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EFFICACY OF FADO1 AGAINST TOMATO MOSAIC VIRUS, *FUSARIUM OXYSPORUM* F.SP. LYCOPERSICI AND *ERWINIA CHRYSANTHEMI* IN DEMINERALIZED WATER

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CONFIDENTIAL

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CONTENTS

SUMMARY

At the Research Station for Floriculture and Glasshouse Vegetables in Naaldwijk research has been performed in 1997 on the efficacy of FAD01 against plant pathogens in demineralized water.

Three experiments were performed with tomato mosaic virus, two experiments with the fungus *Fusarium oxysporum* f. sp. *lycopersici* and one experiment with the bacterium *Erwinia chrysanthemi.* These plant pathogens were applied to demineralized water, after which the product FAD01 was added in several concentrations.
Exposure times were realized by adding the neutralizer sodium sulphite after fixed periods.
The efficacy of FAD01 against tomato mosaic virus was established in a bio assay with tobacco plants. These plants react with local lesions after virus infection. The efficacy of *Fusarium* and *Erwinia* was established on specific culture media. These pathogens produce colonies on the agar plates which can be counted after some days.

The efficacy of FAD01 against tomato mosaic virus is irregular. The reason may be that the protein coat, which protects the infectious RNA, sometimes is insufficiently destructed by the product, leaving the RNA unimpaired.

Fusarium oxysporum f.sp. *lycopersici* is eliminated at a concentration of 0,4% FAD01 during 15 minutes. At higher concentrations a 5 minutes' exposure time was effective.

Erwinia chrysanthemi was eliminated at a concentration of 0,04% FAD01 during 30 minutes. At higher concentrations a 5 minutes' exposure time also resulted in a 100% effect.

The neutralizer greatly affected the results of FAD01 against tobacco mosaic virus. The neutralizer had no effect on *Fusarium.*

1. INTRODUCTION

Research is performed by order of ELF ATOCHEM AGRI B.V. at Vondelingenplaat/Rt with a disinfectant FAD01, a product on the basis of hydrogen peroxide. This product is tested for its efficacy against some of the most important plant pathogens in horticulture; a viral, a fungal and a bacterial pathogen.

Generally speaking products on the basis of hydrogen peroxide are environmentally friendly. Provided that they are effective, they may be an alternative for pesticides which can be harmfull to the environment.

Initially experiments are performed with the plant pathogens applied to demineralized water. When promising results are achieved the next step will be to test the efficacy of the product against plant pathogens in organic material like roots and leaves.

This report presents the results with FAD01 against plant pathogens in demineralized water.

2. MATERIALS AND METHODS

2.1 EXPERIMENTS WITH TOMATO MOSAIC VIRUS

Experiments are performed with tomato mosaic virus (strain SPS), further on indicated as ToMV. Per treatment 1 ml of purified virus was added to 1 litre of demineralized water.

In all experiments the product FAD01 was tested for efficacy against ToMV.
The first experiment was performed on 02-04, the second on 10-06 and the third experiment on 07-07-1997. The temperature of the water was in the first experiment 23-25 °C, in the second experiment 23 °C and in the third experiment 22 °C.
In the first and second experiment exposure times were realized by using the neutralizer sodium sulphite. Because of the negative side effects (temperature rise of suspension, phytotoxicity) the neutralizer was not used anymore in the third experiment. Exposure times were then realized by dilution of the suspension. In all three experiments exposure times were 5 and 30 minutes.

The infectivity of the suspensions after the treatments was established in a bioassay. Water samples of each treatment were rubbed on carborundum-dusted leaves of 4-6 weeks old *Nicotiana tabacum* 'Xanthi' plants. These tobacco plants react with local lesions on ToMV. Three leaves were inoculated on each of three plants per treatment. Four to seven days after inoculation the number of local lesions, produced on tobacco, was counted. By comparison with the untreated control treatment the efficacy could be established.
In the first experiment there was no damage to the plants caused by the neutralizer. In the second experiment however 28 grams of neutralizer or more caused burned tobacco leaves, soon after inoculation. During inoculation of these treatments gloves were used to protect the skin against the neutralizer.

