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RESEARCH ARTICLE

Screening commercial disinfectants to strengthen on-farm biosecurity for the prevention of Fusarium wilt of banana Tropical Race 4 in Ecuador

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Abstract

Bananas (*Musa* spp.), often reported together with plantains, are among the most traded fruit commodities worldwide, particularly in terms of export volume and value, with Ecuador recognized as the leading global exporter, supplying approximately one quarter of the total international trade. However, its production is increasingly threatened by climate change and fungal diseases such as Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (*Foc* TR4). To support preventive management strategies against *Foc* TR4, this study evaluated the *in vitro* efficacy of nine commercial disinfectants available in Ecuador—including quaternary ammonium compounds (QACs), oxidizing agents, aldehydes, and alkaline substances—against conidia and chlamydospores of several *Foc* race 1 strains (VCG0120) as a model organism. Different concentrations and contact times were tested in the presence and absence of organic matter. Chlamydospores exhibited higher resistance than conidia, requiring greater concentrations for inhibition. Aldehyde formulations and QACs achieved the lowest minimum inhibitory concentrations (<1,000 ppm) and remained effective under organic load and variable contact times. These findings emphasize the need to select disinfectants with consistent efficacy under field-relevant conditions to ensure the application of proper biosecurity measures.

Keywords: Plant pathogens; Plant Disease management; Agricultural biosecurity; Bananas; Fusarium Wilt; Disinfectants.

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1. Introduction

Banana is a staple crop of global importance and the most exported fresh fruit worldwide in both volume and value. Production is highest in tropical and subtropical regions, particularly in developing countries such as Ecuador (Croppenstedt, 2025). Although banana is a key food crop in Africa, Asia, and Latin America, only a limited fraction of total production (approximately one-fifth) enters international trade (Evans et al., 2020), underscoring its strategic relevance for domestic markets and its contribution to food security (Olivares Campos, 2023).

The main banana-producing countries in the Americas are Brazil, Ecuador, Guatemala, Costa Rica, Mexico, and Colombia (Zou et al., 2022). Ecuador is the region's leading exporter, reporting approximately USD 3.7 billion in banana export revenues in 2024 (FAO, 2025). The country cultivates around 175,180 hectares (ha) of banana, representing 11.8% of its agricultural land, with production concentrated in the coastal region (Mosquera et al., 2023). Currently, banana production in Ecuador sustains the livelihoods of more than one million families and constitutes one of the country's most important sources of export revenue, surpassed only by shrimp (Corporación Financiera Nacional B.P, 2024).

Climate change exacerbates multiple environmental stresses, such as elevated temperatures, irregular

rainfall patterns, drought, and extreme weather events, which collectively limit plant growth, development, and yield, including in bananas (Abdoussalami et al., 2023). Moreover, these climate-driven stresses can increase crop vulnerability and favor the emergence and spread of pests and diseases. In Ecuador, Fusarium wilt of banana (FWB), also known as Panama disease caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*), devastated the Gros Michel based banana industry (Mendoza et al., 2025). This epidemic, caused by several strains associated with the race 1 of *Foc*, forced a transition to resistant Cavendish cultivars, which today represent approximately 99% of the country's banana exports (Magdama et. al., 2020). However, the industry now faces the looming threat of the more aggressive strain of the same pathogen known as Tropical Race 4 (*Foc* TR4) (Pegg et al., 2019). *Foc* TR4 has spread across several continents, including Latin America, where it was first reported in Colombia in 2019 and was subsequently confirmed in Perú (2021) and Venezuela (2023) (Acuña et al., 2022; García-Bastidas et al., 2020; Mejías Herrera et al., 2023). More recently, *Foc* TR4 was confirmed in Ecuador in December 2025, affecting a farm in Santa Rosa canton, El Oro province (El Comercio, 2025). *Foc* is a soilborne fungus that disperses through asexual spores, primarily microconidia and macroconidia (Dita et al., 2018). This pathogen can obstruct the plant vascular system, thereby impairing the uptake of water and nutrients, ultimately causing wilting and plant death (Özarslandan & Akgül, 2024). In addition, it produces survival structures called chlamydospores that enable it to persist in soil for up to 30 years, which makes disease control particularly challenging (Warman & Aitken, 2018).

To support disease management, *in vitro* sensitivity studies have been carried out to determine the susceptibility of the pathogen to selected chemical compounds (Salacinas et al., 2022) and biological products extracts (Chen et al., 2022; Jing et al., 2020). Management strategies have also focused on preventing and containing pathogen dissemination through biosecurity protocols, which include the use of disinfectants (Izquierdo-García et al., 2021; Nguyen et al., 2019; Ordoñez González et al., 2021).

Research on disinfectants against *Foc* has targeted both reproductive structures (microconidia and macroconidia) and survival spores (chlamydospores) (Izquierdo-García et al., 2021). The most widely used disinfectants to eliminate microorganisms are Quaternary Ammonium Compounds (QACs) (Jones & Joshi, 2021). These disinfectants, also referred as biocides, act as cationic surfactants that disrupt the cell membranes of bacteria, fungi, and certain viruses, leading to protein denaturation, compromising membrane integrity, and ultimately cell lysis and death (Kwaśniewska et al., 2020). In recent years, advances have been reported regarding the inhibitory and biocidal properties of QACs against filamentous fungi and yeast (Fait et al., 2019), including *Foc* TR4 (Arango-Palacio et al., 2024).

The growing need to implement biosecurity measures in banana plantations to manage fungal pathogens has promoted the widespread use of disinfectants. Nevertheless, many of these disinfectants, including QACs, have not been specifically evaluated under local conditions. Therefore, the objective of this study was to evaluate the *in vitro* efficacy of commercial disinfectants available in Ecuador against viable propagules of *Fusarium oxysporum* f. sp. *cubense* Race 1 (conidia and chlamydospores) under different concentrations, contact times, and levels of organic matter, in order to provide science-based guidance for their effective implementation in on-farm biosecurity practices.

2. Methodology

2.1 Disinfectant selection

The *in vitro* efficacy of nine commercial disinfectants available in Ecuador was evaluated. These products belong to the groups of oxidizing agents, QACs, aldehydes (glutaraldehyde), and alkaline/caustic substances. They were tested against the pathogen at different concentrations (Table 1), with contact times of 30 seconds, 5 minutes, 30 minutes, and 24 hours, both in the presence and absence of organic matter.

2.2 Inoculum production

The assay was conducted with four strains of *Foc* race 1 (*Foc* R1), VCG0120, previously isolated from banana pseudostems collected in different coastal provinces of Ecuador (Table 2) (Magdama et. al., 2020). The isolates, preserved in the Microorganism Culture Collection at the Centro de Investigaciones Biotecnológicas del Ecuador (CCM- CIBE) from the Escuela Superior Politécnica del Litoral (Marideueña-Zavala et al., 2021), were subsequently reactivated on Potato Dextrose Agar (Difco™, Becton, Dickinson and company). The four strains were selected to represent pathogen populations collected from four different provinces.

