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Impacts of Deltamethrin Spray on Adults of the Giant Salvinia Biocontrol Agent, *Cyrtobagous salviniae*

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ABSTRACT

Static, short-term bioassays were used to determine the effects of deltamethrin on *Cyrtobagous salviniae*, a biocontrol agent for giant salvinia (*Salvinia molesta* Mitchell). Deltamethrin is a pyrethroid insecticide that kills insects on contact and through digestion. Plastic basins containing weevils and giant salvinia were placed in riverine water and also on the edges of water bodies on the banks where they were exposed to applications of deltamethrin used in the tsetse fly (*Glossina morsitans* Westwood) control program. Reference control basins were placed 30 km outside the sprayed area. Weevil mortalities were determined at fixed periods after aerial application of deltamethrin. Significant mortalities of weevils with a maximum of 47% were evident up to 72 hr after exposure and were related to deltamethrin toxicity. Overall, the toxicity of deltamethrin to weevils was minimal in the field infestations based on data obtained from the field assessments conducted in the post spray periods.

Key words: aerial spraying, insecticide, nagana, salvinia weed, tsetse fly.

INTRODUCTION

Populations of tsetse fly, increased in the Northwest district of Botswana, particularly in the Okavango Delta during 1999 and 2000. It was reported that more than 300 cattle died in 2000 due to nagana, a parasitic disease caused by *Trypanosoma rhodesiense* (gambiense) (tsetse fly is the vector to the parasite) in the delta. The increase in nuisance biting tsetse fly populations affects socio-economic activities in the Okavango Delta. In the years 2001 and 2002, the Tsetse-fly Control Division (TCD) in the Department of Animal Health and Production (DAHP) launched aerial spraying of the insecticide deltamethrin to eradicate tsetse flies in the Okavango Delta. The aerial spray was done in two phases. The first phase in the winter of 2001 was over Seronga in the north and a part of Moremi Game Reserve (MGR), and in the second phase, major parts of the lower ephemeral channels in the MGR were sprayed in winter 2002 (Figure 1). The aerial spraying was accompanied by a comprehensive monitoring program aimed at establishing the impacts of deltamethrin on non-target organisms. The most important beneficial organism monitored was the host-specific biological control agent, *Cyrtobagous salviniae* Calder & Sands, which was introduced in 1986 to control giant salvinia (kariba weed), in the MGR. Forno and Smith (1999) reviewed the progress of salvinia control in the country and described how the physical and biological control methods have been integrated for sustainable long-term management for salvinia weed control. Naidu et al. (2000) demonstrated the establishment of *C. salviniae* in the Moremei Game Reserve and salvinia has been under control in many areas of the wetland systems in the country.

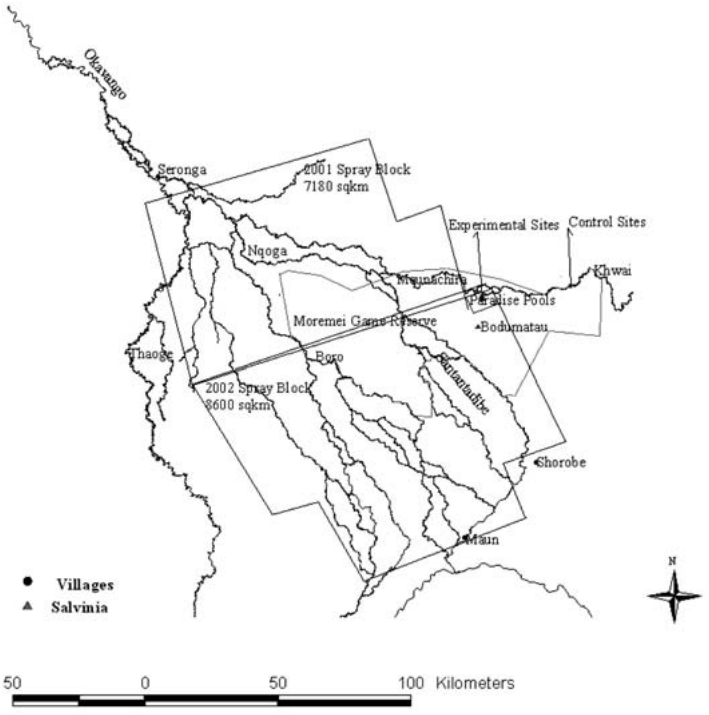

Figure 1. Okavango delta showing deltamethrin spray blocks with major rivers and salvinia weevil experimental and control sites.

