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REPORT

An *in vitro* study to test for possible deterioration of the effectiveness of F10 Germicidal Wound Spray to control *Lucilia cuprina* larvae.

Dr. J.A. Kelly

Senior Researcher, Entomology
Agricultural Research Council
Plant Protection Research Institute
Private Bag X134
Queenswood
0121
Tel: (012) 304-9593
Fax: (012) 325-6998

KellyJ@arc.agric.za

Client: Health and Hygiene
P.O. Box 347
Sunninghill
2157
Tel: (011) 474-1668
Fax: (011) 474-1670

Objective:

This study forms an addition to a previous study done by Prof T.C. van der Linde at the University of the Free State. The objective of that study was "to evaluate the efficacy of two F10 Germicidal formulations with insecticide against *Lucilia cuprina* larvae in an *in vitro* study." This study's objective was to determine if there was any deterioration in the efficiency of F10 Germicidal Wound Spray with Insecticide against *L. cuprina* larvae, after the formula has been stored for prolonged periods of time and at various temperatures.

Study Design, procedures, assessments and analyses:

The study design, procedures, assessments and analyses followed were the same as those done in the previous study conducted at the University of the Free State. This replication was used so that the results of each of these studies could be meaningfully compared.

The parasites used were *Lucilia cuprina* (Diptera: Calliphoridae). These flies are an agricultural pest, causing myiasis on domestic animals.

The experimental units consisted of ten copper tubes (16mm diameter by 23mm high), joined and attached to a plastic base. Ten late second and early third instar *L. cuprina* larvae were released into the tubes and chicken liver pieces were added. The experimental units were maintained at room temperature. After the treatment was applied, the percentages of the dead and incapacitated larvae were recorded after 4, 8 and 24 hours. One experimental unit was used for each treatment, and the experiment was replicated five times. The replications took place over a period of a week.

There were 5 treatments:

- 1) F10-Germicidal Wound Spray with Insecticide. Batch 080917 Initial.
- 2) F10 Germicidal Wound Spray with Insecticide Batch 262 Room Temperature, One Year.
- 3) F10 Germicidal Wound Spray with Insecticide Batch 262 37°C One Year
- 4) F10-Germicidal Wound Spray with Insecticide. Batch 080713. 54°C 2 Weeks
- 5) The control, where no wound spray was applied.

(All F10 Germicidal Wound Spray with Insecticide were supplied by Health and Hygiene (Pty) Ltd. P.O. Box 347, Sunninghill, 2154. Active Ingredients of Quaternary & Biguanide compounds: 0.44%. Cypermethrin: 0.25%, Piperonyl Butoxide: 1.25%.)

The F10 Germicidal Wound Spray was administered by using standard spray canisters. The experimental units were sprayed only once while ensuring there was a good cover of the spray over the whole unit. The treated units were then placed in plastic basins with mesh lids for observations.

Because of the relatively small data set and simplicity of data, it was tested using the student's t-Test: Two-Sample Assuming Equal Variances. This t-test form assumes that the two data sets came from distributions with the same variances. It is referred to as a homoscedastic t-test. This test is used to determine whether the two samples are likely to have similarity or difference.

Results and Discussion:

As soon as the experimental units were treated with F10 Germicidal Wound Spray the *L. cuprina* larvae began to migrate away from the liver. Within half an hour, the larvae became sluggish and their mobility was comprised. This observation was similar to the response seen the initial study done at the university of the Free State.

The results of the 4, 8 and 24 hour observations are given in Table 1.

Table 1: Percentage dead, moribund or live *L. cuprina* larvae after treatments were applied.

Treatment 1: F10-Germicidal Wound Spray with Insecticide. Batch 080917 Initial.						
Replicate	% dead (D) or moribund (M) larvae					
	4 hours		8 hours		24 hours	
	D	M	D	M	D	M
1	87	13	88	12	88	12
2	91	9	91	9	94	8
3	84	16	85	15	88	12
4	94	6	96	4	96	4
5	96	4	100	0	100	0
Average	90.4	9.6	92	8	92.8	7.2
Treatment 2: F10 Germicidal Wound Spray with Insecticide Batch 262 Room Temperature, One Year.						
Replicate	% dead (D) or moribund (M) larvae					
	4 hours		8 hours		24 hours	
	D	M	D	M	D	M
1	92	8	92	8	98	2
2	98	2	100	0	100	0
3	84	16	86	14	88	12
4	80	20	80	20	84	16
5	88	12	92	8	92	8
Average	88.4	11.6	90	10	92.4	7.6
Treatment 3: F10 Germicidal Wound Spray with Insecticide Batch 262 37°C One Year						
Replicate	% dead (D) or moribund (M) larvae					
	4 hours		8 hours		24 hours	
	D	M	D	M	D	M
1	85	16	90	10	94	6

Overall the effectiveness of the F10 Germicidal Wound Spray with Insecticide was 100 % effective across all the treatment as the larval behavior was radically changed. Although a small percentage of the larvae remained alive, they showed such limited movement after 4 hours, they certainly could not actively feed and thus cause any more physical damage to a wound.