Table 1
Commercial disinfectants and concentrations tested against *Foc* R1

Group	Product (Commercial name)	Active ingredient(s)	Formulation concentration	Tested concentration (ppm)	Equivalent active ingredient (%)	% of product used
Aldehydes	Viroguard®	Glutaraldehyde (10%), benzalkonium chloride (10%), formaldehyde (10%)	10%	10 100 1,000 10,000 20,000	0.001–2.0	0.01–20
	ViroShield®	Glutaraldehyde + (30%) Benzalkonium chloride (10%)	30%	30 300 3,000 6,000 12,000	0.003–1.2	0.01–4
Oxidizing agents	ZT®	Hydrogen peroxide (H_2O_2, 27%)	27%	27 270 2,700 27,000 54,000	0.001–5.4	0.01–20
	AgSept Plus®	Elemental silver (60 ppm) + Hydrogen peroxide (2.4%)	2.4%	240 2,400 24,000	0.02–2.4	1–100
	Virex®	Monopersulfate (MPS, 50%)	50%	50 500 5,000 10,000	0.01–1.0	0.01–2
	Neuthox®	Hypochlorous acid (HOCl, 540 ppm)	540 ppm	0.54 5.4 54 270 540	0.001–0.05	0.1–100
Quaternary ammonium compounds (QACs)	Sporekill®	Didecyl dimethyl ammonium chloride (DDAC, 12%)	12%	12 20 1,200 2,400 4,800	0.001–0.48	0.01–4
	ViruKill®	Didecyl dimethyl ammonium chloride (DDAC, 12%)	12%	12 120 1,200 2, 400 4,800	0.001–0.48	0.01–4
Alkaline / Caustic	SuperCal 200 P1®	Calcium oxide (CaO, 62.5%)	62.5%	625 6,250 62,500 125,000 250,000	0.06–25.0	0.1–40

Table 2
Origin of *Foc* isolates used in this study

Isolate	Locality	Cultivar	Tissue
EC-35-G-GM	Guayas	Gros Michel	Pseudostem
EC-19-LR-GM	Los Ríos	Gros Michel	Pseudostem
EC-40-M-GM2	Manabí	Gros Michel	Pseudostem
EC-15-E-GM1	Esmeraldas	Gros Michel	Pseudostem

For inoculum production, isolates with 8 days growth on PDA were used, from which ten mycelial fragments were collected. These fragments were transferred into a 200 mL Erlenmeyer flask containing Potato Dextrose Broth supplemented with streptomycin (300 mg/L) and ampicillin (100 mg/L) and incubated at 28 °C for 8 days under constant agitation at 110 rpm. After incubation, the culture was filtered through four layers of gauze to remove the mycelium, and the filtrate was rinsed with sterile water, yielding a supernatant enriched primarily with conidia.

The supernatant was aliquoted into four 50-mL tubes and centrifuged at 2,000 rpm for 2 min. After centrifugation, the supernatant was discarded and the pellet was resuspended in 5–10 mL of sterile water to obtain a concentrated conidial suspension of $1x10^7$. To initiate the assay, 100 µL of this suspension was added to 900 µL of disinfectant solution at the indicated concentrations, producing a final conidial concentration of 1×10^6 conidia/mL.

2.3 Production and harvest of chlamydospores

In vitro chlamydospore production of *Foc* R1 was carried out following the protocol described by the Corporación Bananera Nacional, CORBANA (Carr et al., 2019). The substrate was prepared with banana root and organic soil in an approximate ratio of 3:1 (w/w) (i.e., 100 g of root, previously blended and dried at 50 °C, with 30 g of organic soil). Healthy banana roots were selected, blended with deionized water, dehydrated at 50 °C for 3 hours, and dried overnight at 29 °C. The material was then blended a second time and sieved to remove large organic (sieve No. 35, 500 µm mesh, 0.0197 inches, S/N 04306776). Substrate sterility was verified by autoclaving the material twice, followed by plating and incubation for 48 h at 28 °C. Samples showing any microbial growth were autoclaved a third time at 121 °C.

For substrate inoculation, a conidial suspension of *Foc* at $1x10^7$ conidia/mL was used. The entire mixture was placed in an Erlenmeyer flask, sealed, and covered with aluminum foil, and incubated for 21 to 28 days in a dark box to avoid light exposure. Alternatively, exposure to black light was used to facilitate subsequent chlamydospore harvest.

At the end of this incubation period, 1 g of the inoculated substrate was weighed and suspended in 48 mL of sterile water plus 1 mL of Tween 20 (1%). The mixture was vortexed for 45 minutes to release the chlamydospores. This suspension was then passed through a sterile gauze layer to obtain a solution mainly composed of free chlamydospores. The chlamydospore concentration was adjusted to 1.9×10^5 CFU/mL by observing and counting chlamydospores under a Neubauer chamber. In addition, 100 µL of the treated suspension was plated on PDA supplemented with streptomycin and ampicillin to assess chlamydospore viability.

To initiate the assay, 100 µL of the chlamydospore suspension was added to 900 µL of disinfectant solution at the desired concentrations, resulting in a final concentration of 1.9×10^4 chlamydospores.

2.4 Evaluation of disinfectants under soil-free conditions

The disinfectant experiment was designed based on the methodology proposed by Nguyen et al., (2019). Viable suspensions of *Foc* conidia and chlamydospores were prepared, and 100 µL aliquots were mixed with 900 µL of disinfectant solutions prepared at the indicated concentrations in 2-mL tubes. The tubes were sealed, vortexed, and pipetted several times to ensure proper mixing. The exposure time of propagules to disinfectants was ≤30 seconds, 5 minutes, 30 minutes and 24 hours. After the exposure time, a 100 µL aliquot was plated on PDA supplemented with streptomycin sulfate (30 mg/L). Plates were incubated at 28 °C ± 1 for 48 to 72 h, after which the number of growing colonies was counted. If no growth was observed after 72 h, plates were incubated for up to seven days to confirm disinfectant effectiveness. Treatments that did not yield colony formation after seven days of incubation were considered effective against *Foc* (Izquierdo-García et al., 2021).

The treatments were arranged in a Completely Randomized Design with five replicates. Each replicate consisted of Petri dishes. The control treatment consisted of sterile water without the addition of any disinfectant.

Results were presented as Minimum Inhibitory Concentration (MIC) values, expressed in parts per million (ppm), comparing active compounds and commercial disinfectants across different exposure times for both chlamydospores and conidia. In addition, the average effective concentration and percentage inhibition were reported.

The efficacy of each disinfectant was calculated using the following equation proposed by Abbott, (1925):

$$E = \left(\frac{a-b}{a}\right) x\ 100$$

Where "a" represents the CFU value in the negative control and "b" represents the CFU value in the disinfectant treatment.