Deltamethrin is a broad-spectrum insecticide and sequential drift sprays of 0.1 to 0.25 g a.i./ha "knock down" a wide range of arthropods in large numbers (Games 1981). Applications of 0.25 g a.i/ha deltamethrin in Zimbabwe increased the mortality rates of a wide range of aquatic invertebrates, but effects were transient and no population declines resulted (Grant and Crick 1987). Schlettwein and Giliomee (1990) found that adult salvinia weevil mortality occurred at concen-

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trations as low as 6 g/ha of endosulfan and 0.1g/ha of alphamethrin. Although considerable data are available on nontarget effects of pyrethroid insecticides (Smith and Stratton 1989), no research work was carried out on the impact of deltamethrin on salvinia weevils during earlier tsetse fly spray operations in Botswana or in other countries.

MATERIALS AND METHODS

Site Selection

A small area of the Khwai River at Xaxanaxa and the stagnant backwater Paradise Pools where salvinia weed is common were selected for the experimental sites during the 2002 tsetse control program (Figure 1). The experiments were conducted along Khwai River at or close to the co-ordinates 19°10'50.9"S; 23°26'22.7"E and 19°11'12.3"S; 23°27'49.4" and at Paradise Pools between the co-ordinates 19°11'35.0"S; 23°27'28.7"E and 19°11'35.0"S; 23°27'28.7"E.

Methods

Distribution of salvinia and weevil populations vary widely under field conditions, so static short-term bioassay methods were established in the field (Reish and Cshida 1987) using plastic containers and designating three categories of habitats. Twenty-four plastic basins (each having 50 cm diameter and 20 cm depth) were placed on the river banks at six randomly selected sites along the watercourses of Khwai River (i.e., 6 sites × 4 basins/site = 24 basins). Each basin was filled with river water and sediments weighing ca. 300 g collected along the riverbanks. Salvinia (500 g) devoid of weevils was placed in each basin. Fifty adult weevils were released onto the salvinia mat in each basin two days before the spray for insect acclimatization and establishment. The set of basins on the banks is hereafter referred to as 'Basin-bank". Tall grass, *Miscanthus junceus* (Stapf) Pilg. and reed, *Phragmites australis* (Cav.) Steud. were cleared to expose the basins directly to the aerial spraying. In the second set of studies, "Basin-water' contained twenty four perforated basins (i.e., 6 sites × 4 basins/site = 24 basins) with same contents as those of Basin-bank, however, six perforations of 1 cm diameter were made in the sidewall at the bottom of the basins so as to dip them directly into the water to enable the basin's flat bottom to sit on the sediment in water while the basins' rim is exposed to the atmosphere. The same sites were used for the Basin-bank and Basin-water studies in all the five treatment cycles. In the third category of 'Field water' experiments, 1 kg fresh salvinia mat was sampled at six random sites in the field infestations in the experimental block of the sprayed zone. Representative reference controls consisting of Basin-bank, Basin-water were maintained in a similar manner approximately 30 km outside the spray block in the Khwai River (Figure 1, 19°10'14.6"S, 23°44'59.2"E). Natural weevil populations were also determined from the control areas of the Khwai River. Deltamethrin was sprayed in a 8600 km^2 block five sequential times with a fixed-wing aircraft between 1800 and 2000 hours on 21 May, 4 June, 6 July, 26 July and 14 August 2002 (Figure 1). Deltamethrin at a dosage rate of 0.3 grams per hectare was sprayed in the first and second cycles and 0.26 grams in the subsequent three cycles. The carrier solvent for the insecticide used in the spray was kerosene.

Sampling

Samples of salvinia with introduced weevils from 6 basins of Basin-bank and from 6 basins of Basin-water at six respective sites were recovered at each sampling time. A total of 6 replicates were obtained on each sample occasion for Basin-bank and Basin-water. The first sampling was conducted 12 hours after deltamethrin aerial application between 0600 and 0800 hours. Subsequent samples were recovered at 24, 48, and 72 hours after the first sample was conducted (i.e., 12 hours after the application). The floating weevils in the basins at the time of each sample event were picked up by hand and released onto a small fragment of unsprayed salvinia in water in small plastic cups for mortality testing. The other weevils in the salvinia mat were completely extracted using Berlese funnels after the collection of samples from the basins (Boland and Room 1983). After extraction, the weevils were transferred onto salvinia mat as described above into another set of plastic cups for determining their survival and mortality. Weevils were also extracted with Berlese funnels from a 1 kg salvinia mat sampled at six field sites in the spray zone two days before spraying and three days after application. Similar methods were followed for sampling salvinia, collecting weevils and determination of mortality from the control basins as well as from field samples in Khwai River.

