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Disinfection Efficacy of Tobamovirus-Contaminated Soil in Greenhouse-Grown Crops

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Abstract: The tobamoviruses tomato brown rugose fruit virus (ToBRFV) and cucumber green mottle mosaic virus (CGMMV) have caused severe crop damages worldwide. Soil-mediated dispersion of the mechanically transmitted tobamoviruses constitute a major hindrance toward mitigating disease spread in crops carefully planted under sanitized conditions. Tobamoviruses are viable for months in soil and plant debris and for more than a year adhere to clay. However, a low percentage of infectious foci occur in soil following a tobamovirus-infected growing cycle, rendering disinfection studies of several contaminated plots inconclusive for large-scale crop productions. We have therefore formulated a rigorous platform for studying disinfectant efficacy in greenhouses by pouring a virus inoculum to planting pits prior to disinfectant treatment and by truncating seedling roots before planting, which was otherwise conducted under sanitized conditions. We have found that chlorine-based Taharan was significantly efficient in preventing disease spread of ToBRFV and CGMMV in tomato and cucumber plants, respectively. KlorBack was often as good as Taharan. In addition, a formulation of chlorinated tri-sodium phosphate used at a nonphytotoxic 3% concentration showed disinfection efficiency similar to Taharan effect on ToBRFV infection only. Our study provided a small-scale platform for disinfectant efficacy evaluation necessary for application in tobamovirus-contaminated soil, which commonly occurs in commercial tomato and cucumber greenhouses.

Keywords: ToBRFV; CGMMV; soil-borne viruses

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1. Introduction

Tobamoviruses are highly stable, mechanically transmitted seed-borne viruses that have caused severe damage to vegetable crops worldwide. The tobamoviruses are preserved and viable in soil for months in plant debris from infected growing cycles [1–7], and virus particles adsorbed to clay are infectious for more than a year [8,9]. The contaminated soil constitutes a primary source of viral spread during successive growing cycles [10]. In particular, soil-mediated disease spread of the tobamoviruses often occurs via mechanical transmission of the viruses at the planting stage, through injured roots, as well as by adherence of contaminated soil to the seedlings. Implementation of careful sanitized planting procedures and the use of either intermediate medium to isolate roots from contacting the contaminated soil or tolerant rootstocks have reduced tobamovirus spread [4,11,12]. However, irrigation water could still mediate disease spread from contaminated soil or infected rootstocks [6,13,14]. Soil steaming, the least hazardous treatment, is sometimes too cumbersome to operate, especially when addition of exothermic chemicals is necessary to achieve high temperatures at the appropriate ground depths in order to inactivate tobamoviruses [15]. We have therefore designed an experimental platform, conducted in research and development (R&D) greenhouses, to test disinfectant efficacy in

mitigating a viral inoculum of two members of the *Tobamovirus* genus, which have reduced fruit quality and have caused great losses to stakeholders worldwide [16–19]. An inoculum of either the tomato brown rugose fruit virus (ToBRFV) or cucumber green mottle mosaic virus (CGMMV) was poured into planting pits prior to the tested disinfectants. Importantly, roots of tomato and cucumber seedlings were truncated before planting, which otherwise was conducted using a cautious sanitized procedure.

The tobamoviruses are single-stranded positive-sense RNA viruses (+ssRNA) encoding six proteins. Four of the proteins are the known viral replicase complex, comprised of 126 kDa and 186 kDa proteins, the movement protein of ~30 kDa, and the coat protein (CP) of ~17 kDa [14]. During infection, virions move in the phloem. Serological detection of the CP indicates viral infection in systemically infected plants, which show symptoms of mottling and mosaic leaves. We have recently shown that the seasonal disease in tomatoes, which were observed primarily during the hot summer weather, has become more severe in the cold weather due to synergism occurring between ToBRFV and a mild potexvirus pepino mosaic virus (PepMV-IL) [19,20]. Regarding CGMMV, we have recently shown a tight correlation between disease manifestations and fluctuating environmental temperatures [21,22].

We have therefore conducted our studies during several months, encompassing the spring and the hot summer, in two consecutive years to obtain rigorous test conditions. In our studies, we have tested the efficacy of several disinfectants. Recent reports have shown that various chlorine-based formulas, such as KlorBack, Virocid, Chlorox, and ChloRun, have been highly effective in tobamovirus inactivation tests and disinfection of planting facilities [13,23]. However, seed and soil disinfections constitute a hindrance toward alleviation of disease spread. Importantly, harsh treatments of seeds with chemicals that could be washed, such as trisodium phosphate (TSP, 10%) [24,25], might be phytotoxic [26] and could not be used in soil disinfection of the greenhouse crops. We have therefore tested various chlorine-based chemicals in the greenhouse experiments, and we have tested a new formulation of chlorinated TSP applied at low nonphytotoxic concentrations, which could still match the effects of the best disinfectant observed in our studies.

2. Materials and Methods

2.1. Tested Disinfectants

Taharan-stabilized chlorine formula containing 60% sodium dichloroisocyanurate ($C_3O_3N_3CL_2Na$) (Luxembourg industries LTD, Tel-Aviv, Israel) 1000 ppm, 2000 ppm; KlorBack, with the active ingredient troclosene sodium ($C_3Cl_3N_3NaO_3$) (Concept for Pharmacy, Kefar Sava, Israel) 1000 ppm, 2000 ppm; ChloRun (sodium dichloroisocyanurate 56%) (ICL, Haifa, Israel) 1000 ppm, 2000 ppm; GreenUp ABV 18% (Green life group, Ashdod, Israel); active oxygen (silver stabilized hydrogen peroxide, Huwa-San TR-50, ROAM technologies, Poort Genk, Belgium) 2%; chlorinated-TSP (97% TSP, 3% Cl) (Sterokem LTD, Haifa, Israel) 3%, 5% were used.

