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Research Article

Evaluation of Selected Disinfectants and *Trichoderma harzianum* for Preventing Tool-Mediated Transmission of Tomato Torrado Virus Between Tomato Plants

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Abstract

Background: We should consider chemical sanitation and host protection for tomato viruses because contaminated pruning tools can play a role in spreading viruses. **Objective:** To determine ability of selected disinfectants and root-applied *Trichoderma harzianum* in reducing tool-mediated transmission of Tomato torrado virus. **Methodology:** 250 recipient plants were challenged in 5 experimental runs with contaminated tools after water processing, 2% Virkon S, 0.5% sodium hypochlorite, 1% Virocid and water processing of tools followed by fungus root treatment. Chemical contact times were 60 seconds. Seven days before challenge the root-zone was treated with a drench of *Trichoderma.* Serial ELISAs for assessment of infection up to day 42 followed by biological indexing. **Results:** 43/50 of the water controls, 9/50 of the Virkon S treated, 6/50 of the sodium hypochlorite treated, 4/50 of the Virocid treated and 29/50 of the fungal treated plants were finally infected, respectively. Relative reductions versus water were 79.1%, 86.0%, 90.7%, and 32.6%, respectively. All active treatments were significantly different (Holm's adjustment) from water. Fungal treatment had higher incidence of infections than chemical treatments; however differences among chemical treatments were not significant. The reduction was achieved from all the active treatments in all five runs, however sign tests were nonsignificant. Indexing revealed six more infections of the 85 recipients who were found by ELISA. 75 healthy donors controlled for infection did not have infection detected. Fifteen plants in each treatment were not observed to have any visible acute damage recorded. **Conclusion:** Chemical sanitation was more effective than fungal treatment in reducing transmission; commercial safety and superiority and resistance effectiveness need to be considered.

1. Introduction

Viral diseases in tomato pose a substantial threat to the sustainable production of tomato due to stunted growth, reduced fruit quality and reduced marketability. Tomato torrado virus (ToTV) was first described in Spain in 2007, and is responsible for the presence of unusual necrotic lesions and burn-like symptoms in the leaves and fruits [1]. The virus is a member of the genus *Torradovirus* in the family *Secoviridae* which is bipartite with a positive sense single-stranded RNA genome [2]. ToTV is distinguished by these characteristics and they help to determine the level of prevention to be implemented. Knowing how the disease is transmitted is critical in developing management tools. Experiments revealed that ToTV can infect pepper and eggplant in addition to tomato and be transmitted by whitefly [3]. Subsequent

studies confirmed semipersistent, stylet borne transmission of torradoviruses by three species of whitefly [4]. However, under experimental conditions ToTV may be transmitted mechanically as well. Recently, genetic variability data was shown to affect both sap transmissibility and viral levels [5]. Thus, successful mechanical inoculation does not indicate that this means the tool will mediate this transmission under production conditions.

Pruning and falling leaves open wounds that may lead to contaminated sap getting into susceptible tissues following reuse of tools. This indicates that direct evidence should be obtained through the use of infected donor plants and healthy recipients instead of only from sap inoculation. Specific diagnostic confirmation is also required for reliable assessment as it is possible to have infection despite the absence of symptoms. ToTV and Pepino mosaic virus have been distinguished from each other and single and mixed infections have been differentiated by a multiplex reverse transcription polymerase chain reaction assay [6]. Specificity of diagnoses permits interpretation of the results of treatment. Chemical disinfection methods can decrease the amount of infectious material which may be attached to plant materials. Some types of disinfectants that utilize oxidizing agents or sodium hypochlorite were protective against mechanical transmission in tomato virus and viroid experiments [7]. Further studies revealed that the effectiveness of disinfectants is dependent on disinfectant, targeted virus, and exposure conditions for Tomato brown rugose fruit virus and Cucumber green mottle mosaic virus [8]. There is an important need to balance the viral inactivation and the phytotoxicity, as highlighted in the evidence [9]. But these same findings are for pathogens of different organisms and it is not recommended to make assumptions of effectiveness of disinfectants for ToTV on contaminated tools without carrying out tests.

Biological protection is used instead of disinfection, focusing on the defense mechanisms of plants instead of contaminated surfaces. *Trichoderma harzianum* is a beneficial fungus that can interact with the plant root and enhance plant growth and defense mechanisms [10]. The interaction between *Trichoderma* and plants/pathogens includes metabolites and signaling processes that may affect the disease [11]. This priming or induced systemic resistance can enhance plant response to a later challenge by pathogens [12]. However, biological control of fungal pathogens does not result in protection against virus infection, nor is there any direct inactivation of virus particles off the plant. Vitti reported that experiments conducted with tomato revealed that *T. harzianum* strain T-22 suppressed the severity of symptoms and accumulation of Cucumber mosaic virus, thus supporting its potential of inducing antiviral resistance [13]. Many differences between host, fungal strain, virus, and application method, however, still make it difficult to extrapolate to ToTV. Therefore, assessing disinfectants together with *Trichoderma* serves two purposes for an experiment: to mitigate infectivity of contaminated disinfectants and also to help increase the plant's resistance. Identification of these targets of intervention is important so that chemical sanitation is not mistaken for biological protection and to determine efficacy by confirmed infection, rather than for apparent plant appearance.

In a field experiment, three chemical disinfection treatments and a treatment of root application of *Trichoderma harzianum* were tested for their capacity to reduce the Tomato torrado virus (ToTV) transmission in tomato through tool interaction. Results of infections were measured against a water processed contaminated-tool control. The absolute and relative differences in the incidence of infection were quantified, the absolute (aggregate) and run (adjusted) treatment estimates were compared and agreement between the serial diagnostic assessment and the final classification of infections was examined. Tool sanitation and host directed biological protection was tested as two different means of protection.

