

Disinfectant Efficacy Testing

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Executive summary

In 2012, VLS undertook testing of various sanitisers on fruit contact and non-fruit contact surfaces including wooden bin, plastic bin and metal and rubber etc to provide the industry with a measure of efficacy against Psa-V. This harvest season, 4 more products including two bio-gro certified products were tested to provide more sanitiser options effective against Psa-V to growers.

The testing regime was similar to last year as outlined below on wood, plastic, mild steel and rubber surfaces and exposure times of 10 seconds, 30 seconds, 1 and 2 minutes.

- 1) Agar susceptibility test
- 2) Minimum inhibitory concentration in saline
- 3) Effect of pH on efficacy of products
- 4) Effect of organic matter on efficacy of products
- 5) Spraying and dipping efficacies.

Table 1 below summarises key results listing the products, their working concentrations and the minimum time required for kill efficacy for each material for both spray and dip applications.

| Summary | | | Sensitive to | | Spray efficacy | | | | Dip efficacy | | | |
|-----------------|------|-----|--------------|----|----------------|--------|-------|---------|--------------|--------|-------|---------|
| Product tested | Conc | pH | pH | OM | Wood | Rubber | Metal | Plastic | Wood | Rubber | Metal | Plastic |
| Harvestcide-gel | 0.1% | 5.5 | NS | NS | 10sec | 10sec | 10sec | 10sec | 10sec | 10sec | 10sec | 10sec |
| Nuron-Biosafe | 0.1% | 7.2 | NS | NS | 10sec | 10sec | 10sec | 10sec | 10sec | 10sec | 10sec | 30sec |
| Citrox 14T | 1% | 3.9 | S | NS | 1 min | 1min | 30sec | 1 min | 10sec | 1 min | 30sec | 1 min |
| Biowash | 1% | 8.8 | NS | S | 1 min | NE | NE | 2 min | 2 min | NE | 1 min | 2 min |

Abbrevs: NE= Not Effective; NS= Not sensitive; S= Sensitive

Table 1: Summary of results

Aim

The purpose of this trial was to determine the efficacy of products used for sanitisation of surfaces.

Background

Previous work has shown the efficacy of sanitising products differs depending on the surfaces to which they are applied. To represent each of these common surfaces, small discs were created from untreated undressed timber, plastic (as used in some bins), rubber (from tyres) and mild steel.

This report presents the evaluation of 4 potential disinfectants tested both in-vitro and in spray and dip applications on all 4 surfaces after each surface had been *spiked* with Psa V. This trial includes a number of different testing approaches. Initial tests were undertaken in solution (0.85 % saline) to determine appropriate concentrations for efficacy. The sensitivity of the product to acidity (pH) was then assessed and we finally attempted to assess whether the efficacy of the disinfectant was affected by the presence of organic matter.

Table 1 below shows the list of products tested and the main active ingredients.

| Products | Active ingredients |
|-----------------|----------------------------------|
| Harvestcide-gel | Bromo-chloro- dimethyl-hydantoin |
| Nuron-Biosafe | Sodium hypochlorite |
| Citrox 14T | Citrus extract |
| Biowash | Chloride dioxide |

Table 2: List of products and active ingredient

Methodology

1. Agar Susceptibility test

This preliminary screening test measures the effect of the product against a target bacteria grown in culture. A standard petri dish containing Mueller-Hinton CLSI agar was used. A 12 mm core of agar was excised from the surface of the plate, and a loopful of aliquots at the application rate of the product were introduced into the excised holes and allowed to diffuse for 10 minutes. An inoculum of Psa was prepared in peptone water to a concentration of 0.5 McFarland Standard and spread evenly over the surface of the plate.

The inoculated plate was incubated for 48 hours and examined for signs of antimicrobial activity (Zone of inhibition).

2. Minimum Inhibitory Concentration (MIC) in 0.85 % normal saline

A Psa-V suspension was made in 0.85% normal saline and quantified in cfu/mL. This assay measures the activity of the chemical agent against Psa-V without the interference of the broth ingredients. The tubes were incubated for 48 hours at $25 \pm 2^\circ\text{C}$ post inoculation after which the tubes were sub-cultured onto Kings B media, incubated for a further 48 hours and the growth of Psa V was characterised. Based on the results obtained in the dilution test, a working concentration of each product was determined for use in the efficacy trial on surfaces.

3. pH sensitivity

The product was tested at progressively higher pH levels by slowly adding phosphate buffer until the desired levels were reached. The tubes were then incubated for 48 hours at $25 \pm 2^\circ\text{C}$. At the end of incubation, the tubes were sub-cultured onto Kings B plates and incubated for 48 hours and growth of Psa V was characterised. The aim of the test was to show the correlation of pH sensitivity to products' efficacy.