Appendix

Table A: Results of t-Test, two-sample assuming equal variances statistical analysis (p-value, one-tailed at 95% confidence interval)

MORTALITY				
4 Hours				
Treatment	2	3	4	5
1	0.46 (p = 0.31)	0.19 (p = 0.9)	-0.22 (p = 0.24)	32.22 (p = 0.00)
2		-0.49 (p = 0.38)	-0.69 (p = 0.46)	25.81 (p = 0.00)
3			-0.24 (p = 0.40)	36.35 (p = 0.00)
4				35.93 (p = 0.00)
8 Hours				
Treatment	2	3	4	5
1	0.52 (p = 0.33)	0.92 (p = 0.5)	0.73 (p = 0.41)	41.01 (p = 0.00)
2		0.32 (p = 0.32)	0.10 (p = 0.26)	24.96 (p = 0.00)
3			-0.26 (p = 0.41)	36.35 (p = 0.00)
4				35.93 (p = 0.00)
24 Hours				
Treatment	2	3	4	5
1	0.21 (p = 0.21)	-0.62 (p = 0.28)	-0.62 (p = 0.28)	33.62 (p = 0.00)
2		-0.74 (p = 0.24)	-0.075 (p = 0.24)	27.20 (p = 0.00)
3			0 (p = 0.5)	35.39 (p = 0.00)
4				35.39 (p = 0.00)
MORIBUND				
4 Hours				
Treatments	2	3	4	5
1	0.31 (p = 0.38)	0.97 (p = 0.18)	0.34 (p = 0.36)	N/A
2		0.73 (p = 0.24)	0.10 (p = 0.46)	
3			-0.52 (p = 0.31)	
8 Hours				
Treatment	2	3	4	5
1	-0.46 (p = 0.33)	0 (p = 0.5)	0.22 (p = 0.41)	N/A
2		0.49 (p = 0.32)	0.69 (p = 0.26)	
3			0.24 (p = 0.41)	
24 Hours				
Treatment	2	3	4	5
1	-0.11 (p = 0.46)	0.74 (p = 0.24)	0.74 (p = 0.24)	N/A
2		0.75 (p = 0.24)	0.75 (p = 0.26)	
3			0 (p = 0.5)	



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SUPPLEMENTARY REPORT

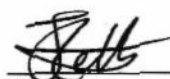
An *in vitro* study to test for possible deterioration of the effectiveness of F10 Germicidal Wound Spray to control *Lucilia cuprina* eggs.

Dr J.A. Kelly

6 December 2006

Senior Researcher, Entomology
Agricultural Research Council
Plant Protection Research Institute
Private Bag X134
Queenswood
0121
Tel: (012) 304-9593
Fax: (012) 325-6998

KellyJ@arc.agric.za


J A Kelly

Client: Health and Hygiene
P.O. Box 347
Sunninghill
2157
Tel: (011) 474-1668
Fax: (011) 474-1670

Objective:

This study supplements previous studies done by Dr J.A. Kelly and Prof. T.C. van der Linde. The objective of those studies was two-fold to evaluate the efficacy of two F10 Germicidal formulations with insecticide against *Lucilia cuprina* larvae in an *in vitro* study, and secondly, to determine if there was any deterioration in the efficiency of F10 Germicidal Wound Spray with Insecticide against *L. cuprina* larvae, after the formula has been stored for prolonged periods of time and at various temperatures. The aim of the present study was to determine if the Germicidal formulations with insecticide had any effect on the eggs of *L. cuprina*.

Study design, procedures, assessments and analyses:

The study design, procedures, assessments and analyses were amended for this study. The parasites used were still *L. cuprina* (Diptera: Calliphoridae). These flies are a veterinary pest, causing myiasis on domestic animals.

Adult *L. cuprina* were raised in a colony, where they were given a constant supply of water and sucrose solution. They were fed on chicken liver to provide a protein source. After 24 hours, the liver was replaced with fresh liver to provide a medium on which the females could lay their eggs. The liver was provided at night and any eggs that were laid during the night were treated early in the morning before they hatched.