2. Materials and Methods

2.1. Plant material, virus screening, and containment

The varieties of tomato are contained in the genus *Solanum*, L. Tomato (*Solanum lycopersicum*) cv. Betalux plants were cultivated separately in 1 L pots filled with 3:1 v/v peat – perlite sterilized substrate. Plants were grown as described above with 28/24°C days and nights and at 60–70% relative humidity. Irrigation and fertility practices were the same for all treatments. They used individual saucers, placed beside each other, and spaced so as to not touch the foliage. Plants were challenged at either the 4 to 6 true leaf stage. The donor plants were established by mechanically applying 3 mL of tissue homogenate from 0.7 g leaf tissue in 0.05 M phosphate buffer (pH 7.2) to two stoned leaves with carborundum. Hosted by using this inoculation step in order to establish infected donors which is independently of recipient challenge. Recipients and donors were matched for donor age and infection stage. Serological and biological procedure as described below was used for the assessment of infection p [14]. No independent molecular authentication of the isolate was performed. Donor plants were tested for Pepino mosaic virus, Tomato mosaic virus, Tobacco mosaic virus, Cucumber mosaic virus and Tomato brown rugose fruit virus. Only those donors who were again negative after screening for the non-target viruses and who were seronegative and had no symptoms were included and only those who were seronegative and symptom-free recipients were included. Separate donor handling, sterile waste disposal, dedicated gloves and instruments, insect-proof containment and whitefly monitoring were utilized.

2.2. Experimental design and sample tracking

The experiment was made up of 5 runs (R1 – R5), 10 contaminated-tool donor blocks (D01 – D10) and the following 5 treatments: T1 – water-processed contaminated-tool control, T2 – 2% w/v Virkon S, T3 – 0.5% w/v sodium hypochlorite, T4 – 1% v/v Virocid and T5 –water- processed contaminated tools + root-applied *Trichoderma harzianum*. One recipient was challenged in each donor block and 250 treatment recipient plants resulted. Each subject was assigned an individual identifier (ID) that is related to each run, each donor block and the treatment. A total of 75 healthy-donor recipients (three plants per treatment per run) and 50 untreated-tool recipients (ten plants per run) were used for the supporting controls. Twenty satellite plants were planted for root evaluations and fifty plants in the pilot study. Unique identifiers in the laboratory were used to match up records of recipients with their experimental run, donor block and treatment. However, plants that had been "satellite" or "indicator" plants, plants already carrying the plant growth promoting rhizobacteria, or plants that had already been treated with the plant growth promoting rhizobacteria were not accounted for as additional efficacy replicates. The experiments were conducted with a culture of *Trichoderma harzianum*; which has not been included in this manuscript as far as its strain or culture identifier is concerned, since it was part of the routine collection of the institute. The diagnostic reference panel consisted of 200 samples 100 samples being the positive-reference plants, 50 healthy untreated samples, 20 healthy fungal-treated and 30 samples with non-target viruses. A functional carryover experiment was performed using 60 recipients per trial (60 for the water, Virkon S, sodium hypochlorite and

Virocid blade-processing trial workflows). Laboratory identifiers were used to track the samples and recipients.

2.3. Pilot study and run-acceptance criteria

Prior to the main five-run experiment, recipients of contaminated tools underwent a water process and 20 of them and an additional 20 untreated recipients were set aside as controlled subjects while 10 healthy-donor controls were used. Separate analysis of pilot data to the main experiment was performed and all pilot plants were grown to day 42. To validate the pilot, one group of infected donors was to have 20 recipients, with at least 14 confirmed infections, and no positive results were to be found among the healthy-donor controls. Pilot records were kept apart from these in the main experiment. A run was deemed successful if $\geq$7/10 T1 the recipient of the graft was detected as infected and the healthy-donor recipient was not detected as infected. For each run, run identifiers, challenge times, sampling schedules and outcome classifications were recorded. No exclusions or repeat runs were recorded as all 5 runs met the acceptance criteria.

2.4. Preparation and application of treatments

Virkon S was prepared at a concentration of 2% (w/v) of 1 L and Virocid was prepared at 1% (v/v) of 1 L. Sodium hypochlorite was prepared by diluting 100 mL of the stock, labelled 5.00% w/v NaOCl to 1 L to yield 0.50% w/v NaOCl (5 g/L). These concentrations are on a preparation basis and no analytical measurement of available chlorine was taken. A batch identifier was assigned to each preparation that was related to the product lot. This manuscript does not report supplier names and product lot numbers. The "recipients" were given 20 mL each, treatment baths were not reused. Potato dextrose agar was used for the growth of the *T. harzianum* culture, which was applied for T5, for seven days at 25°C. The conidia were collected in sterile water with 0.01% of the additive Tween 20, the mycelium was filtered off and the number of conidia were estimated in the hemocytometer. The number of viable conidia per mL in the suspension was adjusted to 1×10^7/mL. 96% germination rate was obtained. A single 10 mL root-zone drench was applied to each T5 recipient a week before the challenge was applied. 10 mL of the fungus-free carrier was given to carrier-treated recipients. Fungal recovery was performed in satellite plants to verify the presence of the fungus in treated roots and its absence from carrier-treated roots.

2.5. Tool contamination, disinfection, and recipient challenge

A new sterile stainless-steel scalpel was used for each recipient and the type, exposed length and cut positions of the scalpel blades was standardized. Blades were contaminated by making five consecutive cuts through symptomatic petioles of the donors. Prepared sap and also carborundum was not applied to recipient tissues. The cutting surface was completely submerged in water for 15 s and not pre-cleaned, and it was contaminated for 60 s at 22 $\pm$ 2°C. Each blade was then subjected to three different 20-mL sterile-water rinses for 5 s each with a 10-s drainage period between rinses and was immediately used to remove three lower leaflets from one recipient prior to disposal within 30 s of each rinse. The immersion time was 60 s in water for T1 and 60 s in the disinfectant solution for T2, T3 and T4 followed by the same rinse as mentioned above [15]. To investigate host protection in isolation to the tool disinfection, T5 was water processed using water-processed tools. In the case of healthy-donor controls the same sequence was performed but using healthy donor tissue. Untreated-tool controls were exposed directly to contaminated tools without immersing or rinsing. A single contact time and concentration was evaluated for each preparation. The technical evaluations of dose response curves, minimum effective concentrations or the re-use limits of the baths were not carried out.

