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Synergic Effects of *Wedelia calendulacea* Less. Plant Extracts with Lambda cyhalothrin on Common Housefly *Musca domestica* L.

[1]M.A. Baki, [2]M.A.A. Rahman, [2]Naznin Ara Khatune,
[1]R.A. Zahid, [4]M. Khalequzzaman, [3]M.M. Husain and [2]G. Sadik
[1]Institute of Biological Silences, [2]Department of Pharmacy,
[4]Department of Zoology, University of Rajshahi, Rajshahi-6205, Bangladesh
[3]BCSIR Laboratory, Rajshahi, Bangladesh

Abstract: Combined effects of *Lambda cyhalothrin* and *W. calendulacea* plant extracts (synergists) were tested against house fly *M. domestica* L. in methanol. Lambda cyhalothrin offered synergism when used with *W. calendulacea* extract. It was observed that the plant extract behaves synergism from 1:1 to 1:5 ratios and above.

Key words: Synergic effects, *W. calendulacea, M. domestica,* Lambda cyhalothrin

Introduction

Challenge to control pests with pesticides is now a very important issue while different synthetic chemicals possessed several side effects, despite the action against the target pest, there have been some problems evolved causing serious damages to non target animals or even soil fertility is disturbed due to heavy use of these detrimental components. As a result, scientists, concerned with pesticide technology, centered their intention to go through further investigation with existing synthetic chemicals to find their mode of action on animal bodies and on the environmental components as well. The effect of pesticides or other synthetic chemicals on biological systems have usually been investigated primarily with single chemical. However, since 1940s increasingly large amounts of insecticides and herbicides have been developed and marketed for insect and weed control. Resistance within or between whole classes of insecticide is an ever-increasing problem for control of major crop pests. Given the tremendous difficulty and investment associated with the development of new, safe and cost-effective insecticides, there is a given need to preserve the efficacy of current insecticides. This may be achieved by the use of proper synergistic chemicals with insecticide (Bhatia, 1990).

Resistant strains of insect pests were developed through the survival and reproduction of individuals after exposure to a given insecticide and as a result insect pests have now developed tolerance to all major classes of insecticides (Saleem and Shakoori, 1993; Saleem *et al.*, 1994; Willkins *et al.*, 1995). The housefly, *Musca domestica,* L. which is responsible for transmission of a variety of diseases in man, was reported to have developed resistance to DDT in Sweden as early as 1946, only two years after its introduction (Brown, 1968). This insect has now developed resistance to different categories of insecticides in several countries (Price, 1991). In particular the development of resistance to organophosphate and pyrethroid-based insecticides has resulted in partial failure of its control (Scott and Georghiou, 1986).

The present way of dealing with this problem is just to increase the dose of the insecticide to eliminate the more resistant populations that ultimately enhances the evolution of resistance in the population and obviously this procedure cannot go on infinitely. Since the presence of pesticide in the environment not only increases the non-target organism mortality, but also their levels can be reached where mammals ultimately can be seriously affected. Therefore the introduction of botanical pesticide or plant extract as synergist could be great beneficial, both economically and ecologically, especially since tests have shown that synergistic increase in toxicity of insecticide is only towards insects and not mammals (Willikins and Metcalfe, 1993).

Wedelia calendulacea Less., (Asteraceae) is a traditional ayurvedie herbs which grow abundantly in the tropical and sub-tropical parts of the world. In ayurvedie medicine *W. calendulacea* is used as best drug for the treatment of liver cirrhosis and infective hepatitis, liver enlargement, jaundice and other ointments of the liver and gall bladder. The present study was aimed at standardizing a protocol for finding out insecticidal or synergistic effect against *Musca domestica* L.

Materials and Methods

The housefly *Musca domestica* L. (local strain) was obtained from the stock culture maintained in the Crop Protection Laboratory, Department of Zoology, University of Rajshahi, Bangladesh. The sub cultures were maintained by the following method of Willikins and Khalequzzaman (1993).

The whole plants of *W. calendulacea* Less. were collected and dried in oven at 40°C for 36 h and powdered in a mortar and pestle separately. Extractions were done in a soxhlet apparatus. The powdered materials were put into the thimble of the soxhlet and extractions were carried out successively with petroleum ether, ethyl acetate, acetone and methanol. The extracted materials were dried in rotary vacuum evaporator under reduced pressure.

Technical grade lambda-cyhalothrin 25EC or 250g/l [(RS)-a-cyano-3-phenozybenzyl (Z)-(1RS, 3RS)-(2-Chloro-3,3,3-trifluropropenyl)-2-dimethyl-cyelopropane carboxylale], commercially available as Karate [A product of ACI Pharmaceutical Company, Bangladesh Limited] was used.

Bioassay procedure: Lambda cyhalothrin and plant extracts were dissolved in methanol to make required volume. The desired serial dilutions were prepared from the stock solutions using methanol. Various concentrations of each chemical were applied to thoracic notum of 5 days old adult flies with a micro syringe (Hamilton gaslight microsyringe # 630). The flies were anaesthetized with diethyl-ether before dosing. Anaesthetized flies were held with padded entomological forceps for treatment. Four concentrations were used in which six replication of flies were treated. One batch of control was maintained in which only methanol was applied topically. Mortality of treated flies was recorded after 24 h.

