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Disinfectants Useful to Manage the Emerging Tomato Brown Rugose Fruit Virus in Greenhouse Tomato Production

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Abstract: Tomato brown rugose fruit virus (ToBRFV) is an emerging tobamovirus infecting tomato and pepper crops. First identified in 2014 in the Middle East, ToBRFV has spread rapidly around the world. Being seed-borne, resistance breaking and easy mechanical transmission, ToBRFV can spread quickly in a greenhouse through plant handling. Thus, selecting an effective disinfectant that is capable of deactivating virus infectivity is important. We aimed to identify these effective disinfectants for ToBRFV management in greenhouse tomato production, particularly for total cleaning. A useful disinfectant should be effective against ToBRFV infectivity without major phytotoxic effect on the test plants. In this study, we evaluated 11 disinfectants at various concentrations and assessed their efficacy in ToBRFV treatment on tomato plants that were pretreated with or without SP2700, a known antiviral plant activator of Ningnanmycin. SP2700 treated-plants generated systemic acquired resistance with a delay in symptom expression for 2–3 weeks in comparison to the mock control. Overall, 1% Virocid, 2% Virkon S, 0.25% sodium hypochlorite (5% Clorox bleach), and 2.5% trisodium phosphate (TSP) achieved complete deactivation of ToBRFV with 15 min exposure. However, TSP presented serious phytotoxicity. Our results offer practical solutions to manage this emerging disease affecting tomato production in greenhouses.

Keywords: tomato; tobamovirus; disinfection; greenhouse

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1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetables grown worldwide. With over $2 billion annual farm gate value in tomato production, the U.S. is one of the world's top tomato producing countries (https://www.fao.org/statistics/en/, access date: 12 February 2022). In the last few years, world tomato production has faced a serious threat from an emerging tobamovirus, tomato brown rugose fruit virus (ToBRFV). First identified in the Middle East [1,2], ToBRFV has caused outbreaks in greenhouse tomatoes around the world [3], including countries in Asia [4–9], Africa [10]; Europe [11–21], and North America [22–26].

ToBRFV is a seed-borne tobamovirus. Due to its ease of mechanical transmission, contaminated seeds can serve as a pathway for global virus spread. We were the first to identify ToBRFV in the U.S. from an outbreak in greenhouse tomato in Southern California in 2018 [25,27]. ToBRFV had also been detected on tomato plants in a community garden in Florida [28]. The emerging ToBRFV is causing serious concerns for the tomato industry due to its ability to break the *Tm-2*^{2} resistance gene in tomato [2,29,30]. The *Tm-2*^{2} gene has been used to confer resistance to tomato mosaic virus (ToMV), a related tobamovirus, for nearly 60 years [31]. Great efforts have been devoted to identifying new sources of genetic resistance to ToBRFV, with promising results [32–35]. Some complexity in genetic resistance to ToBRFV has been reported [32]. However, no commercial ToBRFV-resistant tomato cultivar is available thus far.

Without disease-resistant tomato cultivars, disease management strategies of exclusion and prevention have been implemented to manage this highly contagious and seed-borne

virus. To protect the U.S. tomato and pepper industries, U.S. Department of Agriculture [36] issued a Federal Order in November 2019, with a 2020 amendment, to regulate imports of tomato and pepper seed, transplants, and produce into the U.S. Similarly, the European Union [37] and China [38] also declared the quarantine status of ToBRFV.

In addition to quarantine regulation and use of virus-free seeds, good hygiene practice and disinfection are key weapons for growers to prevent virus transmission. However, screening and selecting different disinfectant application methods are necessary to identify the most appropriate treatments to achieve the optimum management solutions for ToBRFV. Current treatment methods for ToBRFV control focus on seed treatment [39–41], soil disinfection [42], and cloth washing [43], but there is no relevant information available for total greenhouse cleaning and tool dipping. These are key steps in preventing virus spread between crops and among plants during production.

Our previous efforts in screening disinfectants identified several chemicals that are effective after a short time exposure (<60 s) for two common tobamoviruses: ToBRFV and cucumber green mottle mosaic virus (CGMMV) [44]. However, additional improvements are necessary for practical application of these effective disinfectants in ToBRFV management, especially for total greenhouse cleaning, which could use a longer exposure time (>15 min) and lower concentration of disinfectant to achieve effective disinfection. Such treatments will likely lower the corrosive effect of disinfectants on metal structures and equipment in a greenhouse and reduce the potential for phytotoxicity in cases of their splashes to growing plants.

We were also interested in determining whether any of these chemicals could be useful as a disinfectant for direct application on plants. Using tomato plants pretreated with or without SP2700, a bioformulation which induces systemic acquired resistance (SAR) against tobacco mosaic virus [45–47], we aimed to evaluate the efficacy of disinfectants against ToBRFV infectivity through bioassays.

