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Efficacy study on Envirosan, Trifilm, Citrox PWT and Trigene used as disinfecting products on field tools (Secateur).

Aim: The purpose of this experiment was to determine the efficacy of Envirosan, Trifilm, Citrox PWT and Trigene within a short time exposure as disinfectant products for sanitising field tools, packhouse bins and other machinery. This was also done to investigate the efficacy using the soak v/s spray method.

Background: **Citrox PWT** is currently used for disinfecting tools during pruning. This trial was conducted to prove efficacy of **Citrox PWT** and find alternative more effective disinfecting products for sanitising field tools such as secateurs, loppers, handsaws and bins in packhouses which might have been contaminated with a high inoculum of *Pseudomonas syringae* pv *actinidiae* (Psa). To be able to mimic the field conditions when pruning heavily infected vines, secateurs were heavily spiked with a high inoculum of Psa. The Psa broth culture was quantified in cfu/mL before application.

Envirosan, Trifilm and Trigene were tested prior on agar diffusion and broth dilution tests. These products showed efficacy at 1% in broth dilution test but showed poor diffusion on agar susceptibility test. As per label claim, these products are recommended to be used on surfaces for at least 10 minutes exposure time which is not very practical in a field tool sanitisation context.

Citrox PWT was tested at neat and 1 % on agar diffusion test and showed poor inhibition against Psa on plate.

Material and Equipments: 13 clean secateurs, 12 dirty secateurs (from the field), ICMP 18800 (Psa-V strain), Blood agar plates (BAP), Psa-V media, Kings B media, 9 mL and 100 mL Tryptic soy broth (TSB), Spray bottle, sterile distilled water, Envirosan, Trifilm, Trigene and Citrox PWT, spreaders, sterile swabs, disposable tips, Stop watch, PCR and 0.85 % saline.

Methods:

1) A loopful of the ICMP culture 18800 Psa-V was inoculated in 100 mL TSB broth and allowed to incubate for 48 hrs at 25°C ± 2°C.

2) After 48 hrs, the TSB broth showed growth. This was quantified using a serial dilution method using Kings B plates and PCR testing.

3) The TSB broth was sprayed on the secateurs both clean and dirty (from the field) confined within a clean plastic bag. The secateurs were allowed to incubate overnight at 25°C ± 2°C.

4) After 24 hrs, the clean spiked secateurs were then subjected to Envirosan (E), Trifilm (TF), Trigene (TG) and Citrox PWT treatments made up to 1 % concentration (20 mL made up to 2L). This is outlined below:
    - Soak for 30 secs
    - Soak for 2 mins
    - Soak for 5 mins
    - Spray for 30 secs
    - Spray for 2 mins
    - Spray for 5 mins

5) The dirty secateurs were also subjected to Envirosan (E), Trigene (TG) and Citrox PWT treatments made up to 1 % concentration (20 mL made up to 2L). This is outlined below:
    - Soak for 30 secs
    - Soak for 2 mins
    - Spray for 30 secs
    - Spray for 2 mins

6) The secateurs were swabbed carefully and thoroughly on the cutting stainless steel surfaces.

7) The swab was then immersed into 1 mL 0.85 % saline and plated on 1 x Psa-V media, 1 x Kings B media, 1 x BAP media. The swab was inoculated aseptically into TSB broth.

8) The procedure was repeated for all the treatments outlined above soak v/s spray.

9) The plates and broth were incubated at 25°C ± 2°C for 3 days to check for turbidity and growth of Psa-V colonies.

10) A spiked secateur without any treatment was also swabbed and plated as a positive control.

11) One plate of each media and one broth un-inoculated were incubated as a negative control at 25°C ± 2°C for 3 days to check for sterility of media.

RESULTS:

Table 1 shows the results for quantification of ICMP culture 18800. The number of colonies of Psa in 100 mL TSB broth used for spiking the secateurs was 10^7cfu/mL. This is considered to be a high inoculum spiking.

Table 1: Quantification of ICMP 18800 for spiking secateurs

No	Samples	Dilution	Cq value	Direct Broth PCR	Cq value	No of colonies Kings B 1	No of colonies kings B 2	Average no of colonies CFU/mL
1	Q1	Neat	14.74	Q1a	13.95	TNC	TNC	TNC
2	Q2	10^{-1}	19.41	Q2a	16.85	TNC	TNC	TNC
3	Q3	10^{-2}	25.06	Q3a	24.32	TNC	TNC	TNC
4	Q4	10^{-3}	33.48	Q4a	25.08	TNC	TNC	TNC
5	Q5	10^{-4}	N/A	Q5a	27.97	396	353	375
6	Q6	10^{-5}	N/A	Q6a	35.20	55	54	55
7	Q7	10^{-6}	N/A	Q7a	34.13	5	1	3
8	Q8	10^{-7}	N/A	Q8a	N/A	3	0	1.5
9	Q9	10^{-8}	N/A	Q9a	N/A	0	0	0

Figure 1 & 2 show growth in the 3 media and broths. The interpretation of results was based on the presence or absence of Psa-like colonies through morphological identification and using the selective agar Psa-V media as golden standard. For broth, interpretation of growth was based on presence or absence of turbidity. The efficacy for each treatment for Envirosan, Trifilm, Trigene and Citrox PWT has been tabulated below. The positive control swabbed on secateur with no disinfectant treatment had growth on both plates and broth and negative control had no growth.