2.2. Operation of the Experimental Platform

In Western Negev R&D station in Israel (coordinates: 31.271960, 34.387481), greenhouses encompassing three tunnels each were used in our studies for each experiment, excluding the experiment on yield. Flowerbeds of sandy soil served for disinfectant efficacy tests and were used at alternating order of the tested disinfectants in the three tunnels to prevent any possible bias of greenhouse habitation in the results. An inoculum of ToBRFV or CGMMV was prepared by grinding 2 kg of infected tomato or cucumber leaves in 10 L 0.1 M sodium phosphate buffer pH = 7.0. At the test site, each concentrated inoculum was diluted 1:5 with tap water, and the planting pits of ~100 mL each were filled with either ToBRFV or CGMMV inoculum source. When seeping through the sandy soil was fast, up to ~200 mL of each inoculum was poured into the pits. Virion concentrations were determined by extracting virion proteins with 8 M urea-SDS-β-mercapto-ethanol buffer

(USB) using 1.25 dilution factor and running on SDS-PAGE adjacent to a reference bovine serum albumin (BSA) (4–32 μg) followed by coomassie-blue staining. In each planting pit, ~4 g/100 mL virus-infected leaves was ground in 0.05 M sodium phosphate buffer, pH = 7.0, contained ~40 mg and ~20 mg virion CP of ToBRFV and CGMMV, respectively. Considering that tobamovirus virions are composed of 2130 CP units [27] and MW of the genomic RNA is ~2 × 10^6, the calculated total amounts of ToBRFV and CGMMV in each inoculum of 100 mL was ~1 μmoles and ~0.5 μmoles, respectively. Western blot was performed to confirm specificity of ToBRFV and CGMMV CP bands observed in coomassie blue staining using specific antibodies as previously described [13,19]. In each flowerbed, a different disinfectant was poured into the virus contaminated planting pits, until overflow occurred. An hour later, the planting pits were watered to reduce any phytotoxic side effects of the disinfectants, and cautious planting procedure of seedlings with truncated roots was conducted. One person carefully carried a tray with the seedlings, a second person, kneeling, placed the seedlings in the pits, and subsequently a third person covered the roots with the disinfectant-wet soil not touching seedling leaves. Workers used gloves, shoe-covers, and sanitized cloths.

In experiments conducted during April through August, Taharan effect was studied on 90–250 plants/treatment/month. A comparison between Taharan and GreenUp ABV effects was conducted on 120–130 plants/treatment. A comparison between Taharan and KlorBack was conducted on 110–120 plants/treatment. A comparison between Taharan, ChloRun and Klorback was conducted on 90–250 plants/treatment. A comparison between Taharan, GreenUp ABV, and KlorBack, which was performed in a subsequent year in April and June, was conducted on 100–150 plants/treatment. The study of Taharan effect on yield was conducted in a commercial greenhouse containing 960 plants, 25% of which were treated with the disinfectant.

2.3. Monitoring Disinfection Efficacy

Tomato plants cvs. Ikram or Shunit and cucumber plants cvs. Noname or Romi with symptomatic leaves showing mottling and mosaic were counted starting 3–4-weeks post planting, and samples of symptomatic and nonsymptomatic plants were confirmed by testing virus content using enzyme linked immunosorbent assay (ELISA) as previously described [13,19]. Results showing O.D. values of 2.5 times the negative controls were considered positive for the tested virus. Significance of disinfectant efficacy was determined using *t*-Test, paired with two samples for means. For *t*-Test of Taharan effect, data collected from five months were included in the test. For *t*-Test of KorBack effect, four experiments with KlorBack were included in the test.

3. Results and Discussion

3.1. Establishing an Experimental Platform for Studying Disinfectant Efficacy

Studies on greenhouse-grown crops constitute essential steps toward implementation of strategies designed to mitigate viral disease spread. Generally, preventive measures of disease spread should be tested under rigorous, unfavorable conditions to ensure highly promising data application. Regarding the mechanically transmitted tobamoviruses, we and others have previously shown that keeping cautious planting conditions, using disinfected facilities, as well as distribution of chores between workers that wear sanitized clothes, gloves, and shoe covers (thereby preventing any adherence mediated viral infection) have reduced tobamovirus infections, specifically when soil disinfection has been previously performed [11,23]. Efficiency of soil disinfection depends not only on the disinfectant of choice but also on soil types [11]. Gravels in soil could cause extra injuries to the roots and mediate tobamovirus adherence and spread; organic matter in soil consumes the active chlorine and reduces efficacy of chlorinated compounds [28,29]. Sandy soil could reduce disinfectant efficacy due to solute seeping through and the high-clay mineral content, which adsorbs tobamoviruses [8,9] and nucleic acids [30].

In previous studies on soil-mediated CGMMV transmission, we have shown that calculated transmission ratios of sandy and heavily contaminated soils were ~1% and ~2%, respectively, whereas only tuff matrix reached the high 30–70% transmission ratios [4,11]. Under these low-viral transmission ratio conditions, studies of disinfectant efficiency will require large planting areas with thousands of plants to obtain significant results. To accommodate the appropriate conditions for our studies, conducted in several R&D greenhouses, we have designed a rigorous experimental platform that will provide us with the necessary stringent conditions for drawing conclusions.

In our experimental platform for studying disinfection efficiencies in R&D greenhouses, we have included:

(i) Supplementation of viral inoculum. ToBRFV or CGMMV at concentrations of 40 mg/mL infected leaves ground in 0.05 M sodium phosphate buffer, pH = 7.0, were poured into the planting pits prior to the tested disinfectant. A total of ~1.0 μmoles and ~0.5 μmoles of ToBRFV and CGMMV virions, respectively, were in each ~100 mL inoculum.

(ii) Truncated roots. Seedling root edges were cut to increase cellular wounds, thereby ascertaining virus entry via roots.

We have conducted most of our experiments in greenhouses located in sandy soil area while observing cautious planting procedures in order to prevent any foliar infections from contaminated facilities or the ground (Figure 1). Planting pits were filled with an inoculum of either ToBRFV or CGMMV. Virus concentrations in the inoculum were calculated using a BSA reference (Figure 1a,b). Subsequently each of the alternative disinfectants was poured into the contaminated pits until overflow (Figure 1c). An hour later planting of tomato or cucumber seedlings with the truncated roots was carefully performed while observing a cautious planting procedure to prevent any attachment of soil to plant leaves (Figure 1d,e).