2. Materials and Methods

2.1. Virus Inoculum Preparation

The US isolate of ToBRFV (CA18-01) was initially collected from a tomato plant in a greenhouse in Southern California [25] and purified through local lesion passage on tobacco (*Nicotiana tabacum* var. Samsun) plants [30]. The pure culture of ToBRFV-US isolate was maintained on tomato cv. Moneymaker plants in a bug dorm (BioQuip Products, Compton, CA, USA) in a containment greenhouse. For bioassay, virus inoculum ($OD_{405\,nm}$:1.706) was prepared using symptomatic leaf tissue in (1:10 *w*/*v*) of a phosphate-saline solution pH 7.0 (140 mM NaCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 2.7 mM KCl, and 0.8 mM Na_2SO_3) and processed using a Homex-6 tissue homogenizer (BioReba, Reinach, Switzerland). The processed virus inoculum was maintained on ice and used to assess the efficacy of disinfectants.

2.2. Tomato Seedling Preparation

Tomato cv. Paipai seeds (gift from Enza Zaden, Enkhuizen, The Netherlands) were sown in 36-cell seed starter trays filled with Metro-Mix potting soil (SunGro Horticulture, Anderson, SC, USA) and maintained in a greenhouse with natural sunlight for 14 h day/10 h night and the associated temperature of 26 °C in day and 21 °C at night with daily watering and weekly fertilizer applications (All purpose 10-10-10, Scotts Miracle-Gro, Marysville, OH, USA).

2.3. Pre-Treatment with SP2700

Tomato 'Paipai' seedlings at 1–2 leaf stage were used for testing. Two separate experiments were conducted to assess the potential of SAR generated by SP2700/Ninja™ (a novel antiviral plant activator with 2% active ingredients) (SePRO, Carmel, IN, USA) on the test tomato plants. The active ingredient for SP2700 is Ningnanmycin, essentially same as those used in other studies [45–47]. We conducted a comparative study to assess the

potential effect of SAR against ToBRFV infection on tomato test plants that were induced by SP2700. In the first experiment, test plants were treated with SP2700, and the second set of test plants was used as a control by treating with tap water. For the experiment with SP2700 pretreatment, one set of the test plants (156 plants) was treated with SP2700 through spraying each specific SP2700 solution to cover the entire seedling until run-off at the first true leaf stage followed by soil drench around the root zone using 30 mL of 0.043% SP2700 solution per plant. In the control experiment, the same number of test plants (156 plants) was treated with water.

2.4. Disinfectant Treatments

To evaluate the efficacy of disinfectants against ToBRFV infectivity we conducted bioassays on SP2700-pretreated and water-treated control plants. Those effective disinfectants identified from our previous study [44] along with several other disinfectants of interest were used for this study. A total of 11 disinfectants were included in the present study which also included a positive ToBRFV control, buffer mock control and healthy tomato plants. The disinfectants and their concentrations are listed in Supplementary Table S1. For each treatment in an Eppendorf tube, an aliquot of disinfectant (500 µL) was mixed thoroughly with an equal volume of the virus inoculum (500 µL) by hand and incubated at room temperature for 15 min. After reaching the designated exposure time, a bioassay was conducted to assess any remaining virus infectivity in that treatment by mechanical inoculation using a cotton swab soaked in the treated virus inoculum to gently rub inoculum onto six tomato test seedlings (either pretreated with SP2700 for 7 days or water-treated control) that were lightly dusted with carborundum. Inoculated plants were maintained in a greenhouse (25–30 °C with 14 h of sunlight) for observation of potential responses to phytotoxicity at one week post inoculation (wpi) and virus infection with symptom expression at 4–6 wpi. To confirm virus infection, laboratory tests using enzyme-linked immunosorbent assay (ELISA) were carried out using a ToBRFV-specific ELISA kit (Agdia, Elkhart, IN, USA).

2.5. Phytotoxicity Observation

To evaluate the phytotoxicity of disinfectants on tested tomato plants, visual observation of plants inoculated with various concentrations of 11 disinfectants was conducted 1–2 wpi. The test plants were either pretreated with SP2700 or without pretreatment.

2.6. Assessing the Efficacy of Disinfectants against ToBRFV Infectivity

To determine the efficacy of different disinfectants, we used symptom observation on the test plants, followed by a confirmation test using double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). ELISA was conducted following the manufacturer's instructions using a commercial ELISA reagent set kit (catalog No. SRA 66800/0500) with antibody that is specific to ToBRFV (Agdia, Elkhart, IN, USA). The mean $OD_{405\ nm}$ was taken for each sample in duplicate wells and compared to those of the positive, mock control and healthy plant. An absorbent value in at least 3 of the healthy controls and above 0.100 was considered a positive infection. Percent infection rate was calculated using number of diseased plants over total number of inoculated plants in each treatment to assess the efficacy of disinfectants. Percent infectivity = 100 × [(No. of plants infected/No. of total plants)].