At the treatment with 10% product, 900 ml virus suspension was mixed with 100 ml product. In this case the actual virus concentration was 90% in comparison with the control treatment.
By using 50% of the product, 500 ml virus suspension mixed with 500 ml product, the actual virus concentration was reduced to 50% compared with the control treatment. In the efficacy data, mentioned in the tables, this dilution factor is included.

2.2 EXPERIMENTS WITH *FUSARIUM OXYSPORUM* F.SP. *LYCOPERSICI* (FYSIO 1)

This fungal pathogen is further on reported as Fol.
A conidial suspension of Fol was obtained by culturing the fungus one week in an aerated liquid Czapek-Dox medium at room temperature. Per liter demineralized water 1.10^8 conidia were added, resulting in a spore concentration of 1.10^5 spores per ml water. The experiment was performed on 03-02-1997 with FAD01 and the neutralizer sodium sulphite. The water temperature was 22-24 °C.
To establish the effect of the neutralizer sodium sulphite, an additional experiment was performed on 11-02-1997. The neutralizer (0,88 gram) was applied 15 minutes after the application of FAD01 (0,4%).

The efficacy of the treatments was established by plating out 0,5 ml per treatment on each of four agar plates of the *Fusarium*-selective Komada's medium. The plates were incubated at room temperature for about a week, after which the *Fusarium* colonies were counted.

2.3 EXPERIMENT WITH *ERWINIA CHRYSANTHEMI*

This bacterial pathogen was cultured in a liquid medium (Nutrient Broth) at approximately 25 °C for two days. In daytime the flasks with *Erwinia* were placed on an erlenmeyer shaker to prevent clustering. During the night the cultures were placed in an incubator.
The experiment was performed on 29-10-1997. In this experiment sterile demineralized water was used. The water temperature was 20-22 °C.
Per treatment 10 ml bacterial suspension was added, resulting in a final concentration of approximately 6.10^7 bacteria per ml demineralized water. No neutralizer was used in this experiment.
After FAD01 application the pH dropped from 6.0 (0% FAD01) to 4.1 (0.04%), 3.9 (0.1%) and 3.3 (0.4%).
After certain exposure times to several concentrations of FAD01 the bacterial suspensions were diluted with tenfold steps up to 10^9. From each dilution 0,1 ml was pipetted to a nutrient agar plate, on which the suspension was spread over by a Drigalski spatel. Two plates were used for each dilution.
The plates were incubated at 25-26 °C. After five days the colonies on the plates were counted.The most reliable result is achieved when 30 to 300 colonies per plate are counted. Therefore those results are used for drawing conclusions.
At the same time in this experiment the PBG tested a new technique (Spiral Plating Technique) which was compared with the traditional method. The results of this test is also mentioned in this rapport.

3. RESULTS AND DISCUSSION

3.1 EXPERIMENTS WITH TOMATO MOSAIC VIRUS

The pH and neutralizer data of experiment 1 are listed in appendix 1. The results of experiment 1 are mentioned in table 1. From this table it can be concluded that the neutralizer has an effect on the infectivity of ToMV as long as no FAD01 is applied. However when the neutralizer is used after the application of FAD01 the effect of the combination on the infectivity of the virus is less than the effect of the neutralizer alone. Since in tests with human viruses much higher concentrations were examined, a second experiment was set up with higher doses of FAD01.
The pH and neutralizer data of experiment 2 are listed in appendix 2. Table 2 presents the results of this experiment. The results are very irregular: 1% and 50% FAD01 is effective whereas 10% product is only partially effective (1:1 dilution) or not at all (1:10 dilution). Obviously the amount of neutralizer affects the efficacy of FAD01. Because of these results a third experiment was set up to establish the actual effect of FAD01 on ToMV without any influence of the neutralizer. In the third experiment the exposure times were realized by dilution of the suspensions. The pH data of experiment 3 is listed in appendix 3. The results of this experiment are mentioned in table 3. In this experiment there is an effect of FAD01 on ToMV, although the differences between the concentrations sometimes are marginal. Overall the effect of FAD01 against ToMV is very irregular. The reason may be that the virus consists of RNA surrounded by a protein coat. Only the RNA is responsible for the infectivity of the virus which means the RNA must be eliminated by the product. It is very likely that in these experiments the protein coat was not always sufficiently destructed so that sometimes the RNA stayed unimpaired.

