Original Article

The Effects of Oral Application of Cyromazine and Triflumuron on House-Fly Larvae

*B Vazirianzadeh[1], MA Jervis[2], NAC Kidd[2]

[1]Department of Medical Parasitology and Mycology, School of Medicine, Ahwaz Jundishapoor Medical Sciences University, Ahwaz, Iran
[2]Department of Ecology and Biodiversity, School of Biosciences, Cardiff University of Wales, UK

(Received 26 Nov 2006; accepted 13 Oct 2007)

Abstract

Accumulations of large quantities of wastes (manure, used litter, dead birds) which are excellent medium for fly-larvae over poultry houses provide breeding places for different groups of fly pests, with house-flies being the dominant species. This project is a comparative lab study. In this research project the larvicidal effects of cyromazine and triflumuron were studied as two Insect Growth Regulators (IGRs) to reduce the fly population using oral application. Both IGRs had a significant effect on larval mortality compared with their controls among the concentrations ($P< 0.01$, Fisher's LSD with Bonferoni correction) including a dose-dependent relationship. Comparisons among LC_{50} and LC_{90} values, using fiducial limits, showed that cyromazine was significantly more toxic to the larvae of the two strains than triflumuron. It is concluded that cyromazine should be used in a larvicidal programme to control house-fly rather than triflumuron.

Keywords: Housefly, Cyromazine, Triflumuron, Oral application

Introduction

Commercial poultry houses are rapidly expanding worldwide to meet the needs of the increasing human population (Axtell 1999). In modern production systems, poultries are housed in high densities with, consequently, accumulations of large quantities of wastes (manure, used litter, dead birds) which are excellent medium for fly-larvae, consequently adult fly production (Axtell 1986, 1999). This phenomenon provides breeding places for different groups of fly pests, with house-flies being the dominant species.

IGRs are a diverse group of insecticides, with a range of effects on insect specific phenomena, disrupting the growth and development of insects and other arthropods. They mainly affect the development of immature stages, and disrupt metamorphosis and reproduction (Graf 1993, Retnkaran et al. 1985) and are becoming more important in the management of insect pests (Grenier and Grenier 1993). IGRs include various chemical categories including: juvenile hormones, chitin synthesis inhibitors, and triazine derivatives (Retnakaran et al. 1985) with different modes of action.

Cyromazine (CGA 72662, N-cyclopropyl-1, 3, 5-triazine-2, 4, 6-triamine) represents a new class of IGRs derived from aziodotriazine herbicides (Shen and Plapp 1990). It was discovered by Ciba-Giegy Ltd. in the mid 1970s and originally developed under the trade name of 'Vetrazine', a blow-fly control agent. Cyromazine is now also applied topically to control house-fly larvae in manure ('Neporex'), as a feed–through in poultry ('Larvadex'), as well as in crop protection ('Trigard') (Moreno-Mari et al. 1996, Graf 1993).

Ultrastructural studies on larvae of *L. cuprina* have suggested that the pesticide works at the cuticular level (Jimenez-Pyedro 1995). However, Binnington (1985) and Friedel et al. (1988)

*Corresponding author: Dr B Vazirianzadeh, Tel: +98 611 3782956, Fax: +98 611 3332036, E-mail: babakvazir@yahoo.co.uk

suggested that cyromazine did not inhibit chitin formation but might be acting by disruption of the endocrine system.

Triflumuron is the common name for the chemical 2-chloro-N-[[[4-(trifluoromethoxy) phenyl] amino] carbonyl] benzamide. It was first introduced by Bayer in 1979 at the International Plant Protection Congress in Washington (Senior 1998). Triflumuron has been shown to be effective against a variety of insects of medical and agricultural significance, under laboratory and field conditions, belonging to the orders of Diptera, Orthoptera, Siphonaptera, Coleoptera and Lepidoptera. It has been used to control house-flies, mosquitoes, fleas and cockroaches in public and animal health (Retnakaran and Wright 1987, Weaver et al. 1984, Main and Mulla 1982, Miura and Takahashi, 1979). 'Alystin' and 'Starycide' are two of its trade names.

Triflumuron is a benzoylphenyl urea that acts primarily as a stomach poison, but may also have a contact action, depending on the insect species, the developmental stage of the insects, the dose applied, and the method of application (Senior 1998). Its effects are typical of benzoyl phenyl ureas, it acts to inhibit the deposition of chitin in the cuticle of arthropods. It has both ovicidal (Smith and Grigarik 1989, Broadbent and Pree 1984) and larvicidal (Smith and Grigarik 1989, Hejazi and Granett 1986, Asher and Nemny 1984) properties, depending on the species studied. In addition, it induces sterility in adult female house-flies (Howard and Wall 1995, Weaver and Begley 1982).

