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FLAVESONE: A NOVEL INSECTICIDE FOR THE CONTROL OF URBAN PESTS

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Abstract Flavocide® is based on a beta-triketone molecule, flavesone, that is produced by synthetic chemical means. Flavesone exists in nature in a number of plant species but at low abundance. Initial testing suggests a unique mode of action with potential in addressing the increasing problem of resistance to existing classes of insecticides. Flavesone has been initially formulated as an emulsion-in-water (EW) for use in a range of agricultural and public health applications. It is this formulation that was used in urban pest studies. Flavesone was effective as a direct spray in small chamber studies against *Aedes aegypti, Culex quinquefasciatus* and *Musca domestica.* Flavesone also demonstrated residual contact activity against these insects and *Ctenocephalides felis*. Other groups have also shown that flavesone demonstrated residual contact activity against *Anopheles* sp.. Flavesone is a novel insecticide, which has the potential to be useful in the management of insecticide resistant urban pest populations.

Key words *Aedes aegypti, Culex quinquefasciatus*, *Musca domestica,* flavesone.

INTRODUCTION

Bio-Gene Technology Ltd is an Australian company engaged in the development of naturally derived and synthetically produced insecticides. Bio-Gene holds patents covering the use of β-triketones for control of a range of pest groups including insects, arachnids, helminths, molluscs, protozoa and viruses. Flavesone is one of a number of β-triketone compounds that have shown insecticidal activity and expressed what appears to be a novel mode of action. Flavocide® is a commercial formulation of flavesone developed by Bio-Gene Technology Ltd and it is this formulation which is used in these studies. Flavesone has been initially formulated as an emulsion-in-water (EW) for use in a range of agricultural and public health applications. This formulation is compared to a pyrethroid, permethrin. Pyrethroids are used widely for the control of mosquitoes, flies and other urban pests. Resistance to pyrethroids has become an increasing problem in mosquitoes (Liu et al., 2014) and House fly (Liu and Xue, 2014) as well as a wide range of other pests. This resistance can have wider implications than simply resistance to pyrethroids because of the development of cross resistance to other insecticide groups (Zaim and Guillet, 2002). There is now an urgent need for alternative insecticides to manage resistant strains of insects, particularly those that are disease vectors. Zhu et al. (2016) concluded that biopesticides have varied mechanisms of activity that could contribute additional defences against the development of insecticide resistance.

The aim of these studies is to investigate the efficacy of flavesone against two species of mosquito, House fly and Cat flea in the laboratory. In addition a preliminary field study against mosquitoes is reported.

MATERIALS AND METHODS

Insecticides. The insecticide used in these studies was Flavocide 500EW (Active Constituent: flavesone 500 g/l). This was compared to Permethrin 100EC (Active Constituent: 100 g/l permethrin 25:75). Both insecticides were diluted with water. Flavesone 50 mg/ml and 25 mg/ml, and permethrin 2.5 mg/ml for mosquitoes. Flavesone 200 mg/ml and 100 mg/ml, and permethrin 2.5 mg/ml for flies.

Insects. All insects used were laboratory bred susceptible strains. House fly, *Musca domestica,* mixed sex adults 2-5 day old; *Culex quinquefasciatus,* female adults 2-5 day old; *Aedes aegypti,* female adults 2-5 day old. All were supplied by Department of Medical Entomology, ICPMR, Westmead Hospital, Sydney. *Ctenocephalides felis,* mixed sex adults 1-5 days old and first and second instar larvae supplied by VetX Research, Wongaburra, NSW

Small Chamber Studies on Flying Insects. The test chamber consisted of an aluminium frame with glass sides and top. The base of the chamber was constructed from laboratory grade stainless steel. The front of the chamber was hinged to form a door to facilitate cleaning. A small sliding glass door was provided in the front door of the chamber to allow for the introduction of the insects and the spray application. The dimensions of the chamber were 70 cm x 70 cm x 70 cm.

