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Toxicity of Organophosphate Insecticides to Adult Housefly, *Musca domestica*

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Abstract

The present study was made to find out the dose mortality experiments using several most commonly used insecticides applied in agriculture. Interaction between insecticides and insects in mortality offers the chance of evaluation of the level of toxicity. The present study has been done with four organophosphate insecticides, viz. malathion, chlorpyrifos, diazinon and phenthoate. The toxicity test was carried out by the topical application (contact bioassay) on adult housefly in different doses. Insecticides used in this investigation were active against male and female housefly, *Musca domestica.*

The LD_{50} of malathion, chlorpyrifos, diazinon and phenthoate were calculated as 0.823, 0.909; 0.249, 0.276; 2.519; 2.114, 2.151, 1.849 µg/fly-1, respectively. The aim of the study was to find out mortality of general used of insecticides applied in the field for controlling pests.

Keywords

Insecticides, Organophosphate, Toxicity, Housefly, *Musca domestica,* LD_{50}

Introduction

Chemical insecticides have been considered an essential component of insect pest control since the early 1950s. Increasing insecticidal use since world war in the result of several advantages for insect control. Insecticides have brought inestimable benefits to humanity in terms of human lives also saving of public health, sufferings diminished and brought about economic gain (Metcalf, 1968; Smith and Van den Bosch, 1967).

Insecticides are the most powerful tools available for use in pest management. They are highly effective, rapid in curative action, adaptable to most situations, flexible in meeting changing agronomic and ecological conditions, and relatively economical. Insecticides are the only tool for pest management that is reliable for emergency action when insect pest populations approach or exceed the economic threshold. The misuse, overuse and unnecessary use of insecticides (Bailey and Swift, 1968) have been the most important factors in growth of interest in pest management, and indeed this concept seeks to maximize the advantages in their use and minimize the disadvantages. It should always be remembered that the application of insecticides represents purposeful environmental contamination and can be justified only where benefit or risk ratios are clearly titled in favour of insecticide use.

An insecticide can only control an insect pest if it is suitably toxic and applied in such a way that it reaches its intended target. Many interacting factors are involved in the process of ensuring a suitably formulated, toxic, active ingredient is applied to provide adequate coverage and subsequent pickup and mortality of the insect. It is first necessary to ensure that under optimal conditions, the active ingredient is sufficient by efficacious then that the application parameters produce droplets on deposits that can be picked up and transferred to the targeted stage of the insect life cycle.

Laboratory bioassays to establish insecticide efficacy include standardization of insect species, stage, sex, age and physiological and behavioural conditions (Dent, 1995), since all these factors will influence the susceptibility of a pest. Extrinsic factors such as temperature, humidity, feeding and time of treatment, density of treated insects and illumination also have an impact on susceptibility and need to be standardized. The insecticide may be applied topically to the outer surface of the insect using micropipettes or special syringes.

Houseflies may be contaminated with several species of pathogenic bacteria (*Sukontason et al.,* 2000) and are correlated with diseases, such as gastroenteritis, ulcers, nosocomial infections, dysentery, cholera and tuberculosis (Grubel *et al.,* 2000; Sulaiman *et al.,* 2000; Olsen and Hammack, 2000; Fotedar, 2001). Also the potential of houseflies for transmitting viruses was demonstrated by Tan et al., 1997. By transmitting diseases, houseflies cause annoyance to man and animal. The present study was designed to test the lethal effect of different insecticides on adult houseflies with topical method.

The objective of the study is to evaluate efficacy and to compare efficacy of different organophosphate insecticides on adult housefly *(Musca domestica).*

2. Materials & Methods

Test organisms: The adult housefly stock cultures were maintained in the Integrated Pest Management Laboratory, Institute of Biological Sciences, University of Rajshahi. To produce a consistent quality insect at an economical cost, the culture process was conducted by rearing the houseflies according to the methods described by Morgan (1980-81), Morgan and Patterson (1978) and Morgan *et al.* (1978).

The adult houseflies were provided with a medium in which they lay eggs. This medium was presented in plastic pots (250 ml). The medium consisted of 9g milk powder and 5g yeast dissolved in 100ml of water and added to 100g wheat bran, following method of Wilkins and Khalequzzaman (1993). The mixture was then thoroughly stirred and put into the pots leaving 3cm from the top. The pots were placed in the cage for 24 hours. During that time the females laid batches of eggs, each batch containing about 100-150 eggs.

