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QCIDE[®]: NATURAL INSECTICIDE FOR THE CONTROL OF FLYING INSECTS

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QCIDE[®]: NATURAL INSECTICIDE FOR THE CONTROL OF FLYING INSECTS

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Abstract: Qcide is a natural plant extract from a chemotype of *Eucalyptus cloeziana* that is being developed as an insecticide for a number of applications including control of flying insect pests. The primary active compound tasmanone, is a member of the beta-triketone class of compounds that occur in a number of Myrtaceous plant species. Initial testing suggests that beta-triketones have a novel mode of insecticidal action with potential to address the increasing problem of resistance to existing insecticide classes. Qcide has been initially formulated as an oil-in-water emulsion-(EW), alone and in combination with pyrethrins, primarily for control of flying insects in a range of public health and consumer product applications. Qcide has been tested as a direct spray in small chamber studies against *Aedes aegypti* and *Musca domestica* to determine KD and mortality. Other groups have also shown that Qcide is active against a resistant strain of *Aedes aegypti*. Qcide is a novel natural insecticide which has the potential for use in resistance management programs for control of public health pests. Qcide used alone against *Ae. aegypti* achieved

100% mortality at 24 hours after treatment at rates as low as 12.5mg/mL with KD50 and KD90 of approximately 600 and 1080 seconds. KD50 and KD90 at the highest rate of 100mg/mL were 225 and 420 seconds respectively confirming good knockdown activity against this important mosquito species. The activity of Qcide against the larger insect *M. domestica* was understandably less with Qcide rates of up to 200mg/mL required to achieve consistent 100% mortality at 24 hours. Qcide/pyrethrins/PBO combinations demonstrated that control of *M. domestica* could be significantly improved. In particular, noticeable reductions in both KD50 and KD90 were obtained when Qcide at 50g/L and 100g/L concentrations were combined with pyrethrins/PBO.

Key words: *Aedes aegypti*, *Musca domestica*

INTRODUCTION

Bio-Gene Technology Ltd is an Australian company engaged in the development of naturally derived and synthetically produced nature-identical 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. Qcide contains tasmanone as the active ingredient which 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. Qcide is a natural oil product that is extracted from the leaves of a rare chemotype of an Australian eucalypt, the Gympie Messmate (*Eucalyptus cloeziana*), which is grown in plantations in northern Queensland. Qcide has been initially formulated as an oil-in-water emulsion (EW) for use in a range of agricultural and public health applications. Studies reported in this paper compared this formulation to a commercial pyrethrins/PBO formulation and a laboratory formulated pyrethrins/PBO combination. Pyrethrins and pyrethroids are used widely for the control of mosquitoes, flies and other urban pests. Resistance to pyrethrins and pyrethroids has become an increasing problem in control of mosquitoes (Liu et al, 2014) and House fly (Liu and Xue, 2014) as well as a wide range of other pests including stored product pests (Lloyd and Parkin, 1963). This resistance can have wider implications than simply resistance to pyrethrins/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. There are also benefits in identifying natural compounds which can enhance the efficacy of pyrethrins as these are expensive and in short supply (Joffe et al 2012).

The principal aim of the studies undertaken at the University of Technology Sydney (UTS) is to investigate the efficacy of Qcide against *Aedes aegypti* and *Musca domestica* in the laboratory, to compare this to a pyrethrins/PBO

insecticide and to initiate the investigation of Qcide/pyrethrins/PBO combinations at various rates. Studies have also been undertaken to demonstrate the activity of Qcide against resistant strains of insects as indirect supporting evidence of the novel mode of action, including topical dose studies against mosquitoes undertaken at Purdue University USA.

MATERIALS AND METHODS

Insecticides

The insecticide used in the knockdown studies was Qcide 540EW (Active Constituent: Qcide natural oil 540g/l). This was compared to a commercial pyrethrins/PBO product (Active Constituent: 80g/l pyrethrins 25:75, 480g/l piperonyl butoxide) and a laboratory formulated pyrethrins/PBO formulation. Qcide was also tested in combination with this laboratory formulation. All insecticides were diluted with water. Qcide 540EW was also used in the topical dose response studies.

Insects

All insects used were laboratory bred strains.

- House fly, *Musca domestica* (Linnaeus), mixed sex adults 2-5 day old.
- Dengue mosquito, *Aedes aegypti* (Linnaeus), female adults 2-5 day old (knockdown studies)
- *Aedes aegypti* Liverpool (LVP; pyrethroid susceptible) and Puerto Rico (PRS; pyrethroid resistant) strains, 3-5 day old (adult topical dose-response assays)

All insects used in the UTS studies (small chamber and direct spray studies) were supplied by the Department of Medical Entomology, ICPMR, Westmead Hospital, Sydney. Mosquitoes used in the Purdue University topical dose studies were supplied by BEI Resources, Manassas, Virginia, USA.