2.5 Evaluation of disinfectants in the presence of soil

Treatments simulating the presence of organic matter were conducted as described above. To reproduce organic load conditions equivalent to 0.05 and 0.1 of organic matter, 0.5 g and 1 g of clay loam soil were incorporated into every 10 mL of disinfectant solution, respectively. The mixtures were thoroughly homogenized to ensure uniform distribution of the soil particles, and 900 µL aliquots were subsequently dispensed into 2 mL microtubes. Finally, 100 µL of the pathogen inoculum were added to each tube (Arango-Palacio et al., 2024). After immediate exposure, aliquots were plated into Petri dishes containing PDA and incubated as previously mentioned. Although 4 strains were evaluated in preliminary studies, only chlamydospores from strain EC-35-G-GM were used in this assay. MICs of the products used for this experiment were: AgSept® 24,000 ppm, Neuthox® 270 ppm, Sporekill® 1,200 ppm, SuperCal200P1® 250,000 ppm, Virex® 10,000 ppm, Viroguard® 1,000 ppm, ViroShield® 3,000 ppm, ViruKill® 120 ppm and ZT® 2,700 ppm. The results were presented as percentage inhibition.

A second experiment was performed considering the same organic matter load (5 and 10%), but with three different exposure times, 30 s, 24 h and 48 h using only *Foc* chlamydospores.

2.6 Statistical analyses

To evaluate the effects of the treatments, the data were analyzed non-parametrically, as the assumptions of normality of the residuals were not met; this was verified using the Shapiro–Wilk test. When normality assumptions were not met, the Kruskal–Wallis test was used. The susceptibility and average MIC of Foc strains were assessed for each disinfectant and active compound, separating the analysis for conidia and chlamydospores. The inhibition analyses were also conducted by disinfectants, active compound, exposure time, and level of organic matter. If the Kruskal–Wallis test was rejected, Dunn's post-hoc multiple pairwise comparison test was performed with p-values adjusted using the Bonferroni correction, and Spearman's rank correlation was used too. Statistical analyses were carried out using the R statistical software (R v.3.6.3).

3. Results and discussion

3.1 Experiment with disinfectants in absence of soil

3.1.1 Efficacy of treatments against conidia and chlamydospores

The analysis revealed chlamydospores required higher average disinfectant concentration values (up to ~40,000 ppm), whereas conidia lower values (~10,000 ppm or lower) (Figure 1). This suggests that chlamydospores can be effectively controlled with higher disinfectant concentrations, indicating lower susceptibility to chemical treatments compared to conidia. Thus, biosecurity protocols must ensure optimal concentrations and sufficient contact times focused on the efficient elimination of chlamydospores, particularly on tools, soils, and surfaces where they may persist for extended periods. Chlamydospores are resistant structures due to a thickened cell wall, capable of surviving under adverse environmental conditions and persisting in soil for years (Bennett, 2012; Gordon, 2017). Stover (1962) considered that chlamydospores of *Foc* are highly resistant to chemicals, heat, and UV radiation, which allows them to remain viable in the absence of the host. Accordingly, considering their resilience and long-term persistence, both conidia and chlamydospores should be included in disinfectant efficacy trials to ensure biosecurity protocols effectively target the most resistant propagules. Variability among strains was also observed. Strain EC-35-G-GM1, for instance, exhibited higher significant resistance to disinfectants in chlamydospores but intermediate values in conidia assays when exposed to the different treatments. In contrast, strain EC-19-LR-GM3 showed higher susceptibility in both chlamydospores and conidia experiments. Variability in resistance among strains may be associated with genetic differences in cell wall structure, melanin production, and detoxification mechanisms. Moreover, different races and variants of *F. oxysporum* may exhibit differential adaptations to chemical stress conditions (Leslie & Summerell, 2006).

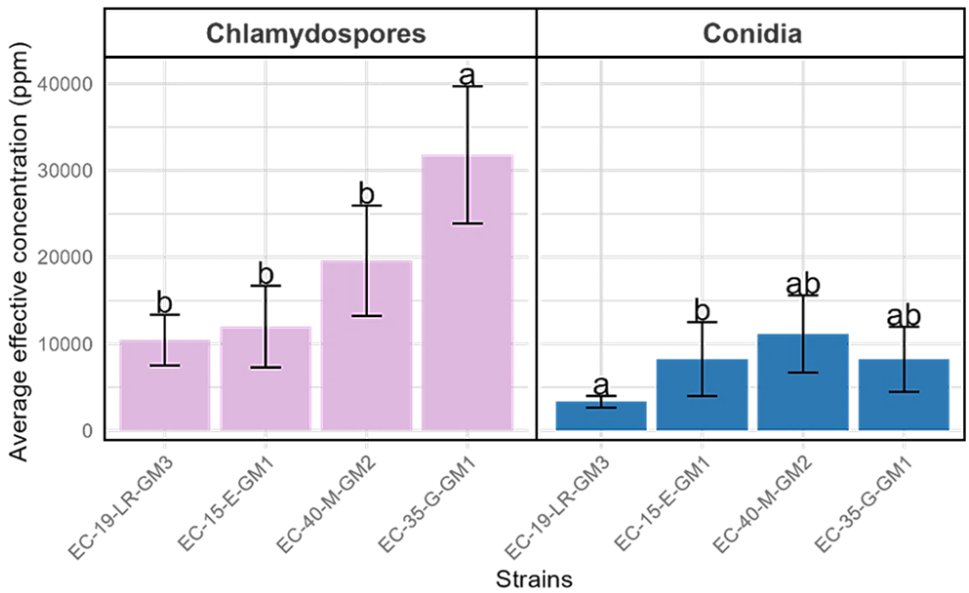

Figure 1. Average effective concentration (ppm) required to inhibit chlamydospores and conidia of different *Foc* strains. Bars with different lowercase letters within each group indicate significant differences among treatments according to the Kruskal–Wallis rank-based test (α = 0,05).

3.1.2 MIC values against chlamydospores and conidia

The results of MIC showed significant differences among the disinfectants by active compound group. The quaternary ammonium group had the highest efficiency among active compounds; it presented significantly the highest inhibitory effect on conidia and chlamydospores in all strains, followed by the aldehydes group. For this assay, disinfectants formulated with quaternary ammonium and aldehydes exhibited MIC values below 1,000 ppm against both spore types (conidia and chlamydospores). In contrast, oxidizing agents required concentrations exceeding 1,000 ppm to inhibit growth, while alkaline and caustic compounds showed MIC values above 100,000 ppm (Figure 2).

Overall, Neuthox®, Sporekill®, and ViruKill® demonstrated the highest inhibitory activity, achieving reductions of >3 log units in viable propagules within a short contact time (30 s) and exhibiting MIC values between 100 and 1,000 ppm. In contrast, AgSept Plus®, SuperCal200P1®, and Virex® showed MIC values between 10,000 and 100,000 ppm even after 24 h, suggesting poor antifungal activity under the tested conditions. When comparing propagule types, conidia were consistently more susceptible than chlamydospores across most disinfectants and time points, underscoring the higher resilience of chlamydospores to chemical treatments (Figure 3 and Appendix 1).

According to previous experimental studies, disinfectants formulated with QACs have demonstrated a greater capacity to inhibit the growth of various *Foc* strains, including race 1 and TR4 (Meldrum et al., 2013). QACs are rapidly absorbed onto *Fusarium* conidia and hyphae, affecting the hydrophobicity of the conidia surface, altering the cell wall composition, and disrupting its structural integrity (Zhang et al., 2023). QACs can interfere with DNA and RNA synthesis by indirectly affecting enzymes such as DNA polymerase and can induce DNA fragmentation due to loss of cellular integrity. Moreover, didecyldimethylammonium chloride (DDAC) can also disrupt cellular redox metabolism by increasing the production of reactive oxygen species (ROS), leading to lipid, protein, and DNA damage through lipid peroxidation and oxidation of sulfhydryl groups (Knauf et al., 2018; Rowe et al., 2008).