Bioassay to evaluate the efficacy of Virocid and Prevento to deactivate ToBRFV infectivity was conducted by inoculating six tomato 'Moneymaker' seedlings (2–3 leaf stage) using a freshly prepared ToBRFV inoculum (1:10 w/v) mixed with a serial dilution of Virocid solution (0.5% to 2.0%) for an exposure time of 1 min before inoculation. Inoculated plants were maintained in a greenhouse for symptom observation at 4 wpi. A confirmation test for the presence of ToBRFV in test plants was performed using a ToBRFV ELISA kit (Agdia). The mean absorbance values are the average of individual readings or $OD_{405\ nm}$ readings from bulked samples from 6 test plants per treatment.

2.7. Statistical Analysis

Descriptive statistics were generated using the option 'Tabulate' of JMP Genomics®7 (SAS Institute Inc., Cary, NC, USA). Means and standard deviations were obtained for each treatment. Data were exported to excel and used to generate graphs to visualize the data.

3. Results

3.1. Systemic Acquired Resistance of SP2700 against ToBRFV

Tomato plants pre-treated with SP2700 initially showed enhanced resistance to ToBRFV in comparison to those plants without pre-treatment with SP2700 at 3 wpi (Figure 1). Such initial promising SAR in those SP2700-treated plants did not last and was overcome by aggressive ToBRFV infection after more than 3 wpi. By 6 wpi, symptom expression between SP2700-treatment and those from the mock control were similarly severe on the test tomato plants with no significant difference (Figure 1).

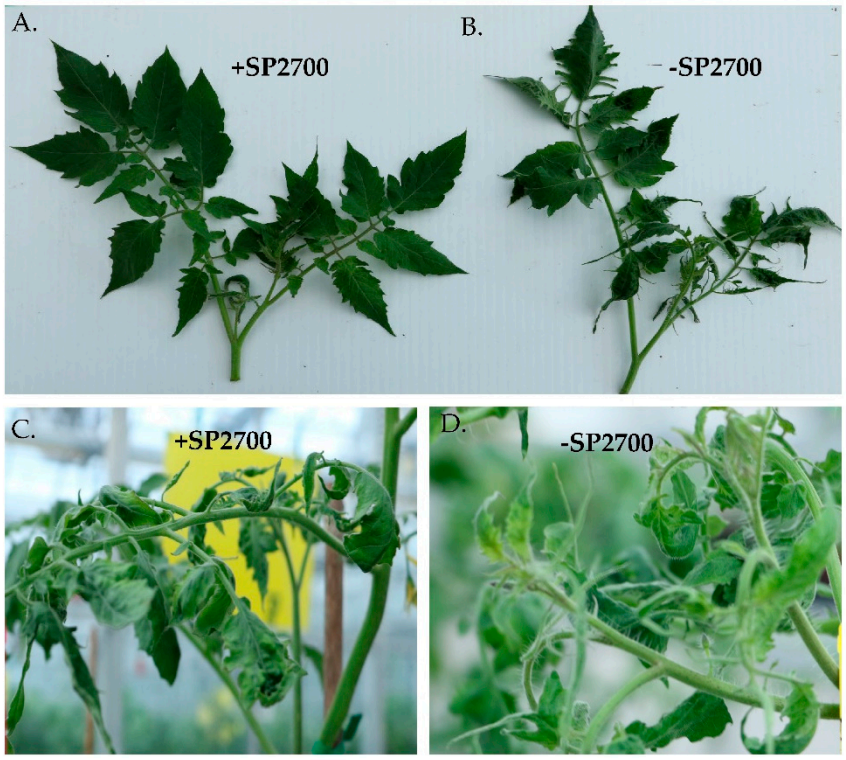

Figure 1. Symptom expression on tomato 'Paipai' plants that were pretreated with or without SP2700 upon inoculation with ToBRFV. (**A**) A tomato plant with pre-treatment with SP2700 showed normal appearance 3 weeks post inoculation (wpi) with ToBRFV. (**B**) A tomato plant without SP2700 pre-treatment displayed mottling and narrow leaves 3 wpi (right). (**C**) Symptom expression on ToBRFV-infected tomato plants that were pre-treated with SP2700 at 6 wpi. (**D**) +Symptom expression on ToBRFV-infected tomato plants without SP2700 pre-treatment at 6 wpi.