Table 1 - Efficacy of FAD01 against tomato mosaic virus (ToMV) ; experiment 1

Treatment	Dilution	Number of local lesions				Efficacy (%)
		plant 1	plant 2	plant 3	total	
0% + ToMV	1:10	869	242	342	1453	0
0% + ToMV + Na_2SO_3	1:10	45	50	11	106	93
0,1% - 5 min + Na_2SO_3	1:10	148	251	307	706	51
0,1% - 30 min + Na_2SO_3	1:10	176	245	99	520	64
0,4% - 5 min + Na_2SO_3	1:10	292	683	469	1444	1
0,4% - 30 min + Na_2SO_3	1:10	209	533	977	1719	negative
1,6% - 5 min + Na_2SO_3	1:10	703	947	843	2493	negative
1,6% - 30 min + Na_2SO_3	1:10	482	791	282	1555	negative
0% + ToMV	1:1	ca 2300	ca 2500	ca 3000	ca 7800	0
0% + ToMV + Na_2SO_3	1:1	1003	604	281	1888	76
0,1% - 5 min + Na_2SO_3	1:1	1630	1485	1110	4225	46
0,1% - 30 min + Na_2SO_3	1:1	1613	777	1411	3801	51
0,4% - 5 min + Na_2SO_3	1:1	1820	ca 2100	ca 2000	ca 5920	ca 24
0,4% - 30 min + Na_2SO_3	1:1	1252	1592	1486	4330	44
1,6% - 5 min + Na_2SO_3	1:1	ca 1300	1565	1116	ca 3981	ca 49
1,6% - 30 min + Na_2SO_3	1:1	643	689	ca 1870	ca 3202	59

Table 2 - Efficacy of FAD01 against tomato mosaic virus (ToMV) ; experiment 2

Treatment	Dilution	Number of local lesions				Efficacy (%)
		plant 1	plant 2	plant 3	total	
0% + ToMV	1:10	121	175	294	590	0
0% + ToMV + Na_2SO_3*	1:10	0	7	0	7	99
1% - 5 min . no Na_2SO_3	1:100	0	0	0	0	100
1% - 30 min no Na_2SO_3	1:100	0	0	0	0	100
1% - 5 min no Na_2SO_3	1:10	0	0	0	0	100
1% - 30 min no Na_2SO_3	1:10	0	0	0	0	100
1% - 5 min + Na_2SO_3	1:10	0	0	0	0	100
1% - 30 min + Na_2SO_3	1:10	3	3	3	9	99
10% - 5 min + Na_2SO_3	1:10	559	818	390	1767	negative
10% - 30 min + Na_2SO_3	1:10	259	178	161	598	negative
50% - 5 min + Na_2SO_3	1:10	0	0	0	0	100
50% - 30 min + Na_2SO_3*	1:10	0	0	0	0	100
0% + ToMV	1:1	ca 1800	ca 1800	ca 1900	ca 5500	0
0% + ToMV + Na_2SO_3*	1:1	86	92	62	240	96
1% - 5 min + Na_2SO_3	1:1	0	0	0	0	100
1% - 30 min + Na_2SO_3	1:1	0	0	0	0	100
10% - 5 min + Na_2SO_3	1:1	629	752	504	1885	62**
10% - 30 min + Na_2SO_3*	1:1	72	86	177	335	93**
50% - 5 min + Na_2SO_3	1:1	0	0	0	0	100
50% - 30 min + Na_2SO_3*	1:1	0	0	0	0	100

