The efficacy of insecticides (Fendona and Malathion) against larvae and adult stages of *Musca domestica*

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Abstract. Malathion and Fendona® (alphacypermethrin) insecticides were tested against larvae and adult stages of house flies, *Musca domestica* Linnaeus. The percentage mortality of House flies larvae range between 0% and 100% at different exposed dose rate of tested insecticide. Probit analysis was used to determine the $LD_{50.}$ The value LD_{50} for malathion without food medium was 0.833 µl/larvae and treatment with food medium was 0.895 µl/larvae respectively. The LD_{50} for Fendona® without food medium was 0.545 µl/larvae and 0.870 µl/larvae for treatment with food medium. The LD_{50} values for Fendona® in both treatment was lowers compared with malathion, which means that alphacypermethrin is more toxic than malathion. Different with the test on adult house flies, the knockdown time values for alphacypermethrin at KT_{50} is 9.876 minutes and KT_{95} is 20.495 minutes against female adult house flies and were significantly lower than malathion (KT_{50} is 75.17 minutes and KT_{95} is 103.38 minutes respectively). Result also showed that a total of 1.0µl of Fendona® is reacting more effective than 1.0µl of malathion. Therefore, we can conclude that Fendona® is the most effective insecticide towards larvae and adult stage of *M. domestica*. The effectiveness of both chemicals was affected when treated larvae was in the rearing medium.
Keywords: Insecticides, Fendona®, alphacypermethrin, Malathion, *Musca domestica*

Introduction

The house-fly, *Musca domestica*, not only is a nuisance pest, but can also transport important disease-causing organisms. Although this fly species does not bite, its populations can irritated people and animals by flying around and landing on them or on their food and leave regurgitation and fecal spots on the surface (Hinkle 2002, Steenberg and Jespersen 2002, Winpisinger *et al*. 2005). They can mechanically transmit bacteria, protozoa, worms, fungi and viruses amongst humans and animals. Ventures to control *M. domestica* have been aimed via various methods, but essentially at controlling the adult populations. Among the control approaches, chemical control or insecticide are the most preferred by farmers (Yap *et al*., 2003). The use of insecticides to reduce housefly populations (Lee *et al.,* 2003) worked well in some cases, but only for limited periods and has resulted in resistance development. House fly resistance to organochlorine, organophosphate, carbamate and pyrethroid insecticides has been reported in several regions of peninsular Malaysia. Due to the high resistance of house flies to various insecticides, the spectrum of the effective ones has shrunken and as a consequence, it is of utmost importance to look for new strategies to manage *M. domestica.*

The feeding behavior of the house flies are on almost any organic material such as sugar, milk, rotting vegetables, animal feces and carcasses and the mode of feeding depends on the physical state of the food (Lee *et al*. 2003). In Malaysia, *M. domestica* is always found in association with humans or activities of humans and is a serious nuisance pest. Its nuisance role is obvious especially in the North-South highway restaurants in Peninsular Malaysia. This species is the most common species found poultry farms and village areas cutting across the highways. In such areas, flies commonly develop in large numbers and this is a serious problem requiring control. As most house flies, *M. domestica* lay eggs on animal feces and garbage, in close proximity, leading to large masses of larvae and pupae. Maggots feed on and develop in the material where the eggs are laid. When the maggots are full grown, they crawl to a drier region of the breeding material and transform to the pupal stage. The crawling behavior is central to the successful application of an insecticide-based control of house flies. During crawling, an infected larva can reduce the amount of product sprayed onto its body and this has the potential to reduce insecticide efficacy. Surprisingly, the role of crawling as it affects susceptibility to insecticides has attracted little research interests.

Rather than simply investigating the susceptibility of adults to insecticides currently in use against *M. domestica* in Malaysia (Malathion and Alphacypermethrin), we also attempted to find out whether crawling effects on larval vulnerability to these chemicals.

Materials and Methods

Sample collection and colonization

M. domestica used in this study was collected as pupae from a poultry farm located at Kampung Lima Kongsi, Penang, Malaysia. Pupae were placed in insect adult cage made from plywood (40 cm x 40 cm) and provided with water, sugar and milk powder. Two hundred grams of dog food was mix with water at the ratio 1:1 and placed into adult cage as breeding medium for adult flies. Adult flies will lay their eggs on breeding medium and eggs hatched as 1st instar larvae after 8 to 12 hours. The larva rearing tray contained larvae were removed from the cage and placed in laboratory at the temperature of 26 ± 2°C and 70% relative humidity. After 5 days the larva rearing medium containing larvae were transferred into a tray containing vermiculite. Matured 3rd instar larvae will crawl out from larva rearing tray into pupa tray containing vermiculite for pupation. House fly pupae from pupa tray in vermiculite will be place into adult cage for rearing to next generation. Adult flies in second generation will be used for insecticide testing in laboratory.