Batches of approximately 100 eggs were separated out and transferred to similar pot containing the same mixture and fitted with plastic lids with gauze centers. These were then placed in an incubator at 28? ± 0.5?. Within 8-12 hours' eggs were hatched and larvae developed into pupae from 5 to 8 days. Pupae were collected and kept in separate pots at the same temperature and newly emerged into adults approximately of same age.

When imagoes became adult, they were sexed by examining the adult's abdominal tips. The abdomen of the males is somewhat smaller than that of females. The male bears large black spot with bristles, but the female bears smaller black spot having no bristles. The male has no ovipositor, but the female has an ovipositor.

Selection of Insecticides : Four organophosphate insecticides used in this investigation were malathion, diazinon, chlorpynifos and phenthoate. Malathion was procured as Limithion 57EC by Cheminova, Denmark. Chlorpyrifos was procured as Classic 20EC by ACI Limited of Bangladesh (Lupin Agrochemicals). Diazion was procured as Diazinol 60EC by ACI Limited of Bangladesh (The Limit Agro-products Ltd.). Phenthoate commercially available as Cidial by ACI Limited of Bangladesh was used. The insecticides were diluted in acetone and different doses were made. Bioassay method including a control dose of only acetone for each insecticide were used in this study.

All experiments were carried out using *Musca domestica* reared at 28? ± 3?, 70% relative humidity and fed on milk and sugar cubes. The flies were sexed and treated with insecticide separately.

Contact bioassay: Bioassays were carried out by topical application of a 1µl drop of an insecticide solution of different concentration to the thoracic notum of 5 days old adult flies (anaesthetized flies) with a Hamilton gas tight micro syringe (No. 630) . Different concentrations were used in which eight replications of flies (each having 10 flies) were treated. One batch of control flies were maintained in which only solvent was applied topically.

A set of ahoc experiments of each insecticide with a particular dose before the final experiment were done, to obtain the dose in which mortality rate was 10-90%. Then the actual experiments were carried out with the appropriate doses.

The treated flies were kept in a beaker (500 ml) with a wad of cotton soaked with sugar solution as food. Mortality of test insects was recorded 24 hours after treatment. Moribund insects were counted as dead. The insects, which could not walk and failed to respond even after touching with an entomological forceps were also considered as dead.

Mortality of the treated flies was recorded after 24 hours of treatment. Corrected mortality percentage was calculated using Abbott's formula (Abbott, 1925; Busvine, 1971)

Each trail was repeated 2-3 times to obtain more uniform results and a smaller error. Probit analysis was done according to Busvine (1971) and Finney (1971) using a software developed in the Department of Agricultural and Environmental Science, University of Newcastle Upon Tyne, United Kingdom. The program also calculates confidence limits for LD_{50}.

3. Results

The results obtained from the toxicity experiments were shown in tabular form and probits were shown in graphical presentation. (fig.1)

Initial bioassays were carried out to establish the effect of the insecticides. Different doses of malathion, chlorpyrifos, diazinon and phenthoate were applied topically on adult housefly *Musca domestica*. The calculated LD_{50} value on adult houseflies were 0.824, 0.250, 2.519, 2.151 µg/fly-1 for male and 0.909, 0.276, 2.114, 1.849 µg/fly-1 for female treated on malathion, chlorpyrifos, diazinon and phenthoate respectively.

The results indicate that chlorpyrifos was the most toxic and diazinon was the least toxic to both male and female houseflies. The order of toxicity of the insecticides was chlorpyrifos> malathion> phenthoate> diazinon for both male and female houseflies. The regression equations, 95% confidence limits and? 2 (df) values are presented in the table 1 below.

Table1 LD_{50}, 95% confidence limits and regression equations of the tested insecticides (topical application) on adult houseflies (*Musca domestica*) after 24 hours of treatment

Male Houseflies

Insecticide	LD50 (µg/cm^2)	95% confidence lower limit (µg/fly^{-1})	95% confidence upper limit (µg/fly^{-1})	Regression equation	χ2 (3 df)
Malathion	0.823	0.695	0.975	Y=3.257242+1.9031X	0.749
Chlorpyrifos	0.249	0.212	0.293	Y=2.250086+ 1.968246X	0.723
Diazinon	2.519	2.129	2.979	Y=2.356537+ 1.886506X	0.691
Phenthoate	2.151	1.841	2.513	Y=2.208063+ 2.094941X	1.605