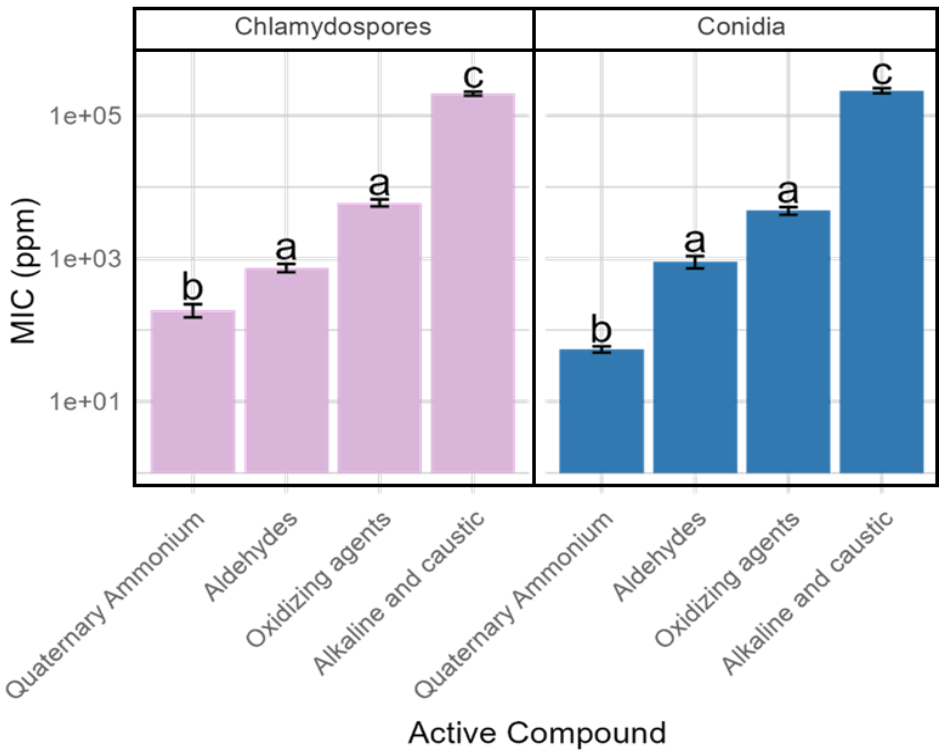

Figure 2. Average MIC of different groups of disinfectants against chlamydospores and conidia. Bars with different lowercase letters within each group indicate significant differences among treatments according to the rank-based Kruskal–Wallis test (α = 0.05).

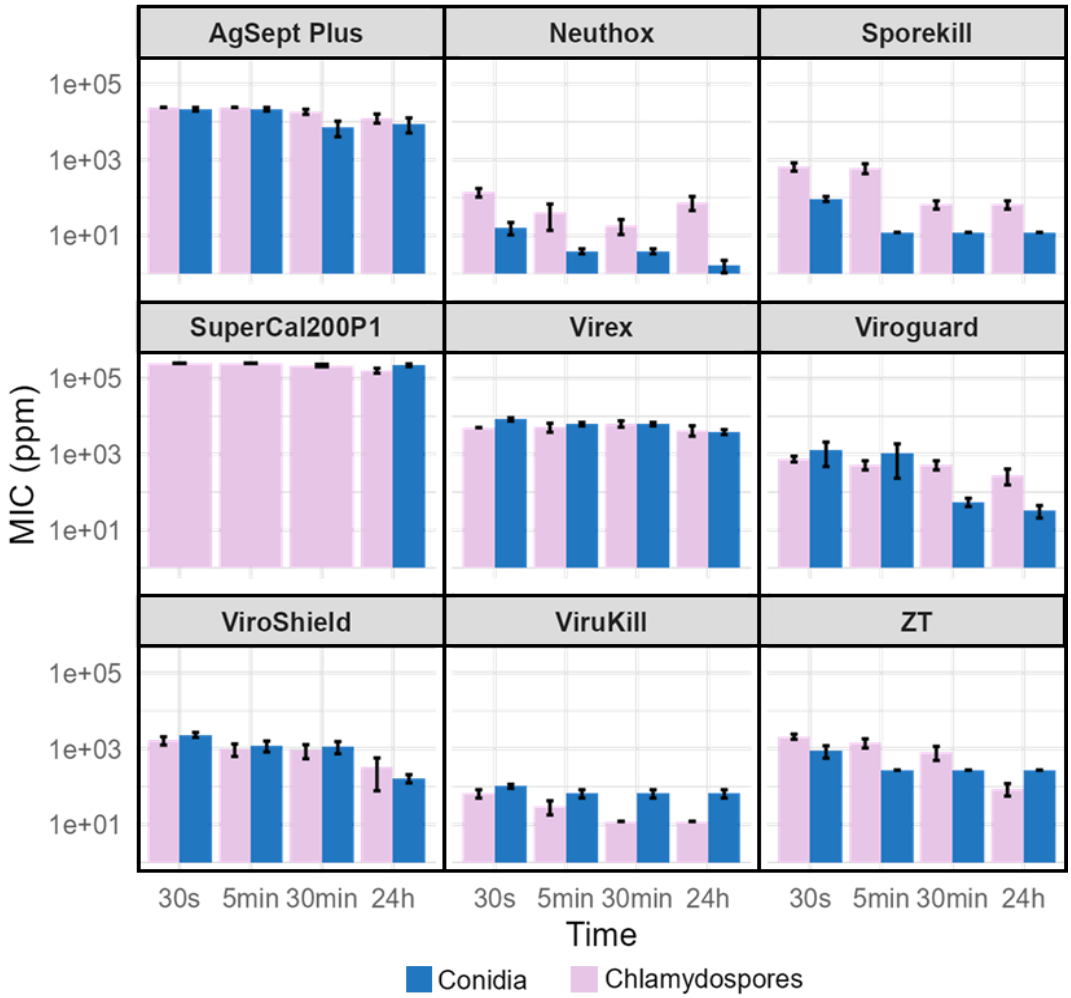

Figure 3. Average MIC of different disinfectants against chlamydospores and conidia. Bars with different lowercase letters within each group indicate significant differences among treatments according to the rank-based Kruskal–Wallis test (α = 0.05).

Previous studies have assessed several DDAC-containing formulations; however, their reported efficacy has been variable across products and experimental conditions. For example, Farmcleanse® applied to *F. oxysporum* f. sp. *vasinfectum* (*Fov*), the causal agent of *Fusarium* wilt in cotton, achieved complete inhibition of conidial germination when used at the recommended 10% rate (Moore & O'Neill, 2000). In another study, comparing Farmcleanse®, Sporekill®, and Domestos® showed that the highest conidial germination inhibition was achieved by Sporekill® preventing germination of microconidia after the minimum exposure time of 30 s. (Meldrum et al., 2013).