There have been several previous investigations using cyromazine, of triazine derivatives, as a larvicide against house-fly larvae (Keiding et al. 1991, Keiding et al. 1990, Kelly et al. 1987, Iseki and Georghiou 1986) but no comparisons have ever been made with triflumuron. The benzoylphenyl ureas, to which triflumuron belongs, are mainly used as larvicides (Reynolds 1987), but very little work has been done on the larvicidal effects of triflumuron on house-fly larvae (Weaver and Begley 1982).

In this study the toxicity effects of cyromazine and triflumuron were evaluated and compared using the oral application.

Materials and Methods

IGRs

Both IGRs were of technical grade: cyromazine (99.9% *a.i.*, Novartis) and triflumuron (99.6% *a.i.*, Bayer).

Insects

House-fly larvae were of two strains: Rentokil, a non-resistant laboratory strain, and Chicken house, a wild strain. The house-flies were reared at Insect Investigations Ltd. (Cardiff University of Wales) in constant environmental chambers (25 °C, 55%-60% RH and 16hL: 8hD). The rearing method was that of Cetin et al. (2006) and Kristensen and Jespersen (2003) with some modifications. The larvae were reared in a medium made by mixing the following ingredients: 170g dog food pellets, 20g-yeast extract (Sigma) and 30g milk powder. The pellets were placed into a plastic container, and sufficient water added to soften them. The pellets were then soaked in the water for at least one hour. The pellets, milk powder and yeast extract were together placed into a food mixer, and blended thoroughly. The resultant mixture was left for another hour, after which its time consistency was checked, and more water added if necessary. The mixture should be not much watery or thick.

Oral Application (OA)

To examine the effects of OA, second-instar larvae were used, because of their higher food consumption rate. The larvae were provided with: 0.125, 0.25, 0.5 and 1 mg/kg larval medium (Kelly et al. 1987). Distilled water was used as the solvent for cyromazine and acetone was used for triflumuron.

Twenty-five of the second-instar larvae were placed in a 250cm glass-jar containing 70gr of IGR-contaminated larval medium. The latter was treated with the highest volume of acetone, 1000µl/Kg diet, as this was the volume used in

the treated control (see above). A non-treated control did not receive any IGR or acetone. In all cases three replicates were used for both strains. The jars containing larvae were kept in a constant environmental chamber and monitored for two weeks. Emergence of the adults usually took< 10 days. The numbers of emerged house-flies at the end of experiment was counted to record the rate of survival which was then converted to the rate of mortality.

All the experiments were conducted in School of Biosciences, Cardiff University of Wales, UK.

Data analysis

All percentage mortality data were arcsine square root-transformed. They were checked for the homogeneity of their variances using Bartlett or Levene tests and for the normality of their residuals using the Ryan-Joyner test, which is similar to the Shapiro-Wilks test. One-way ANOVAs were then performed on the data followed by the Least Significant Difference (Fisher's LSD and Bonfferoni correction) method for distinguishing between the means to find any significant differences in mortality between the different concentrations and control, also amongst the concentrations. If the ANOVA table indicates a significant difference between the means, then pairs of means were compared, using LSD method to determine if they are significantly different from each other. Then concentration-mortality regression was performed by probit analysis (Chi package, 2000) to determine the LC_{50} and LC_{90} for each treatment. The Chi package is based on Finney's probit analysis (Finney, 1978) and takes Abbott's formula (Abott 1925) as a common correction. Differences in toxicity at LC_{50} and LC_{90} were considered to be significant when fiducial limits did not overlap (Sheppard et al. 1992, Stark et al. 1991).

Results

Overall one way-ANOVAs revealed significant differences in mortality among the treatments and controls ($P< 0.001$). Moreover both IGRs had a significant effect on mortality of larval mortality compared with their controls among the concentrations ($P< 0.01$, Fisher's LSD with Bonfferoni correction). The results are summarised in Table 1 and 2. There was a dose-dependent relationship in the rate of mortality of larvae with both IGRs. This was confirmed using probit analysis. Comparisons among LC_{50} and LC_{90} values, using fiducial limits, showed that cyromazine was significantly more toxic to the larvae of the two strains than triflumuron. Besides, there was no significant difference in the susceptibility of the two strains to either cyromazine or triflumuron. There was no difference in mortality between treated and un-treated controls. Acetone had no side effects on the larval mortality.