Preliminary Screening Small chamber studies on flying insects

Ae. aegypti - Qcide 100mg/ml, 50mg/ml, 25 mg/ml and 12.5mg/ml, and Pyrethrins/PBO 0.08 mg/ml.

M. domestica - Qcide 200mg/ml, 100 mg/ml 50mg/ml, 25 mg/ml, and Pyrethrins/PBO 0.08 mg/ml.

The aim of this chamber study was to establish baseline rates for Qcide and to compare it to a commercial pyrethrins/PBO formulation.

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 internal dimensions of the chamber were 70cm x 70cm x 70cm.

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 for *Ae. aegypti*. The study on *M. domestica* was extended to 3600 seconds with knockdown noted at 3000 seconds and 3600 seconds. Insects were considered knocked down when they were incapable of coordinated movement. At the conclusion of the experiment 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 3 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 3 replicates for the control. The study took place in a room with a temperature of $22\pm 2^{\circ}\text{C}$. and at approximately 50% RH.

Direct spray study on *M. domestica*

Qcide 200mg/ml, 100 mg/ml 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml, Pyrethrins/PBO 0.08 mg/ml.

A Preval[®] Spray System was used to dispense all insecticides in this study. The Preval Spray system consists of an aerosol can with a dip tube into a connected glass container. The glass container can be unscrewed from the aerosol can and the candidate insecticide can be introduced into the glass container, and the container re-attached. When the nozzle on the aerosol is depressed the insecticide is sucked up the dip tube and passes out of the nozzle as an aerosol spray. The Preval spray system was placed in an automatic spray device that can be set for the required spray time.

Qcide and Pyrethrins/PBO were diluted in water to the dilutions detailed above.

Ten mixed sex *M. domestica* were aspirated into a holding container. The holding container was a transparent plastic container 115mm in diameter and 110mm high. The base of the container was removed and replaced by a fine nylon mesh. The same mesh was placed on the top of the container. A Whatman filter paper was placed in the base of the container to prevent pooling on the container base and trapping of flies in the liquid.

The holding container as described above was placed on a plastic ring, 100mm in diameter with the bottom of the ring cut at an angle of 30° to the horizontal. The automatic spraying device was suspended by supports also at 30° to the horizontal. The aerosol can was fitted to the spraying device to give a distance of 45cm from the nozzle of the spray can to the mesh on the bottom of the holding container. The automatic aerosol sprayer, and the holding container,

were both set at an angle of 30° to the horizontal so there was no ‘shadow’ effect of the aerosol spraying into the holding container.

A 2.0 second burst of the formulation was sprayed onto the *M. domestica* from a distance of 45cm using an automatic solenoid aerosol sprayer. The fume hood was left operating during the spray procedure.

The number of *M. domestica* knocked down was noted at 5, 10, 15 and 20 minutes after spray application. Insects were considered knocked down when they were incapable of flight and coordinated movement, on their back or side or not able to walk if gently touched with a paint brush. At the conclusion of the experiment 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. *M. domestica* was considered dead when there was no obvious movement after being observed for 3 seconds.

All the above was repeated for the remaining aerosol formulations. There were five replicates of each formulation. The weight of aerosol sprayed was recorded for each replicate. The control consisted of five replicates of 10 *M. domestica* mixed-sex adults sprayed with water and held in the same laboratory as the treated flies for 24 hours.

Small chamber studies on *M. domestica* using insecticides in combination

Qcide 100 mg/ml and 50mg/ml alone or in combination with Pyrethrins/PBO 0.08 mg/ml at various rates.

The aim of these studies was to investigate the benefits of combining Qcide with pyrethrins/PBO formulations. Pyrethrins are expensive, and there is a limited supply. A combination could enable a reduction in the amount of pyrethrins but give a similar efficacy. Qcide has the added advantage of being a natural product which would complement pyrethrins, particularly where insects resistant to pyrethrins are present. The combinations evaluated are detailed in Table 3.

The method was similar to that used in the preliminary small chamber study detailed above.

The insecticides were applied using the Preval aerosol spray, fitted to a UTS solenoid spray system. The nozzle of the Preval spray system was pointed slightly upwards to aim at the middle of the upper half of the back wall of the chamber. The automatic spraying system was activated to give a spray duration of 2.0 seconds. After spraying the weight of the spray discharge was noted. Housefly knockdown was noted at various timed intervals up to a maximum period of 1,800 seconds (30 minutes).