Female Houseflies

Insecticide	LD50 (µg/cm^2)	95% confidence lower limit (µg/fly^{-1})	95% confidence upper limit (µg/fly^{-1})	Regression equation	χ2 (3 df)
Malathion	0.909	0.755	1.094	Y=3.362447+1.70838X	0.041
Chlorpyrifos	0.276	0.236	0.322	Y=1.987593+2.089705X	0.297
Diazinon	2.114	1.808	2.471	Y=2.244117+2.079653X	0.550
Phenthoate	1.849	1.577	2.168	Y=2.421213+2.035317X	0.869

For the doses of malathion, the offered LD_{50} was 0.823 µg/fly-1 for male and 0.909 µg/fly-1 for female with 95% confidence limits as 0.695 to 0.975 µg/fly-1 for male and 0.755 to 1.094 µg/fly-1 for female. The regression equation for male flies is

Y=3.257242+ 1.9031X and for female flies is Y=3.362447+1.70838X. With the ? 2 value of 0.749 for male and 0.041 for female houseflies at 3 degrees of freedom. No significant heterogeneity was found in male and female house flies.

In cases of chlorpyrifos, the offered LD_{50} was 0.249 µg/fly-1 for male and 0.276 µg/fly-1 for female with 95% confidence limits as 0.212 to 0.293 µg/fly-1 for male and 0.236 to 0.322 µg/fly-1 for female. The regression equation for male flies is Y=2.250086+1.968246X and for female flies is Y=1.987593+2.089705X. With the ? 2 value of 0.723 for male and 0.297 for female houseflies at 3 degrees of freedom. No significant heterogeneity was found in male and female house flies. It was found that chlorpyrifos was highly toxic to houseflies.

Furthermore, diazinon, the offered LD_{50} was 2.519 µg/fly-1 for male and 2.114 µg/fly-1 for female with 95% confidence limits as 2.129 to 2.979 µg/fly-1 for male and 1.808 to 2.471 µg/fly-1 for female. The regression equation for male flies is Y= 2.356537+ 1.886506X and for female flies is Y=2.244117+2.079653X. With the ? 2 value of 0.691 for male and 0.550 for female houseflies at 3 degrees of freedom. No significant heterogeneity was found in male and female house flies. It was found that diazinon was a less toxic insecticide to the housefly.

Lastly, for the doses of phenthoate the offered LD_{50} was 2.151 µg/fly-1 for male and 1.849 µg/fly-1 for female with 95% confidence limits as 1.841 to 2.513 µg/fly-1 for male and 1.577 to 2.168 µg/fly-1 for female. The regression equation for male flies is Y=2.208063+ 2.094941X and for female flies is Y=2.421213+ 2.035317X. With the ?2 value of 1.605 for male and 0.869 for female

houseflies at 3 degrees of freedom. No significant heterogeneity was found in male and female house flie.

