation was performed on the the samples taken when the fruits were in the third cluster stage. Total nucleic acid extraction was performed during the DNA extraction process. This process was performed with the dellaporta method [4]. Molecular tests were carried out by RT-PCR (Reverse Transcription- Polymerase Chain Reaction) method to diagnose whether ToBRFV, an RNA virus, is present in tomato plants. Primer pair specific to ToBRFV was used during RT-PCR testing [3].

For each of the 3 disinfectants used in the experiment, 3 separate doses were applied and 2 plants were selected from each dose. Since the 18 selected plants were in 4 blocks, a total of 72 plants were selected and tested with the RT-PCR method.

The PCR products obtained after each RT-PCR step were run on a 1.5% agarose gel at 70 Volts for 40minutes.The gels were then stained with ethidium bromide and visualized under UV light.

Table 1. Primers used in molecular testing

ToBRFV1	ToBRFV1 F 5´-CTTCCAAACGTGTACGCACC-3´ToBRFV1R5´-ATGCATCTTCCATTGCGCTG-3´	475 bp Fidanvd. 2021

Table 2. RT-PCR components

Chemical components	Amount (µL)
Verso Enzim	1
2X 1-Step RT-PCR ReddyMix	25
RT Enhancer	2.5
Forward primer	1
Reverse primer	1
Kalıp RNA	2
Distile su	17.5
TOTAL VOLUME	**50**

Table 3. RT-PCR cycling protocol

Stages	Temperature	Period	Cycle
Initial denaturation	50°C	15minutes	1
	95°C	3 minutes	1
Denaturation	95°C	45 seconds	
Annealing	59°C	45 seconds	38
Extension	72°C	45 seconds	
Final extension	72°C	3 minutes	1
Final	12°C	∞	

3. Results and Discussion

The symptoms in the control plants were first observed 16 days after the infection.

Observation of the symptoms of the disease was continued from the 16th day until the third cluster fruits of tomato plants ripened. In seedlings such as mosaic patterns, narrowing of the leaf blades, nodules on the leaf surface and yellowing in the leaf veins were detected. During the ripening period of the fruits, brown lesions, yellow spots causing necrosis, immature, deformed and wrinkled fruits were observed in the fruit as a result of ToBRFV infection.

In the control group plants, the same practices (rope picking, seat picking) were conducted at different times until the fruit ripened. Although disinfectant is applied once a week, if there is a source of infection, virus infections will be detected in most healthy plants. The first symptoms were observed in plants close to the control group when cultural treatments were carried out in the greenhouse. It later spread to other plants. Although leaf symptoms were not observed in some plants, variegation was observed in the fruits.

Within the scope of our study, molecular testing was also carried out to support the phenotypic assessments results in determining the effectiveness of disinfectants. Of the 72 randomly selected plants from different dose-treated plants that were subjected to molecular testing, 70 were confirmed to be infected with ToBRFV.

If these products, which are used for disinfection, are used at intervals of 1 week, very clear symptoms occur in some plants until the third cluster, while in others, symptoms were not seen until the red coloration of the fruit begins. This shows that the disinfectant have an influence on the spread of the disease, on the spread of the disease, but does not stop the virus infection initiation. The tests performed and the symptomatic observations agree with each other.

It was determined that the symptom in plants that were treated with less dose of developed faster than plants that were treated with the target dose. Testing and symptomatic observations were compatible with each other in target dose applications. In the upper dose application of HCl, phytotoxicity occurred in plants. The virus status in the plant could not be evaluated properly because the green parts of the plant were dried.

Figure 3. ToBRFV fruit and leaf symptoms in positive control.

4. Conclusion

In the study to determine the effectiveness of disinfectants against ToBRFV, the virus development could not be stopped in the plants infected with the virus under the application conditions in the greenhouse experiment, however, the spread of the virus was partially slowed down.

Until the 3rd cluster of the tomato plants, 98% of the crops in the greenhouse was infected. As a result of its regular

use once a week, it has a partial inhibitory effect on the spread of the virus. However, the use of the chemicals for more than once a week will increase the protection of the crop against the disease and the cost of production will be high. According to the results obtained from this trial, it was concluded that disinfectants did not have a direct effect on the initial infection capacity of ToBRFV, but have a role in the decrease in the spread of the epidemic.

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