***Ae. aegypti* adult topical dose response assays**

Dose mortality adult topical dose response assays were conducting using Purdue standard operating procedures (SOP) for this assay to determine the lethal concentration (LC) of test material. The assays comprised five-point dose of Qcide

540EW, three technical replicates (10 mosquitoes/replicate) per dose with negative control vehicle of acetone only. Positive control was technical grade synthetic pyrethroid (SP) diluted in acetone.

Using these procedures, treatment dilutions are delivered using a syringe micro-applicator to apply a volume of 0.25µL to the dorsal thorax of each tested mosquito. After treatment mosquitoes are transferred to a labelled paper cup on cold plate, and the treatment process repeated nine times to obtain n=10 treated mosquitoes. After treatment the mosquitoes are sealed in the cup with a mesh square secured with a rubber band, and transferred to a growth chamber under constant temperature/RH conditions on a 12h day/12h night cycle. This process is repeated twice to obtain three technical replicates (30 mosquitoes total).

Phenotypic endpoint was scored for death/paralysis at 24 and 48 hours. Mosquitoes were scored as “dead/non-responsive” if they show lack of movement, defined as lying on one side or on the back, with the inability to fly when the cup is gently tapped.

Data was corrected for control mortality using the modified Abbott’s formula as follows:

$$\text{Mortality \%} = (X - Y) * 100 / (100 - Y), \text{ where } X = \text{the percent mortality in the treated sample, and } Y = \text{the percent mortality in the control.}$$

The results were displayed as a histogram or an exponential curve and the LC value calculated for the test material relative to the control. The assay was repeated using separate batches of mosquitoes to obtain n = 3 biological replicates.

RESULTS AND DISCUSSION

Preliminary screening small chamber studies on flying insects

Results are presented in Table 1. This table summarises the estimated KD50 and KD90 points, and 24-hour mortality after exposure. There were only 3 replicates thus no statistical analysis was carried out.

There was a clear dose response in KD50 values for Qcide against *Ae. aegypti*. At the lowest concentration of 12.5mg/ml, Qcide achieved a KD50 of approximately 600 seconds. At the highest concentration of 100.0mg/ml, Qcide achieved a KD50 of approximately 225 seconds. The remaining concentrations of Qcide had KD50 values between the above values. Pyrethrins/PBO at the label recommended dose of 0.08mg/ml achieved a KD50 of 300 seconds.

The KD90 of Qcide at 12.5mg/ml & 25.0mg/ml was 1080 and 1200 seconds respectively. Qcide at 50.0mg/ml & 100.0mg/ml achieved a KD90 of 540 seconds & 420 seconds respectively. Pyrethrins/PBO at 0.08mg/ml achieved a KD90 of 500 seconds. All treatments reached 100% mortality after 24 hours. Qcide at 25.0mg/ml & 50.0mg/ml did not reach the KD50 by 3,600 seconds against *M. domestica* which was the time limit for the study. Qcide at a 100

mg/ml and 200mg/ml achieved a KD50 of 3,200 seconds and 2600 seconds respectively. Pyrethrins/PBO at 0.08mg/ml did not reach the KD50 in 3,600 seconds.

Pyrethrins/PBO and Qcide did not reach the KD90 by 3,600 seconds against *M. domestica*. There was a clear dose response for Qcide in the 24-hour mortality of *M. domestica*. Qcide at 25.0mg/ml achieved a 24-hour mortality of 11.7% and at 50.0mg/ml, a 24-hour mortality of 15.0%. Qcide. At 100mg/ml and 200mg/ml Qcide achieved a 24-hour mortality of 56.7% and 100% respectively. Pyrethrins/PBO at 0.08mg/ml achieved a 24-hour mortality of 5.0% indicating it has poor killing capacity to *M. domestica*. Thus Qcide 24-hour *M. domestica* mortality was superior to Pyrethrins/PBO when Pyrethrins/PBO was used at the label rate.

The KD50 and KD90 results were much slower for *M. domestica* than for *Ae. aegypti*. The 24-hour mortality was also inferior for *M. domestica*. For these reasons it was decided to conduct future studies on *M. domestica*.

All formulations were introduced into the chamber using a simple consumer pump spray device. Pump sprays have limitations in producing the correct particle size to contact mosquitoes. If the particle size is too small it will not contact the mosquito during flight, as it may be affected by the slipstream of the mosquito. If the particle size is too big, it falls out of the air quickly and settles on the base of the chamber. There was evidence that some particles were too large as they fell down and wetted the floor of the chamber. Flying Insect Killer (FIK) aerosols are designed to provide the correct particle size to contact flying insects, predominantly, mosquitoes and flies and it was decided, after these preliminary studies, future studies would be carried out with an aerosolized formulation.