Table1. Probit analysis, using oral application of 2nd instar, for both IGRs against both strains of house-fly larvae, mortality of larvae to adults

Treatment	LC_{50} mg/kg diet	FL* of LC_{50}	LC_{90} mg/kg diet	FL* of LC_{90}	chi-squared values	df	SD of slope
cy/Re	0.207	0.176-0.238	0.566	0.473-0.727	0.362	2	0.342
cy/Ch	0.216	0.190-0.241	0.566	0.473-0.727	0.249	2	0.349
tr/Re	0.531	0.475-0.602	3.455	2.567-5.132	0.085	2	0.267
tr/Ch	0.594	0.541-0.659	3.905	3.024-5.390	0.005	2	0.272

cy/Re= cyromazine # Rentokil, cy/Ch= cyromazine # Chicken, tr/Re= triflumuron # Rentokil, tr/Ch= triflumuron # Chicken, FL*= Fiducial Limits

Table 2. Toxicity of two IGRs against house-fly larvae, using Fiducial Limits (mortality of larvae to adults), topical application of 2nd instar

Comparisons	Ratio of toxicity (LC_{50})	Significant	Ratio of toxicity (LC_{90})	Significant
trCh=trRe	1.12	ns	1.10	ns
cyCh=cyRe	1.04	ns	1.00	ns
trRe<cyRe	2.57	sig	6.10	sig
trCh<cyCh	2.75	sig	6.90	sig

trCh= triflumuron # Chicken, trRe= triflumuron # Rentokil, cyCh= cyromazine # Chicken, cyRe= cyromazine # Rentokil

Discussion

The mortality effects of OA of cyromazine agree with the results of Kelly et al. (1987) and those of Iseki and Georghiou (1986) but do not accord with the results of Scotte et al. (2000). The reason for these mismatches in the latter case is unclear. The obtained results of cyromazine in this study have been confirmed by using of 50% cyromazine to larval foci followed by cultural and biological control methods as a field trial in poultry houses (Crespo et al. 2002). They obtained satisfactory reduction on the house flies population to overcome on insecticide resistance population. Kocisova et al. (2004) obtained a 74-81% reduction in the population of house flies over a 5 weeks field trial, using Neporex (*a.i*. 2% cyromazine) against second and third larval instars which confirmed the larvicidal effects of cyromazine. Novartis technical brochure (2006) explains a 100% efficacy in the mortality of house fly larvae in the different instar larvae by using an oral application. This result is similar to the results of current study.

A dose-dependent relationship in the rate of mortality of larvae with cyromazine in the current study agrees with the results of Tomberlin et al. (2002). They obtained similar results using cyromazine in an oral application against black soldier fly, *Hermetia illucens* (L.).

The obtained rates of mortality with OA of triflumuron confirm the results of Weaver and Begley (1982), as they reported general dose-dependent of mortality. However they used percentages of puparium formation to calculate the rate of mortality, whereas in this study, successful emergence of adults was used. In addition, the results of Howard (1995) based on topical application of adults show a dose- dependent for mortality of house-fly larvae, using triflumuron. The results of the present study are confirmed by study of Tong and Hui (2004), using a similar application method against housefly larvae too,

The significant larvicidal properties of triflumuron is confirmed by studies of Batra et al. (2005), Sulaiman et al. (2004) and Ladonni and Farashiani (2000) against *Aedes aegypti* and *Anopheles stephensi* larvae in field trials of India, Malaysia and Iran, respectively. These agree with larvicidal effects of triflumuron in the current study. The obtained results using novularon, an IGR of benzoylurea, in a laboratory feeding larvicidal application against house fly larvae, by Cetin et al. (2006) agree the results of present study using triflumoron as an IGR of benzoylurea.

Results of the present study reveal that, both IGRs, can affect significantly on mortality of house-fly larvae (Table 1 and 2), and that cyromazine was more toxic than triflumuron in all cases. Cyromazine was 2.57 and 2.75-fold more effective than triflumuron at LC_{50} and 6.10, 6.90-fold more effective at LC_{90} against Rentokil and Chicken house strains, respectively, than triflumuron.

Furthermore, using OA both strains showed the same degree of susceptibility to each IGR (Table 1 and 2). Therefore it is concluded that

cyromazine should be used in a larvicidal programme to control house-fly.

Also, several researchers have found a lack of cross-resistance between cyromazine and other IGRs: lufenuron (Wilson 1997), diflubenzuron (Farkas and Plapp 1991), methoprene (Bloomcamp et al. 1987). Furthermore, no cross-resistance was reported between cyromazine and conventional insecticides, organophosphorous, pyrethroids and organochlorines (Keiding et al. 1991, Shen and Plapp 1990) and cyromazine as a larvicide. Keiding (1991) reported no increase in LC_{50} and LC_{90} values of cyromazine as a larvicide, after five or six selections.

In conclusion according to the above mentioned results and facts using cyromazine in a larvicidal program is preferred to triflumuron.

Acknowledgements

We are very thankful to the Ministry of Health, Iran, and Department of Ecology and Biodiversity, School of Biosciences, Cardiff University of Wales, UK, for finance supporting and providing the facilities to carry on this research project, respectively.

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