The quaternary ammonium-based product Sporekill®, has been generally considered as a fast-acting disinfectant at low concentrations and a highly effective compound for the sterilization of *Foc*-contaminated equipment, vehicles, and shoes in the field, showing efficacy against *Foc* conidia at an exposure time of only 30 s at 1200 ppm. (Nel et al., 2007).

Besides QACs, the effectiveness of other disinfectants has been investigated, including those based on glutaraldehyde, oxidizing agents, and alkaline compounds (Ordoñez González et al., 2021) Previous studies have documented the sporicidal activity of glutaraldehyde against different *formae speciales* of *Fusarium oxysporum*, including *F. oxysporum* f. sp. *narcissi* and *F. oxysporum* f. sp. *cubense*. In these studies, concentrations of 0.25% (Linfield, 1991) and 500–2,000 ppm (Izquierdo-García et al., 2021) were effective in eliminating spores, which is consistent with the results obtained in the present study.

The biocidal action of glutaraldehyde targets intracellular proteins and nucleic acids. Its mode of action is based on the alkylation of key functional groups such as hydroxyl, amino, sulfhydryl, and carboxyl groups, interfering particularly with protein synthesis in microorganisms including viruses, fungi,

bacteria, and mycobacteria. This confers antifungal and antibacterial activity (Brenes et al., 2011), as well as potent virucidal capacity (Figueroa et al., 2017).

ViroKill®, a commercial disinfectant whose active ingredient is didecyldimethylammonium chloride (DDAC) (a fourth-generation quaternary ammonium compound; QAC), showed higher efficacy against chlamydospores than against conidia at 120 ppm, particularly under longer exposure times. Previous studies have evaluated DDAC-based commercial formulations and validated their effectiveness, showing superior performance compared with other QAC generations when tested against *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (*Foc* TR4) at concentrations ≥1,200 ppm (Izquierdo-García et al., 2021). This highlights the need to conduct trials with a wider range of commercially available QACs disinfectants in Ecuador to strengthen biosafety protocols.

In this study, quaternary ammonium compounds (QACs) and aldehydes were the most effective disinfectant groups overall. However, Neuthox®—an oxidizing agent—showed also a strong inhibitory effect on propagule germination, particularly on conidia, achieving high efficacy at 270 ppm when applied for an extended exposure period (24 h). Previous studies evaluating hypochlorite-based disinfectants have also reported strong activity against fungal pathogens, with effective concentrations of 50–200 ppm HOCl and short contact times (1–5 min), resulting in >99% reduction of *Fusarium oxysporum* conidia and chlamydospores in water (Copes & Ojiambo, 2021; Guerra-Fuentes et al., 2019; Ordoñez González et al., 2021).

Previous studies reported that Neuthox® inhibited conidial germination (1–5 min) at <270 ppm, whereas effective control at lower concentrations required longer exposure. In contrast, chlamydospores required higher concentrations (>270 ppm) and longer contact times for inhibition (Ordoñez et. al., 2021). The effectiveness of this disinfectant largely due to its ability to induce oxidative damage in fungal cellular structures, including the cell wall and spores, through the oxidation of key cellular targets such as proteins, membrane lipids, and nucleic acids (Copes & Ojiambo, 2021).

In the present work, chlamydospores exhibit greater structural resistance to chemical treatment, highlighting the need for more aggressive or complementary strategies for their elimination (Zhang et al., 2024). Disinfectants are widely used as emulsifying and dispersing agents, which facilitates their combination with more active substances, such as glutaraldehyde-based compounds, thereby enhancing the disinfectant efficacy of QACs (Christen et al., 2017; Pedreira et al., 2024; Visconti et al., 2021). Consequently, aldehyde and quaternary ammonium-based products are the most suitable candidates to be incorporated into biosecurity protocols for the management of Fusarium wilt of banana (Dita et al., 2018; García-Bastidas et al., 2020; Pegg et al., 2019). Nonetheless, their biocidal activity may decrease on *Foc* propagules due to the presence of organic matter (OM) (Gélinas & Goulet, 1983; Nguyen et al., 2019).

3.2 Experiment with disinfectants in the presence of soil

3.2.1 Effect of organic matter on the efficacy of disinfectants

The disinfectant efficacy of nine commercial products against *Fusarium oxysporum* f. sp. *cubense* (*Foc*) race 1 propagules was evaluated in the presence of organic matter (0.05 and 0.1 g mL^{-1}) using the previously determined Minimum Inhibitory Concentrations (MICs) for each formulation. Despite being tested at their respective MIC levels, ranging from 120 ppm to 250,000 ppm, marked differences were observed in their tolerance to organic load.

Products containing oxidizing compounds such as Neuthox® (270 ppm, hypochlorous acid) and AgSept Plus® (24,000 ppm, stabilized silver/peroxide complex) retained activity in the presence of organic matter, showing minimal reduction in log-kill values (Figure 4). However, it is important to note that the total active ingredient concentration in these formulations, especially for AgSept Plus®, was substantially higher than that of some quaternary ammonium-based products, which achieved comparable or superior reductions at much lower MICs.

Indeed, disinfectants based on quaternary ammonium compounds, such as ViruKill® (120 ppm), Viroguard® (1,000 ppm), and Sporekill® (1,200 ppm), displayed strong biocidal performance and maintained moderate stability in the presence of organic matter. Considering their significantly lower MIC values, these products demonstrated greater efficacy per unit of active ingredient, indicating a more efficient chemical mode of action against *Foc* propagules. Among them, Sporekill® and ViruKill® exhibited only partial inhibition at 0.1 g/mL organic load, confirming a measurable but not complete neutralization of activity. *In vitro* studies have shown that Sporekill® inhibited microconidial germination after 30 seconds of exposure (Meldrum et al., 2013; Nel et al., 2007). According to Stewart-Wade, (2011),

many disinfectants lose effectiveness in the presence of organic matter, especially those acting through oxidation, as organic compounds can react with the active agent before it targets the microorganisms, suggesting that their use should be carefully evaluated in real field situations with high organic loads. Disinfectants that are unstable in the presence of organic matter should be applied only on previously cleaned surfaces, as their activity is drastically reduced by proteins, lipids, or cellular debris (Rutala & Weber, 2016). Similarly, calcium-based (SuperCal200P1®) and complex surfactant formulations (ZT®) experienced severe efficacy loss when exposed to organic matter, with log-reductions dropping below −2 and −3, respectively. This indicates that their active compounds are either rapidly bound or neutralized by organic molecules, rendering them less suitable for field conditions where soil and organic debris are prevalent. This is consistent with previous studies reporting that its disinfectant effect can be rapidly neutralized by organic compounds present in the environment, thereby compromising its effectiveness (Copes & Ojiambo, 2023; Maillard, 2002, 2018; Nasiłowska et al., 2024). ViroShield® (3,000 ppm) and Virex® (10,000 ppm) maintained intermediate performance, with reductions near −0.5 log, reflecting moderate tolerance to organic interference.