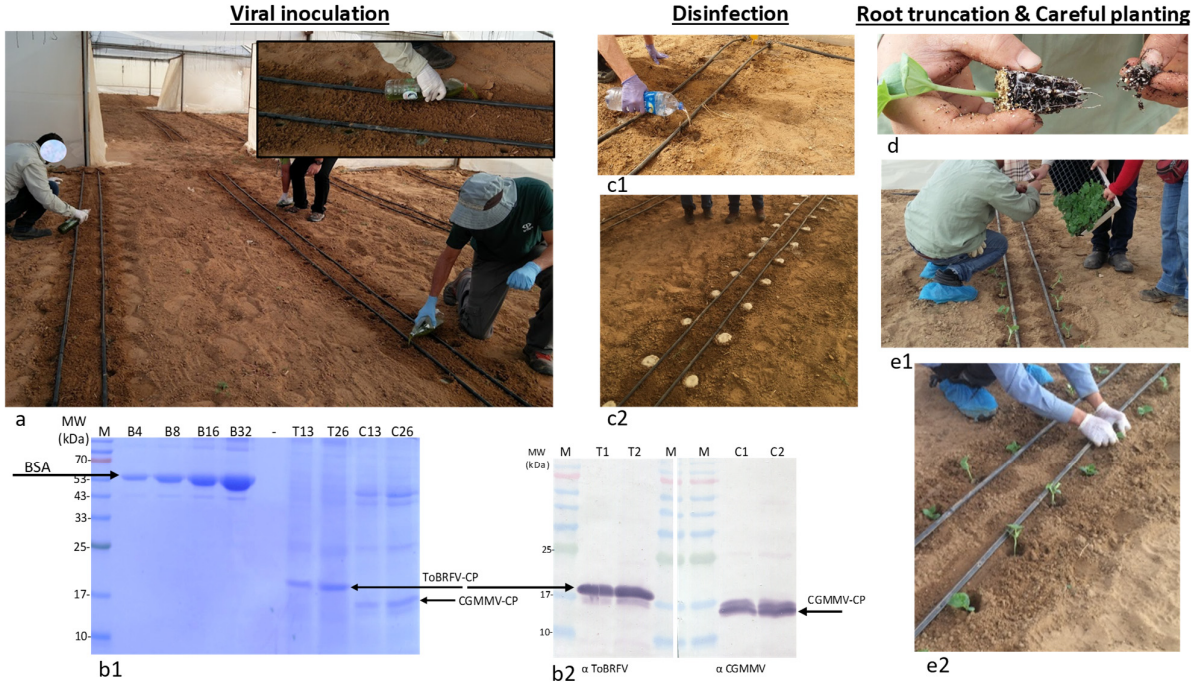

Figure 1. A platform for disinfectant efficacy tests in greenhouses: (**a**) a viral inoculum of ToBRFV or CGMMV infected leaves ground in 0.05 M sodium phosphate buffer pH = 7.0 at a concentration of 40 mg/mL was poured (100–200 mL) into planting pits; (**b1**) evaluation of viral concentrations in each inoculum by SDS-PAGE followed by coomassie blue staining. ToBRFV or CGMMV inoculum

were subjected to extraction with US and run adjacent to a BSA reference; (**b2**) western blot of ToBRFV and CGMMV confirming the marked viral CP in the SDS-PAGE using specific antibodies. B, BSA; T, ToBRFV; C, CGMMV; 4–32 are μg of BSA; 13 and 26 are μL of 1.25 times diluted inoculum; (**c1**) disinfectant was poured into the planting pits until overflow occurred; (**c2**) bubbling peroxide; (**d**) root truncation; (**e1**,**e2**) careful planting procedure depiction showing one person carrying the root-truncated seedlings, a second person placing the seedlings into the planting pits, and a third person carefully covering the roots without touching seedling leaves.

3.2. Disinfectant Efficacy

The experiments that encompassed five months during spring and the hot summer in Israel (April through August) tested Taharan disinfection efficiency on ToBRFV- and CGMMV-associated disease spread in tomatoes and cucumbers, respectively (Figure 2). A highly significant disinfection efficiency was observed for Taharan, tested on ToBRFV or CGMMV inoculum, with $p < 0.01$ and $p < 0.05$ t-Test, and paired with two samples for means, respectively (Figure 2a1). Concurrently, studies were conducted to compare between the four disinfectants: Taharan, Chloran, KlorBack, and GreenUp ABV (Figure 2a2–a4). In all the experiments, Taharan treatment showed a consistent positive correlation with high-disinfection efficiency of the tobamovirus inoculum. (Figure 2). In one experiment, testing the special formula of GreenUp ABV, disinfection efficiency of GreenUp ABV was as effective as that of Taharan.