3.2. Efficacy of Disinfectants against ToBRFV

Some benefits were observed with a delay in symptom expression for 2–3 wpi on tomato plants in pre-treatment with SP2700 in comparison to mock control plants. However, by 6 wpi, the intensity of symptom expression on those SP2700-treated plants was in the same level of severity as those plants without SP2700 pretreatment (Figure 1). Therefore, we focused our attention on evaluating the efficacy of inactivating ToBRFV infectivity using different concentrations of 11 disinfectants. Two independent experiments were conducted. In one experiment, test tomato plants were pre-treated with SP2700, and in the other experiment, plants with no pretreatment. Results from the two experiments agreed and showed similar trend, therefore the data from both experiments were combined for analysis.

The results indicated that average means for selected concentrations of 11 disinfectants ranged from 0% to 100%. Among these treatments, Virkon S 2%, TSP 10%, TSP 5%, and TSP 2.5% showed mean values of "0", indicating their complete efficacy against ToBRFV infectivity (Figure 2; Tables 1, S2 and S3). TSP achieved a complete deactivation of ToBRFV infectivity at all concentrations (2.5%, 5% and 10%), however, there was severe phytotoxicity to the test plants at all three concentrations. In comparison to those plants treated with SP2700, plants treated with TSP were severely stunted (Figure 3). While there was 100% efficacy for deactivating ToBRFV using 2% Virkon S, there was nearly no effect when using Virkon S at two lower concentrations (0.1% and 0.5%). Clorox bleach solution at 5% (0.25% sodium hypochlorite) had nearly full control against ToBRFV infectivity whereas no significant effects were observed at lower concentrations (0.1% and 0.25%). Similarly, Virocid at 0.5% had only partial effect (50%), while the lower concentrations (0.01%, 0.05%, and 0.025%) showed no effect against ToBRFV infectivity. Our previous results showed near complete efficacy to ToBRFV infectivity when using Virocid in higher concentrations at 0.5% and 2% [44]. On the other hand, several other chemicals tested (including SP2700, Proferrin, Physan 20, Menno Florades, and KleenGrow) had no effect on ToBRFV infectivity at all the concentrations used (Figure 2, Tables 1, S2 and S3).

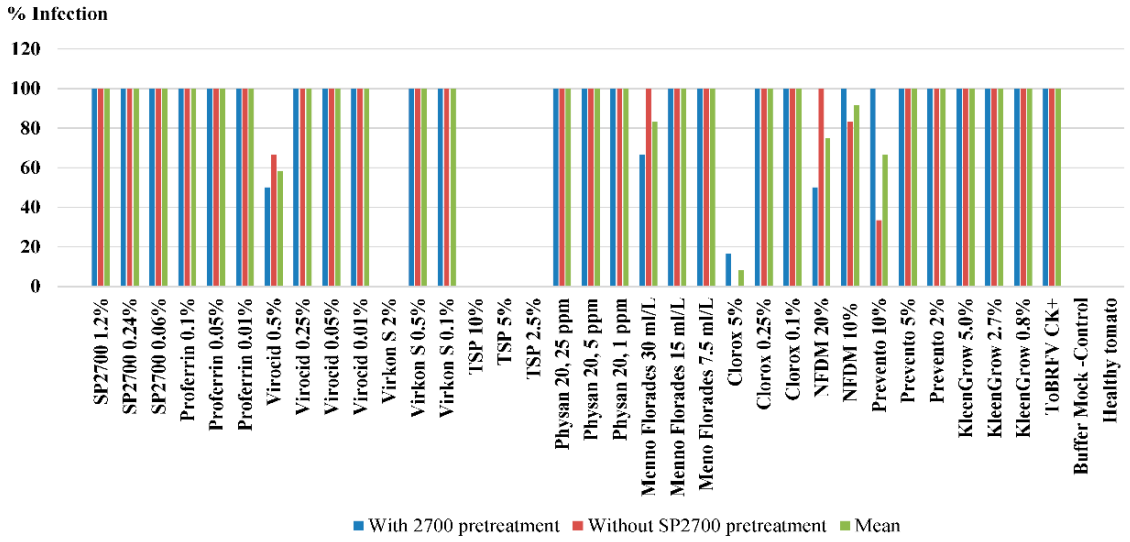

Figure 2. Mean percent infection rates on test tomato plants infected by ToBRFV in two bioassay experiments to assess efficacies of disinfectants at their specified concentrations. In two separate experiments, similar effects in disinfection against ToBRFV infectivity were observed on various disinfectants using test plants that were either pre-treated with SP2700 or without pre-treatment. The positive control (ToBRFV CK+) showed 100% infection, whereas the Mock treatment with buffer as well as tomato plants without inoculation remained healthy. TSP: trisodium phosphate, NFDM: nonfat dry milk.