4. Organic matter sensitivity

In order to test the sensitivity of each product to a quantifiable level of organic matter 0.1% tannic acid was used to create the working concentrations of each product (rather than water) in solution with concentrations of 1, 5, 10 and 20ppm organic matter. The mixture was then tested as per the routine procedure against Psa V: tubes were incubated for 48 hours at $25 \pm 2^\circ\text{C}$ and then sub-cultured onto Kings B plates and incubated for 48 hours and growth of Psa V characterised.

5. Spray and dip applications

The product was made up to its working concentration prior to spray and dip applications. For each product and material, the prepared spiked discs (1×10^8 cfu/mL) were either sprayed or soaked for 10sec, 30sec, 1 minute and 2 minutes in the test products. Following this test the discs were carefully swabbed with a sterile pre-moistened swab. The swab was then immersed into 1 mL 0.85 % saline and plated onto both 1 x media H and 1 x Kings B media. The procedure was repeated for all spray and dip treatments and for each material type. These plates were incubated at $25^\circ\text{C} \pm 2^\circ\text{C}$ for 2 days before checking for growth of Psa V. A set of discs of each material was spiked without any treatment and swabbed and plated as a positive control.

Results

1. Agar susceptibility test

Harvescide gel and Citrox14T showed zone of inhibition on agar susceptibility test as per Figure 1. Biowash showed no inhibition on agar susceptibility test. This may be due to poor diffusion of the product through the agar. Refer to Figure 2. Nuron-Biosafe had been tested in agar susceptibility prior to this trial and was not repeated in this study.

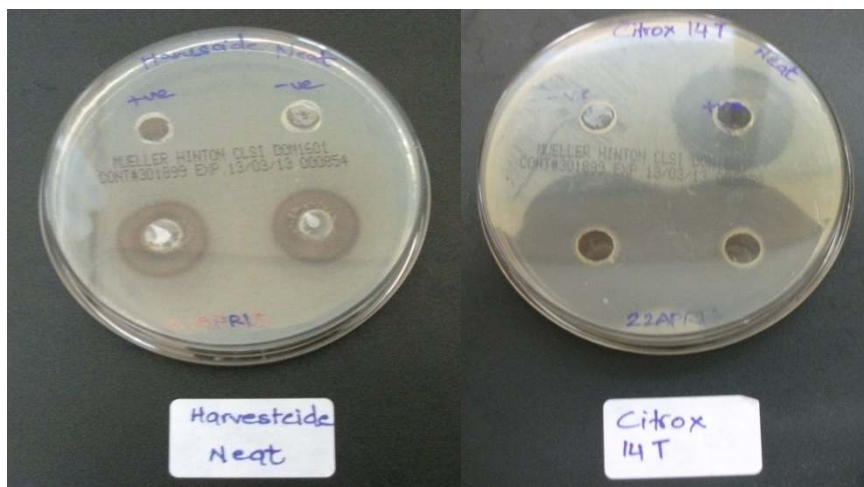


Figure 1: Agar susceptibility test for harvescide gel and citrox 14T -Zone of diffusion



Figure 2: Agar susceptibility test biowash –no zone of inhibition

2. Minimum Inhibitory Concentration (MIC) in 0.85 % normal saline

The minimum inhibitory concentration of each product tested from 0.01 % to 10% dilution. A range of concentrations where both growth and no-growth of Psa-V was obtained and a working concentration for each product derived from the MIC was used for subsequent testing as per Table 1 below.

| Product under test | Quantification in cfu/mL | 0.01% | 0.05% | 0.1% | 1% | 2% | 5% | 10% | Working conc |
|--------------------|--------------------------|-------|-------|------|----|----|----|-----|--------------|
| Harvescide gel | 1 x 10 ⁷ | G | NG | NG | NG | NG | NG | NG | 0.1% |
| Nuron -Biocide | 1 x 10 ⁷ | G | NG | NG | NG | NG | NG | NG | 0.1% |
| Citrox14T | 1 x 10 ⁷ | G | G | NG | NG | NG | NG | NG | 1% |
| Biowash | 1 x 10 ⁷ | G | G | G | NG | NG | NG | NG | 1% |

Table 3: MIC in 0.85 % saline suspension

Key: **G – Growth** **NG- No growth**

3. Effect of pH on efficacy of products

We attempted to adjust the pH of the product solution to achieve 3 different pH levels from the range 4, 5, 6, 7, 8 depending on the pH of the product diluted in Psa-V suspension. The pH of the Psa solution is neutral. A fourth undisturbed treatment was left as a control. Measured pH levels are recorded since small volumes and different buffering meant that final pH levels varied. The product was tested at its working concentration determined from the earlier MIC tests.

