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Potentiation of Malathion by Other Insecticides Against Adult Housefly

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Abstract: With a view to potentiate malathion by other insecticides, experiments were carried out with eight insecticides like, dichlorovos, pirimiphosmethyl, phenthoate, monocrotophos, diazinon, cypermethrin, lambda-cyhalothrin, and propoxur. All tests were done by the topical application of the insecticide mixtures on adult housefly, *Musca domestica* L. Toxicity of malathion was determined when applied alone and low dose of each insecticide was mixed with malathion in ratios of 1:1, 1:2, 1:5, 1:10 and 1:20. Separately. The mortality data after 24 hours of application showed that nearly all insecticides offered increased toxicity of malathion. But when it was applied jointly with lambda-cyhalothrin and cypermethrin the potentiation results were maximum.

Key words: Malathion, insecticide, co-toxicity coefficient, *Musca domestica*

Introduction

Resistance to pesticides is one of the most serious problems facing agriculture today. Resistance within or between whole classes of insecticides is an ever-increasing problem for control of major crop pests (Kanga *et al.*, 1999; Bues *et al.*, 1999; Graham and Janet, 2000; McKenzie, 2000). Resistance can lead to increased application rates, increased frequency of pesticide use, and ultimately loss of efficacy. We cannot assume there will be an unending supply of new insecticides to replace current compounds, due to the increased difficulty in discovering pesticides and the tremendous cost of their development (Georghiou, 1986; Hammock and Soderlund, 1986). The present way of dealing this problem is just to increase the dose of the insecticide to eliminate the more resistant populations. However, the result of this is to apply a greater selection pressure on the population. This ultimately enhances the evaluation of resistance in the population and obviously this procedure can not go on infinitely, since increasing the dose and thus the presence in the environment of a pesticide not only increases the non-target organisms mortality, but level could reach where mammals, ultimately man would be seriously affected. Therefore, the introduction of synergists to these systems could be of great benefit both economically and ecologically, especially since tests have shown that synergistic increases in toxicity of insecticides is only towards insects and not mammals (Metcalfe, 1992).

These interactions may be viewed as beneficial if the control of target pests is accentuated by unintended joint action. It has also been suggested that the advent of resistance to insecticides may be forestalled through the use of potentiations (Knowles and El-Sayed, 1985), that the control of recalcitrant pests may be increased through the use of joint action (El-Sayed and Knowles, 1984a), and that resurgence of certain pests could be prevented with potentiations (El-Sayed and Knowles, 1984b). Insecticide resistance in the housefly, *Musca domestica* (L.), has been recognized for many years. The rapid life cycle of houseflies in livestock farms gives a high potential for the selection of resistance when insecticides are applied intensively; such as through the repeated use of residual sprays (Keiding and Jespersen, 1986). The objective of this study was to assess the ability of malathion to act as a potentiation with a series of insecticides belonging to the organophosphorus, pyrethroids and carbamate classes.

Material and Methods

A consistent quality of common housefly was reared in the Crop Protection Laboratory, Department of Zoology, Rajshahi University at an economical cost following the methods of Morgan (1981), Morgan and Patterson (1978) and Morgan *et al.* (1978). The adults were provided with a medium to lay eggs. The medium was presented in plastic cups approximately 10cm deep. The medium consisted of 9 gm milk powder and 5 gm fresh yeast dissolved in 100 ml of water and added to 100 gm bran (Wilkins and Khalequzzaman, 1993). The mixture was then thoroughly stirred and put into the pots leaving 3 cm from the top. The pots were placed in the fly rearing cage (50 x 30 x 20cm^3) for 24 h. The adults were provided with petridish (90 ml) containing cotton wool soaked in the solution of milk powder with sugar for a food source. After that time the females had laid batches of eggs. Batches of approximately 100 eggs were separated out and transferred to similar pots containing the same mixture and fitted with plastic lids with gauze centers. They were then placed in an incubator at 28°C ± 0.5°C. Pupa were collected and kept in separate pots (kept in the incubator under the same temperature) for emergence of the adults.