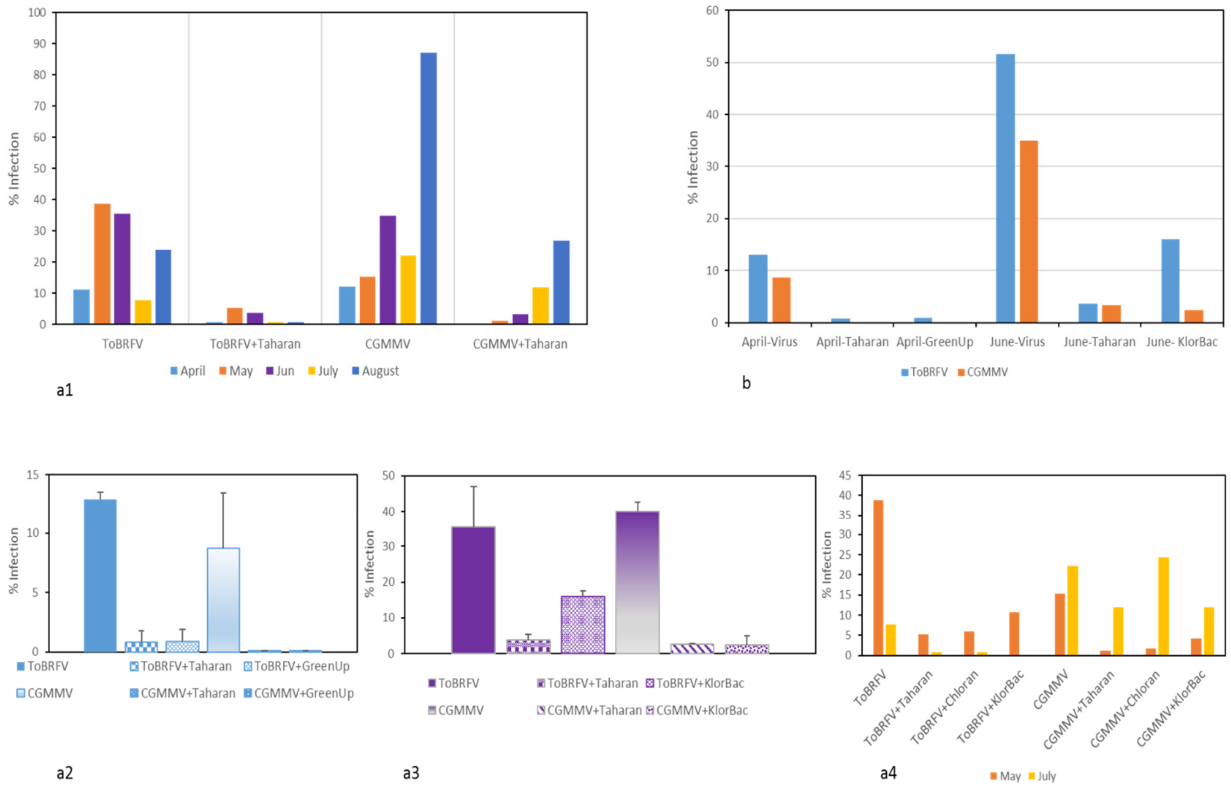

Figure 2. Disinfection efficacy in greenhouse studies during spring and hot summer in consecutive years, using an inoculum of either ToBRFV or CGMMV: (**a1**) Taharan effect, studied between April and August, was significant for ToBRFV and CGMMV with $p < 0.01$ and $p < 0.05$, respectively, using *t*-Test, paired with two samples for means. Number of plants was 90–250/treatment/month; (**a2**–**a4**) GreenUp ABV Chloran, and KlorBack were compared to Taharan (shown in (**a1**)) tested between April and June; (**a2**) Taharan and GreenUP ABV disinfection efficacy were compared in April, n = 120–130/treatment; (**a3**) a comparison between Taharan and KlorBack in June, n = 110–120/treat-

ment; (**a4**) a comparison between Taharan Chloran and KlorBack in May and July, n = 90–250/treatment; (**b**) a comparison between Taharan, GreenUp ABV and KlorBack in April and June in a subsequent year, n = 100–150/treatment. KlorBack disinfection efficacy tested on ToBRFV or CGMMV was significant with $p < 0.016$ and $p < 0.024$, respectively, using *t*-Test, paired with two samples for means.

In a subsequent year, similar results were obtained when Taharan, GreenUp ABV, and KlorBac disinfection efficiencies were tested on ToBRFV and CGMMV disease spread, conducted in April and June (Figure 2b). KlorBac efficacy was as high as that of Taharan, showing significant disinfection of either ToBRFV or CGMMV with $p < 0.016$ and $p < 0.024$ t-Test, paired with two samples for means, respectively. Interestingly, although our platform provided consistent results, root-mediated virus infections were not always high. This phenomenon could be the result of the different resistance mechanisms operating in roots compared to leaves as well as root-mediated signaling to neighboring plants of systemic acquired resistance [31,32]. A possible additional means to increase root-mediated viral infection is to increase soil contamination sources by operating our platform on soil contaminated from a previous growing cycle. We have conducted an experiment in a commercial greenhouse that was ToBRFV infected in a previous growing cycle, and Taharan disinfection efficacy on a ToBRFV inoculum was tested. In that experiment, we have tested the effect of Taharan on tomato yield as well. However, due to trellising, pruning, and the fact that the disinfection treatment was conducted in only 25% of the plants, ToBRFV infection occurring in the untreated side of the greenhouse had spread to all the plants by the 54th day. Under these unfavorable conditions, we have found that there was no difference in fruit yield between the treated and untreated areas in the greenhouse (Figure 3). Importantly, plants grown in the disinfectant-treated soil (following ToBRFV inoculation) were exposed to the additional foliar infection. Nevertheless, the rate of infection spread in the plants grown in the disinfected soil up until the 38th day was less than half the rate of the infection occurring in the positive control plants planted in the soil contaminated with ToBRFV (Figure 3a).