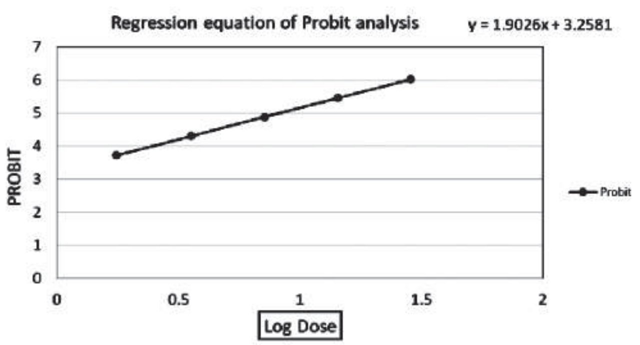

Figure : 1-Toxicity of Malathion treated on Male Housefly after 24 hours

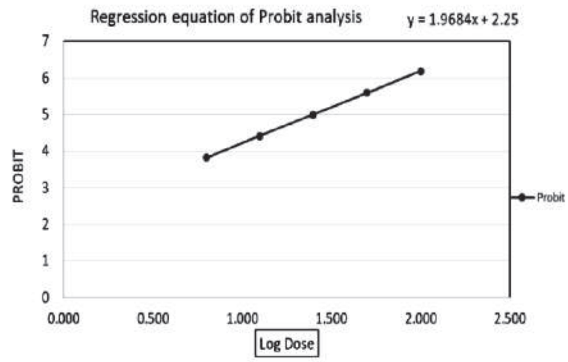

Figure : 2-Toxicity of Chlorpyrifos treated on Male Housefly after 24 hours

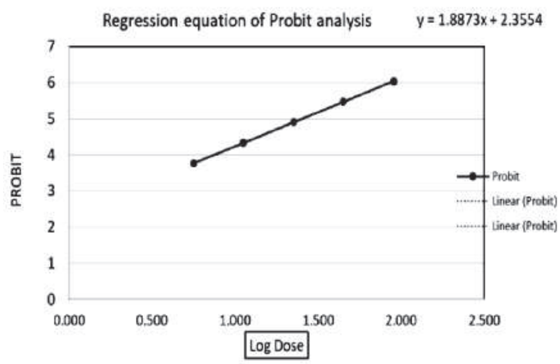

Figure : 3-Toxicity of Diazinon treated on Male Housefly after 24 hours

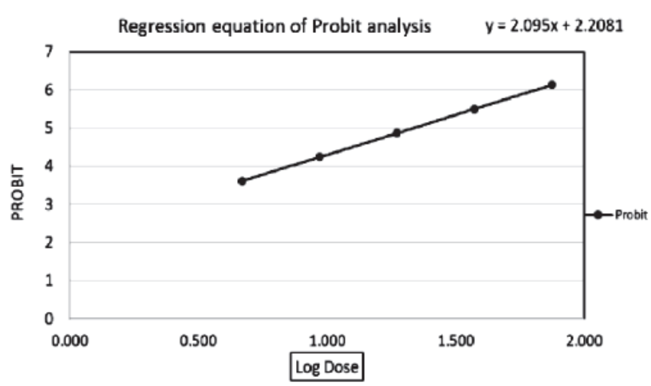

Figure : 4-Toxicity of Phenthoate treated on Male Housefly after 24 hours

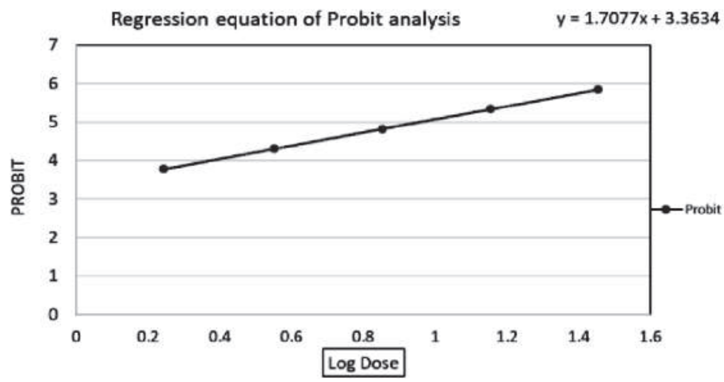

Figure : 5-Toxicity of Malathion treated on Female Housefly after 24 hours

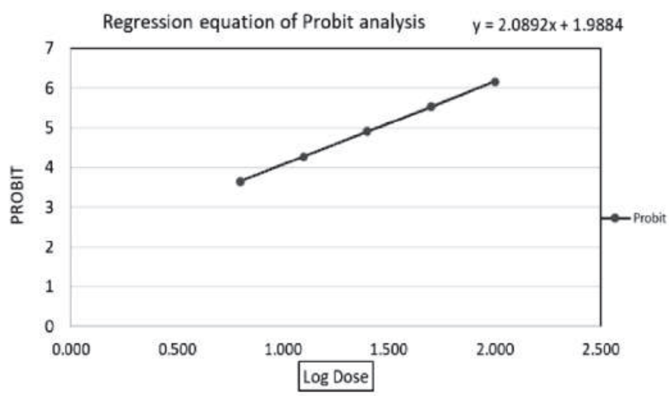

Figure : 6-Toxicity of Chlorpyrifos treated on Female Housefly after 24 hours

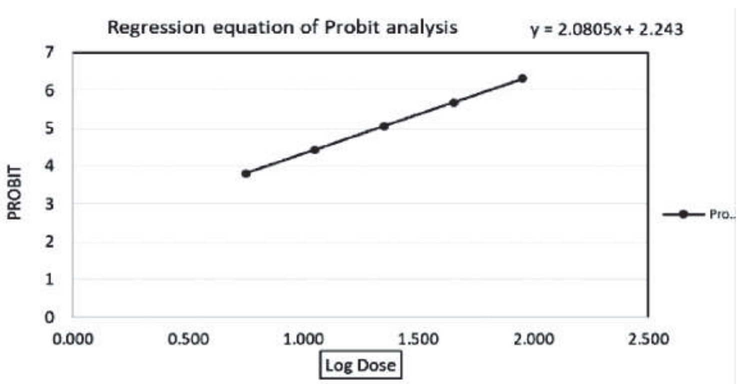

Figure : 7-Toxicity of Diazinon treated on Female Housefly after 24 hours

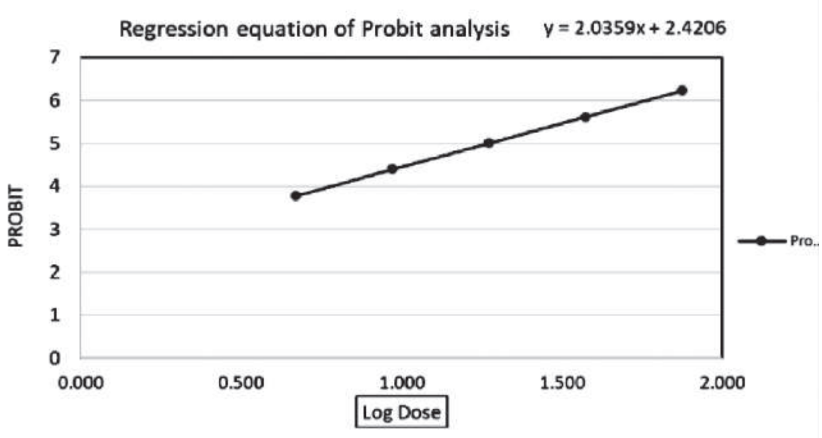

Figure : 7-Toxicity of Phenthoate treated on Female Housefly after 24 hours

Figure : 1-Graphical Presentation of Probit Analysis

4. Discussion

The common housefly has been extensively utilized as a test organism to screen candidate insecticides, chemosterilants and insect growth regulators by scientists in public or private research institutions, since the immature stadia can survive in various substrates, entomologists have normally used materials that are available locally (Spiller, 1964,1966; Louw, 1964; Sawicki, 1964; Keiding and Arevad, 1964; Schoof, 1964). Organophosphate insecticides are of great importance in this regard. In this investigation organophosphate insecticide; malathion, chlorpyrifos, diazinon and phenthoate was against adult housefly *Musca domestica*. The present investigation revealed that from organophosphate insecticides, chlorpyrifos was more active than other insecticides to control flies. According to the calculated topical LD_{50} values, chlorpyrifos was the most toxic (LD_{50} 0.249 µg/fly-1 and 0.276 µg/fly-1 for male and female respectively) while diazinon was the least toxic (LD_{50} 2.519 µg/fly-1 and 2.114 µg/fly-1 for male and female respectively).