Surface water temperatures were measured at 6 cm depth at 0800 and 1800 hours in the basins as well as in field waters. Dissolved oxygen was determined following the modified azide method (APHA 1995) in the three categories of habitats two days prior to and three days after application.

Mortality Determination

Only those weevils that remained alive for more than 12 hours after their recovery were considered to have survived the spray treatment (Schlettwein and Giliomee 1990). Controls were also subjected to the same methods. The best method to determine mortality is to wait and observe if the insects respond when the weevils on the host plant are exposed to sunlight.

Preparation and Collection of Target Aluminium Foils

Six replicated sheets of aluminium foil (42 × 42 cm, 1764 cm^2) were spread across the bottom of empty plastic basins to obtain the insecticide spray drift and placed near salvinia containers in all five applications. Three replicated sheets of aluminium foils of similar size were prepared at control sites. The targeted foils were collected and folded in the early hours of the following day 10-12 hours after application and immediately placed in a plastic box over ice to preserve the samples.

Extraction and Analysis

The deltamethrin in the aluminium target foils was extracted using the solvent mixture of acetone and n.hexane (1:1) and measured using Gas Liquid Chromatography with

TABLE 1 WEEVILS' MEAN SURVIVAL AND CORRECTED % MORTALITY IN RESPONSE TO DELTAMETHRIN SPRAY DRIFT IN BASIN-BANK AND BASIN-WATER.

Sampling-Hours	Survival—Basin-bank		Corrected % mortality	Survival—Basin-water		Corrected % mortality
	Control	Sprayed		Control	Sprayed	
Cycle 1						
12	45.7 ± 0.3	30.3 ± 1.7*	33.5	48.7 ± 0.3	32.0 ± 1.3*	34.3
24	46.0 ± 0.6	29.3 ± 0.9*	36.3	48.0 ± 0.6	30.5 ± 1.4*	36.4
48	46.0 ± 0.6	27.0 ± 0.9*	41.3	45.0 ± 0.6	25.0 ± 1.0*	44.4
72	46.7 ± 0.9	24.8 ± 1.1*	46.8	49.0 ± 0.6	26.5 ± 1.1*	45.9
	% Mean mortality		39.5 ± 3.0	% Mean mortality		40.2 ± 2.9
Cycle 2						
12	48.3 ± 0.3	42.2 ± 1.4	12.6	48.3 ± 0.3	31.7 ± 0.9**	34.6
24	48.7 ± 1.0	32.7 ± 0.8**	33.0	48.7 ± 0.3	38.2 ± 1.0*	21.6
48	46.0 ± 0.6	32.5 ± 0.9	29.3	48.3 ± 1.0	38.8 ± 1.3	19.7
72	47.3 ± 1.0	38.7 ± 1.8	18.2	46.0 ± 0.6	36.7 ± 0.9	20.2
	% Mean mortality		23.2 ± 4.8	% Mean mortality		24.2 ± 3.6
Cycle 3						
12	46.0 ± 0.6	37.8 ± 1.6	17.8	47.3 ± 1.0	42.3 ± 0.6	10.6
24	46.7 ± 0.3	37.5 ± 1.1	19.7	45.7 ± 0.3	33.5 ± 0.9	26.7
48	47.0 ± 1.2	32.2 ± 0.4	31.5	45.0 ± 0.6	31.0 ± 1.4	31.1
72	48.3 ± 0.7	35.5 ± 0.9	26.5	46.7 ± 0.9	38.0 ± 0.9	18.6
	% Mean mortality		23.9 ± 3.2	% Mean mortality		21.7 ± 4.6
Cycle 4						
12	49.0 ± 0.6	44.5 ± 1.1	09.2	46.7 ± 0.3	37.0 ± 1.1	20.8
24	45.0 ± 0.6	41.8 ± 1.6	07.1	47.3 ± 0.9	44.7 ± 1.3	05.5
48	45.7 ± 0.3	35.8 ± 1.3	21.7	47.0 ± 0.6	37.0 ± 1.4	21.3
72	47.7 ± 0.3	38.0 ± 1.2	20.3	47.3 ± 0.9	36.0 ± 1.1	23.9
	% Mean mortality		14.6 ± 3.8	% Mean mortality		17.9 ± 4.2
Cycle 5						
12	48.7 ± 0.3	35.3 ± 0.8*	27.5	45.0 ± 0.6	30.3 ± 0.7*	
24	49.3 ± 0.3	29.0 ± 0.9*	41.2	45.3 ± 0.3	30.5 ± 0.9*	
48	45.3 ± 0.3	29.3 ± 0.9*	35.3	46.0 ± 0.6	32.2 ± 1.4*	
72	45.3 ± 0.7	26.3 ± 1.1**	41.7	48.7 ± 0.3	30.8 ± 1.1*	
	% Mean mortality		36.4 ± 3.3	% Mean mortality		32.9 ± 1.4