*Actual pH refers to the unadjusted solution pH

**Adjusted pH is what was achieved by adding small amounts of HCl or phosphate buffer (to increase pH)

| Harvescide gel (0.1 %) | Actual pH | Adjusted pH | Growth |
|------------------------|-----------|-------------|--------|
| 1 | 5.5 | N/A | NG |
| 2 | 5.5 | 3.6 | NG |
| 3 | 5.5 | 6.2 | NG |
| 4 | 5.5 | 7.1 | NG |

Table 4(a): Natural pH is acidic. No pH sensitivity.

| Nuron- Biosafe (0.1 %) | Actual pH | Adjusted pH | Growth |
|------------------------|-----------|-------------|--------|
| 1 | 7.2 | N/A | NG |
| 2 | 7.2 | 6.1 | NG |
| 3 | 7.2 | 5.2 | NG |
| 4 | 7.2 | 3.1 | NG |

Table 4(b): Natural pH is almost neutral. No pH sensitivity.

| Citrox14T (1 %) | Actual pH | Adjusted pH | Growth |
|-----------------|-----------|-------------|--------|
| 1 | 3.9 | N/A | NG |
| 2 | 3.9 | 6.2 | G |
| 3 | 3.9 | 5.2 | G |
| 4 | 3.9 | 7 | G |

Table 4(c): Natural pH is highly acidic. No efficacy was seen when pH was raised.

| Biowash (1 %) | Actual pH | Adjusted pH | Growth |
|---------------|-----------|-------------|--------|
| 1 | 8.8 | N/A | NG |
| 2 | 8.8 | 3.0 | NG |
| 3 | 8.8 | 6.3 | NG |
| 4 | 8.8 | 7.1 | NG |

Table 4(d): Natural pH is basic, no pH sensitivity

4. Effect of organic matter on efficacy of products

This trial is an attempt to indicate any intrinsic sensitivity of the product to the presence of organic matter using a simple, but repeatable, test. Each product was tested at its working concentration determined from the earlier MIC tests. We expected that any product which reacted in a purely chemical way with organic matter might simply react with the entire active ingredient leaving none to react with Psa-V. The results obtained for the 4 products tested are as per Table 3 below.

| Product | Product concentration | Concentration of organic matter | | | |
|----------------|-----------------------|---------------------------------|--------|-------|-------|
| | | 20 ppm | 10 ppm | 5 ppm | 1 ppm |
| Harvescide gel | 0.1% | NG | NG | NG | NG |
| Nuron -Biosafe | 0.1% | NG | NG | NG | NG |
| Citrox14T | 1% | NG | NG | NG | NG |
| Biowash | 1% | G | G | G | G |

Table 5: Effect of organic matter on efficacy of product.

Key: **G – Growth** **NG- No growth**

5. Spraying and Dipping Efficacy

A Table of results is presented for each product at its working concentration showing efficacy on each surface for each application method and for each media type and exposure time.

| Harvescide gel | 0.1% concentration | Media -H (Psa selective media) | | | | Kings B media | | | |
|----------------|--------------------|--------------------------------|-------|------|------|---------------|-------|-------|-------|
| Surface | Application method | 10sec | 30sec | 1min | 2min | 10sec | 30sec | 1 min | 2 min |
| Wood | Spray | NG | NG | NG | NG | NG | NG | NG | NG |
| | Dip | NG | NG | NG | NG | NG | NG | NG | NG |
| Rubber | Spray | NG | NG | NG | NG | NG | NG | NG | NG |
| | Dip | NG | NG | NG | NG | NG | NG | NG | NG |
| Metal | Spray | NG | NG | NG | NG | NG | NG | NG | NG |
| | Dip | NG | NG | NG | NG | NG | NG | NG | NG |
| Plastic | Spray | NG | NG | NG | NG | NG | NG | NG | NG |
| | Dip | NG | NG | NG | NG | NG | NG | NG | NG |

Table 6a: Harvescide gel

Key: **G – Growth** **NG- No growth**

Harvescide gel showed efficacy on all surfaces tested at 10 seconds and longer contact time in both spray and dip application.