Overall, while oxidizing disinfectants (e.g., Neuthox®, AgSept®) were chemically stable under organic load, quaternary ammonium formulations demonstrated the best balance between potency and resilience, achieving high antifungal efficacy at far lower MIC concentrations. This suggests that cationic surfactants remain among the most efficient options for biosecurity applications against *Fusarium oxysporum* f. sp. *cubense* (*Foc*) in banana production systems, particularly when the goal is to maximize antimicrobial activity while minimizing chemical input. Consistent with the findings of Dita et al. (2018) and Pegg et al. (2019), disinfectants formulated with stable active ingredients, such as next-generation quaternary ammonium compounds and glutaraldehyde, can retain their efficacy even under organic matter interference, especially when applied to porous surfaces or those contaminated with plant residues. Collectively, these results underscore the importance of preventive strategies, including disinfection units and footbaths, as effective measures to reduce the risk of *Foc* TR4 introduction into banana plantations (Salacinas et al., 2022).

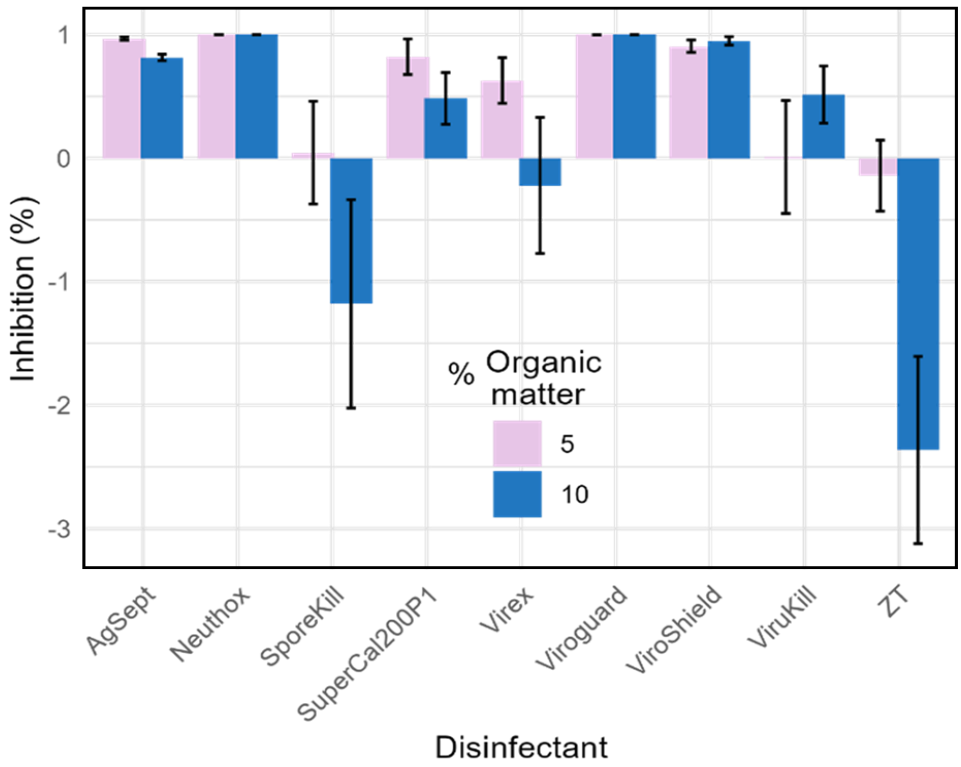

Figure 4. Efficacy of disinfectants against *Foc* chlamydospores in the presence of organic matter, significant differences among treatments according to Spearman's rank correlation (*rho)* (α = 0,05).

3.2.2 Efficacy of disinfectants under different OM and time conditions

The results revealed significant differences among treatments according to product exposure time (30 seconds, 24 and 48 hours), organic matter (OM) content (5% and 10%), and disinfectant chemical group (aldehyde, QACs, and oxidants, excluding the least efficient alkaline and caustic formulations from previous experiments) (Figure 5).

In the case of aldehyde disinfectants, inhibition values remained positive or only slightly reduced as the OM content increased, showing relatively stable performance over time. These findings agree with previous studies reporting that glutaraldehyde-based formulations retain partial activity even in the presence of organic loads during *Fusarium oxysporum* assays. However, this apparent stability does not imply full resistance; efficacy can still be influenced by substrate composition and environmental parameters such as pH, temperature, or oxidative stress (Izquierdo-García et al., 2021; Nguyen et al., 2019).

For the quaternary ammonium compounds (QACs), a marked decline in inhibitory activity was observed under higher OM conditions, with inhibition values dropping below zero, particularly after 48 hours of exposure. This behavior suggests that the active cationic compounds were progressively neutralized or adsorbed by the organic matrix, leading to loss of efficacy and, in some cases, potential fungal regrowth. These results are consistent with recent findings by Arango-Palacio et al. (2024), who reported that QACs were fully effective against *F. oxysporum* f. sp. *cubense* in the absence of soil but showed substantial loss of activity in the presence of organic matter. Notably, only one QAC formulation (QAC4) maintained complete control under such conditions, confirming that the chemical composition and surfactant blend critically determine the persistence of QAC activity (Arango-Palacio et. al., 2024). Nguyen (2019) showed that differences in OM content could explain differences in disinfectant efficacy, especially among QACs, which also agrees with the results presented in this study. It has also been reported that OM can affect the effectiveness of commercial glutaraldehyde-based disinfectants in suspensions with low or no organic matter content (Chandler-Bostock & Mellits, 2015; Izquierdo-García et al., 2021). The mechanisms explaining the negative effect of organic matter on biocidal activity are that organic matter may act as a physical barrier protecting microorganisms, and that organic matter interacts with the disinfectant molecule due to its cationic charge, affecting the disinfectant's availability to the microorganism (Iñiguez-Moreno et al., 2018).