* = leaf burning by neutralizer
** = dilution factor included

Table 3 - Efficacy of FAD01 against tomato mosaic virus (ToMV) ; experiment 3 (no Na_2SO_3 added)

Treatment	Dilution	Number of local lesions				Efficacy (%)
		plant 1	plant 2	plant 3	total	
0% - 5 min	1:100	124	166	131	421	0
0,1% - 5 min	1:100	71	48	94	213	49
1% - 5 min	1:100	162	143	109	414	2
10% - 5 min	1:100	64	57	57	178	58*
0% - 5 min	1:10	1278	1446	1249	3973	0
0,1% - 5 min	1:10	227	237	526	990	75
1% - 5 min	1:10	154	317	269	740	81
10% - 5 min	1:10	76	67	50	193	95*
0% - 30 min	1:100	56	158	246	460	0
0,1% - 30 min	1:100	37	38	39	114	75
1% - 30 min	1:100	89	41	41	171	63
10% - 30 min	1:100	40	51	11	102	75*
0% - 30 min	1:10	885	742	601	2228	0
0,1% - 30 min	1:10	341	471	284	1096	51
1% - 30 min	1:10	116	135	118	369	83
10% - 30 min	1:10	137	67	112	316	84*

* = dilution factor included

3.2 EXPERIMENTS WITH *FUSARIUM OXYSPORUM* F.SP. *LYCOPERSICI*

The pH and neutralizer data of the first experiment, with FAD01 and neutralizer, are listed in appendix 4. The results of this experiment are mentioned in table 4 whereas table 5 presents the results of the second experiment, where the effect of the neutralizer was tested. Table 4 shows that the tested concentrations FAD01 are very effective against *Fusarium*; apart from the treatment 0,4% FAD01 during 5 minutes all treatments are 100% effective. In the second experiment, listed in table 5, the efficacy is somewhat lower (98,5-98,9) in comparison with the first experiment (100%). On the other hand the control treatment (with neutralizer) was more viable in the second experiment (82650 viable spores) than in the first experiment, without neutralizer (19360 viable spores).
The neutralizer sodium sulphite has no killing effect on the *Fusarium*-spores.
The neutralizer has really stopped the activity of FAD01 after 15 minutes, which can be concluded from the efficacy results; without neutralizer the effect is 99,9% and with neutralizer 98,5-98,9%. Obviously the product is not completely inactivated within 15 minutes. For that reason an exposure time of about 30 minutes is preferable to realize an optimal effect.

Table 4 - Efficacy of FAD01 against *Fusarium oxysporum* f.sp. *lycopersici* (Fol)

Treatment	Dilution	Number of *Fusarium* colonies per 0.5 ml						Fol per ml undiluted	Efficacy (%)
		plate 1	plate 2	plate 3	plate 4	plate 5	average		
0% + Fol + Na_2SO_3	1:100	91	114	88	98	93	96,8	19360	0
0,4% 5 min + Na_2SO_3	1:1	3	1	0	0	0	0,8	1,6	99,992
0,4% 15 min + Na_2SO_3	1:1	0	0	0	0	0	0	0	100
1,6% 5 min + Na_2SO_3	1:1	0	0	0	0	0	0	0	100
1,6% 15 min + Na_2SO_3	1:1	0	0	0	0	0	0	0	100
6,4% 5 min + Na_2SO_3	1:1	0	0	0	0	0	0	0	100
6,4% 15 min + Na_2SO_3	1:1	0	0	0	0	0	0	0	100

Table 5 - Effect of neutralizer* on *Fusarium oxysporum* f.sp. *lycopersici* (Fol)