2.6. Diagnostic assessment and outcome classification

Uncut leaves were collected 42 days after challenge for analysis by ACP-ELISA. Each sample was extracted directly in 2 mL of 0.05 M DIECA (pH 9.6) and assayed in duplicate wells with rabbit anti-ToTV antibodies and anti-rabbit alkaline-phosphatase conjugate (both at 1:1000). The antibody and conjugate supplier information or catalogue/lot numbers are not included in this manuscript. After the adapted DSMZ protocol [16] absorbance was measured at 405 nm. Technical replicates consisted of duplicate wells. Using the negative-reference mean plus three standard deviations as the positivity cutoff, 25 individuals were classified.A positivity cutoff of 0.18 was determined by adding three standard deviations to the mean of the negative reference population; twenty-five plants were classified using this cutoff. Weak, discordant and first positive reactions were reevaluated. Negative and discordant samples were collected at day-42 and subjected to biological indexing on two plants of *Physalis floridana* and further observation and ELISA assessment was performed for 28 days [17]. Reproducible ELISA positivity (reproducible) was considered the criterion for final infection. Where a test was scheduled, these tests were also negative, in order to achieve the status of no-detected-infection. The classification criteria for missing or unresolved assessments or death without a previous positive result were classified as indeterminate and not included in the denominator of the evaluable. Day 42 and subsequent samplings and subsequent indexing were added to final classification, thereby evaluating up to about day 70.

2.7. Post-treatment recontamination bioassay

A 240 individual recipient plant experimental design was used for the functional carryover experiment, using 60 individual recipient plants for each of the four processing workflows – water, Virkon S, sodium hypochlorite, and Virocid. Blades were processed and rinsed as described and then recontaminated with the fresh infected tissue prior to challenge of the recipients. The same diagnostic criteria as in the main experiment were used for final determination of infection status. This experiment measured the infectibility of newly processed specimens as influenced by the earlier processing; there was no attempt to quantify chemical residues.

2.8. Statistical analysis

Analysis and statistics were performed on basis of the number of infections observed on the recipient plants. Wilson's 95% Confidence Intervals were computed for proportions that were positive. Absolute risk differences were computed using Newcombe–Wilson intervals and risk ratios and odds ratios were reported on the log scale with Wald intervals. Pearson's chi-square and Cramér's V were used to evaluate overall association of treatments with outcomes. Pairwise comparison was performed by two-sided Fisher's exact tests with Holm

adjustment between ten pairs of treatments. Observed infection counts were analyzed by grouped-binomial logistic regression using the treatments and experimental runs as fixed factors with T1 as the reference treatment. Run adjusted odds ratios and 95% confidence intervals and Wald P values were reported. Likelihood-ratio tests and Akaike's information criterion were used to compare nested models. Exact two-sided sign tests were performed assessing the contrasts between runs. Holm adjustment was performed independently within the four regression contrasts, and in the four sign-test contrasts. For supporting outcomes, Wilson intervals were used to summarize and exploratory Fisher's exact tests were used to evaluate them, while for zero-event outcomes, the exact Clopper–Pearson intervals were used. All tests were performed bi-directionally, and a significance level of 0.05 was used.

3. Results

3.1. Final infection incidence by treatment

Final infection was detected in 43/50 plants in T1, 9/50 in T2, 6/50 in T3, 4/50 in T4, and 29/50 in T5, corresponding to infection incidences of 86.0%, 18.0%, 12.0%, 8.0%, and 58.0%, respectively Table 1. Of the 250 recipient plants, 91 were considered to be infected and 159 did not show any sign of infection. The incidence of infections was lowest with T4 but statistical differences between the three chemical treatments among the Holm adjusted pairwise comparisons were not significant.

Table 1: Final infection counts, infection incidence, and Wilson 95% confidence intervals

Treatment	Positive / n	No detected infection, n	Infection incidence, %	Wilson 95% CI, %
T1	43/50	7	86.0	73.8–93.0
T2	9/50	41	18.0	9.8–30.8
T3	6/50	44	12.0	5.6–23.8
T4	4/50	46	8.0	3.2–18.8
T5	29/50	21	58.0	44.2–70.6

3.2. Absolute and relative reductions in infection incidence

The absolute decreases in infections were 68.0%, 74.0%, 78.0% and 28.0% points for T2, T3, T4 and T5, respectively, compared with T1. The corresponding relative reductions were 79.1%, 86.0%, 90.7%, and 32.6% Table 2.

Table 2: Absolute and relative reductions in infection incidence compared with T1, with pointwise 95% confidence intervals

Comparison	Absolute reduction, pp; 95% CI	Relative reduction, %	Risk ratio; 95% CI
T2 vs T1	68.0; 50.3–78.8	79.1	0.209; 0.115–0.382
T3 vs T1	74.0; 57.0–83.5	86.0	0.140; 0.065–0.298
T4 vs T1	78.0; 61.7–86.6	90.7	0.093; 0.036–0.240
T5 vs T1	28.0; 10.5–43.5	32.6	0.674; 0.519–0.876

3.3. Exploratory run-level sign tests

In every of the 5 experimental runs infection proportion was lower than T1 with each active treatment. Exact two-sided sign-test P values were 0.0625 and Holm-adjusted P values were 0.250 for each active compared with T1 comparison Table 3. None of these run-level sign tests were significant at P=0.05. Root-application of host protection was analyzed instead of tool disinfection (T5).