Direct spray study on *M. domestica*

The aim of this study was to establish some baseline rates for Qcide and the commercial formulation of Pyrethrins/PBO with the Preval system.

Results are presented in Table 2. Qcide at 6.25mg/ml gave 46% knockdown at 20 minutes and there was some recovery at 24 hours (32% mortality). Qcide at 12.5 mg/ml and Qcide at 25 mg/ml gave 56% and 64% knockdown at 20 minutes respectively, with no recovery at 24 hours. Qcide 50 at mg/ml achieved 48% knockdown at 10 minutes and 94% knockdown at 20 minutes. All Houseflies were dead after 24 hours. Qcide at 100 mg/ml had 84% knockdown at 10 minutes and 100% knockdown at 20minutes. Mortality at 24 hours was 100%. Pyrethrins/PBO at 0.08 mg/ml gave 100% knockdown at 5 minutes and 100% mortality at 24 hours. It appeared from this study that a Qcide rate of 50mg/ml to 100mg/ml were good rates to study further

Small chamber studies on *M domestica* using Qcide in combination with Pyrethrins/PBO

The aim of these studies was to identify any benefits of using Qcide in combination with pyrethrins/PBO either in speed of knockdown or in subsequent mortality. Details of combinations tested and results obtained are summarised

in Table 3. KD90 value for 3.5g/L Pyrethrins/16g/L PBO was improved from 1200 seconds to 820 seconds with the addition of Qcide 50g/L and to 450 seconds with the addition of Qcide 100g/L. Mortality at 24 hours was also improved marginally from 98.3% to 100% with the addition of Qcide at both concentrations. In addition, reductions in knockdown times from KD90 of 1200 seconds to 920 seconds of were achieved with Qcide addition of 50g/L to lower pyrethrins/PBO concentrations of 1.75g/L pyrethrins/8g/L PBO.

***Ae. aegypti* adult topical dose response assays**

The aim of these studies was to determine lethal concentrations (LC50) of Qcide at 24 and 48 hours post topical exposure against SP-susceptible and SP-resistant *Ae. aegypti* female adult mosquitoes in order to provide evidence that Qcide has a mode of action different to that of pyrethroids. Results are presented in Table 4 and showed that Qcide LC50s against susceptible (LVP) strain were 8.1 mg/mL (24h) and 7.9 mg/mL (48h) compared to LC50s against resistant (PRS) strain of 8.5 mg/mL (24h) and 8.3 mg/mL (48h). This indicated little difference between the effects of Qcide on susceptible and SP-resistant strains of *Ae. aegypti* thus exhibiting no cross-resistance and providing evidence in support of Qcide's novel mode of action. In contrast, and as expected, the LC50s for permethrin against susceptible strain were 542.9 ng/mL (24h) and 504.6 ng/mL (48h), and for resistant strain were much higher at 0.13 mg/mL (24h) and 0.12 mg/mL (48h).

CONCLUSION

In these studies Qcide demonstrated its ability to knockdown and kill *Ae. aegypti* and *M. domestica* in both small chamber studies and direct contact residual studies when used alone and in combination with pyrethrins/PBO. Qcide used alone against *Ae. aegypti* achieved 100% mortality at 24 hours after treatment at rates as low as 12.5mg/mL with KD50 and KD90 of approximately 600 and 1080 seconds. KD50 and KD90 at the highest rate of 100mg/mL were 225 and 420 seconds respectively confirming good knockdown activity against this important mosquito species. The activity of Qcide against the larger insect *M. domestica* was understandably less with Qcide rates of up to 200mg/mL required to achieve consistent 100% mortality at 24 hours. This led to testing of Qcide/pyrethrins/PBO combinations where the level of control of *M. domestica* could be significantly improved by combining Qcide and pyrethrins/PBO products. In particular, noticeable reductions in both KD50 and KD90 were obtained when Qcide at 50g/L and 100g/L concentrations were combined with pyrethrins/PBO. Although possible synergy was not investigated in these studies, this is the subject for further investigation.

Initial studies on the mode of action of Qcide and other β -triketone insecticides suggest that this class of chemistry has a different mode of action to pyrethroids. This was supported in the dose response assays against SP-resistant and susceptible strains of *Ae. aegypti* where Qcide controlled both strains at similar rates. The widespread issue of

insecticide resistance therefore presents significant opportunities for Qcide to act as a “resistance breaker” in integrated pest management (IPM) programs including against *Ae. aegypti* and other flying insect pests.

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