Insecticides and Larval bioassay

The organophosphate Malathion and the pyrethroid Alphacypermethrin were used for larval and adult bioassays. Malathion interferes with the nervous system by blocking an enzyme that normally acts as an off switch by ending the signal. Without the enzyme, the nerve keeps firing and eventually the nervous system fails. Pyrethroids such as Alphacypermethrin, called also Fendona® are synthetic forms of pyrethrins which act as contact poisons, affecting the insect's nervous system (Smith and Stratton 1986).

Malathion and Alphacypermethrin are the insecticides that were used to test against larva stage. Four replicates for each evaluation which are control, 0.2µl, 0.4µl, 0.6µl and 0.8µl. were used in this study with two sets of evaluation. Thirty third instar larvae were used in this study. Different dose of insecticide were applied topically on each of house fly larva using micropipette. The larvae were then let to be dried in petri-dish. For first treatment, 15g of dog food as rearing medium while the other treatment was without rearing medium. The rearing medium was used to test the residual effect of insecticide left in the rearing medium or dilution effect of chemical due to exposure to rearing medium that might influenced the mortality rate of house fly larvae. The mortality of larvae was observed after 24 hours and all experiments were done in laboratory with the temperature of 26ºC and relative humidity of 70%.

Adult bioassay and Data collection and analysis

The mature adult flies were reared in laboratory for two generation before being used in this study. Adult fly in the cage was placed in refrigerator at 0 ºC for 30 minutes for anesthesia effect. The flies were removed from refrigerator and placed individually on ice cube cover with a layer of plastic sheet. 1.0 µl of Alphacypermethrin or Malathion was applied topically on the thorax of adult fly using micropipette. For the control experiment, 1.0 µl of distilled solvent were used to treat adult fly using similar method as above. Treatment and control group were placed in adult cage in the laboratory at 26^{o}C and 70% relative humidity. The mortality rate of treated and control group were observed for 24 hours.

SPSS program with version 17.0 was used to obtain the probit analysis of LD_{50} for each experiment while SigmaPlot was used to create a graph to show the mortality rate of the larvae. Knockdown (KT_{50}) is the time when 50% of tested insect unable move or dead. The data on knockdown time of Alphacypermethrin and Malathion were analyzed by the probit analysis program 17.0 for window.

Data on survival rate of house flies on ice cube were analyzed using Student's t-test. The treatment group was compared with control group to show any significant in survival rate. Weight of house flies population was calculated by using the mean of the sample and correction of time and effect of chemicals on insect will be analyzed by Bivariate Correlation at 95% confidence level.

Results and Discussion

Larvacidal activity

House fly larvae treated with Alphacypermethrin showed high mortality rate (80.53%) at dose rate of 0.8µl/ larva without rearing medium compare with Malathion treated group with rearing (0% mortality). LD_{50} value for Alphacypermethrin without rearing medium was 0.545 µl/larvae (χ^2 = 25.878, df = 14; p >0.15) and it was significantly lowest compare with other treatment. LD_{50} for Alphacypermethrin with rearing medium was 0.870 µl/larvae, LD_{50} for Malathion treated with rearing medium was the higher, 0.895 µl/larvae (χ^2 = 9.576, df = 14; p > 0.15) and LD_{50} Malathion without rearing medium was 0.833 µl/larvae (χ^2 = 8.119, df = 14; p > 0.15)(Table 1).

Table. The LD_{50} values for Alphacypermethrin and Malathion treated groups.

	Sample (n)	LD_{50} (%)	Regression Coefficient ± Standard error	X^2 (df)
Alphacypermethrin with food medium	120	0.870	2.400 ± 0.328	9.576 (14)
Alphacypermethrin without food medium	120	0.545	3.669 ± 0.336	25.878 (14)
Malathion with food medium	120	0.895	4.575 ± 0.652	7.147 (14)
Malathion without food medium	120	0.833	2.574 ± 0.339	8.119 (14)

Adultcidal activity

Mortality of house flies after paralyzed on ice cube at different time

The survival rate of flies treated on ice cube was high(100%) and no significant different (t = 0.05, P > 0.05) compare with control group at time of 5 minutes, 10 minutes and 15 minutes treatment. The result shown that adult house flies still survived or recover to normal condition when it was paralyzed from 5 to 15 minutes on the ice cube and do not influence mortality of insect or the result of insecticides in bioassay treatment.