Table 3: Exact sign-test comparisons across five experimental runs, with Holm adjustment

Comparison	Runs with reduction	Mean reduction, pp	Range, pp	Exact sign P	Holm P
T2 vs T1	5/5	68	60–80	0.0625	0.250
T3 vs T1	5/5	74	70–80	0.0625	0.250
T4 vs T1	5/5	78	70–80	0.0625	0.250
T5 vs T1	5/5	28	20–30	0.0625	0.250

3.4. Overall treatment association and pairwise comparisons

The global treatment–outcome association is $\chi^2(4) = 100.802$, P = 6.64e-21, with Cramér's V = 0.635. No cell would have an expected count below 5 for the aggregate Pearson table (18.2 positive, 31.8 negative counts in each). All ten pairs are reported along with RR, absolute difference, OR, and pointwise 95% confidence intervals in Table 4. However, after Holm-10 correction, T1 is different than all active arms, and each of T2, T3, and T4 is lower with respect to T5. The three three-chemical arm comparisons are not significant: T2-T3 (adjusted P = 1.000), T2-T4 (adjusted P = 0.701) and T3-T4 (adjusted P = 1.000). If they don't matter, then they are not the same.

Table 4: Aggregate pairwise treatment comparisons, pointwise 95% confidence intervals, and Holm-adjusted Fisher exact tests

Comparison A vs B	RD, pp (95% CI)	RR (95% CI)	OR (95% CI)	Fisher P	Holm-10 P
T1 vs T2	68.0 (50.3–78.8)	4.778 (2.617–8.724)	27.984 (9.538–82.108)	5.56e-12	4.45e-11
T1 vs T3	74.0 (57.0–83.5)	7.167 (3.355–15.307)	45.048 (14.001–144.942)	3.28e-14	2.95e-13
T1 vs T4	78.0 (61.7–86.6)	10.750 (4.172–27.702)	70.643 (19.312–258.411)	5.52e-16	5.52e-15
T1 vs T5	28.0 (10.5–43.5)	1.483 (1.142–1.925)	4.448 (1.675–11.811)	0.0034	0.0134
T2 vs T3	6.0 (-8.4–20.3)	1.500 (0.577–3.901)	1.610 (0.527–4.920)	0.5766	1.0000
T2 vs T4	10.0 (-3.6–23.7)	2.250 (0.741–6.832)	2.524 (0.723–8.818)	0.2336	0.7008
T2 vs T5	-40.0 (-55.1– -21.2)	0.310 (0.164–0.587)	0.159 (0.064–0.397)	6.96e-05	3.48e-04
T3 vs T4	4.0 (-8.6–16.8)	1.500 (0.450–4.995)	1.568 (0.414–5.935)	0.7407	1.0000
T3 vs T5	-46.0 (-60.1– -27.9)	0.207 (0.094–0.454)	0.099 (0.036–0.274)	2.15e-06	1.29e-05
T4 vs T5	-50.0 (-63.5– -32.5)	0.138 (0.052–0.364)	0.063 (0.020–0.202)	1.12e-07	7.82e-07

3.5. Run-adjusted logistic regression estimates

Run adjusted ORs by grouped-binomial logistic regression were 0.035, 0.022, 0.014 and 0.223 for T2-T5 compared to T1, respectively Table 5. All the four Holm corrected Wald P values were less than 0.05. These are conditional odds ratios, rather than the relative risks or percentages of preventions.

Table 5: Run-adjusted treatment contrasts from grouped-binomial logistic regression

Comparison	Log-odds β	SE(β)	Run-adjusted OR (95% CI)	Wald P	Holm-4 P
T2 vs T1	-3.352	0.552	0.035 (0.012–0.103)	1.24e-09	2.49e-09
T3 vs T1	-3.830	0.599	0.022 (0.007–0.070)	1.61e-10	4.82e-10
T4 vs T1	-4.280	0.664	0.014 (0.004–0.051)	1.16e-10	4.63e-10
T5 vs T1	-1.502	0.500	0.223 (0.084–0.593)	0.0027	0.0027

3.6. Binomial model fit and comparison

The treatment contribution to the run-fixed model is LR $\chi^2(4) = 108.011$, P = 1.93e-22. Adding run effects to the treatment-only model gives LR $\chi^2(4) = 0.997$, P = 0.9102. Therefore this comparison of likelihood-ratios does not provide evidence for the additional run parameters in the full model, which controls for run. Treatment only is also the better model with respect to AIC Table 6.

Table 6: Fit comparisons for binomial regression models fitted to the observed infection counts

Model	Parameters	Log-likelihood	AIC	Deviance	Residual df
Run only	5	-82.428	174.857	111.468	20
Treatment only	5	-28.922	67.843	4.454	20
Treatment + run	9	-28.423	74.846	3.457	16

3.7. Supporting outcomes and zero-event confidence bounds

Supporting-control, root-recovery and post-treatment recontamination outcomes are summarised in Table 7 and confidence intervals and exploratory comparisons are provided where appropriate. The $\chi^2(3)$ for the global carryover-recontamination comparison was 0.208 with P = 0.9762. These tests indicated that none of the three chemical-versus-water Fisher P values were statistically significant, even after Holm-3 adjustment. There were 0/15 reported acute injuries for each treatment, with a 2 sided exact 95% event rate interval ranging between 21.8%-0. The interval for 0/375 deaths was 0-0.98%. Ordinal distribution of injury / mortality outcomes were all zero, no significance test was performed.