The experiments were carried out during July to December 1999. Nine insecticides, viz., malathion (Limithon 57 EC of ACI Ltd.), dichlorovos (Nogos 100 EC of Novartis Bd. Ltd.), pirimiphosmethyl (Actellic of ACI Ltd.), phenthoate (Cidial of ACI Ltd.), monocrotophos (Novacron 40 EC of Novartis Bd. Ltd.), diazinon (Diazinon 60 EC of Novartis Bd. Ltd.), cypermethrin (Cymbush 10 EC of ACI Ltd.), lambda-cyhalothrin (Karate 25 EC of ACI Ltd.) and propoxur (Acekro 20 EC of McDonald Ltd) were used for this study. The insecticides were diluted in acetone and the treatments were done by topical application with 1 µl of solution of insecticide on thoracic notum of each fly with the help of a micro syringe (Hamilton gas tight micro syringe no. 630). The actual dose was calculated from the amount of active ingredient present in 1 µl of the solution. At first malathion was applied singly. Then the low doses of dichlorovos (6.25 ng/fly), pirimiphosmethyl (62.5 ng/fly), phenthoate (62.5 ng/fly), monocrotophos (25 ng/fly), diazinon (25 ng/fly), cypermethrin (1.25 ng/fly), lambda-cyhalothrin (1.25 ng/fly) and propoxur (50 ng/fly) were mixed with malathion in mass ratio of 1:1, 1:2, 1:5, 1:10 and 1:20 and applied. The treated flies were kept in the food cup with cotton soaked in glucose solution. Each trial was repeated 3 times to obtain more uniform results with a smaller error. The mortality of the flies was recorded after 24 hours of treatment.

The mortality percentage was corrected using Abbott's formula (Abbott, 1925) and observed data was subjected to probit analysis according to Finney (1947) and Busvine (1971) using a software developed in the Department of Agricultural and Environmental Science, University of Newcastle Upon Tyne, UK. The co-toxicity coefficient values were calculated by the method of Sun and Johnson (1960a,b). When the co-toxicity coefficient of a mixture is 100, the effect of this mixture indicates probability of similar action. If the mixture gives a coefficient significantly greater than 100, it indicates a synergistic action. On the other hand, when a mixture gives a co-toxicity coefficient less than 100, the effect of the mixture indicates an antagonistic action. In those cases where potentiation occurred the co-toxicity coefficient of mixture was divided by one hundred to obtain the degree of potentiation of malathion in that mixture (Gera and Gupta, 1978).

Results

The combined LD_{50} with 95% confidence limits and regression equations of log doses of different mixtures on probit mortality of the adult housefly after 24 hours of topical application are

Table 1: LD_{50}, confidence limits (CL) and regression equation of combined doses of different insecticides and malathion on adult housefly (*M. domestica)* after 24 hours of treatment.

Insecticide mixtures	Ratio	LD_{50} (ng/fly)	95% CL (ng/fly) Lower	Upper	Regression equation	χ^2 (df)
Dichlorovos + Malathion	1:1	38.756	10.379	144.718	Y = 2.541 + 1.548X	0.109(1)
	1:2	59.222	18.468	189.914	Y = 2.650 + 1.325X	0.273(2)
	1:5	82.367	40.203	168.755	Y = 2.242 + 1.439X	0.273(2)
	1:10	91.311	60.244	138.397	Y = 2.020 + 1.519X	0.699(3)
	1:20	118.483	86.549	162.198	Y = 1.759 + 1.563X	0.179(3)
Pirimiphosmethyl + Malathion	1:1	253.285	126.827	505.833	Y = 1.118 + 1.615X	0.213(2)
	1:2	262.760	159.493	432.890	Y = 1.017 + 1.646X	0.245(2)
	1:5	304.629	215.927	429.768	Y = 1.173 + 1.541X	0.366(3)
	1:10	279.957	190.832	410.706	Y = -0.693 + 2.327X	4.412(3)
	1:20	321.788	268.141	386.168	Y = -0.859 + 2.337X	4.518(2)
Phenthoate + Malathion	1:1	578.251	137.696	2428.357	Y = 1.720 + 1.187X	0.056(2)
	1:2	593.415	193.518	1819.686	Y = 1.588 + 1.230X	0.300(2)
	1:5	415.864	277.992	622.115	Y = 0.765 + 1.617X	1.104(3)
	1:10	306.400	255.798	367.012	Y = -0.627 + 2.263X	7.582(3)
	1:20	333.595	275.416	404.065	Y = -0.624 + 2.229X	3.298(2)
Monocrotophos + Malathion	1:1	187.896	64.050	551.209	Y = 1.912 + 1.358X	0.488(3)
	1:2	203.348	86.512	477.979	Y = 1.671 + 1.442X	0.400(3)
	1:5	276.162	147.573	516.799	Y = 1.555 + 1.411X	0.445(3)
	1:10	303.416	196.636	468.182	Y = 1.345 + 1.472X	1.487(3)
	1:20	299.186	239.672	373.479	Y = 0.050 + 1.999X	4.403(3)
Diazinin + Malathion	1:1	160.680	58.798	439.097	Y = 2.300 + 1.224X	0.303(3)
	1:2	184.875	78.904	433.169	Y = 2.235 + 1.220X	0.676(3)
	1:5	209.549	130.588	336.254	Y = 1.498 + 1.508X	0.689(3)
	1:10	242.259	182.269	321.993	Y = 0.874 + 1.730X	2.477(3)
	1:20	245.718	203.099	297.280	Y = -0.213 + 2.181X	6.604(3)
Cypermethrin + Malathion	1:1	10.296	2.129	49.777	Y = 2.249 + 1.367X	0.026(1)
	1:2	12.580	3.149	50.251	Y = 2.183 + 1.342X	0.165(1)
	1:5	15.200	7.610	30.360	Y = 1.477 + 1.615X	0.213(2)
	1:10	22.004	13.397	36.140	Y = 2.859 + 1.595X	0.032(2)
	1:20	27.081	18.682	39.255	Y = 2.954 + 1.428X	0.287(3)
Lamba-cyhalothrin + Malathion	1:1	4.585	2.345	8.965	Y = 2.506 + 1.501X	0.528(2)
	1:2	6.415	3.321	12.391	Y = 2.428 + 1.423X	1.685(2)
	1:5	10.880	6.228	19.007	Y = 1.944 + 1.500X	0.060(2)
	1:10	15.020	9.784	23.059	Y = 1.754 + 1.491X	0.111(3)
	1:20	23.239	16.199	33.336	Y = 2.879 + 1.552X	0.151(3)
Propoxur + Malathion	1:1	250.997	107.627	585.350	Y = 1.489 + 1.463X	0.099(2)
	1:2	296.625	144.723	607.963	Y = 1.384 + 1.463X	0.396(2)
	1:5	305.139	210.048	443.280	Y = 0.896 + 1.652X	0.758(3)
	1:10	295.337	237.091	367.892	Y = 0.135 + 1.969X	7.600(3)
	1:20	341.583	269.894	432.313	Y = -0.122 + 1.022X	2.247(2)