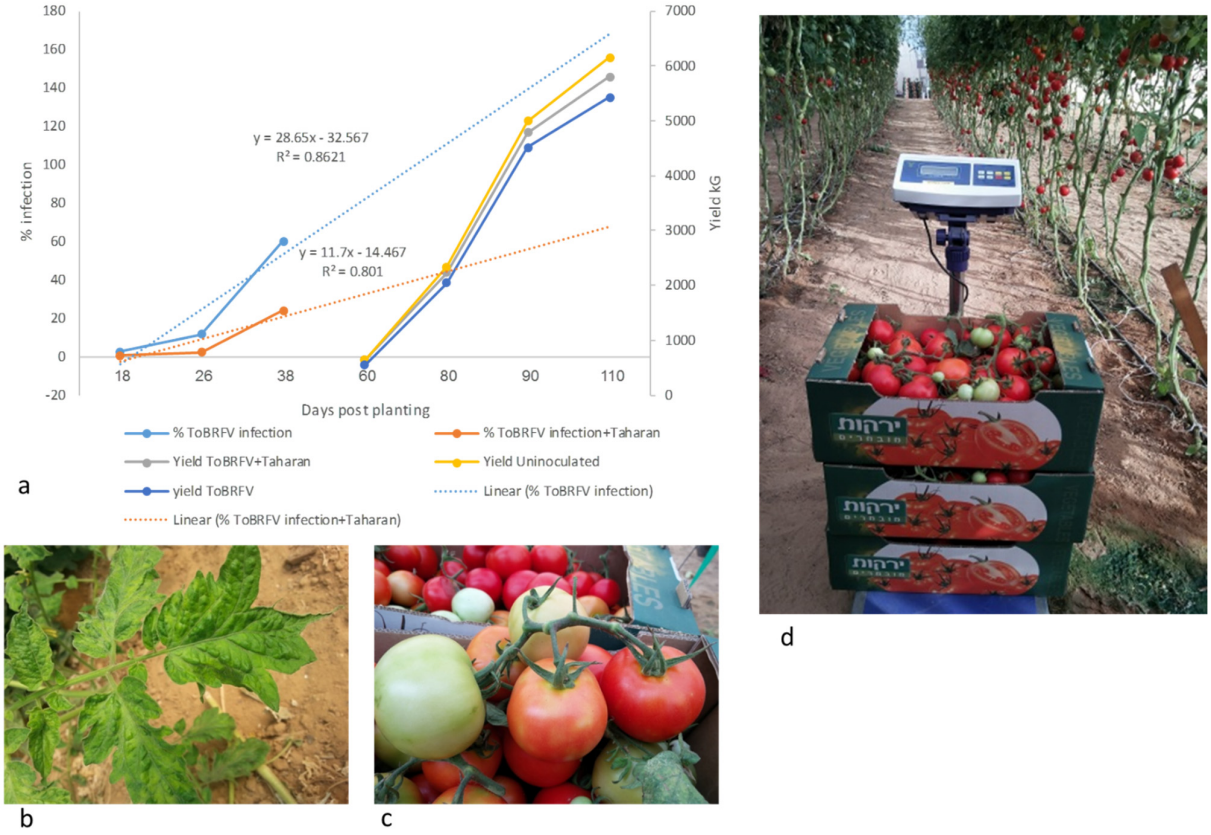

Figure 3. Taharan reduces ToBRFV infection rate: (**a**–**d**) in a large-scale greenhouse experiment conducted in soil contaminated with ToBRFV from a previous growing cycle, the area was divided between disinfectant-treated and untreated soil, following ToBRFV inoculation, and plants were grown for yield measurements. Total contamination of the greenhouse plants was inevitable due to pruning and trellising; (**a**) Taharan reduced, by a factor of ~2.5, the infection rate by ToBRFV. There was no effect on yield accumulation rate: Negative control, y = 116x, R^2 = 0.9; ToBRFV, y = 103x, R^2 = 0.9; ToBRFV + Taharan, y = 109x, R^2 = 0.9; (**b**) ToBRFV-infected plants showing yellowing and mosaic pattern; (**c**) ToBRFV infected fruits showing yellow patches; (**d**) yield assessments in the greenhouse.

Concomitant with the large-scale experiments, we have conducted several small-scale experiments and compared the relative efficiencies of additional disinfectants as well as additional disinfectant concentrations in reducing tobamovirus disease spread from virus-inoculated soil. In one experiment, we have added hydrogen peroxide, which was efficient in disinfecting CGMMV-contaminated sowing trays and caused only a transient phytotoxic effect on cucumber leaves [12,13]. Tables 1 and 2 summarize the soil disinfection efficiencies of the tested disinfectants compared to Taharan activity.

Table 1. Relative disinfection efficiencies of ToBRFV infested sandy soil.

Treatment	Infection Ratios (%) *				Total Number of Plants				Relative Disinfection Efficiencies **			
ToBRFV	38.8	7.8	17.9	29.8	250	108	160	47				
ToBRFV + Taharan	13.7	0.9	0.0	10	150	101	156	50	1	1	1	1
ToBRFV + Chloran	15.5	0.9			150	107			0.98	1		
ToBRFV + KlorBack	27.6	0.0		6	150	109		50	0.84	1.13		1.2
ToBRFV + GreenUp-ABV			0.0				149				1	

ToBRFV + Hydrogen Peroxide	8.2	151	0.54

* Each column represents an experiment conducted in three tunnels; ** Taharan disinfection defined as 1.

Table 2. Relative disinfection efficiencies of CGMMV infested sandy soil.

Treatment	Infection Ratios (%)*					Total Number of Plants					Relative Disinfection Efficiencies **				
CGMMV	20.0	15.4	22.2	15.2	12.5	30	275	117	109	72					
CGMMV + Taharan	6.7	7.8	11.9	0.0	0	30	165	91	149	40	1	1	1	1	1
CGMMV + Chloran	13.3	11.7	24.3			30	165	98			0.5	0.96	0		
CGMMV + KlorBack	3.3	27.3	11.9		0	30	165	91		40	1.3	0.79	1		1
CGMMV + GreenUp-ABV				1.4					144					0.91	
CGMMV + Hydrogen Peroxide				6.0					147					0.61	

* Each column represents an experiment conducted in three tunnels; ** Taharan disinfection defined as 1.