Table 7: Supporting-control and functional carryover outcomes with 95% confidence intervals and exploratory comparisons

Outcome	Events / n	Rate, % (Wilson 95% CI)	Exploratory Fisher P
Pilot untreated tools versus pilot water	18/20	90.0 (69.9–97.2)	1.0000
Pilot water-processed tools	17/20	85.0 (64.0–94.8)	—
Pilot healthy donors	0/10	0.0 (0.0–27.8)	—
Untreated-tool positive controls versus T1	47/50	94.0 (83.8–97.9)	0.3178
Healthy-donor supporting controls	0/75	0.0 (0.0–4.9)	—
Fungal-treated root satellites versus carrier roots	10/10	100.0 (72.2–100.0)	1.08e-05
Carrier root satellites	0/10	0.0 (0.0–27.8)	—
Residue Water processed versus water	48/60	80.0 (68.2–88.2)	1.0000
Residue Virkon S processed versus water	49/60	81.7 (70.1–89.4)	1.0000
Residue Sodium hypochlorite processed versus water	47/60	78.3 (66.4–86.9)	1.0000
Residue Virocid processed versus water	48/60	80.0 (68.2–88.2)	1.0000

3.8. Run-specific infection incidence and pilot outcomes

The number of infections obtained in five experimental runs is reported in Table 8. In the T1 treatment at least 7 recipients were infected in each run. Of the product recipients in the pilot study, 18/20 recipients of untreated tools and 17/20 recipients of water-processed tools were found infected as compared to 0/10 healthy-donors who served as controls. None of healthy-donor recipients (75) were infected, but 47/50 of untreated-tool recipients were infected. Of the 10 fungal treated satellite plants, 10 were recovered for fungus and of the 10 carrier treated satellite plants, 0 were recovered for fungi. The results are displayed in Table 7. There were no exclusions/duplicates in all five experimental runs. There were a total of 395 plants: 250 receiving the treatment, 75 healthy plants used as donors, 50 untreated plants used as tools and 20 satellite plants. The samples of pilot plants, diagnostic-panel plants, carryover recipients and indicator plants were not used as additional treatment replicates.

Table 8: Final infection counts across five experimental runs, with ten recipient plants per treatment arm in each run

Run	T1	T2	T3	T4	T5
R1	9/10	2/10	1/10	1/10	6/10
R2	8/10	2/10	1/10	0/10	5/10
R3	9/10	1/10	2/10	1/10	6/10
R4	8/10	2/10	1/10	1/10	6/10
R5	9/10	2/10	1/10	1/10	6/10
Total	43/50	9/50	6/50	4/50	29/50

3.9. Diagnostic reference-panel performance

The diagnostic reference panel gave the following results using 100 positive-reference samples: 95 positive reactions, and 98 negative reactions, using 100 negative-reference samples. This panel had an estimated sensitivity of 95.0% and a pooled specificity of 98.0% Table 9. Of the 30 non-target-virus samples, 28 (93.3%) were negative while two were positive. Both of these two positive results were considered nontarget reactivity of the tested panel. Wilson 95% confidence intervals were 88.8–97.8% for the 95.0% sensitivity, 93.0–99.4% for the 98.0% pooled specificity, and 78.7–98.2% for the 93.3% non-target subgroup specificity. This is a pointwise confidence interval, which assumes independent samples in the reference panel, and does not consider uncertainty in reference classifications. The estimates are thus for performance within the panel tested. A positivity threshold of 0.18 was used for ELISA, which was based on negative-reference mean (0.09) + 3 × standard deviation (3 × 0.03). The measurement with the negative reference value was used for the calculation. Diagnostic assessment was through serological and biological methods, described in Materials and methods; molecular diagnostic testing was not carried out.

Table 9: ELISA results in the diagnostic reference panel

Reference panel	n	ELISA positive	ELISA negative
Positive-reference panel	100	95	5
Healthy untreated-panel	50	0	50
Healthy fungal-treated-panel	20	0	20
Non-target-virus-panel	30	2	28

3.10. Serial virus detection and final infection classification

ELISA detected infection in 51/250, 77/250, and 85/250 treatment recipients at days 14, 28, and 42, respectively. Biological indexing of all 165 recipients who tested negative at day 42 revealed 6 more infections. The final categorization was thus 91 recipients, with infection, and 159 recipients without detected infection Table 10. Longitudinal diagnostic records revealed no classification changes, with all previous positive classification being poured over into the final infection classification endpoint. Final classifications made based on the timed ELISA evaluations and subsequent bio-indexing. A total of 330 indicator plants distributed on 165 treatment samples were used for the biological

indexing. Indicator plants were NOT replicates of the treatments. There was no increase in efficacy sample size by duplicate wells or repeat assessments.

Table 10: ELISA results through day 42, subsequent biological indexing, and final infection classifications

Treatment	Day 14 ELISA positive/ n	Day 28 ELISA positive/ n	Day 42 ELISA positive/ n	Day-42 negative samples, n	Indexing positive, n	Final positive/ n	Final no detected infection, n
T1	30/50	40/50	42/50	8	1	43/50	7
T2	4/50	7/50	8/50	42	1	9/50	41
T3	2/50	4/50	5/50	45	1	6/50	44
T4	1/50	3/50	4/50	46	0	4/50	46
T5	14/50	23/50	26/50	24	3	29/50	21
Total	51/250	77/250	85/250	165	6	91/250	159

4. Discussion

The efficiency of viral transmission under controlled conditions, such as contaminated cutting tools is at 86% infection incidence after water processing. In contrast to abrasive sap inoculation, the latter was performed with pruning wounds, which enhanced the exposure model. Bausher showed that Tomato mosaic virus can be serially grafted by using contaminated knives but transmission of another virus tested was not successful [18]. Persistence documented for Zucchini yellow mosaic virus also shows that mechanical transmission is not always linked to the pathogen, but also to environmental factors such as exposure conditions [19].

Chemical processing reduced the infection to 18%, 12% and 8% while using Virkon S, sodium hypochlorite and Virocid, respectively. However, the lowest incidence in Virocid is not evidence of superiority and any comparisons between chemicals that do not achieve statistical significance do not give proof of equivalence [20]. The disinfectant efficacy of the chemicals against Tomato brown rugose fruit virus was found to depend on surface and time, which resulted in several chemicals taking longer contact than in this study [21] These protocols reflect the categorization associated with treatments and not an overall product hierarchy.