| Nuron-Biosafe | 0.1 % concentration | Media -H (Psa selective media) | | | | Kings B media | | | |
|---------------|---------------------|--------------------------------|-------|------|------|---------------|-------|-------|-------|
| Surface | Application method | 10sec | 30sec | 1min | 2min | 10sec | 30sec | 1 min | 2 min |
| Wood | Spray | NG | NG | NG | NG | NG | NG | NG | NG |
| | Dip | NG | NG | NG | NG | NG | NG | NG | NG |
| Rubber | Spray | NG | NG | NG | NG | NG | NG | NG | NG |
| | Dip | NG | NG | NG | NG | NG | NG | NG | NG |
| Metal | Spray | NG | NG | NG | NG | NG | NG | NG | NG |
| | Dip | NG | NG | NG | NG | NG | NG | NG | NG |
| Plastic | Spray | NG | NG | NG | NG | NG | NG | NG | NG |
| | Dip | NG | NG | NG | NG | G | NG | NG | NG |

Table 6b: Nuron-Biosafe

Key: **G – Growth** **NG- No growth**

Nuron-biosafe showed efficacy on all surfaces at 10 seconds and above except for dip application on plastic where longer exposure time of at least 30 seconds was required for absolute kill.

| Citrox 14T | 1 % concentration | Media -H (Psa selective media) | | | | Kings B media | | | |
|------------|--------------------|--------------------------------|-------|------|------|---------------|-------|-------|-------|
| Surface | Application method | 10sec | 30sec | 1min | 2min | 10sec | 30sec | 1 min | 2 min |
| Wood | Spray | G | G | NG | NG | G | G | NG | NG |
| | Dip | NG | NG | NG | NG | NG | NG | NG | NG |
| Rubber | Spray | G | G | NG | NG | G | G | NG | NG |
| | Dip | G | G | NG | NG | G | G | NG | NG |
| Metal | Spray | G | NG | NG | NG | G | NG | NG | NG |
| | Dip | G | NG | NG | NG | G | NG | NG | NG |
| Plastic | Spray | G | G | NG | NG | G | G | NG | NG |
| | Dip | G | G | NG | NG | G | G | NG | NG |

Table 6c: Citrox14T

Key: **G – Growth** **NG- No growth**

Citrox14T showed efficacy on most surfaces at 1 minute exposure time in both spray and dip applications except for wood (dip application) and metal (spray and dip applications) where absolute kill rate was observed at 10 seconds and 30 seconds respectively.

| Biowash | 1 % concentration | Media -H (Psa selective media) | | | | Kings B media | | | |
|---------|--------------------|--------------------------------|-------|------|------|---------------|-------|-------|-------|
| Surface | Application method | 10sec | 30sec | 1min | 2min | 10sec | 30sec | 1 min | 2 min |
| Wood | Spray | G | G | NG | NG | G | G | NG | NG |
| | Dip | G | G | G | NG | G | G | G | NG |
| Rubber | Spray | G | G | G | G | G | G | G | G |
| | Dip | G | G | G | G | G | G | G | G |
| Metal | Spray | G | G | G | G | G | G | G | G |
| | Dip | G | G | NG | NG | G | G | NG | NG |
| Plastic | Spray | G | G | G | NG | G | G | G | NG |
| | Dip | G | G | G | NG | G | G | G | NG |

Table 6d: Biowash

Key: **G – Growth** **NG- No growth**

Biowash showed efficacy in wood, plastic and metal (dip application) at longer exposure times of 1 minute and 2 minutes. No efficacy was seen on rubber and metal (spray application).

Summary

A brief summary of the characteristics and results for each product tested is shown in Table 5. Harvescide gel and Nuron- Biosafe showed efficacy at the lower exposure time of 10- 30 seconds on all surfaces in both applications.

| Summary | | | Sensitive to | | Spray efficacy | | | | Dip efficacy | | | |
|----------------|------|-----|--------------|----|----------------|--------|-------|---------|--------------|--------|-------|---------|
| Product tested | Conc | pH | pH | OM | Wood | Rubber | Metal | Plastic | Wood | Rubber | Metal | Plastic |
| Harvescide-gel | 0.1% | 5.5 | NS | NS | 10sec | 10sec | 10sec | 10sec | 10sec | 10sec | 10sec | 10sec |
| Nuron-Biosafe | 0.1% | 7.2 | NS | NS | 10sec | 10sec | 10sec | 10sec | 10sec | 10sec | 10sec | 30sec |
| Citrox 14T | 1% | 3.9 | S | NS | 1 min | 1min | 30sec | 1 min | 10sec | 1 min | 30sec | 1 min |
| Biowash | 1% | 8.8 | NS | S | 1 min | NE | NE | 2 min | 2 min | NE | 1 min | 2 min |

Abbrevs: NE= Not Effective; NS= Not sensitive; S= Sensitive

Table 7: Summary of results.

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