Treatment with FAD01	Dilution	Number of *Fusarium* colonies per 0,5 ml					Fol per ml undiluted	Efficacy (%)
		plate 1	plate 2	plate 3	plate 4	average		
0% + Fol	1:100	284	294	267	281	281,5	56300	0
0% + Fol	1:1000	38	33	37	36	36	72000	0
0% + Fol + Na_2SO_3	1:100	426	411	384	432	413,25	82650	0
0% + Fol + Na_2SO_3	1:1000	55	49	59	57	55	110000	0
0,4%	1:1	24	21	8	24	19,25	38,5	99,9**
0,4%	1:10	8	1	3	3	3,75	75	99,9**
0,4% + Na_2SO_3	1:1	443	446	417	493	449,75	899,5	98,9***
0,4% + Na_2SO_3	1:10	56	64	69	58	61,75	1235	98,5***

* = the neutralizer Na_2SO_3 is applied after 15 min
** = efficacy in comparison with 0% FAD01 and 1:100 dilution
*** = efficacy in comparison with 0% FAD01 + Na_2SO_3 and 1:100 dilution

3.3 EXPERIMENTS WITH *ERWINIA CHRYSANTHEMI*

The results of the experiment with *Erwinia chrysanthemi* are listed in table 6 (0% FAD01), table 7 (0,04%), table 8 (0,1%) and table 9 (0,4%).
From these tables it can be concluded that all tested concentrations FAD01, apart from 0,04% during 5 minutes, are 100% effective against the bacteria. An exposure time of 30 minutes to 0,04% FAD01 was also 100% effective.
Comparable results were achieved with the spiral plater system. *Erwinia chrysanthemi* was not eliminated completely by 0,04% FAD01 when the exposure time was 5 minutes; 17000 spores per ml survived the treatment which is 48% in comparison with the control treatment. However exposure to 0,04% FAD01 for 30 minutes proved to be 100% effective, just as all other treatments.

Table 6 - Efficacy of FAD01 (0%) against *Erwinia chrysanthemi*

TREATMENT	NUMBER OF COLONIES			EFFICACY
concentration FAD01 - time - dilution factor 10^n	plate 1 = 0,1 ml	plate 2 = 0,1 ml	per ml	in %
0% - 5 min - 10^0	i.*	i.	i.	0
0% - 5 min - 10^1	approx. 800	approx. 600	approx. 70 000	0
0% - 5 min - 10^2	53	33	43 000^{C5}	0
0% - 5 min - 10^3	8	23	155 000	0
0% - 5 min - 10^4	4	4	400 000	0
0% - 5 min - 10^5	0	1	n.d.**	n.d.
0% - 5 min - 10^6	0	0	n.d.	n.d.
0% - 5 min - 10^7	0	0	n.d.	n.d.
0% - 5 min - 10^8	1	0	n.d.	n.d.
0% - 5 min - 10^9	0	0	n.d.	n.d.
0% - 30 min - 10^0	i.	i.	i.	0
0% - 30 min - 10^1	approx. 500	approx. 1000	approx. 75 000	0
0% - 30 min - 10^2	155	221	188 000^{C30}	0
0% - 30 min - 10^3	not tested			
0% - 30 min - 10^4	2	1	150 000	0
0% - 30 min - 10^5	0	0	n.d.	n.d.
0% - 30 min - 10^6	1	0	n.d.	n.d.
0% - 30 min - 10^7	0	0	n.d.	n.d.
0% - 30 min - 10^8	0	0	n.d.	n.d.
0% - 30 min - 10^9	0	0	n.d.	n.d.

* = innumerable
** = not determined
C5 = this control treatment is used as reference for all concentrations FAD01 with 5 minutes' exposure time
C30 = this control treatment is used as reference for all concentrations FAD01 with 30 minutes' exposure time