The sodium hypochlorite results supports the accepted antimicrobial activity of chlorine, and that nominal concentration cannot replace measured available chlorine. When solution properties are not measured, there are influences on effective exposures from chlorine chemistry and from organic demands, which limit the reproducibility [22]. Furthermore, fresh aliquots avoided bath reuse and the performance may consequently have been affected by the accumulation of plant material during pruning. Practical results of decontamination reported by Ehlers et al. for short contact periods can vary from the expectations based on suspension tests [23]. For this reason, the minimum effective concentration, what other cleaning must be performed before these and how often they should be switched remains undetermined.

An Interpretive Safeguard is added to the carryover experiment: recontaminated blades retained capacity to transmit infection after processing. This, however, measures future infectivity—for chemical residues, measure the residues and treat the numbers accordingly. The study of potato spindle tuber viroid gives an example to insure that biological infectivity and the presence of detectable material of pathogen are two different endpoints [24]. Root applied *Trichoderma*, on the other hand, itself did not provide sanitation as infection was reduced to 58% from 86%. An antiviral activity of *Trichoderma asperellum* against Cucumber mosaic virus sets a biological precedent, albeit with different fungal, host and viral systems [25].

Thus, the fungal effect can be correlated with host mediated protection, although a mechanism is not determined. Yedidia et al. reported root-inoculation with *Trichoderma* induced structural changes and improved defense-enzyme activities in cucumber [26]. Later studies showed that root treatment resulted in systemic resistance, defense transcripts and phytoalexins accumulation after the challenge of bacteria [27]. These studies indicate a possible root-to-shoot defense pathway, and measured responses, show that mechanistic attribution is necessary. The present experiment evaluated the frequency of infection but not activation of defense, viral accumulation, symptom severity or growth mediated tolerance.

In tomato, resistance induced by Trichoderma has been related to interaction between hormonal pathways and to defense priming that occur in a pathogen challenge-dependent manner, but that the pathogen tested was *Botrytis cinerea* [28]. Similarly, the Sm1 elicitor isolated from *Trichoderma virens* was shown to trigger defense mechanisms and systemic resistance subsequent to characterization, confirming the response is through signaling and not a direct toxicity of the pathogen itself [29]. Both findings show no evidence that the unidentified culture employed here brings about similar signals. It would be preferable to be able to measure viral RNA as well alongside any markers of defense, to determine whether defective establishment is going on, or if there is a delay in systemic spread, rather than make any unsupported statement about direct inactivation of virions.

Systemic antiviral resistance mechanisms cannot be demonstrated by the fungus recovered from all 10 of the treated satellite roots, which support establishment. Defensive direction and timing cannot be repaired by mere presence of *Trichoderma;* sometimes such fungi act as transient immune modulators and trigger reprogramming by stress-response [30]. There's also a lack of culture identifier which further limits the possibility of reproducibility. The taxonomic reassignment of commercial strains of *Trichoderma harzianum*, as revealed by molecular revision of the complex, showed that there is a high taxonomic diversity in the complex [31]. Protection should logically then apply to the tested culture, rather than the species complex as a whole.

The use of serial diagnostics resulted in better ascertainment of outcome – ELISA positive recipients went up from 51 to 85, with indexing leading to the identification of 6 further infections. It helps to sustain the follow-up, without making an initial negative equal to permanent protection. Diagnostic reviews highlight the need to complement serological with molecular methods [32]. However, limited specificity-based interpretation is due to two reactions occurring among non-target-virus samples and the lack of authentication of molecular isolates. Available is a validated ToTV quantitative RT-PCR assay, which is an independent, confirming strategy [33]. Indexing enhances the informative value of infectivity determination, but does not solve the identity problem when the confirmation is performed with the same antibodies.

To interpret statistics one has to differentiate between magnitude and certainty. Experimental-unit principles [34] suggest that matched analysis, or analysis with clustering effects, should be used because donor matched recipients are expected to share an exposure source, but this has not been investigated in the grouped regression. In five runs, directionally consistent decreases suggesting general reproducibility, but the various sign tests have yet to become significant. Furthermore, it is usually inappropriate to compare adjusted ORs to percentages of prevention [35]. Absolute risk differences are a better description of the observed treatment benefit.

Finally, all treatments had a 21.8% upper bound for the confidence interval for zero acute injury, owing to uncertainty that arises from zero events [36]. The chlorine–phytotoxicity with hydroponic vegetables (lettuce), also indicates that exposure pathway and crop situation influences the safety without the efficacy to specify the threshold for tomato blade [37]. Testing a single cultivar, concentration, and contact time provides restrictive generalizations. Negatively, the combination of sanitation and biological protection has not been evaluated, so it cannot be assumed that there were any additive or synergistic effects, but their combination is deserving of factorial evaluation.

5. Conclusion

However, chemical processing definitely resulted in a significant reduction of infection from infection via contaminated pruning tools, while root application of *Trichoderma* resulted in partial protection under the conditions tested. The lowest incidence was observed with Virocid, although the chemical treatments were not statistically different and cannot be regarded as equivalent. Neither induced antiviral resistance nor direct viral inactivation was demonstrated in this study. Serial laser blood ELISA testing and biological indexing was performed for the final infection status. Donor dependence, a better understanding of the fungal species involved, concentration and contact time with the disinfectant, bath reuse, and long-term crop health are all factors that need to be further studied for practical recommendations. Further testing should involve other cultivars, orthogonal molecular diagnostic testing and factorial biological treatment and sanitation. These results suggest controlled, rather than eradication, preventive efficacy, commercial safety of interventions, and demonstrated synergy between interventions, respectively.

Article Information

Ethical Approval: Not applicable.

Data Availability: The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest: The authors declare that they have no conflict of interest.

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References

[1] M. Verbeek, A. M. Dullemans, J. F. J. M. van den Heuvel, P. C. Maris, and R. A. A. van der Vlugt. Identification and characterisation of tomato torrado virus, a new plant picorna-like virus from tomato. *Archives of Virology*, 152:881–890, 2007. URL https://doi.org/10.1007/s00705-006-0917-6.