**1% significance.
*5% significance.

an Electron Capture Detector (ECD HP 6890 Instrument, NRI 1995). The lowest limit of detection on each sample reported by Natural Resources Institute, United Kingdom is 0.05 µg/l.

Data Analysis

Students' t-test comparing two groups was applied for the experimental data collected from basins and paired t-test (Snedecor & Cochran 1989) was applied to the data collected from the field infestations. Survival of weevils in treatments was corrected to 100% with respect to survival of weevils in controls to obtain corrected % mortality. Analysis of Variance (ANOVA) performed in SPSS version 14.0 using corrected % mortalities in Basin-bank and Basin-water was used to determine the weevil mortality between the five cycles. Corrected % mortalities obtained at time intervals in five cycles of 12, 24, 48 and 72 hours were used as independent variables to obtain the relationship between mortality and exposure time periods

RESULTS AND DISCUSSION

During the 2001 tsetse control program, the range between the mean minimum and maximum water temperatures in initial Basin-bank studies was high and hence, Basin-water studies were introduced as an additional treatment during the 2002 spray periods to reduce the range in water temperatures. Southeast winds dominated during the first three deltamethrin application cycles and north to northeast winds dominated cycles 4 and 5.

In the Basin-bank studies, the mean minimum temperature in five cycles was 11.1°C and mean maximum temperature was 26.4°C compared to Basin-water (Mean Min. 14.1°C to Mean Max. 23.3°C). The range of temperatures in field water was in between 14.7 C, and 23.1°C. The wide variation

in temperatures experienced in the Basin-bank treatments did not increase the deltamethrin toxicity to the weevils as suspected in 2001 spray program (Kurugundla 2001). However, Cesida (1980) suggests that pyrethroid toxicities increased at lower temperatures. Dissolved oxygen (DO) measured in the experimental period did not show significant changes. The DO content was in the range of 4.4 to 8.6 mg/l and is typical of earlier observations (UNDP/FAO 1977) and possibly reflect the relative stages of flood progression in the Delta (Cronberg et al. 1996).

Mean survival of the 50 adult salvinia weevils in the control basins was generally in the range of 45 to 49 (90% to 98%) while their mean survival in basins exposed to deltamethrin varied from 25 to 45 (50% to 90%) (Table 1). Cycles 1 and 5 were the only applications that resulted in significant mortality differences between control and treatment basins (Table 1, Figure 2). The location of the basins, either in the river or on the riverbank, had no effect on survival of adult weevils. There were significant differences in mortalities between cycles ($P < 0.0001$). Tukey's post hoc tests analyses showed that corrected % mortalities for cycles 1 and 5 were different compared to cycles 2, 3, and 4. However, there were no significant differences in corrected % mortalities between cycles 2, 3 and 4 and between cycles 1 and 5 (Figure 3). The aluminium foil sheets placed in the control sites of Khwai did not yield any deltamethrin residue.

Although the striking rate of deltamethrin to the ground (foil) was higher in the 5th cycle at 6.9% (±1.3) than in the 1st cycle at 3.7% (±1.0), there was not significant variation in the % mean mortalities between these two cycles (Figure 2). The spray deposition in the 2nd cycle was 2.3% (±0.3) and much lower in 3rd and 4th cycles affecting the weevil mortality at 24% (±4.2) and 18% (±3.2) respectively (Table 1, Figure 2). This indicates that application of about 2% did not decrease the survival of weevils in the present experiments. Semple and Forno (1990) found in lab experiments that adult weevils were highly susceptible to deltamethrin reporting an LC_{50} of 0.038 µg/l). The floating behavior of weevils we observed in basins at the time of sampling was due to the initial knock down followed by recovery or death in response to pyrethroids (Hill 1985).