The primary Parasympathomimetic effects of organophosphate insecticides, in both the pest organisms and in mammals, are attributes in part or entirely to phosphorylation of a serine residue to the active site of acetylcholinesterase (AChE), a critical enzyme in the nervous system. It was hypothesized by Johnson (1975) that organophosphate compound induced delayed neurotoxicity is attributable to a large degree of inhibition of an enzyme characterized as neurotoxic esterase (NTE) in nervous system, but not to inhibition of AChE.

Arrange of mechanisms have been implicated in resistance of houseflies (Devonshire, 1975; Oppenoorth, 1982; Scott et al., 2000) organophosphate compounds undergo various metabolic reactions in living organisms. Recent studies revealed that these enzymes show clear stereo selectivity in the metabolism of topically active organophosphate compounds (Ohkawa, 1982). In the present investigation four organophosphate insecticides showed different toxicity levels to adult housefly. Malathion is a non-systemic insecticide and acaricide of low mammalian toxicity. Metabolism is by hydrolysis of the carboxylate and phosphorodithioate esters or oxidation to the phosphorodithioate (sometimes known as malaxon); methods for their separation have been reported (Worthing and Walker, 1987).

The LD_{50} of malathion has been recorded in the present investigation as 0.825 and 0.909 µg/fly-1 for male and female flies, respectively. Nicholson and Sawicki (1982) recorded the LD_{50} of malathion as 0.27 µg/fly-1 on cooper stain and 30 µg/fly-1 on lapis stain of housefly after 24 hours of topical application. Acute oral LD_{50} of malathion for rats is 2800 mg/kg.

5. Conclusion

Present investigation reveals the influence of chemical insecticides on adult housefly toxicity (mortality) to find efficient ways to chemically control this pest. The goal was to search for insecticides to be used in the field that follow the best available profile; rapidly kills adults and decreases egg laying. The effectiveness of an insecticidal treatment is influenced not only by the toxicity of the insecticide but also by the primary response of the insect to its mode of application. The objectives when attempting to eradicate pest infestation is to obtain maximum pick up of toxicant by the pest and the chances of this are reduced when the dust or spray repels the pest. Each successful use of such a substance will decrease the need for toxic pesticides and will ultimately be of benefit to the environment, ecology and mankind.

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