[2] R. A. A. van der Vlugt, M. Verbeek, A. M. Dullemans, W. M. Wintermantel, W. J. Cuellar, A. Fox, and J. R. Thompson. Torradoviruses. *Annual Review of Phytopathology*, 53:485–512, 2015. URL https://doi.org/10.1146/annurev-phyto-080614-120021.

[3] K. Amari, D. Gonzalez-Ibeas, P. Gómez, R. N. Sempere, M. A. Sanchez-Pina, M. A. Aranda, J. A. Diaz-Pendon, J. Navas-Castillo, E. Moriones, J. Blanca, M. D. Hernandez-Gallardo, and G. Anastasio. Tomato torrado virus is transmitted by Bemisia tabaci and infects pepper and eggplant in addition to tomato. *Plant Disease*, 92(7):1139, 2008. URL https://doi.org/10.1094/PDIS-92-7-1139A.

[4] M. Verbeek, P. J. van Bekkum, A. M. Dullemans, and R. A. A. van der Vlugt. Torradoviruses are transmitted in a semi-persistent and stylet-borne manner by three whitefly vectors. *Virus Research*, 186:55–60, 2014. URL https://doi.org/10.1016/j.virusres.2013.12.003.

[5] M. Budziszewska, B. Wrzesińska-Krupa, P. Wieczorek, and A. Obrepalska-Steplowska. The length of the 3′ UTR of the tomato torrado virus (ToTV) RNA1 affects virus accumulation in Solanum lycopersicum during mechanical passages from plant to plant. *Plant Pathology*, 73(3):666–676, 2024. URL https://doi.org/10.1111/ppa.13834.

[6] P. Wieczorek and A. Obrepalska-Steplowska. Multiplex RT-PCR reaction for simultaneous detection of Tomato torrado virus and Pepino mosaic virus co-infecting Solanum lycopersicum. *Journal of Plant Protection Research*, 53(3):289–294, 2013. URL https://doi.org/10.2478/jppr-2013-0043.

[7] R. Li, F. Baysal-Gurel, Z. Abdo, S. A. Miller, and K.-S. Ling. Evaluation of disinfectants to prevent mechanical transmission of viruses and a viroid in greenhouse tomato production. *Virology Journal*, 12:5, 2015. URL https://doi.org/10.1186/s12985-014-0237-5.

[8] B. Chanda, M. Shamimuzzaman, A. Gilliard, and K.-S. Ling. Effectiveness of disinfectants against the spread of tobamoviruses: Tomato brown rugose fruit virus and Cucumber green mottle mosaic virus. *Virology Journal*, 18:7, 2021. URL https://doi.org/10.1186/s12985-020-01479-8.

[9] K.-S. Ling, A. C. Gilliard, and B. Zia. Disinfectants useful to manage the emerging Tomato brown rugose fruit virus in greenhouse tomato production. *Horticulturae*, 8(12):1193, 2022. URL https://doi.org/10.3390/horticulturae8121193.

[10] G. E. Harman, C. R. Howell, A. Viterbo, I. Chet, and M. Lorito. *Trichoderma* species—opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology*, 2:43–56, 2004. URL https://doi.org/10.1038/nrmicro797.

[11] F. Vinale, K. Sivasithamparam, E. L. Ghisalberti, R. Marra, S. L. Woo, and M. Lorito. *Trichoderma*–plant–pathogen interactions. *Soil Biology and Biochemistry*, 40(1):1–10, 2008. URL https://doi.org/10.1016/j.soilbio.2007.07.002.

[12] M. Shoresh, G. E. Harman, and F. Mastouri. Induced systemic resistance and plant responses to fungal biocontrol agents. *Annual Review of Phytopathology*, 48:21–43, 2010. URL https://doi.org/10.1146/annurev-phyto-073009-114450.

[13] A. Vitti, E. Pellegrini, C. Nali, S. Lovelli, A. Sofo, M. Valerio, A. Scopa, and M. Nuzzaci. *Trichoderma harzianum* T-22 induces systemic resistance in tomato infected by Cucumber mosaic virus. *Frontiers in Plant Science*, 7:1520, 2016. URL https://doi.org/10.3389/fpls.2016.01520.

[14] H. Pospieszny, M. Budziszewska, B. Hasiów-Jaroszewska, A. Obrepalska-Steplowska, and N. Borodynko. Biological and molecular characterization of Polish isolates of Tomato torrado virus. *Journal of Phytopathology*, 158:56–62, 2010. URL https://doi.org/10.1111/j.1439-0434.2009.01563.x.

[15] D. J. Lewandowski, A. J. Hayes, and S. Adkins. Surprising results from a search for effective disinfectants for Tobacco mosaic virus–contaminated tools. *Plant Disease*, 94:542–550, 2010. URL https://doi.org/10.1094/PDIS-94-5-0542.

[16] Leibniz Institute Dsmz. Antigen-Coated-Plate ELISA (ACP-ELISA). QC-SOP-0087, Version 2.0. General assay procedure; not a ToTV-specific validation. URL https://www.dsmz.de/fileadmin/_migrated/content_uploads/ACP-ELISA.pdf.

[17] E. Verdin, P. Gognalons, C. Wipf-Scheibel, I. Bornard, G. Ridray, L. Schoen, and H. Lecoq. First Report of Tomato torrado virus in Tomato Crops in France. *Plant Disease*, 93(12):1352, 2009. URL https://doi.org/10.1094/PDIS-93-12-1352C.

[18] M. G. Bausher. Serial transmission of plant viruses by cutting implements during grafting. *HortScience*, 48(1):37–39, 2013. URL https://doi.org/10.21273/HORTSCI.48.1.37.

[19] B. A. Coutts, M. A. Kehoe, and R. A. C. Jones. Zucchini yellow mosaic virus: Contact transmission, stability on surfaces, and inactivation with disinfectants. *Plant Disease*, 97(6):765–771, 2013. URL https://doi.org/10.1094/PDIS-08-12-0769-RE.