Weevil populations are generally low in winter (Naidu et al. 2000) and the weevils' breeding and feeding would be normal above 26°C (Forno et al. 1983). In the 1st cycle, abundance of weevils were statistically significant in the field infestations of sprayed areas of Xakanaxa compared to the control areas of Khwai River while control areas had higher weevil density in 3rd cycle than in the sprayed areas reflecting the natural variations of weevils' abundance in time and space.

Direct surface contact of the insecticide with the adult weevils would be minimal during this study because they normally hide in buds, roots and beneath the leaves on cold nights. However, deltamethrin aerosols that drifted into the basins could eventually come into contact with the weevils to increase toxicity as there was no chance for the insecticide to escape from the basins.

Regression of time intervals against corrected percent mortality ($n = 10$, $r^2 = 0.84$) showed an increasing trend in mortality over time period of 72 hr (Figure 4). In the field, deltamethrin could be diluted, partitioned and adsorbed to various organic sediments (Muir et al. 1985), which would reduce the dose on the weevils in field infestations compared to the containers.

The weevils deposit eggs in buds and underneath the leaves and new emerging larvae normally feed inside the rhizome and therefore the eggs and larvae would be protected against contact with the insecticide (Schlettwein and Giliomee 1990). The deltamethrin spray to control tsetse fly in any given area is normally done in one winter of a year and it is not a continuous process. It was observed that Paradise Pools and Bodumatau hippo pools became fully covered by salvinia four months following the spray period in December

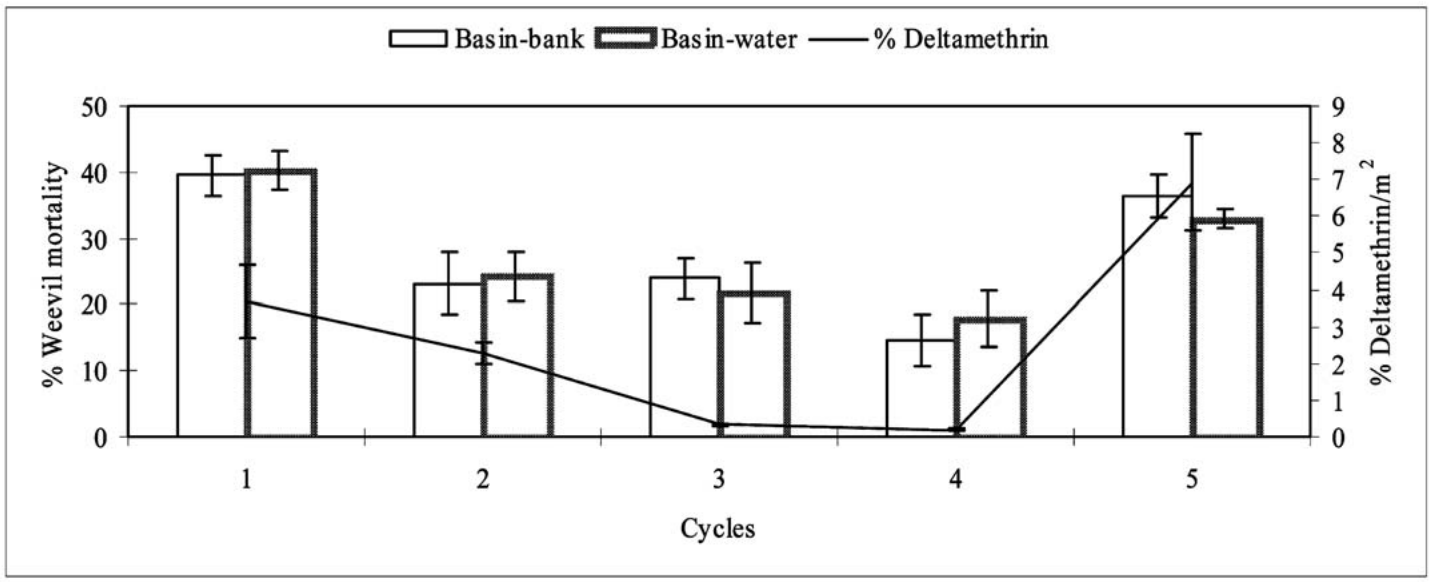

Figure 2. Percent mean weevil mortality in response to deltamethrin in Basin-bank and Basin-water studies.