Prior to testing, the chamber was cleaned using detergent and water. The chamber was then dried completely. Twenty laboratory cultured insects were introduced into the chamber. A 0.5 litre pump spray was used to spray the insecticide. The nozzle of the pump spray was pointed slightly upwards to aim at the middle of the upper half of the back wall of the chamber. The spray was pumped once to deliver one gram of diluted insecticide. The weight of the spray discharge was recorded. Knockdown was noted at 30 second intervals to 300 seconds, 60 second intervals to 600 seconds, 120 second intervals to 1200 seconds and 300 second intervals up to a maximum period of 2,400 seconds. Insects were considered knocked down when they were incapable of coordinated movement. After 2,400 seconds or after all insects were knocked down the insects were collected and placed in a clean plastic holding container with a 10% sucrose pad, to check for recovery. Mortality was observed at 24 hours post treatment. The above was repeated to give a total of 5 replicates per rate. The control consisted of 20 insects (as above) handled in the same manner as for the active treatment; only water was sprayed into the chamber. Knockdown was noted as above for the active treatments. There were 5 replicates for the control. The study took place in a room with a temperature of 22±2° C. and 50% RH.

Residual Contact Studies on Flying Insects. *Ae. aegypti* - flavesone 12.5 mg/ml and 6.25 mg/ml and permethrin 0.625 mg/ml; *C. quinquefasciatus* - flavesone 50 mg/ml and 25 mg/ml and permethrin 2.5 mg/ml; *M. domestica* - flavesone 200 mg/ml and 100 mg/ml and permethrin 2.5 mg/ml.

Insecticide treatments were applied to the glazed tiles from a distance of 20 cm. The substrate was placed on a 0.5 m^2 grid and the entire 0.5 m^2 area, including the plate, was sprayed using a handheld pump sprayer. The spray rate was 50 ml/m^2. The pump spray was weighed to check that the correct spray weight was applied. The plates were left for 2 hours post-treatment to allow the plates to dry prior to the start of the exposure period.

A plastic petri dish, perforated with small holes for ventilation, and one larger hole for introduction of insects was place on a treated glazed tile. Ten insects were placed into the plastic petri dish using a powered aspirator. A cotton wool plug soaked in sugar solution was used to plug the hole and provide moisture. The 10 insects were constantly exposed to the treated surface for thirty minutes. The exposure took place in a room with a temperature of 22±2° C. and approximately 50% RH. After 30 minutes exposure a piece of printing paper was introduced between the petri dish and the treated tile surface to prevent further mosquito contact. The insects continued to be held at 22±0.5° C and approximately 50% RH for 24 hours. Knockdown was noted after 15 minutes and 1 hour post initial exposure. Mortality was noted at 24 hours post initial exposure. This was repeated with a further four batches of insects to give five replicates per active treatment. The control consisted of 5 replicates of 10 insects placed on untreated glazed tiles.

Residual Contact Studies on *C. felis*. Insecticide treatments were applied to 32 mm diameter nylon carpet discs or 32 mm cotton fabric discs. Carpet and fabric discs were alternatively placed in a 0.25 m^2 grid and the entire area, including the discs, was sprayed using a hand held pump sprayer at the rate of 100 ml/m^2. The pump spray was weighed to check the correct spray weight was applied. The discs were then left to dry for 30 mins. At 30 minutes post treatment, three 32 mm carpet discs were placed in separate plastic vials. The vials were 32 mm diameter and 9 cm high and had a perforated screw cap.

Ten adult fleas were placed in each vial by a power aspirator and the screw cap closed. Vials were stored in an incubator at 27° C and 75% relative humidity for 24 hours. This was repeated with a further two batches of insects to give three replicates per active treatment. The control consisted of three replicates of 10 insects placed on untreated carpet discs. Mortality was noted after 24 hours exposure. Mortality included all fleas that were showing no movement and not maintaining normal posture.

All the above was repeated for flea larvae. Flea larvae were picked up using a small soft paint brush and placed on the treated cotton fabric in the plastic vials. Larvae were examined at 24 hours post exposure and considered dead when they displayed no movement when touched with a fine paint brush. Controls consisted of carpet discs or cotton fabric discs treated with water alone and handled as above.

Preliminary Field Study on *C. quinquefasciatus*. Flavesone 50 mg/ml, 25 mg/ml and 12.5 mg/ml. Ten *C. quinquefasciatus* adults were placed in a plastic container. The container was 10 cm high and 11cm diameter and had mesh over one end to allow penetration of the insecticide fog. The container was placed out in an urban garden at a height of one metre. The area was sprayed using a Dynafog Trijet Fogger at a rate of 500 ml/hectare. This was repeated to provide three replicates of each of the three rates of flavesone. There were three untreated controls. Knockdown was noted at 0.5 and 2 hours, and mortality at 24 hours after fogging. The mosquitoes were transported to the laboratory after the 0.5 hour assessment and held at 22±2° C. and 50% RH.