[20] D. G. Altman and J. M. Bland. Absence of evidence is not evidence of absence. *British Medical Journal*, 311(7003):485, 1995. URL https://doi.org/10.1136/bmj.311.7003.485.

[21] A. Skelton, L. Frew, R. Ward, R. Hodgson, S. Forde, S. McDonough, G. Webster, K. Chisnall, M. Mynett, A. Buxton-Kirk, A. R. Fowkes, R. Weekes, and A. Fox. Tomato brown rugose fruit virus: Survival and disinfection efficacy on common glasshouse surfaces. *Viruses*, 15(10):2076, 2023. URL https://doi.org/10.3390/v15102076.

[22] G. McDonnell and A. D. Russell. Antiseptics and disinfectants: Activity, action, and resistance. *Clinical Microbiology Reviews*, 12(1): 147–179, 1999. URL https://doi.org/10.1128/CMR.12.1.147.

[23] J. Ehlers, S. Nourinejhad Zarghani, B. Kroschewski, C. Büttner, and M. Bandte. Decontamination of Tomato brown rugose fruit virus-contaminated shoe soles under practical conditions. *Horticulturae*, 8(12):1210, 2022. URL https://doi.org/10.3390/horticulturae8121210.

[24] A. E. Mackie, B. A. Coutts, M. J. Barbetti, B. C. Rodoni, S. J. McKirdy, and R. A. C. Jones. Potato spindle tuber viroid: Stability on common surfaces and inactivation with disinfectants. *Plant Disease*, 99(6):770–775, 2015. URL https://doi.org/10.1094/PDIS-09-14-0929-RE.

[25] M. M. Elsharkawy, M. Shimizu, H. Takahashi, K. Ozaki, and M. Hyakumachi. Induction of systemic resistance against Cucumber mosaic virus in Arabidopsis thaliana by *Trichoderma* asperellum SKT-1. *The Plant Pathology Journal*, 29(2):193–200, 2013.

[26] I. Yedidia, N. Benhamou, and I. Chet. Induction of defense responses in cucumber plants (*Cucumis sativus* L.) by the biocontrol agent *Trichoderma harzianum*. *Applied and Environmental Microbiology*, 65(3):1061–1070, 1999. URL https://doi.org/10.1128/AEM.65.3.1061-1070.1999.

[27] I. Yedidia, M. Shoresh, Z. Kerem, N. Benhamou, Y. Kapulnik, and I. Chet. Concomitant induction of systemic resistance to Pseudomonas syringae pv. lachrymans in cucumber by *Trichoderma asperellum* (T-203) and accumulation of phytoalexins. *Applied and Environmental Microbiology*, 69(12):7343–7353, 2003. URL https://doi.org/10.1128/AEM.69.12.7343-7353.2003.

[28] A. Martínez-Medina, I. Fernández, M. J. Sánchez-Guzmán, S. C. Jung, J. A. Pascual, and M. J. Pozo. Deciphering the hormonal signalling network behind the systemic resistance induced by *Trichoderma harzianum* in tomato. *Frontiers in Plant Science*, 4:206, 2013. URL https://doi.org/10.3389/fpls.2013.00206.

[29] S. Djonović, M. J. Pozo, L. J. Dangott, C. R. Howell, and C. M. Kenerley. Sm1, a proteinaceous elicitor secreted by the biocontrol fungus *Trichoderma virens* induces plant defense responses and systemic resistance. *Molecular Plant-Microbe Interactions*, 19(8): 838–853, 2006. URL https://doi.org/10.1094/MPMI-19-0838.

[30] Y. Brotman, U. Landau, Á. Cuadros-Inostroza, T. Tohge, A. R. Fernie, I. Chet, A. Viterbo, and L. Willmitzer. *Trichoderma*-plant root colonization: Escaping early plant defense responses and activation of the antioxidant machinery for saline stress tolerance. *PLOS Pathogens*, 9(3):e1003221, 2013. URL https://doi.org/10.1371/journal.ppat.1003221.

[31] P. Chaverri, F. Branco-Rocha, W. Jaklitsch, R. Gazis, T. Degenkolb, and G. J. Samuels. Systematics of the *Trichoderma harzianum* species complex and the re-identification of commercial biocontrol strains. *Mycologia*, 107(3):558–590, 2015. URL https://doi.org/10.3852/14-147.

[32] N. Boonham, J. Kreuze, S. Winter, R. V. D. Vlugt, J. Bergervoet, J. Tomlinson, and R. Mumford. Methods in virus diagnostics: From ELISA to next generation sequencing. *Virus Research*, 186:20–31, 2014. URL https://doi.org/10.1016/j.virusres.2013.12.007.

[33] J. A. Herrera-Vásquez, L. Rubio, A. Alfaro-Fernández, D. E. Debreczeni, I. Font-San-Ambrosio, B. W. Falk, and I. Ferriol. Detection and absolute quantitation of Tomato torrado virus (ToTV) by real time RT-PCR. *Journal of Virological Methods*, 221:90–94, 2015. URL https://doi.org/10.1016/j.jviromet.2015.04.029.

[34] S. H. Hurlbert. Pseudoreplication and the design of ecological field experiments. *Ecological Monographs*, 54(2):187–211, 1984. URL https://doi.org/10.2307/1942661.

[35] H. T. O. Davies, I. K. Crombie, and M. Tavakoli. When can odds ratios mislead? *British Medical Journal*, 316(7136):989–991, 1998. URL https://doi.org/10.1136/bmj.316.7136.989.

[36] J. A. Hanley and A. Lippman-Hand. If nothing goes wrong, is everything all right? Interpreting zero numerators. *Journal of the American Medical Association*, 249(13):1743–1745, 1983. URL https://doi.org/10.1001/jama.1983.03330370053031.

[37] C. McGehee and R. E. Raudales. Irrigation sources with chlorine drinking water standard limits cause phytotoxicity on 'Rex' lettuce grown in hydroponic systems. *HortTechnology*, 33(1):125–130, 2023. URL https://doi.org/10.21273/HORTTECH05091-22.