In order to ascertain that the disinfectant efficacy results were not exclusive to sandy-soil, we have conducted a greenhouse experiment in heavy soil enriched with organic compounds, which reduce the effect of chlorinated chemicals as disinfectants in order to establish the effectivity of our platform to study disinfection efficiencies of the tested chlorine-based chemicals. Chlorine-based formulas used in this and other studies, such as Taharan, KlorBack, Virocid, and Chlorox, are apparently promising disinfectants [13,23], but residual ingredients might be absorbed by the plants. A recent finding of the tobamovirus inactivation effect suggests that Lactoferrin would be a preferable ingredient in soil disinfection studies [23]. Reducing phytotoxicity potential of an available ingredient might be a good criterion for a preferable disinfectant. We have included in our experiments a new formulation of chlorinated TSP (Cl-TSP, 3%) that improved the disinfection effect of TSP while avoiding the phytotoxic effects of 5–10% solutions of the original chemical (Figure 4, Table 3). Figure 4 depicts phytotoxic effects of 5–10% TSP-treated soil on cucumber and tomato plants. In field experiments, inhibition of cucumber plant growth was recorded in 7 and 26 days post soil disinfection and planting (Figure 4(a1,a2), comparing left side cucumber control plant growth to right side plants planted in 10% TSP-treated soil). Cucumber and tomato plants tested in an experimental greenhouse showed phytotoxic effects of 5–10% TSP-treated soil at 24–48 h post planting (Figure 4b,c1–c3,). Apparently, 10% TSP-treated soil has caused, in 48h post planting, a prominent reduction in growth of cucumber plants (Figure 4b) and manifestations of burning-yellow spotted leaves in tomato plants (Figure 4c2 compared to the control in Figure 4c3). The new formulation of chlorinated TSP was tested at 3% concentrations in both sandy and heavy soil. The results showed that compared to Taharan, 3% Cl-TSP disinfection effect on ToBRFV-contaminated soil was highly efficient, but the solution showed only ~60% of Taharan effect on disinfection of CGMMV-contaminated soil (Table 3).

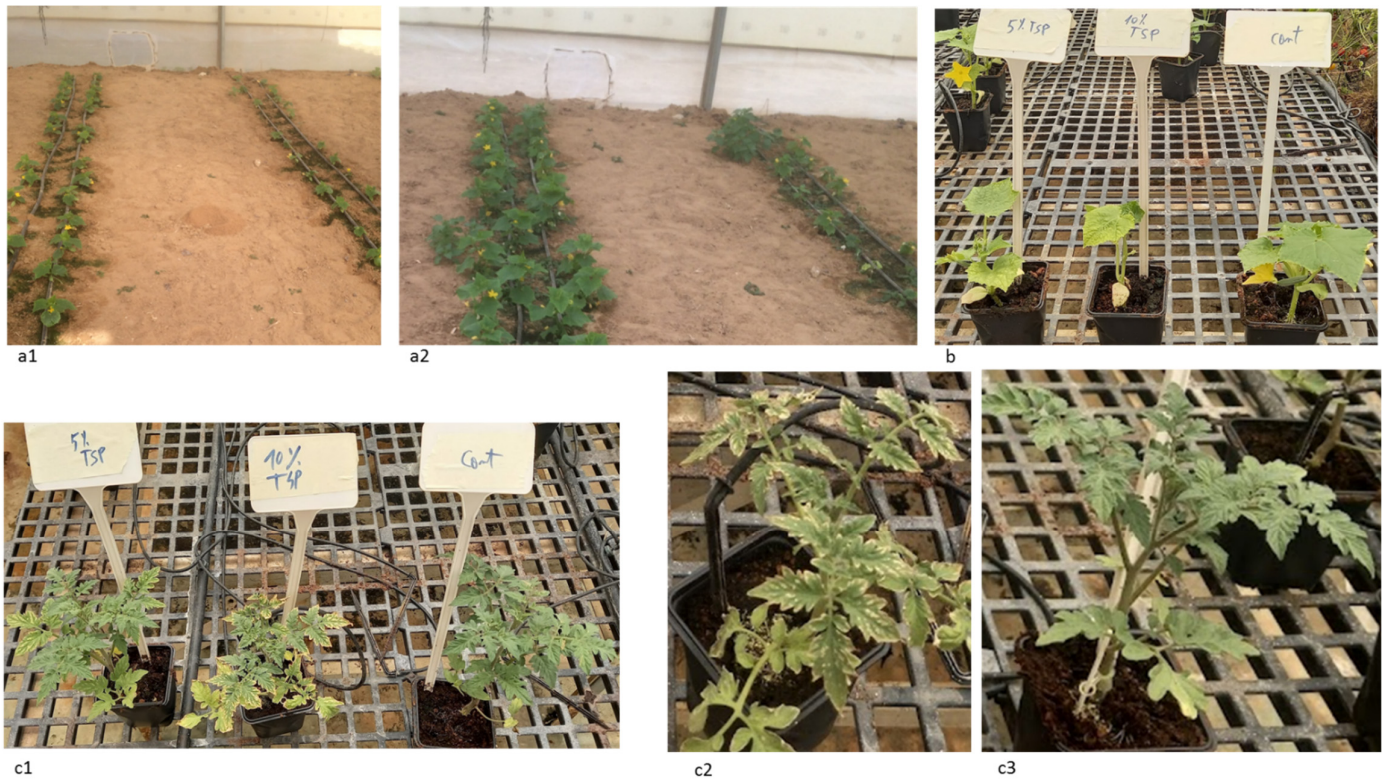

Figure 4. Phytotoxicity of tri-sodium phosphate (TSP)-treated soil: (**a1**,**a2**) inhibition of growth in greenhouse grown cucumber plants exposed to soil disinfection with 10% TSP (left side control plants compared to the right side plants planted in 10% TSP-treated soil); (**a1**) 7 days post soil disinfection and planting; (**a2**) 26 days post soil disinfection and planting; (**b**) cucumber plants at five days post exposure to 5–10% TSP. A total of 50 mL of 5–10% TSP were poured into the planting pits before cucumber seedlings without root truncation were planted; (**c1**) tomato plants at five days post exposure to 5–10% TSP. A total of 50 mL of 5–10% TSP were poured into the planting pits before tomato seedlings without root truncation were planted; (**c2**) a strong phytotoxic effect of burning-yellow spotted leaves on a tomato plant grown in 10% TSP-treated soil for 48 h; (**c3**) a control tomato plant, planted in untreated soil, at 48 h post planting.

Table 3. Relative disinfection efficiencies of ToBRFV- and CGMMV-infested heavy or sandy soils.