This process was repeated 5 times to provide enough eggs for each treatment. The liver and the eggs were treated / sprayed as a unit. No attempt was made to remove the eggs from the liver as this could damage the eggs and provide a false result. After the treatment was applied, the eggs were left for 6 days to ensure that any hatching events were recorded. It was assumed that due to warmer weather experienced at the time, the eggs were not viable after this period. This was based on observations made during the previous experiments during which the eggs hatched within 3 days after they were laid.

There were 5 treatments:

- 1) F10-Germicidal Wound Spray with Insecticide. "Batch 080917 Initial" (21/11/2006).
- 2) F10 Germicidal Wound Spray with Insecticide. "Batch 262 Room Temperature, One Year." (22/11/2006).
- 3) F10 Germicidal Wound Spray with Insecticide. "Batch 262 37°C One Year" (23/11/2006)

4) F10-Germicidal Wound Spray with Insecticide. "Batch 080713. 54°C 2 Weeks"
(26/11/2006)

5) The control, where no wound spray was applied (27/11/2006).

(All samples of the F10 Germicidal Wound Spray with Insecticide were supplied by Health and Hygiene (Pty) Ltd. P.O. Box 347, Sunninghill, 2154. Active Ingredients of Quaternary & Biguanide compounds: 0.44%. Cypermethrin: 0.25%, Piperonyl Butoxide: 1.25%.)

The F10 Germicidal Wound Spray was administered by using standard spray canisters. The experimental units (container with approximately 50 grams of liver and egg batches) were sprayed only once while ensuring there was a good cover of the spray over the whole surface. The treated units were then placed in plastic basins with mesh lids for observation.

Because of the relatively small data set and unquestionable results, the data were not tested statistically.

Results and Discussion

None of the treated eggs hatched in any of the treatments where the F10 Germicidal Wound Spray was applied. The control eggs hatched within 24 hours after being laid. Only 8% of the egg masses were not viable. This percentage was expected as the control unit was the last one given to the *L. cuprina* colony. The females in the colony were at the end of their laying cycle and only a few eggs were laid on the liver given to them the next day to maintain the colony.

These results showed that F10 Germicidal Wound Spray with Insecticide was extremely effective with 100% mortality of the eggs. It should be noted that the experimental egg batches were of average sizes. However, in the field, the egg batches may be larger, which may influence the effectiveness of the Germicidal Wound Spray. These results showed that the F10 Germicidal Wound Spray with Insecticide does not appear to be affected significantly by temperature or time.



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Enquiry Report

Re: Enquiry as to the effectiveness of F10 Germicidal Wound Spray to control species of blow flies other than *L. cuprina*.

The species used during the previous experiments regarding the effectiveness of F10 Germicidal Wound Spray with Insecticide was *Lucilia cuprina* (Diptera, Calliphoridae). This is one of the common widely distributed blow flies in South Africa. The other common species known to occur in South Africa all belong to the Suborder Cyclorrhapha, families Calliphoridae and Sarcophagidae.

Family Calliphoridae

Genus: *Chrysomya* Robineau-Desvoidy, 1830

1) *Chrysomya chloropyga* (Wiedemann 1818)

2) *Chrysomya albiceps* (Wiedemann, 1819)

3) *Chrysomya marginalis* (Wiedemann, 1830)

Genus: *Lucilia* Robineau-Desvoidy, 1830

4) *Lucilia sericata* (Meigen, 1826)

5) *Lucilia cuprina* (Wiedemann, 1830)

Family Sarcophagidae

Genus: *Sarcophaga* Meigen, 1826

6) Various species

The maggots of all the species of this suborder are apodous acephalous in morphology. In other words, they have no legs or appendages. They also have no discernible head capsule, with the mouth parts modified into hooks. Because of their almost identical structure, it is often difficult to tell the maggots of the various species apart. This similarity in morphology and general life-cycles would make it more than probable that these species would be susceptible to the F10 Germicidal Wound Spray with insecticide as in the case of *L. cuprina*.

There is an exception namely *C. albiceps*, that has fleshy protrusions on its body which may provide it with a little more protection from pesticides.

Dr. J.A. Kelly

21 February 2007

Senior Researcher, Entomology
Agricultural Research Council
Plant Protection Research Institute
Private Bag X134
Queenswood
0121
Tel: (012) 304-9593
Fax: (012) 325-6998
KellyJ@arc.agric.za