Table 7 - Efficacy of FAD01 (0,04%) against *Erwinia chrysanthemi*

TREATMENT	NUMBER OF COLONIES			EFFICACY
concentration FAD01 - time - dilution factor 10^n	plate 1 =0,1 ml	plate 2 = 0,1 ml	per ml	in %
0,04% - 5 min - 10^0	0	0	0	100*
0,04% - 5 min - 10^1	5	10	750	98.3
0,04% - 5 min - 10^2	12	6	9000	79.1
0,04% - 5 min - 10^3	0	0	0	100
0,04% - 5 min - 10^4	0	0	0	100
0,04% - 5 min - 10^5	0	0	0	n.d.**
0,04% - 5 min - 10^6	0	1***	n.d.	n.d.
0,04% - 5 min - 10^7	0	0	0	n.d.
0,04% - 5 min - 10^8	16****	0	n.d.	n.d.
0,04% - 5 min - 10^9	0	0	n.d.	n.d.
0,04% - 30 min - 10^0	0	0	0	100
0,04% - 30 min - 10^1	0	0	0	100
0,04% - 30 min - 10^2	0	0	0	100
0,04% - 30 min - 10^3	0	0	0	100
0,04% - 30 min - 10^4	0	1	n.d.	n.d.
0,04% - 30 min - 10^5	0	0	0	n.d.
0,04% - 30 min - 10^6	1	0	n.d.	n.d.
0,04% - 30 min - 10^7	0	18****	n.d.	n.d.
0,04% - 30 min - 10^8	0	1	n.d.	n.d.
0,04% - 30 min - 10^9	0	0	0	n.d.

* = unreliable result
** = not determined
*** = numbers regarded as pollution
**** = possibly clustering of *Erwinia*

Table 8 - Efficacy of FAD01 (0,1%) against *Erwinia chrysanthemi*

TREATMENT	NUMBER OF COLONIES			EFFICACY
concentration FAD01 - time - dilution factor 10^n	plate 1 =0,1 ml	plate 2 =0,1 ml	per ml	in %
0,1% - 5 min - 10^0	0	0	0	100
0,1% - 5 min - 10^1	0	0	0	100
0,1% - 5 min - 10^2	0	2*	n.d.**	n.d.
0,1% - 5 min - 10^3	1	1	n.d.	n.d.
0,1% - 5min - 10^4	0	0	0	n.d.
0,1% - 5 min - 10^5	1	1	n.d.	n.d.
0,1% - 5 min - 10^6	0	1	n.d.	n.d.
0,1% - 5 min - 10^7	0	1	n.d.	n.d.
0,1% - 5 min - 10^8	1	0	n.d.	n.d.
0,1% - 5 min - 10^9	0	0	0	n.d.
0,1% - 30 min - 10^0	0	0	0	100
0,1% - 30 min - 10^1	0	1	n.d.	n.d.
0,1% - 30 min - 10^2	0	0	0	100
0,1% - 30 min - 10^3	0	0	0	100
0,1% - 30 min - 10^4	0	0	0	100
0,1% - 30 min - 10^5	0	0	0	n.d.
0,1% - 30 min - 10^6	1	0	n.d.	n.d.
0,1% - 30 min - 10^7	0	0	0	n.d.
0,1% - 30 min - 10^8	0	1	n.d.	n.d.
0,1% - 30 min - 10^9	0	0	0	n.d.

* = numbers regarded as pollution
** = not determined

Table 9 - Efficacy of FAD01 (0,4%) against *Erwinia chrysanthemi*

TREATMENT	NUMBER OF COLONIES			EFFICACY
concentration FAD01 - time - dilution factor 10^n	plate 1 = 0,1 ml	plate 2 = 0,1 ml	per ml	in %
0,4% - 5 min - 10^0	0	0	0	100
0,4% - 5 min - 10^1	0	1*	n.d.**	n.d.
0,4% - 5 min - 10^2	1	0	n.d.	n.d.
0,4% - 5 min - 10^3	0	1	n.d.	n.d.
0,4% - 5 min - 10^4	0	0	0	100
0,4% - 5 min - 10^5	0	1	n.d.	n.d.
0,4% - 5 min - 10^6	0	0	0	n.d.
0,4% - 5 min - 10^7	0	0	0	n.d.
0,4% - 5 min - 10^8	2	0	n.d.	n.d.
0,4% - 5 min - 10^9	1	0	n.d.	n.d.
0,4% - 30 min - 10^0	0	0	0	100
0,4% - 30 min - 10^1	0	1	n.d.	n.d.
0,4% - 30 min - 10^2	0	0	0	100
0,4% - 30 min - 10^3	0	0	0	100
0,4% - 30 min - 10^4	1	0	n.d.	n.d.
0,4% - 30 min - 10^5	0	0	0	n.d.
0,4% - 30 min - 10^6	0	0	0	n.d.
0,4% - 30 min - 10^7	0	1	n.d.	n.d.
0,4% - 30 min - 10^8	0	0	0	n.d.
0,4% - 30 min - 10^9	0	0	0	n.d.