Weight of house fly and Knockdown Time (KT) of house flies

The average weight of adult house fly was 0.0067mg. The weight of fly is very importance to determine the dose rate of treatment. From probit analysis the knockdown time of adult house fly for Malathion treated group was 75.168 minutes (KT_{50}) and 103.378 minutes (KT_{95}) much higher compared with Alphacypermethrin group 9.876 minutes (KT_{50}) and 20.496 minutes (KT_{95}). Both insecticides were very effective against adult house flies and causing 100% mortality after 24 hours treatment.

In this study, we noted topical amount of Alphacypermethrin caused higher mortality in *Musca domestica* larvae tested without rearing medium. However, Alphacypermethrin used with the food medium did not show higher mortality. LD_{50} value for Alphacypermethrin tested without food medium is the lower compared with Malathion especially with food medium. LD_{50} for Malathion without food medium is lower than Alphacypermethrin with food medium. This showed that rearing medium is influence the efficacy of larvicide. As a result, Alphacypermethrin is the most effective larvicide treated without rearing medium.

The insecticide residual left over in the rearing medium seems does not effective in killing house fly larvae and insecticides treated with rearing medium may be diluted the dosage of insecticide causing not effective in killing the larvae. According to Chandre *et al*., 2000, the mortality rate of larvae was directly correlated with the degree of contact between insects and the insecticides. The degree of contact may be achieved in the field but is difficult to predicted, but it is always less than theoretical amount (WHO, 1996).

There was no larva mortality after 24 hours insecticide exposure at lower dosage of Malathion. This could be caused by lower toxicity of insecticide or the flies developed resistance to Malathion. According to Service, 1996, house flies have developed resistance to Malathion in certain areas**.** Widespread of resistant to Organochlorines have forced the change of this insecticide to Organophosphate to ensure the effectiveness of control programme (WHO, 1984). The same problem occurred to organophosphate where they have to use Pyrethroid to overcome the problem (Brogdon & McAllister, 1998). One of the major resistance mechanisms in insects is due to the elevated level of non-specific esterases (Lee *et al.,* 2003)**.** The role of acetyl cholinesterase enzyme is to clear the system before another nerve transmission occurs (Pedigo, 2002). As a consequences, rapid nerve firing occurs, producing symptoms of restlessness, tremors and convulsions, paralysis and death to insects due to exhaustion of energy system (Lee *et al.,* 2003).

Insecticides resistance can occur when a population if insects has been subjected to insecticide selection pressure for a very long time or the frequency of usage or exposure rate are very high (Lee *et al.,* 2003). Resistance to insecticide also can occur due to cross-resisrence to other group of insecticide such resistant to Dieldrin also have cross-resistance to Heptachlor and Tocaphene. The result of this study showed high survival rate of insect when the house fly treated on ice cube for 15 minutes and similar with study by Feldmen-Muhsam B. (1944) when the house flies exposed to temperature of $0^{o}C$ up to 12 hours, and the insect are able to recover quickly from their state of paralysis. This treatment proved that paralysis of house flies on ice cube during the experiment was not effecting the mortality of the house flies during bioassay test.

Different body weight of house fly used in this experiment (6.7 mg) as compared with Palacios *et al*., 2009 study (10mg) but the result shown similar patent. The weight of flies does not affecting the result because dose rate of insecticide are calculated base on the weight of the house flies. The insecticides are very effective in killing the house flies even the knowkdown was not immediate in this study. The 100% mortality was observed in Alphacypermethrin treated insect after 24 hours exposure as compare with Malathion treated insect. This study proved the residual effect of chemical and remain effective even after long hours used. The overall analysis of the data can be concluded that Alphacypermethrin is more effective and provide faster knockdown effect compared with Malathion. The knockdown values at KT50 and KT95 for Alphacypermethrin is higher than Malathion. According to the report from WHO, (2003), the knockdown time KT_{50} of adult house flies using Alphacypermethrin is 9.9 minutes similarly with this experiment 9.876 minutes. The result from Lane *et al.* 1978 study showed KT_{50} of house flies by using Malathion is 22 minutes to 30 minutes but much lower than this study (75.168 minutes).

Conclusions

This study proved Alphacypermethrin is highly toxic and more efficient than Malathion in controlling house flies problems in the poultry farms and it also can be summarized that Pyrethroid insecticide is more efficient than Organophosphate group insecticides at larvae and adult stage of house fly. Both chemical Malathion and Alphacypermethrin still suitable to be used in the poultry farms since both can provide 100% mortality at different concentrations. The appropriate chemical formulation and fast dispersal increased the efficacy of the insecticide and resulting in faster knockdown time and Alphacypermethrin has been proven better and effective as fly repellent that provided rapid knockdown and suitable at different environmental conditions and the best insecticide to be used in poultry farm.

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