3.3. Effect of Different Virocid Concentrations against ToBRFV Infectivity

To further characterize the efficacy of Virocid for its ability to deactivate ToBRFV infectivity, we conducted two additional experiments with a small increment of concentration of Virocid, including 0.5%, 0.75%, 1.0%, 1.25%, 1.50%, 1.75%, and 2.0%. As shown in Figure 4, Tables 2, S4 and S5, results from two experiments were in general agreement, suggesting that treatments were reliable. Although there was a complete deactivation of ToBRFV at all concentrations (0.5% to 2.0%) in the experiment 1, a small number of test plants in experiment 2 showed partial efficacies in two lower concentrations, 0.5% (50%) and 0.75% (36.7%). However, those treatments using higher concentrations (1–2%) achieved complete or near complete deactivation of ToBRFV infection, with one of the six plants infected at 1.75% (Figure 4; Tables 2, S4 and S5). Taken together, these results demonstrate that 1% Virocid was reliable for complete deactivation of ToBRFV infectivity with less than 1 min

exposure time. In addition to Virocid, we also included Prevento for evaluation in these experiments. The efficacy of Virocid and Prevento in serial dilutions from two independent experiments demonstrated that Virocid (1.0%), Virocid (1.25%), Virocid (1.50%), and Virocid (2.00%) were fully effective in treating the ToBRFV infectivity (Figure 4, Tables 2, S4 and S5). Although Prevento was not fully effective against ToBRFV, some reduction in infection was observed in both experiments (Figure 4, Tables 2, S4 and S5).

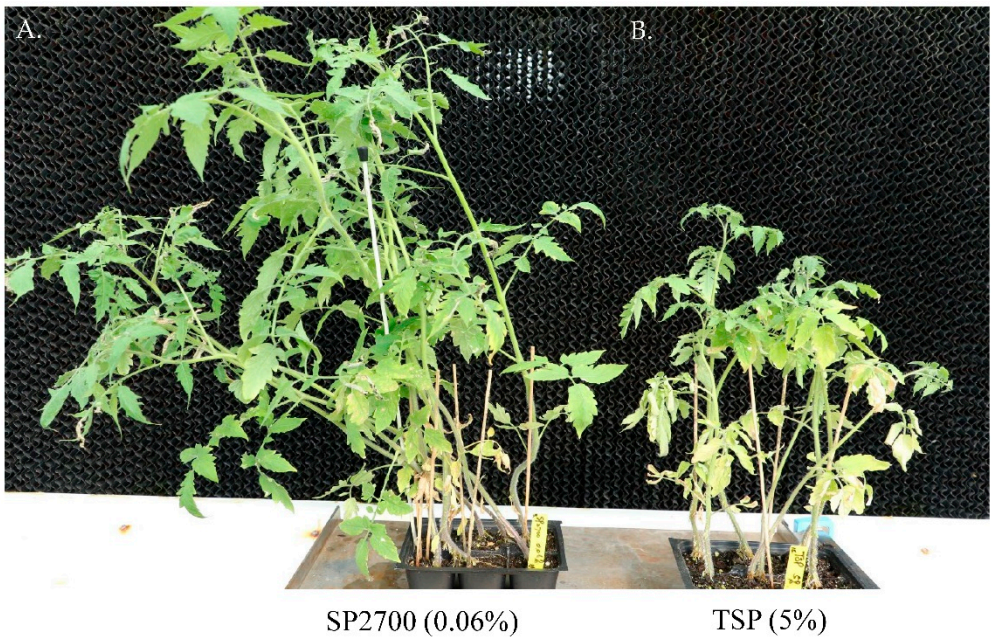

Figure 3. Comparison of plant growth enhancement on tomato 'Paipai' plants treated with SP2700 and plant growth suppression on other tomato plants treated by trisodium phosphate (TSP). (**A**) tomato plants treated with SP2700 (0.06%) had enhanced plant growth. (**B**) Tomato plants treated with TSP (5%) had stunted growth at 6 weeks post inoculation using ToBRFV inoculum that was treated with respective disinfectants.