TABLE 2. MEAN WEEVILS' POPULATION AND VARIABILITY IN KG. FRESH WEIGHT OF SALVINIA IN THE SPRAYED (XAKANAXA) AND IN CONTROL (KHWAI) AREAS.

	Cycle 1		Cycle 2		Cycle 3		Cycle 4		Cycle 5	
Days	Control	Sprayed	Control	Sprayed	Control	Sprayed	Control	Sprayed	Control	Sprayed
1	4.5 ± 1.0**	22.8 ± 2.1	7.8 ± 1.2	7.5 ± 1.2	7.3 ± 0.9	3.0 ± 0.7*	3.5 ± 0.7	2.0 ± 0.5	3.5 ± 0.7	3.5 ± 0.4
2	5.0 ± 1.7**	19.8 ± 3.2	9.5 ± 1.2	7.5 ± 1.1	6.0 ± 0.7	1.8 ± 0.6*	4.0 ± 0.9	1.7 ± 0.5	4.0 ± 0.9	2.2 ± 0.8
3	6.0 ± 1.2**	19.8 ± 2.5	8.5 ± 1.5	8.5 ± 1.0	5.7 ± 0.9	2.3 ± 0.5	3.8 ± 0.8	2.3 ± 0.5	2.8 ± 0.6	2.5 ± 0.4
4	3.3 ± 1.1**	18.0 ± 1.9	9.0 ± 1.7	7.3 ± 1.6	6.8 ± 1.4	2.2 ± 0.5*	3.0 ± 1.0	2.2 ± 0.5	3.0 ± 1.0	2.8 ± 0.9
5	4.3 ± 0.9**	20.8 ± 2.5	10.0 ± 1.1	6.7 ± 0.9	7.0 ± 0.6	1.8 ± 0.3*	4.5 ± 1.1	1.8 ± 0.3	4.5 ± 1.1	2.4 ± 0.6

**1% significance.
* 5% significance.

2002 and was controlled by the weevils by January 2003. It is, therefore, unlikely that the biological control agent of salvinia would be affected in large numbers in the field and it is concluded that the response of any organism to the toxicant may differ in closed systems as demonstrated in the containers from the open field conditions.

ACKNOWLEDGMENTS

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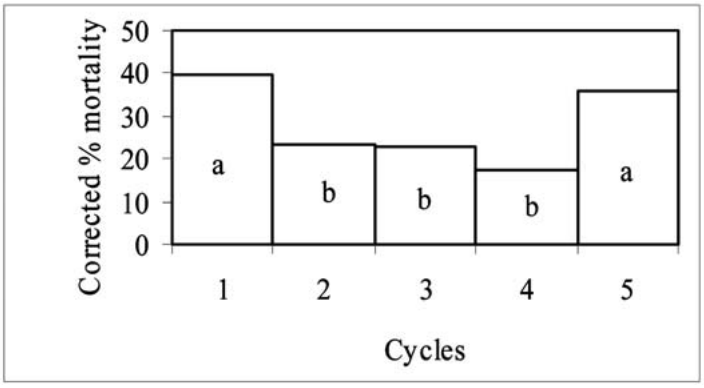

Figure 3. Analysis variance of corrected % mortalities in between cycles. Note that bars with the same letters are not significantly different.

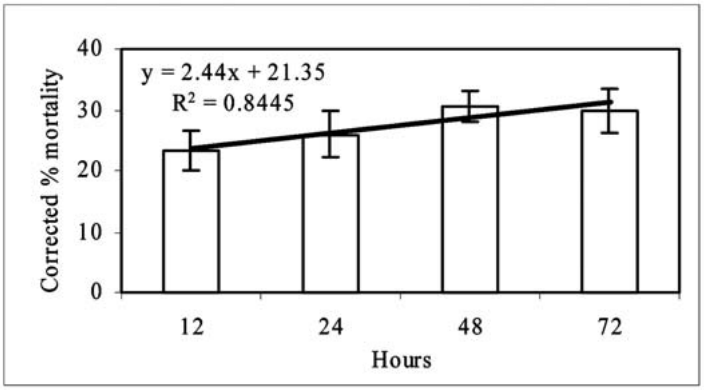

Figure 4 Relationship between corrected % mortality and time intervals after the spray application (n = 10, r^2 = 0.84).

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