Table 2: LD_{50} of the combined doses and co-toxicity coefficient at different ratios of insecticides and malathion applied topically on adult housefly (*M. domestica)* after 24 hours of treatment

Insecticide mixtures	Ratio	LD_{50} of malathion in mixture (ng/fly)	LD_{50} of malathion in mixture (ng/fly)	Co-toxicity coefficient Degree of potentiation	Degree of potentiation
Dichlorovos + Malathion	1:1	19.378	19.378	2237.87	22.38
	1:2	19.741	39.481	1098.39	10.98
	1:5	13.728	68.639	631.79	6.32
	1:10	8.301	83.010	522.41	5.22
	1:20	5.642	112.841	384.31	3.84
Pirimiphosmethyl + Malathion	1:1	126.642	126.642	342.42	3.42
	1:2	87.587	175.173	247.56	2.48
	1:5	50.771	253.858	170.82	1.71
	1:10	25.451	254.506	170.39	1.70
	1:20	15.323	306.465	141.50	1.42
Phenthoate + Malathion	1:1	289.125	289.125	149.99	1.50
	1:2	197.805	395.610	109.62	1.10
	1:5	69.311	346.553	125.13	1.25
	1:10	27.855	278.545	155.68	1.56
	1:20	15.885	317.710	136.49	1.36
Monocrotophos + Malathion	1:1	93.948	93.948	461.59	4.62
	1:2	67.783	135.565	319.89	3.20
	1:5	46.027	230.135	188.43	1.88
	1:10	27.583	275.833	157.22	1.57
	1:20	14.247	284.939	152.19	1.52
Diazinin + Malathion	1:1	80.340	80.340	539.77	5.40
	1:2	61.625	123.250	351.85	3.52
	1:5	34.925	174.624	248.34	2.48
	1:10	22.024	220.235	196.90	1.97
	1:20	11.701	234.017	185.31	1.85

Table 2: Continued

Insecticide mixtures	Ratio	LD_{50} of malathion in mixture (ng/fly)	LD_{50} of malathion in mixture (ng/fly)	Co-toxicity coefficient Degree of potentiation	Degree of potentiation
Cypermethrin + Malathion	1:1	5.148	5.148	8423.76	84.24
	1:2	4.193	8.387	5170.56	51.71
	1:5	2.533	12.667	3423.50	34.24
	1:10	2.000	20.004	2167.84	21.68
	1:20	1.280	25.791	1681.42	16.81
Lamba-cyhalothrin + Malathion	1:1	2.292	2.292	18920.3	189.20
	1:2	2.138	4.277	10139.2	101.39
	1:5	1.813	9.067	4782.78	47.83
	1:10	1.365	13.655	3175.80	31.76
	1:20	1.107	22.132	1959.40	19.59
Propoxur + Malathion	1:1	125.498	125.498	345.55	3.45
	1:2	98.875	197.750	219.29	2.19
	1:5	50.857	254.282	170.54	1.71
	1:10	26.849	268.488	161.52	1.62
	1:20	16.266	325.317	133.30	1.33