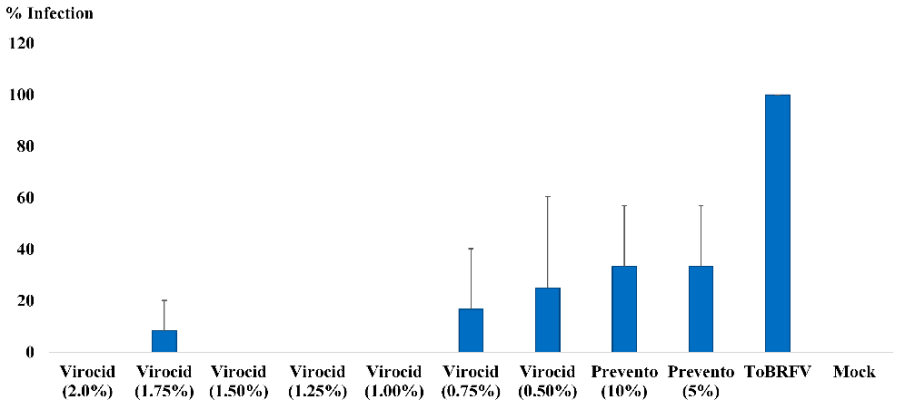

Figure 4. Average percent infections and standard deviations on test plants used for bioassays to assess efficacy of different concentrations of Virocid in a serial dilution (0.5% to 2%) against ToBRFV infectivity in two separate experiments. In addition, Prevento in 5% and 10% was also included in the test to show some partial effects against ToBRFV infectivity, whereas the positive control (ToBRFV) showed 100% infection and the Mock treatment with inoculation buffer had no infection.

Table 1. Bioassays to assess the efficacy of disinfectants against ToBRFV infection with or without SP2700 pretreatment.

Treatment *	Plants with 2700 Pretreatment		Plants without SP2700 Pretreatment	
	Infected/Total Plants	Mean $OD_{405\ nm}$	Infected/Total Plants	Mean $OD_{405\ nm}$
SP2700 1.2%	6/6	1.43	6/6	1.25
SP2700 0.24%	6/6	1.48	6/6	1.27
SP2700 0.06%	6/6	1.44	6/6	1.27
Proferrin 0.1%	6/6	1.49	6/6	1.30
Proferrin 0.05%	6/6	1.43	6/6	1.30
Proferrin 0.01%	6/6	1.48	6/6	1.31
Virocid 0.5%	3/6	0.95	4/6	0.83
Virocid 0.25%	6/6	1.57	6/6	1.20
Virocid 0.05%	6/6	1.37	6/6	1.20
Virocid 0.01%	6/6	1.40	6/6	1.09
Virkon S 2%	0/6	0.15	0/6	0.43
Virkon S 0.5%	6/6	1.37	6/6	1.33
Virkon S 0.1%	6/6	1.24	6/6	1.21
TSP 10%	0/6	0.07	0/6	0.18
TSP 5%	0/6	0.08	0/6	0.05
TSP 2.5%	0/6	0.00	0/6	0.41
Physan 20, 25 ppm	6/6	1.57	6/6	1.27
Physan 20, 5 ppm	6/6	1.60	6/6	1.27
Physan 20, 1 ppm	6/6	1.62	6/6	1.25
Menno Florades 30 mL/L	4/6	1.25	6/6	1.34
Menno Florades 15 mL/L	6/6	1.57	6/6	1.17
Meno Florades 7.5 mL/L	6/6	1.52	6/6	1.31
Clorox 5%	1/6	0.47	0/6	0.13
Clorox 0.25%	6/6	1.54	6/6	1.26
Clorox 0.1%	6/6	1.54	6/6	1.26
NFDM 20%	3/6	0.97	6/6	1.26
NFDM 10%	6/6	1.70	5/6	1.14
Prevento 10%	6/6	1.58	2/6	0.51
Prevento 5%	6/6	1.72	6/6	1.30
Prevento 2%	6/6	1.83	6/6	1.32
KleenGrow 5.0%	6/6	1.60	6/6	1.31
KleenGrow 2.7%	6/6	1.65	6/6	1.29
KleenGrow 0.8%	6/6	1.74	6/6	1.32
ToBRFV CK+	6/6	1.48	6/6	1.60
Buffer Mock-Control	0/6	0.00	0/6	0.02
Healthy tomato	0/6	0.01	0/6	0.00

* TSP: trisodium phosphate, NFDM: nonfat dry milk.

Table 2. Bioassay to assess the efficacy of Virocid and Prevento at serial dilutions for short exposure time to treat ToBRFV infectivity.

