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Simple Solid Dose Bioassay for Insecticides Using the Fruit Fly (*Drosophila melanogaster*)

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Synthetic insecticides differ from their natural counterparts in many ways. One of the most dramatic differences is that synthetic insecticides were originally developed with the assumption that they would be used as contact poisons whereas most naturally occurring chemicals with insecticidal properties would be ingested by the insect. It is not surprising therefore that most bioassays that have been developed for insecticides have been based on the assumption that the insect will be brought into contact with the test chemical via surface or via the vapour phase (Keddy *et al.*, 1995).

Insecticides such as imidacloprid are neurotoxins, used mainly in the treatment of sucking insects working by contact and via stomach action from oral uptake (Extoxnet 2000). In many bioassays such as those described in Grant (2001) that use more direct exposure routes for rapid response, measuring trace concentrations of chemicals that primarily work by feeding / oral uptake can be problematic (Nauen and Elbert 1997). For example, the chemistry of the residue being tested such as its surface tension in solution can affect how well a bioassay performs. Where attempts have been made to cause ingestion of a chemical, such as spraying the chemical onto leaves it is not always simple to control the dose (Chiao-Cheng *et al.*, 1988; Wiles and Jepson 1992; Herron *et al.*, 1996; Brogdon and McAllister 1998; Akkerhuis *et al.*, 1999). Where the dose has been carefully controlled, measurements of insect and food mass are required for mortality to dose results (Alonsoamelot *et al.*, 1994; Nigg *et al.*, 1994) making the process more complex.

The purpose of this research was to design a simple bioassay using a food solid containing the insecticide where the primary exposure route would by feeding. This would negate the problems with aqueous samples and provide a known sample concentration (although not a known dose per insect).

MATERIALS AND METHODS

All laboratory chemicals used came from Sigma-Aldrich except for Oxoid technical agar. Nipagin (an anti-fungal agent) is a solution of p-hydroxybenzoic

acid methyl ester dissolved in 100% ethanol to a concentration of 100g l^{-1}. The 30ml universal tubes are produced by Philip Harris Scientific.

Technical cypermethrin (SP compound (72.6% pure) was donated by Zeneca Agrochemicals. Ecofleece® Non-OP sheep dip (contains 10% cypermethrin SP) was donated by Bimeda. Bayticol® Scab & Tick dip (contains 6% flumethrin SP) was provided by Bayer. Rapid Greenfly Killer® (contains 50g l^{-1} of pirimicarb) is made by Miracle Garden Care Limited and Bio Fruit Spray® (contains 178g l^{-1} of the OP fenitrothion) is made by pbi Home & Garden Limited. The Provado® (5% imidacloprid is made by Bayer.

The technical cypermethrin compound was prepared by dissolving 50mg of compound into 500µl 100% ethanol to produce a 10% w/v solution and this was then diluted to the required concentrations using water. Ethanol acts as a carrier for the SP in water.

The *Drosophila melanogaster* stocks were bred and kept at 25°C on bottles containing standard fruit fly food mixture (Carpenter 1950). ry^{506} wild type flies were used as a wild type genus and then used throughout to keep continuity

44.61ml of water, 0.75g technical agar and 5g sucrose were microwaved together until boiling. Upon cooling to hold in the hand 0.39ml nipagin solution and 5ml of the test solution was added. This gave a one tenth concentration of the test solution. 5ml of the mixture was then placed into the bottom of a universal tube and left to set and dry. 10 Fruit flies were added, the top screwed on and the timing of the test begun. The $Knockdown_{50}$ and $_{100}$, and the Lethal $Time_{50}$ and $_{100}$ times were recorded. These were taken as the point when the number of flies no longer left the solid (knockdown) or showed no movement response to tapping the bioassay tube (mortality). Additionally, the dead flies could usually be identified by other visible methods. Controls were carried out with water as the test solution and each assay test was replicated 5 times. The mean results with each insecticide were calculated. The log-probit analysis is not appropriate here because the data points are not independent.

RESULTS AND DISCUSSION

The results obtained are shown in Table 1.

Exposure of fruit flies to low doses of known insecticides in their diet causes a response that is dose dependant (Figure 1). Although contact exposure will have occurred along with possible fumigant action, this will have been of minimal significance at the low concentrations used. The fruit flies spend there time at the top of the bioassay tube unless feeding; a behaviour seen with all chemicals and controls. The control bioassay showed nothing but the natural response of ageing and death with the flies.