RESULTS AND DISCUSSION

Small Chamber Studies on Flying Insects

Results are presented in Tables 1 to 3. An analysis of variance was performed to determine the differences between treatments at KD50 and KD90 points, and 24 hours after exposure. Treatments were tested against each other using Ryan's Q test if the assumption of equality of variance was met or Dunnett T-test if the assumption was not met.

***Ae. aegypti*.** Flavesone 50 mg/ml and 25 mg/ml gave 100% mortality at 24 hours and this was equivalent to the mortality achieved by permethrin 2.5 mg/ml. The KD50 and KD90 of both rates of flavesone were both slightly slower than those for permethrin. Flavesone 50 mg/ml KD90 was 633 seconds and permethrin was 510 seconds.

C. quinquefasciatus. Flavesone 50 mg/ml and 25 mg/ml gave 100% mortality at 24 hours and this was equivalent to the mortality achieved by permethrin 2.5 mg/ml. The KD50 and KD90 of both flavesone 50 mg/ml were both slightly faster than those for permethrin. Flavesone 50 mg/ml KD90 was 1431 seconds and permethrin was 1745 seconds. Flavesone KD50 and KD90 25 mg/ml were slightly slower than permethrin.

M. domestica. Flavesone 200 mg/ml and 100 mg/ml gave 100% mortality at 24 hours and this was equivalent to the mortality achieved by permethrin 2.5 mg/ml. The KD50 and KD90 of both rates of flavesone were both slightly slower than those for permethrin. Flavesone 200 mg/ml KD90 was 637 seconds and permethrin was 386 seconds.

Residual Contact Studies on Flying Insects

The results are presented in Tables 4 to 6. No statistical analysis was conducted on mosquito data as most results were 100%. An analysis of variance was performed to determine the differences between treatments for *M. domestica.* Treatments were tested against each other using a Dunnett T-test as the assumption was not met.

Ae. aegypti. Flavesone 12.5 mg/ml and 6.25 mg/ml gave 100% knockdown at 15 minutes and 2 hours and 100% mortality at 24 hours and this was equivalent to knockdown and mortality for permethrin 0.625 mg/ml.

C. quinquefasciatus. Flavesone 50 mg/ml and 25 mg/ml gave 100% knockdown at 15 minutes and 2 hours and 100% mortality at 24 hours and this was equivalent to knockdown and mortality for permethrin 2.5 mg/ml. Other collaborators have also shown that flavesone demonstrated residual contact activity against *Anopheles* sp.

M. domestica. Flavesone 200 mg/ml and 100 mg/ml gave 100% knockdown at 15 minutes and 2 hours and 100% mortality at 24 hours. Both rates of flavesone appeared superior to permethrin 2.5 mg/ml which gave 70% and 92% knockdown at 15 minutes and 2 hours, respectively, and 72% mortality at 24 hours, however these differences were not statistically significant. There was some recovery at 24 hours with permethrin but this did not occur with flavesone. The comparative finding in contact studies in *M. domestica* that flavesone was able to induce 100% mortality versus 72% for permethrin highlights the ongoing issue of increasing reduced efficacy of the synthetic pyrethroids.

Residual Contact Studies on *C. felis*

The results are presented in Table 7. No statistical analysis was conducted on *C. felis* data as most results were 100%. Against adult *C. felis* on a nylon carpet the 3 higher rates of flavesone (150 mg/ml, 62.5 mg/ml and 23.8 mg/ml) resulted in 100% mortality after 24 hours exposure. Flavesone at 2.38 mg/ml achieved 10% mortality after 24 hours exposure. Permethrin 2.5 mg/ml gave 100% mortality. Control adult flea mortality was nil. Against *C. felis* larvae on cotton fabric the 3 higher rates of flavesone (150 mg/ml, 62.5 mg/ml and 23.8 mg/ml) resulted in 100% mortality after 24 hours exposure. Flavesone at 2.38 mg/ml achieved 3.3% mortality after 24 hours exposure. Permethrin 2.5 mg/ml gave 100% mortality. Control larval flea mortality was nil.

Preliminary Field Study on Mosquitoes

The results are presented in Table 8. No statistical analysis was conducted for this pilot study. Flavesone at 50 mg/ml gave 80% knockdown after 15 minutes and mortality was 76.7% at 24 hours, thus there was very little recovery. Based on these early promising results two further field studies are planned in Cairns, Queensland. The first study will investigate further the efficacy of flavesone for knockdown and kill of mosquitoes. The second study will look at the ability of flavesone to protect humans from mosquito bites (bite inhibition).