Treatment	Exp-1		Exp-2	
	Disease/ Total Plants	Mean $OD_{405\ nm}$	Disease/ Total Plants	Mean $OD_{405\ nm}$
Virocid (2.0%)	0/6	0.00	0/6	0.01
Virocid (1.75%)	0/6	0.13	1/6	0.52
Virocid (1.50%)	0/6	0.01	0/6	0.02
Virocid (1.25%)	0/6	0.01	0/6	0.02
Virocid (1.00%)	0/6	0.00	0/6	0.01
Virocid (0.75%)	0/6	0.04	2/6	0.84
Virocid (0.50%)	0/6	0.05	3/6	0.54
Prevento (10%)	1/6	0.50	3/6	1.53
Prevento (5%)	3/6	1.38	1/6	0.42
ToBRFV + control	6/6	2.63	6/6	2.67
Mock control	0/6	0.00	0/6	0.00

3.4. Phytotoxicity

There were some minor phytotoxic effects observed on inoculated plants for some disinfectants, particularly those at higher concentrations. However, affected plants were able to recover and grow normally in most cases. Only in those plants treated with TSP were permanent phytotoxic effects observed from all three concentrations (2.5%, 5%, and 10% TSP) (Figure 3). For Virocid, we observed some general phytotoxic effect on inoculated cotyledons and/or leaves (with necrotic lesions) from test tomato plants at each of those concentrations (Figure 5). The injured plants were able to recover as the plant grew. Similarly, plants exhibiting minor phytotoxicity effects with necrotic lesions as those from Virocid (Figure 5) were treated by SP2700 (0.24% and 1.2%), Virkon S (2%), Clorox (5.0%), Prevento (10%), and KleenGrow (5%).

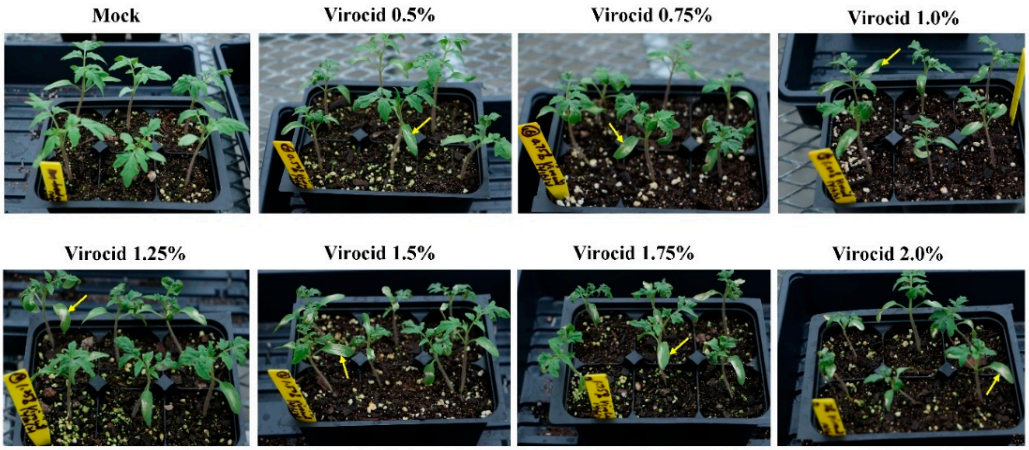

Figure 5. Phytotoxicity was observed on inoculated leaves in test plants treated with Virocid in serial dilutions (0.5% to 2%) on cotyledon and/or first leaf, with necrotic lesions. These injured plants were able to recover as plants continued to grow.

4. Discussion

ToBRFV is an emerging virus and breaks the resistance gene (*Tm*-2^2) in tomato that has been incorporated in many tomato cultivars to manage tomato mosaic virus (ToMV), a common tobamovirus infecting tomato plants. Without a resistance cultivar available against ToBRFV, we aimed to develop an integrated pest management system to control this emerging disease through a combination of systemic acquired resistance and effective disinfectants. We conducted experiments to assess SAR induced by SP2700 on tomato plants against ToBRFV infectivity. SP2700 is a bioformulation product of Ningnanmycin which imparts SAR against TMV [45–48]. Some delay in symptom expression for up to three weeks was observed on SP2700-treated plants. However, SP2700 did not offer sufficient immunity against ToBRFV infection as plants grew older. When used at higher concentrations (2.4%), it was an effective disinfectant with antiviral activity against tobamoviruses, including ToBRFV and CGMMV [44]. When used at a lower concentration (0.06%) as spray on seedlings and/or root drench, we observed a delay in symptom expression on test plants infected by ToBRFV. This is a promising observation, although the mechanism of SAR by SP2700 against ToBRFV on tomato plants would require further investigation, which is beyond the scope of the present study.