Treatment	Infection Ratios (%)	Total Number of Plants	Relative Disinfection Efficiencies *
ToBRFV	29.8	47	
ToBRFV + KlorBack 1000 ppm	14.3	42	0.78
ToBRFV + KlorBack 2000 ppm	6	50	1.2
ToBRFV + Taharan 1000 ppm	15	40	0.75
ToBRFV + Taharan 2000 ppm	10	50	1
ToBRFV + Cl-TSP 3%	8	50	1.1
ToBRFV + Cl-TSP 5%	2	50	1.5
SS//ToBRFV	46	41	
SS//ToBRFV + Cl-TSP 5%	0	40	1
SS//ToBRFV + TSP 5%	0	39	1
SS//ToBRFV + Taharan 2000 ppm	0	37	1
CGMMV	12.5	72	
CGMMV + KlorBack 1000 ppm	5	40	0.6
CGMMV + KlorBack 2000 ppm	0	40	1

CGMMV + Taharan 1000 ppm	5	40	0.6
CGMMV + Taharan 2000 ppm	0	40	1
CGMMV + Cl-TSP 3%	7.5	40	0.4
CGMMV + Cl-TSP 5%	0	40	1
SS//CGMMV	12.6	41	
SS//CGMMV + Cl-TSP 5%	0	40	1
SS//CGMMV + TSP 5%	0	39	1
SS//CGMMV + Taharan 2000 ppm	0	37	1

* Taharan disinfection effect defined as 1; SS, sandy soil.

4. Conclusions

A low percentage of infectious foci (1–2%) occur in soil following a tobamovirus-infected growing cycle, rendering disinfection studies of several contaminated plots inconclusive unless thousands of plants were tested. Our study provided the platform for disinfectant efficacy evaluation, a necessary stage prior to a large-scale application in tobamovirus-contaminated soil, which commonly occurs in commercial tomato and cucumber greenhouses. In that platform, the viral inoculum and the truncated roots ensured equal exposure of seedlings to the tested tobamovirus, thereby reducing the plant number necessary for drawing a conclusion regarding the preferable disinfectants. In Israel, the obtained data using the described platform have served growers, and following a highly contaminated growth cycle, chlorine-based disinfectants were added to the irrigation system 1–2 days prior to planting.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Broadbent, L. The epidemiology of tomato mosaic VIII. Virus infection through tomato roots. *Ann. Appl. Biol.* **1965**, *55*, 57–66. https://doi.org/10.1111/j.1744-7348.1965.tb07867.x.
2. Lanter, J.M.; McGuire, J.M.; Goode, M.J. Persistence of Tomato Mosaic Virus in Tomato Debris and Soil Under Field Conditions. *Plant Dis.* **1982**, *66*, 552–555. https://doi.org/10.1094/PD-66-552.
3. Pares, R.D.; Gunn, L.V.; Keskula, E.N. The role of infective plant debris, and its concentration in soil, in the ecology of tomato mosaic tobamovirus—A non-vectored plant virus. *J. Phytopathol.* **1996**, *144*, 147–150. https://doi.org/10.1111/j.1439-0434.1996.tb01505.x.
4. Reingold, V.; Lachman, O.; Belausov, E.; Koren, A.; Mor, N.; Dombrovsky, A. Epidemiological study of *Cucumber green mottle mosaic virus* in greenhouses enables reduction of disease damage in cucurbit production. *Ann. Appl. Biol.* **2016**, *168*, 29–40. https://doi.org/10.1111/aab.12238.
5. Dombrovsky, A.; Smith, E. Seed Transmission of Tobamoviruses: Aspects of Global Disease Distribution. In *Advances in Seed Biology*, InTech: 2017; Zagreb, Croatia; pp. 233–260, doi.org/10.5772/intechopen.70244.