Table 1. Knockdown and mortality of *Aedes aegypti*.

Treatment and Rate	**KD50 (seconds)**	**KD90 (seconds)**	**24 hours mortality (%)**
Permethrin 2.5 mg/ml	352.8a	510.0a	100a
Flavesone 50 mg/ml	488.0b	633.0b	100a
Flavesone 25 mg/ml	570.2c	788.0c	100a

Treatments with same letter do not differ significantly from each other.

Table 2. Knockdown and mortality *Culex quinquefasciatus.*

Treatment and Rate	**KD50 (seconds)**	**KD90 (seconds)**	**24 hours mortality# (%)**
Flavesone 50 mg/ml	1025.1a	1431.4a	100a
Permethrin 2.5 mg/ml	1284.1b	1745.0b	100a
Flavesone 25 mg/ml	1606.4c	1932.9c	100a

Treatments with same letter do not differ significantly from each other.

Table 3. Knockdown and mortality of *Musca domestica.*

Treatment and Rate	**KD50 KM (sec)**	**KD90 KM (sec)**	**24 hours mortality# (%)**
Permethrin 2.5 mg/ml	253.3a	386.0a	100a
Flavesone 200 mg/ml	467.5b	637.2b	100a
Flavesone 100 mg/ml	1051.7c	1376.0c	100a

Table 4. Percentage knockdown and Mortality of *Aedes aegypti*

Treatment and Rate	**Percentage Knockdown and Mortality at Three Exposure Times**		
	15 minutes	**4 hours**	**24 hour mortality**
Flavesone 12.5 mg/ml	100	100	100
Flavesone 6.25 mg/ml	100	100	100
Permethrin 0.625 mg/ml	94	100	100
Control	0	0	12

*Treatments with same letter do not differ significantly from each other.

Table 5. Percentage knockdown and mortality of *Culex quinquefasciatus*

Treatment and Rate	Percentage Knockdown and Mortality at Three Exposure Times		
	15 minutes	2 hours	24 hour mortality
Flavesone 50 mg/ml	100	100	100
Flavesone 25 mg/ml	100	100	100
Permethrin 2.5 mg/ml	100	100	100
Control	0	0	4

Table 6. Percentage Knockdown and Mortality of *Musca domestica*

Treatment and Rate	Percentage Knockdown and Mortality at Three Exposure Times		
	15 minutes	2 hours	24 hour mortality
Flavesone 200 mg/ml	100a	100a	100a
Flavesone 100 mg/ml	100a	100a	100a
Permethrin 2.5 mg/ml	70a	92a	72a
Control	0	0	4

Treatments with same letter do not differ significantly from each other.

Table 7. *Ctenocephalides felis* mortality after 24 hour exposure.

Treatment and Rate	Flea Mortality After 24 Hours Exposure (n=10)	
	Adults	Larvae
Flavesone 2.38 mg/ml	10	3.3
Flavesone 23.8 mg/ml	100	100
Flavesone 62.5 mg/ml	100	100
Flavesone 150 mg/ml	100	100
Permethrin 2.5 mg/ml	100	100
Control	0	0

Table 8. Percentage knockdown and mortality of *Culex quinquefasciatus* at Three exposure times (Pilot Field Trial)

Concentration mg/ml	Percentage Knockdown and Mortality at Three Exposure Times		
	15 minutes KD	2 hours KD	24 hours mortality
Flavesone 50 mg/ml	80.0	76.7	76.7
Flavesone 25 mg/ml	43.3	40.0	20.0
Permethrin 12.5 mg/ml	53.3	10.0	6.6
Permethrin 2.5 mg/ml	20.0	13.3	10.0
Control	0	0	6.7

CONCLUSION

In our studies flavesone demonstrated its ability to knockdown and kill *Ae. aegypti, C. quinquefasciatus* and *M. domestica* in both small chamber studies and direct contact residual studies. It was also very effective against *C. felis*. Initial studies on the mode of action of flavesone suggest that flavesone has a different mode of action to pyrethroids. The widespread issue of insecticide resistance therefore presents significant opportunities for flavesone to act as a "resistance breaker" in integrated pest management programs including against urban pests.

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