Table 1. Knockdown and lethal times for different insecticides.

Cypermethrin	100 mg L^{-1}	10 mg L^{-1}	1 mg L^{-1}	0.1 mg L^{-1}
$Knockdown_{50}$	12m	22m	45m	19h 45m
LT_{50}	14m	40m	55m	28h 45m
$Knockdown_{100}$	15m	25m	60m	40h 45m
LT_{100}	18m	45m	70m	45h 45m
Ecofleece®	1000 mg L^{-1}	100 mg L^{-1}	10 mg L^{-1}	1 mg L^{-1}
$Knockdown_{50}$	5m	14m	27m	1h 59m
LT_{50}	8m	19m	37m	2h 9m
$Knockdown_{100}$	7m	19m	41m	2h 29m
LT_{100}	10m	23m	50m	2h 49m
Bayticol®	1000 mg L^{-1}	100 mg L^{-1}	10 mg L^{-1}	1 mg L^{-1}
$Knockdown_{50}$	58m	4h 48m	7h 47m	20h
LT_{50}	1h 1m	5h 18m	8h 47m	24h 1m
$Knockdown_{100}$	1h 1m	5h 28m	11h 47m	24h 1m
LT_{100}	1h 5m	5h 38m	12h 47m	26h 46m
R. G. K.®	1000 mg L^{-1}	100 mg L^{-1}	10 mg L^{-1}	1 mg L^{-1}
$Knockdown_{50}$	2h 20m	46h	89h	100h
LT_{50}	2h 30m	52h	90h 30m	120h
$Knockdown_{100}$	2h 30m	54h	92h	120h
LT_{100}	3h	55h	116h	144h
B. F. S.®	1000 mg L^{-1}	100 mg L^{-1}	10 mg L^{-1}	1 mg L^{-1}
$Knockdown_{50}$	40m	50m	96h	140h
LT_{50}	1h	100h	*	*
$Knockdown_{100}$	1h	100h	123h	212h
LT_{100}	1h 30m	120h	*	*
Provado®	1000 mg L^{-1}	100 mg L^{-1}	10 mg L^{-1}	1 mg L^{-1}
$Knockdown_{50}$	2h 30m	7h 30m	92h	144h
LT_{50}	4h	14h	*	*
$Knockdown_{100}$	3h 30m	11h	180h	240h
LT_{100}	7h	17h	*	*

*No difference to the control. Times are based upon 5 replicates. The variation is approximately 10% based on the times of checking with regard to the overall time measurement. Control bioassays had a $Knockdown_{100}$ of approximately 336h and an LT_{100} of approximately 672h.

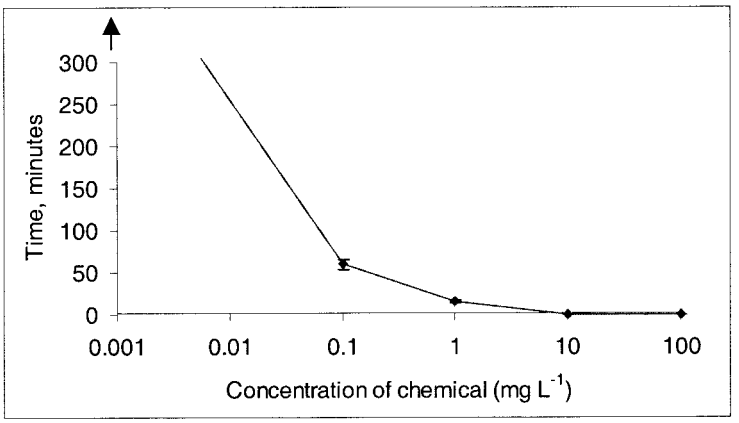

Figure 1. The Knockdown$_{100}$ response with cypermethrin. 10% error bars are shown which are greater than the calculated variances.

Figure 1 shows the Knockdown$_{100}$ result with cypermethrin. When other chemicals were tested they showed similar dose-response curve shapes but the chemical.