* = numbers regarded as pollution
** = not determined

4. CONCLUSION

In the virus tests initially the efficacy of FAD01 against tomato mosaic virus was very irregular. The neutralizer sodium sulphite was kept responsible for these results. In a latter test without neutralizer however the results stayed irregular. The reason may be that at the concentrations tested (highest 10% FAD01) the protein coat of the virus is not sufficiently destructed by which the infectious RNA stayed unimpaired.

The fungus *Fusarium oxysporum* f.sp. *lycopersici* could be completely eliminated with 0,4% FAD01 in combination with an exposure time of 15 minutes. At higher concentrations (1,6% and 6,4%) a 5 minutes' exposure time was also 100% effective. The neutralizer had no effect on the efficacy of FAD01 against the fungal spores.

All bacteria of *Erwinia chrysanthemi* were killed at a concentration of 0,04% FAD01 during 30 minutes. After 5 minutes at 0,04% FAD01 some bacteria survived. At higher concentrations (0,1% and 0,4%) a 5 minutes'exposure time also resulted in a 100% effect.

APPENDIX 1. pH and neutralizer data first virus experiment

Treatment FAD01	pH demi-water + virus	pH after application FAD01	pH after application Na_2SO_3	grammes Na_2SO_3
0%	4.8	-	9.7	4,28
0,1%	4.5	3.9	4.2 - 7.8	0,18
0,4%	4.5	3.8	4.0 - 4.1	0,88
1,6%	5.3	3.9 - 4.0	4.1 - 4.2	4,28

APPENDIX 2. pH and neutralizer data second virus experiment

Treatment FAD01	pH demi-water + virus	pH after application FAD01	pH after application Na_2SO_3	grammes Na_2SO_3
0%	6.8	-	9.9	27,96
1%	6.8	3.2	3.7	2,21
10%	6.8	2.6	4.6	27,96
50%	6.8	2.3	3.7	139,8

APPENDIX 3. pH data third virus experiment

Treatment FAD01	pH demi-water + virus	pH after application FAD01
0%	6.4	-
0,1%	6.4	4.2
1%	6.4	4.1
10%	6.4	3.4

APPENDIX 4 pH and neutralizer data first *Fusarium* experiment

Treatment FAD01	pH demi-water + *Fusarium*	pH after application FAD01	pH after application Na_2SO_3	grammes Na_2SO_3
0%	5.6	-	-	0
0,4%	5.6	4.2	4.2	0,88
1,6%	5.6	3.6	3.8	4,28
6,4%	5.6	3.1	3.6	17,89

Ministerie van Landbouw, Natuurbeheer en Visserij

APPENDIX 5 **Certificate Plant Protection Service**

PLANTENZIEKTENKUNDIGE DIENST

This is to declare that, in conformity with the request 957528 of October 25, 1995

PROEFSTATION VOOR BLOEMISTERIJ EN GLASGROENTE

residing Kruisbroekweg 5, Naaldwijk and Linnaeuslaan 2 a, Aalsmeer, the Netherlands

HAS OFFICIALLY BEEN RECOGNIZED AS AN ORGANIZATION FOR EFFICACY TESTING

commencing September 25, 1996.

as has been laid down in the 'Regulation for the Authorization of Pesticides' of March 1, 1995.

This recognition will expire on September 25, 1997.

Wageningen, September 25, 1996

For the Minister of Agriculture,
Nature Management and Fisheries,

Drs.ing. H.A. Duringhof
Director Plant Protection Service