presented in Table 1. The LD_{50} of malathion was 433.655 ng/fly when applied singly on adult housefly but the combined LD_{50} showed variation in different insecticide-malathion mixtures. The combined LD_{50} was the lowest indicating high toxicity in all ratios of lambda-cyhalothrin:malathion and they were 4.585, 6.415, 10.880, 15.020 and 23.239 ng/fly in 1:1, 1:2, 1:5, 1:10 and 1:20 ratios respectively. The second highest toxicity was observed for cypermethrin:malathion mixtures followed by mixtures of dichlorovos, diazinon, monocrotophos, pirimiphosmethyl and propoxur with malathion. Phenthoate: malathion showed the highest LD_{50} value indicating less toxicity to flies and the LD_{50} values were 250.997, 296.625, 305.139, 295.337 and 341.583 in the above mentioned ratios respectively.

Table 2 contains segregated LD_{50} as per ratio in mixture of insecticides, co-toxicity coefficient and degree of potentiation of malathion by other insecticides. Here also lambda-cyhalothrin showed the highest co-toxicity coefficient and degree of potentiation. It may be noted that lambda-cyhalothrin and cypermethrin when mixed separately with malathion in ratios of 1:1, 1:2 and 1:5 gave the maximum co-toxicity coefficient value. It was 18920.3, 10139.2 and 4782.78 for lambda-cyhalothrin and 8423.76, 5170.56 and 3423.50 for cypermethrin with malathion in the above mentioned ratios respectively. The lowest values were observed for phenthoate: malathion mixtures indicating low level of potentiation.

Discussion

Interactions between chemicals in insects are well known. A variety of pesticides, applied in binary mixtures, cause a toxic effect in insects, which is different than the sum of the two alone. For instance, the formamidine chlorodimeform while itself less toxic has been shown to potentiate the activity with a variety of insecticides belonging to other organophosphorus, pyrethroid and carbamate classes (Scott *et al.*, 1990; Rahim *et al.*, 1994). In this case, the toxicity of the mixture generates a response which is greater than the additive effects of the two compounds alone (Sprague, 1969). Joint interactions are also important from an environmental standpoint. Pesticide residues are found both at the site of application and, due to dissemination by leaching, runoff, and erosive events, in remote destinations which include ground and surface waters. Some contaminants found in surface waters include parathion, malathion, carbaryl, carbofuran, atrazine, simizine, and 2,4-D (Crathorne *et al.*, 1984). It is not known whether these chemicals will interact jointly in nontarget biota in terrestrial and aquatic environments or how environmental quality parameters can affect such interactions.

Toxicities of two triazine herbicides (atrazine and cyanazine) and an organophosphate insecticide (chlorpyrifos) were evaluated individually and in binary combinations using the fourth instars of the aquatic midge, *Chironomus tentans* by Jin-Clark (2001) and observed that atrazine and cyanazine produced significant synergistic effects on the toxicity of chlorpyrifos. Although atrazine and cyanazine were not effective inhibitor of acetylcholinesterase (AChE) *in vitro*, the synergism of atrazine and cyanazine to chlorpyrifos was associated with increased *in vivo* inhibition of AChE in the midges. Parimi *et al.* (2001) observed that the joint action seen between atrazine and methyl parathion, carbaryl, fipronil and indoxcarb may be due to atrazine induction of detoxification enzymes involved in biotransformation of the insecticides. The mixture of Delfin with conventional insecticides also killed diamondback moth *Plutella xylostella* faster than Delfin alone (Saranthoy *et al.*, 1997).

Eighteen tris-substituted derivatives of phosphoric acid were evaluated as synergists for malathion and for several other organophosphate insecticides against resistant strains of the housefly (*M. domestica*) and the mosquito *Culex trasalis* (Coqulilett). Many of the materials markedly synergized malathion against resistant insects, but caused little or no increase in its toxicity to susceptible insects. In most cases with the insect populations studied the synergists did not overcome resistance present to organophosphates other than malathion (Plapp *et al.*, 1963).

It was found that nearly all insecticides offered increased toxicity of malathion when applied jointly on housefly and cypermethrin and lambda-cyhalothrin were the best in this regard. It can safely be said from the present study that pyrethroid insecticides like lambda-cyhalothrin and cypermethrin can be used to potentiate malathion significantly for its toxicity.

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