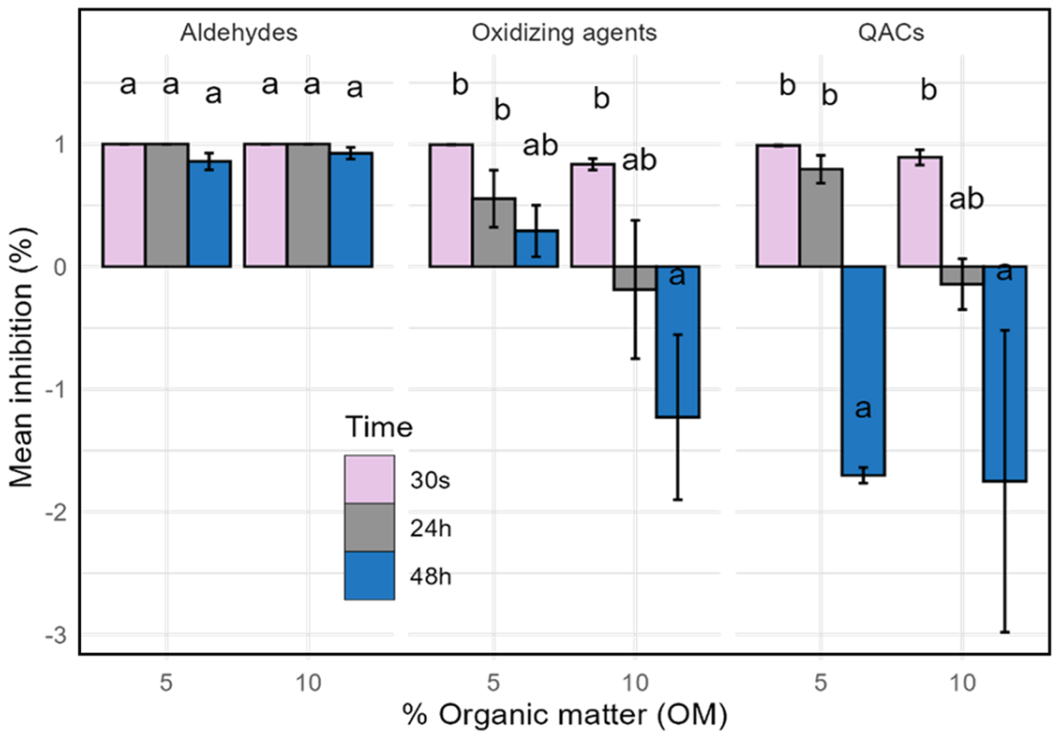

Figure 5. Inhibition (%) of *Foc* (chlamydospores or conidia) as a function of disinfectant type, organic matter percentage, and exposure time. Bars with different lowercase letters within each group indicate significant differences among treatments according to the Kruskal–Wallis rank-based test (α = 0,05).

Within the oxidant group (mainly hypochlorous acid and related oxidizing agents), efficacy was drastically reduced at 10% OM, indicating a strong neutralization effect caused by organic compounds. Longer exposure times did not compensate for this decline, suggesting that oxidative inhibition was irreversibly compromised. Similar patterns have been documented in hypochlorite and hypochlorous acid disinfectants, where organic load rapidly consumes the oxidant, reducing redox potential and thereby diminishing antimicrobial power (Jo et al., 2018).

Moreover, products formulated with sodium hypochlorite, QACs, or citrus-based oils (limonene) have shown notable antimicrobial activity in aqueous suspension; however, their efficacy decreases substantially in soil, where they fail to suppress *Fusarium* survival structures such as chlamydospores (Bennett et al., 2011). Continued development of novel QAC-based mixtures and blends has yielded diverse commercial products with variable antifungal performance, raising concerns about their reliability for controlling phytopathogenic fungi, particularly *F. oxysporum* (Copes & Ojiambo, 2023).

Given that most disinfectants were not fully effective against *Foc* propagules in soil-containing conditions, on-farm decontamination should incorporate complementary biosecurity practices, such as complete soil removal from tools and equipment. Therefore, thorough washing to remove dirt from vehicles, tools, and boots, among other items, is essential before disinfection (Izquierdo-García et al., 2021). Moreover, the use of higher concentrations of QACs, aldehydes, or oxidizing agents requires consideration of additional criteria, including product cost, contact time, type of surface/application (e.g., boot or tool soaking), corrosiveness, persistence, and environmental impact (Izquierdo-García et al., 2021).

4. Conclusions

The *in vitro* evaluation of commercial disinfectants available in Ecuador demonstrated that chlamydospores of *Foc* exhibit greater resistance than conidia, requiring higher concentrations for inhibition. Formulations based on aldehydes (glutaraldehyde) and quaternary ammonium compounds achieved the lowest MICs and maintained high efficacy across different exposure times and even in the presence of organic matter. These findings support the prioritization of stable, high-efficacy formulations in biosecurity protocols, with optimized concentration and contact time to ensure the elimination of highly persistent structures such as chlamydospores, particularly in agricultural contexts where the pathogen remains prevalent. Similarly, continuous monitoring of disinfectant solutions is required to accurately assess their efficacy, as organic matter contamination occurs frequently under field conditions. The rate of efficacy loss is further influenced by the frequency of worker passage in each plantation, which accelerates the accumulation of organic debris and consequently diminishes disinfectant activity. The findings of this study provide robust experimental evidence for the strategic use of locally available disinfectants, particularly those formulated with quaternary ammonium compounds (QACs) and glutaraldehyde, as effective tools to reduce the risk of *Foc* dissemination. Although evaluated against race 1 isolates, the demonstrated efficacy highlights the broader relevance of these disinfectants in preparedness and response strategies for other races of the pathogen, including the highly destructive Tropical Race 4 (TR4). Incorporating these products into routine farm biosecurity protocols will not only reduce pathogen transmission through human and equipment movement but also contribute to strengthening the overall resilience of banana production systems against current and emerging phytosanitary threats.

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Conflicts of Interest

There are no conflicts of interest.

Author Contributions

L. Monserrate-Maggi: Methodology, Formal analysis, Investigation, Writing – original draft. **A. Quevedo:** Methodology, Investigation, Writing – review & editing. **L. Serrano:** Methodology, Investigation, Writing – review & editing, Formal analysis. **M. Vera-Morales:** Formal analysis, Investigation, Writing – original draft. **M. Villavicencio:** Methodology. **M. Marcial:** Methodology. **J. Yánez:** Methodology. **F. Magdama:** Resources, Funding acquisition, Writing – review & editing.