Several disinfectants have been identified against tobamoviruses using short exposure time (<1 min) [44]. In the present study, we were interested in their effect with extensive period of exposure (15 min) to mimic floor cleaning process during greenhouse total cleaning. Although the efficacy of effective disinfectants against ToBRFV infectivity with longer exposure (15 min) did not improve significantly in comparison to those with shorter exposure (<1 min) [44], optimum concentrations for each specific disinfectant were selected through this extensive screening. Using 11 chemicals at various concentrations for treatment against ToBRFV, we assessed first the phytotoxicity from each disinfectant on test

plants 1–2 wpi. The efficacy of disinfectants against ToBRFV infection was then determined by symptom expression, followed by a confirmation test using ELISA for the target virus at 4 wpi. Although some minor phytotoxicity from various disinfectants in higher concentrations was observed, the injured plants were generally able to recover as plants continued to grow. One exception was TSP. Even though TSP achieved complete deactivation of ToBRFV, severe phytotoxicity on test plants was observed from all three concentrations (2.5%, 5% and 10%). In two independent experiments, test plants in one experiment had a pretreatment with SP2700 for two weeks prior to the disinfectant evaluation and another experiment using water as a mock control. The most effective disinfectants were 2% Virkon S and 5% Clorox bleach solution (0.25% sodium hypochlorite) in longer exposure time (15 min). Virocid at 0.5% only had a partial effect (50%), although our earlier study using 0.5% had near complete efficacy on ToBRFV [44].

However, Virocid at 1% achieved complete deactivation of ToBRFV infectivity. The lesser effect in using 0.5% Virocid in the present test prompted us to take a closer examination of Virocid, as 2% Virocid is not currently supported by the product label. To achieve better assessment on the efficacy of Virocid, a serial dilution of Virocid in 0.25% increments from 0.5% to 2% (0.5%, 0.75%, 1%, 1.25%, 1.5%, 1.75% and 2%) was used. Although the efficacies against ToBRFV from two lower concentrations (0.5% and 0.75%) were not complete, Virocid at higher concentrations (1%, 1.25%, 1.5%, 1.75% and 2%) achieved full protection against ToBRFV infection. There was only one outlier with less than 100% efficacy, but still highly effective at 1.75%.

In the present study, TSP was highly effective against ToBRFV with nearly complete deactivation of ToBRFV using three concentrations tested (2.5%, 5.0%, and 10.0%), although phytotoxicity and adverse effects on plant growth were observed. Dombrovsky et al. (2022) used 3% chlorinated TSP to achieve a successful application through soil disinfection against ToBRFV and CGMMV. These studies support that TSP could be an effective disinfectant for ToBRFV, some preventative measures would be necessary to avoid a direct contact of TSP to the test plants.

With the 15 min exposure time used in the present study, we observed no major effect in disinfection against ToBRFV by Menno Florades. However, with prolonged exposure (16 h), Ehlers and colleagues [43] were able to demonstrate that Menno Florades, in combination with detergents (Fadex H+ and Menno Hortisept Clean Plus), achieved a near complete removal of ToBRFV from contaminated fabrics. In the present study, the 15 min exposure time was selected based on consultation with several major greenhouse tomato growers in the U.S. and Canada to mimic the general time that is needed for a disinfectant staying to remain with a concrete floor or other surface areas during total greenhouse cleaning. It is therefore important to understand the growers' needs for disinfection and to design the experiments tailored their specific application and solution needs.

Several attempts in seed treatment on tomato against ToBRFV have yielded some promising results using common chemicals, including hydrochloric acid and sodium hypochlorite [39,40]. With several other disinfectants (Virocid, Virkon S and TSP) that are effective against ToBRFV in the present study, it is now possible to evaluate their efficacies for seed treatments against this and other seed-borne tobamoviruses.

5. Conclusions

With ToBRFV outbreaks being reported around the world and the lack of resistance cultivars, there is an urgent need to develop an integrated pest management system capable to effectively control this highly contagious and emerging disease. In the present study we assessed the SAR by SP2700 against ToBRFV on tomato and determined the effectiveness of disinfectants against ToBRFV infectivity at longer exposure time (>15 min). The most effective disinfectants against ToBRFV were 1% Virocid, 2% Virkon S, and 5% Clorox bleach (0.25% sodium hypochlorite). This work complemented other disinfectant studies on seed treatment [39–41], soil disinfection [42], and cloth washing [43] by filling the gap to potentially use these effective disinfectants for tool dipping, surface disinfection, equipment

spraying and total greenhouse cleaning. Further study is needed to evaluate the mechanism of SAR induced by SP2700 on tomato plants and to sustain the immunity level useful for practical disease management.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8121193/s1, Supplementary Table S1. List of disinfectants and their application rates used in this study; Supplementary Table S2. Virus infection status to assess the efficacy of treatment against ToBRFV using various disinfectants on tomato plants with SP2700 pretreatment; Supplementary Table S3. Virus infection status to assess the efficacy of treatment against ToBRFV using various disinfectants on tomato plants without SP2700 pretreatment; Supplementary Table S4. Virus infection status to assess the effectiveness of treatment against ToBRFV using serial dilutions of Virocid and Prevento, Experiment-1; Supplementary Table S5. Virus infection status to assess the effectiveness of treatment against ToBRFV using serial dilutions of Virocid and Prevento, Experiment-2.

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