Figure 1: Absence v/s presence of Psa-V in TSB broth

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Figure 2: Growth on Psa-V, Kings B and BAP media

Table 1: Results for efficacy of Envirosan on secateurs

Secateurs	Treatments	Psa-V media	Kings B	BAP	TSB
Spiked clean	Soak for 30 secs	NG	NG	NG	NT
	Soak for 2 mins	NG	NG	NG	NT
	Soak for 5 mins	NG	NG	NG	NT
	Spray for 30 secs	NG	1 colony	NG	NT
	Spray for 2 mins	NG	NG	NG	NT
	Spray for 5 mins	NG	NG	NG	NT
Spike Dirty	Soak for 30 secs	NG	NG	1 x fungi	NT
	Soak for 2 mins	NG	NG	1 x fungi	NT
	Spray for 30 secs	NG	NG	1 x fungi	NT
	Spray for 2 mins	NG	NG	1 x fungi	NT

Key NG: No growth G: Growth T: Turbid NT: No turbidity

Table 2: Results for efficacy of Trifilm on secateurs

Secateurs	Treatments	Psa-V media	Kings B	BAP	TSB
Spiked clean	Soak for 30 secs	G	G	G	T
	Soak for 2 mins	NG	NG	NG	NT
	Soak for 5 mins	NG	NG	NG	NT
	Spray for 30 secs	G	G	G	T
	Spray for 2 mins	NG	NG	NG	T
	Spray for 5 mins	NG	NG	G	T

Key NG: No growth G: Growth T: Turbid NT: No Turbidity

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Table 3: Results for efficacy of Trigene on secateurs

Secateurs	Treatments	Psa-V media	Kings B	BAP	TSB
Spiked clean	Soak for 30 secs	NG	NG	NG	NT
	Soak for 2 mins	NG	NG	NG	NT
	Soak for 5 mins	NG	NG	NG	NT
	Spray for 30 secs	G	G	G	T
	Spray for 2 mins	G	G	G	T
	Spray for 5 mins	NG	NG	1 colony	NT
Spiked Dirty	Soak for 30 secs	NG	G	G	T
	Soak for 2 mins	NG	NG	NG	NT
	Spray for 30 secs	G	G	G	T
	Spray for 2 mins	G	G	G	NT

Key NG: No growth G: Growth T: Turbid NT: No turbidity

Table 1: Results for efficacy of Citrox PWT on secateurs

Secateurs	Treatments	Psa-V media	Kings B	BAP	TSB
Spiked clean	Soak for 30 secs	NG	1 colony	NG	NT
	Soak for 2 mins	NG	NG	NG	NT
	Soak for 5 mins	NG	NG	NG	NT
	Spray for 30 secs	G	G	G	T
	Spray for 2 mins	G	G	G	T
	Spray for 5 mins	NG	1 colony	NG	NT
Spiked Dirty	Soak for 30 secs	G	G	G	T
	Soak for 2 mins	G	G	G	T
	Spray for 30 secs	G	G	G	T
	Spray for 2 mins	G	G	G	T

Key NG: No growth G: Growth T: Turbid NT: No turbidity

CONCLUSION:

- **Envirosan** showed efficacy at 1 % concentration both on clean and dirty spiked secateurs.
- **Trifilm, Trigene and Citrox PWT** showed poor or no efficacy at 1 % concentration on dirty spiked secateurs.

RECOMMENDATION:

Envirosan seems a better product to be used as an alternative disinfectant as the minimum contact time for efficacy was proven to be **30 secs** both on clean and dirty spiked secateurs. Soaking seems a better option as the whole surface area is fully covered as compared to spraying.

Citrox PWT did not show efficacy at 1 % concentration on dirty secateurs and <u>is not recommended</u> to be used for field tool sanitisation.

Disclaimer

This test result reflects the findings for the sample received and tested by VLS. Every effort has been made to collect and test a representative sample however, as only a sample of the population has been tested, the results may not be conclusive in respect to the population collected. If you have collected and provided the sample yourself, there may be inaccuracies in the result based on the method of taking the sample and its storage before being provided to VLS. Seeka Kiwifruit Industries Limited shall have no liability to the addressee of this test result, or to any third party, for the test services it provides, including in respect of any negligent acts.

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