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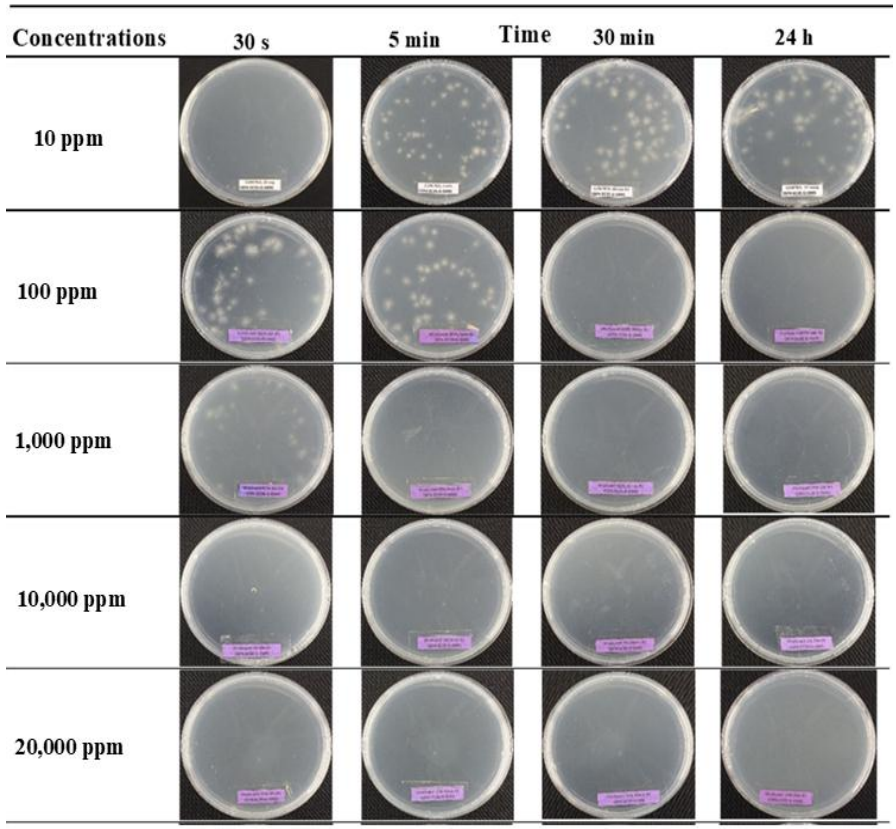
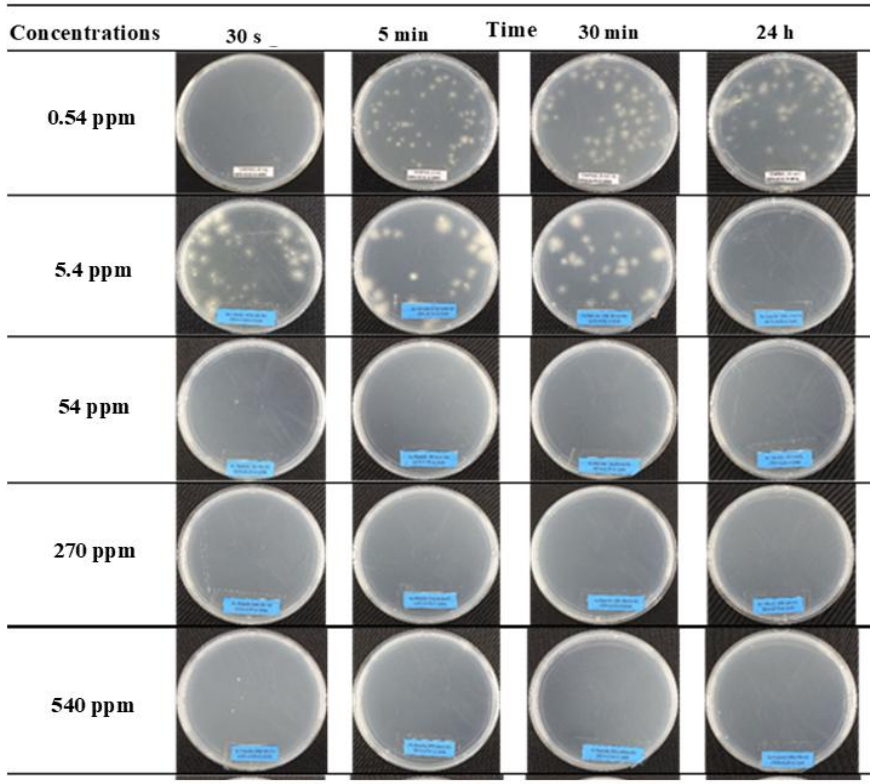
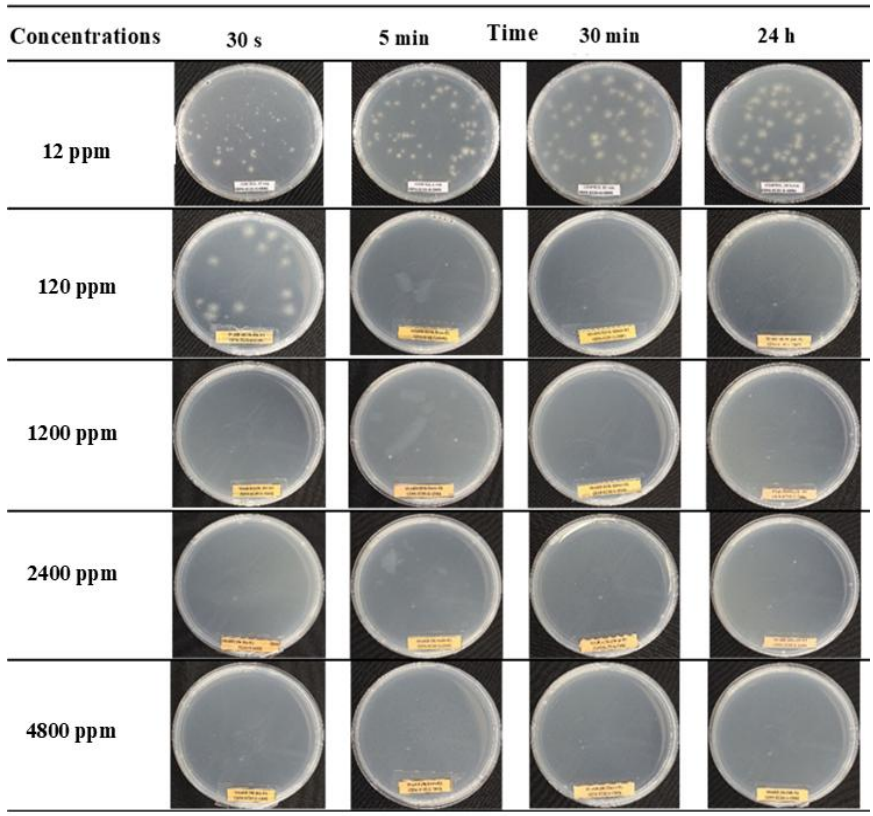
Appendix 1



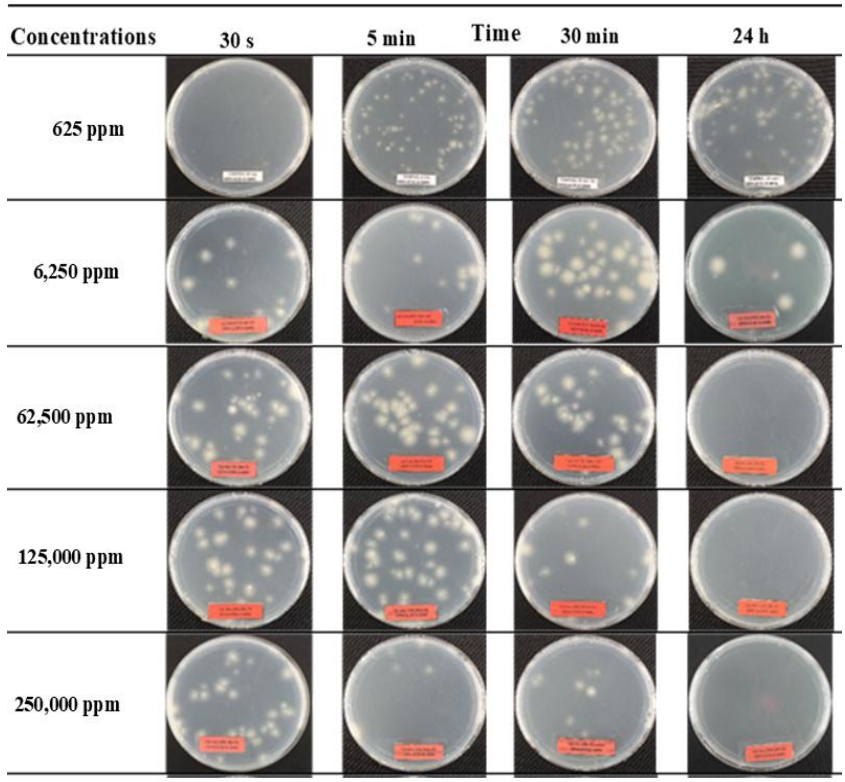
Viroguard® (Aldehydes)



Neuthox® (Oxidizing Agent)



ViruKill® (QAC)



SuperCal 200 P1® (Alkaline / Caustic)