6. Li, J.X.; Liu, S.S.; Gu, Q.S. Transmission efficiency of Cucumber green mottle mosaic virus via seeds, soil, pruning and irrigation water. *Journal of Phytopathology* **2016**, *164*, 300–309. https://doi.org/10.1111/jph.12457.
7. Takeuchi, S.; Hikichi, Y.; Kawada, Y.; Okuno, T. Detection of tobamoviruses from soils by non-precoated indirect ELISA. *J. Gen. Plant Pathol.* **2000**, *66*, 153–158. https://doi.org/10.1007/PL00012938.
8. Dornai, D.; Mingelgrin, U.; Frenkel, H.; Bar-Joseph, M. Direct quantification of unadsorbed viruses in suspensions of adsorbing colloids with the enzyme-linked-immunosorbent-assay. *Appl. Env. Microb.* **1993**, *59*, 3123–3125. https://doi.org/10.1128/aem.59.9.3123-3125.1993.
9. Gülser, C.; Yılmaz, N.K.; Candemir, F. Accumulation of Tobacco mosaic virus (TMV) at different depths clay and loamy sand textural soils due to tobacco waste application. *Environ. Monit. Assess.* **2008**, *146*, 235–242. https://doi.org/10.1007/s10661-007-0075-7.
10. Lister, R. Soil-borne virus diseases. *Sci. Hortic.* **1959**, *14*, 90–96. Available online: https://www.jstor.org/stable/45126127 (accessed on 21 Jun. 2022).
11. Smith, E.; Luria, N.; Reingold, V.; Frenkel, O.; Koren, A.; Klein, E.; Bekelman, H.; Lachman, O.; Dombrovsky, A. Aspects in tobamovirus management in modern agriculture: Cucumber green mottle mosaic virus. In Proceedings of the XXX International Horticultural Congress IHC2018: International Symposium on Tropical and Subtropical Vegetable Production, Istanbul-Turkey from 12-17 August 2018; 1257; pp. 1–8.
12. Ellouze, W.; Mishra, V.; Howard, R.J.; Ling, K.-S.; Zhang, W. Preliminary Study on the Control of Cucumber Green Mottle Mosaic Virus in Commercial Greenhouses Using Agricultural Disinfectants and Resistant Cucumber Varieties. *Agronomy* **2020**, *10*, 1879. https://doi.org/10.3390/agronomy10121879.
13. Darzi, E.; Lachman, O.; Smith, E.; Koren, A.; Klein, E.; Pass, N.; Frenkel, O.; Dombrovsky, A. Paths of cucumber green mottle mosaic virus disease spread and disinfectant-based management. *Ann. Appl. Biol.* **2020**, *177*, 374–384. https://doi.org/10.1111/aab.12629.
14. Smith, E.; Dombrovsky, A. Aspects in Tobamovirus management in intensive agriculture. In *Plant Diseases-Current Threats and Management Trends*, IntechOpen: 2019; London, UK; pp. 31–47. https://doi.org/10.5772/intechopen.87101.
15. Luvisi, A.; Panattoni, A.; Materazzi, A. Heat treatments for sustainable control of soil viruses. *Agron. Sustain. Dev.* **2015**, *35*, 657–666, doi.org/10.1007/s13593-014-0258-x.
16. Ainsworth, G.C. Mosaic disease of cucumber. *Ann. Appl. Biol.* **1935**, *22*, 55–67, doi.org/10.1111/j.1744-7348.1935.tb07708.x.
17. Dombrovsky, A.; Tran-Nguyen, L.T.; Jones, R.A. Cucumber green mottle mosaic virus: Rapidly Increasing Global Distribution, Etiology, Epidemiology, and Management. *Annu. Rev. Phytopathol.* **2017**, *55*, 231–256. https://doi.org/10.1146/annurev-phyto-080516-035349.
18. Salem, N.; Mansour, A.; Ciuffo, M.; Falk, B.; Turina, M. A new tobamovirus infecting tomato crops in Jordan. *Arch. Virol.* **2016**, *161*, 503–506, doi.org/10.1007/s00705-015-2677-7.
19. Luria, N.; Smith, E.; Reingold, V.; Bekelman, I.; Lapidot, M.; Levin, I.; Elad, N.; Tam, Y.; Sela, N.; Abu-Ras, A.; et al. A New Israeli Tobamovirus Isolate Infects Tomato Plants Harboring Tm-22 Resistance Genes. *PLoS ONE* **2017**, *12*, e0170429. https://doi.org/10.1371/journal.pone.0170429.
20. Klap, C.; Luria, N.; Smith, E.; Hadad, L.; Bakelman, E.; Sela, N.; Belausov, E.; Lachman, O.; Leibman, D.; Dombrovsky, A. Tomato Brown Rugose Fruit Virus Contributes to Enhanced Pepino Mosaic Virus Titers in Tomato Plants. *Viruses* **2020**, *12*, 879, doi.org/10.3390/v12080879.
21. Molad, O.; Smith, E.; Luria, N.; Sela, N.; Lachman, O.; Bakelman, E.; Leibman, D.; Dombrovsky, A. New early phenotypic markers for cucumber green mottle mosaic virus disease in cucumbers exposed to fluctuating extreme temperatures. *Sci. Rep.* **2021**, *11*, 1–16. https://doi.org/10.1038/s41598-021-98595-4.
22. Molad, O.; Smith, E.; Luria, N.; Sela, N.; Lachman, O.; Bakelman, E.; Leibman, D.; Dombrovsky, A. Plant Disease Symptomatology: Cucumber Green Mottle Mosaic Virus (CGMMV)-Infected Cucumber Plants Exposed to Fluctuating Extreme Temperatures, Biol. Life Sci. Forum **2022**, *11*, 58.
23. Chanda, B.; Shamimuzzaman, M.; Gilliard, A.; Ling, K.-S. Effectiveness of disinfectants against the spread of tobamoviruses: Tomato brown rugose fruit virus and Cucumber green mottle mosaic virus. *Virol. J.* **2021**, *18*, 1–12. https://doi.org/10.1186/s12985-020-01479-8.
24. Taylor, R.H.; Grogan, R.G.; Kimble, K.A. Transmission of tobacco mosaic viras in tomato seed. *Phytopathology* **1961**, *51*, 837–842.
25. Broadbent, L. The epidemiology of tomato mosaic. XI. Seed transmission of TMV. *Ann. Appl. Biol.* **1965**, *56*, 177–205, https://doi.org/10.1111/j.1744-7348.1965.tb01227.x.
26. Czajkowski, R.; De Boer, W.; Van der Wolf, J. Chemical disinfectants can reduce potato blackleg caused by 'Dickeya solani'. *Eur. J. Plant Pathol.* **2013**, *136*, 419–432. https://doi.org/10.1007/s10658-013-0177-8.
27. Lu, B.; Stubbs, G.; Culver, J.N. Coat protein interactions involved in tobacco mosaic tobamovirus cross-protection. *Virology* **1998**, *248*, 188–198. https://doi.org/10.1006/viro.1998.9280.
28. Pryor, A.K.; Brown, R.S. Quaternary ammonium disinfectants: An updated perspective. *J. Environ. Health* **1975**, *37*, 326–330. Available online: www.jstor.org/stable/44546816 (accessed on 21 Jun. 2022).
29. Lambert, R.; Johnston, M. Disinfection kinetics: A new hypothesis and model for the tailing of log-survivor/time curves. *J. Appl. Microbiol.* **2000**, *88*, 907–913. https://doi.org/10.1046/j.1365-2672.2000.01060.x.
30. Yu, W.H.; Li, N.; Tong, D.S.; Zhou, C.H.; Lin, C.X.C.; Xu, C.Y. Adsorption of proteins and nucleic acids on clay minerals and their interactions: A review. *Appl. Clay Sci.* **2013**, *80*, 443–452. https://doi.org/10.1016/j.clay.2013.06.003.

31. Andika, I.B.; Kondo, H.; Sun, L. Interplays between soil-borne plant viruses and RNA silencing-mediated antiviral defense in roots. *Front. Microbiol.* **2016**, *7*, 1458. https://doi.org/10.3389/fmicb.2016.01458.
32. Cheol Song, G.; Sim, H.-J.; Kim, S.-G.; Ryu, C.-M. Root-mediated signal transmission of systemic acquired resistance against above-ground and below-ground pathogens. *Ann. Bot.* **2016**, *118*, 821–831. https://doi.org/10.1093/aob/mcw152.

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