If the concentrations of test chemical were plotted against the Knockdown $_{50}$, thei Lethal time $_{50}$, then Knockdown$_{100}$, and finally Lethal time$_{100}$, equidistantly apart, then straight lines are produced for the majority of chemicals and concentrations. The exceptions produce straight lines if Lethal time$_{50}$ and Knockdown$_{100}$ are swapped.

One advantage of this bioassay as described is that the flies being studied survive for long periods. For example, control bioassays showed no mortality or obvious signs of loss of fitness for at least 10 days but after that time there is some fly mortality and survivorship declines.

This solid exposure bioassay has been shown as an alternative to other direct toxicity bioassays, with a particular propensity to insecticides that have a feeding route of exposure. Depending on the insecticide used the response time can vary greatly, with the cypermethrin clearly being much quicker to react. The lower concentrations are of the most interest in this bioassay as it is here where the feeding becomes more important, rather than direct epidermal exposure. There does not appear to be any published literature relating to simple ingestion bioassays using fruit flies, perhaps because of the organism themselves or the method used for this bioassay.

With regards to the methodology, the amount of test chemical will be limited by the concentration and its dispersal in the solid. The quantity actually ingested by each fly is not measured which could be problematic for a definitive dose

response although with a standard population measuring the effect on half of the flies should remove extreme results. It is possible that the lethal concentration point will be passed and so there will be a limit to the sensitivity of the bioassay, although the knockdown measurements mean that any effect could be seen and thus providing there is a difference to the controls, and a previous calibration to compare with, the concentration of chemical present could be estimated. This does rely on knowing the compound being tested, otherwise it is a toxicity test estimating the potential toxicity.

Adding the chemical to the food mixture instead of directly into the bioassay gets around many problems presented with aqueous samples. This bioassay provides a simple methodology for testing oral poisons which could have use as a preliminary test before more conventional methods are applied.

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REFERENCES

Akkerhuis G, Kjaer C, Damgaard C, Elmegaard N (1999) Temperature-dependant time-dose-effect model for pesticide effects on growing, herbivorous arthropods: Bioassays with dimethoate and cypermethrin. Environ Toxicol Chem 18: 2370-2378

Alonsoamelot ME, Avila JL, Otero LD, Mora F, Wolff B (1994) A new bioassay for testing plant extracts and pure compounds using red flour beetle *Tribolium-castaneum herbst.* J Chem Ecol 20: 1161-1177

Brogdon WG, McAllister JC (1998) Simplification of adult mosquito bioassays through use of the time-mortality determinations in glass bottles. J American Mosquito Cont 14: 159-164

Carpenter JM (1950) A new semi-synthetic food medium for Drosophila. Drosophila Information Service 24: 96-97

Chiao-Cheng JH, Reagan BM, Bresse RR, Meloan CE, Kadoum AM (1998) Carbamate insecticide removal in laundering from cotton and polyester fabrics. Arch Environ Contam Toxicol 17: 87-94

Extoxnet (2000) Pesticide Information Profile. Imidacloprid. http://ace.orst.edu/info/extoxnet/pips/pyrethri.htm. University of California at Davis, Environmental Toxicology Department.

Grant RJ (2001) A bioassay for the measurement of insecticide concentration. Arch Environ Cont Toxicol 41: 319-324

Herron G, Jiang L, Spooner-Hart R (1996) A laboratory-based method to measure relative pesticide and spray oil efficacy against broad mite, Polyphagotarsonemus latus (Banks) (Acari: Tarsonemidae). Exp Appl Acarol 20: 495-502

Keddy CJ, Greene JC, Bonnell MA (1995) Review of whole-organism bioassays; Soil, freshwater assessment in Canada. Ecotoxicol Environ Saf 30: 221-251

Nauen R, Elbert A (1997) Apparent tolerance of a field-collected strain of Myzus nicotianae to imidacloprid due to strong antifeeding responses. Pestic Sci 49: 252-258

Nigg HN, Mallory LL, Fraser S, Simpson SE, Robertson JL, Attaway JA, Callaham SB, Brown RE (1994) Test protocols and toxicity of organophosphate insecticides to Caribbean fruit fly (Diptera: Tephritidae) J Econ Entomol 87: 589-595

Wiles JA, Jepson PC (1992) In situ bioassay techniques to evaluate the toxicity of pesticides to beneficial invertebrates in cereals. Aspects Appl Biol 31: 61- 68