Analysis of data: The mortality data was corrected using Abbott's formula (1925), Pr-Po-Pc, where Pr = corrected mortality %, Po= observed, Pc= control. Probit analysis were carried out as described by Busvine (1971), using apple computer. Co-toxicity coefficients were calculated as described by Sun and Johnson (1960):

Corresponding Author: Md. Aziz Abdur Rahman, Lecturer, Department of Pharmacy, University of Rajshahi, Rajshahi-6205, Bangladesh

Abbott's formula: $P_r = \frac{P_o - P_c}{100 - P_c}$

$$\text{Co-toxicity coefficient} = \frac{LD_{50} \text{ of toxicant alone}}{LD_{50} \text{ of toxicant in mixture}} \times 100$$

If the mixture gives a co-efficient significantly greater than 100, it indicates a synergistic action. On the other hand, when a mixture gives a co-toxicity co-efficient less than 100, the effect of the mixture indicates an antagonistic action.

Results and Discussion

The extraction was done with four solvents for the separation of different compounds from the whole plant of *W. calendulacea*. Petroleum ether extract separates oil, fats and fatty acids whereas ethyl acetate separates terpenes, alkaloids, flavonoids and steroids. Acetone separates chlorophylls, dyes and other alkaloids and methanol extracts separates all the remaining alkaloids and acidic compounds.

Toxic effect of lambda-cyhalothrin and methanolic extract of *W. calendulacea* (as synergists) on common adult housefly are shown in Tables 1 and 2.

Table 1: Effect of lambda cyhalothrin and *Wedelia calendulacea* plant extract against house fly *Musca domestica* L. after 24 hours of application

Name of toxicants	Dose (ng/fly)	LD_{50} (ng/fly)	95% confidence limit Lower	upper	Regression line	χ^2 value (df)
Lambda cyhalothrin	100.00					
	50.00					
	25.00	29.667	24.08	36.541	Y = 1.653+2272X	4.861 (4)
	15.00					
	10.00					
W. calendulacea	7.68					
Methanol extract	3.84					
	1.92	7.783	4.485	13.506	Y = 1.894+16422X	0.808 (2)
	0.96					
Ethyl acetate extract	7.06					
	3.53					
	1.76	-	-	-	-	-
	0.88					
	Control					
Acetone extract	17.96					
	8.98					
	4.49	-	-	-	-	-
	2.24					
	Control					
Pet ether extract	96.25					
	48.12					
	24.06	-	-	-	-	-
	12.03					
	Control					

(-) Indicates no sensitivity

Table 2: Toxicity of combined doses of lambda-cyhalothrin in mixture of *Wedelia calendulacea* plant extract in methanol to adult house fly after 24 hours application

Ratio (L. cyhalothrin: extract)	Combined doses (ng/fly)	Lambda cyhalothrin	Extract	Mortality %	Combined LD-mg (ng/fly)	LD_{50} of lamda cyhalothrin (ng/fly)	LD_{50} of Extract	95% confidence Lower	Upper	Regression line	χ^2 value (df)
1:1	30.000	15.000	15.000	63.33							
	15.000	7.500	7.500	43.33							
	7.500	3.750	3.750	36.66							
	3.750	1.875	1.875	36.66	38.55	19.227	19.276	16.02	92.73	Y=3.278+ 1.0854X	2.301 (3)
	1.875	0.938	0.938	30.00							
	0.937	0.469	0.469	23.33							
1:2	45.000	15.000	30.000	76.66							
	22.560	7.500	15.000	53.33							
	5.625	3.750	7.000	46.66							
	2.813	1.500	3.000	43.33	19.27	6.425	12.843	11.87	31.29	Y=2.465+ 1.109 X	3.106 (5)
	1.406	0.938	1.800	36.66							
	0.703	0.469	0.938	23.33							
1:5	90.000	15.000	75.000	86.66							
	45.000	7.500	37.500	86.66							
	22.500	3.750	18.730	90.00							
	11.250	1.875	9.375	73.00	3.683	0.614	3.069	1.697	8.088	Y=4.508+ 0.867X	4.184 (4)
	5.625	0.938	4.690	60.00							
	2.812	0.469	2.340	56.66							
1:10	165.000	15.000	150.000	100.00							
	82.000	7.500	75.000	100.00							
	41.250	3.750	37.500	93.00							
	20.625	1.875	18.750	86.66	1.114	0.101	1.012	0.096	12.81	Y=4.96+ 0.830X	0.421 (2)
	10.322	0.938	9.370	86.60							
	5.156	0.469	4.687	76.60							

Table 3: Co-toxicity coefficient of lambda-cyhalothrin and *Wedelia calendulacea* plant extract in methanol applied in different ratios on adult house fly *M. domestica* L.

Ratio (lambda cyhalothrin: methanolic extract)	LD_{50} of combined dose (ngm/fly)	LD_{50} of lambda cyhalothrin (ngm/fly)	Co-toxicity coefficient
1:1	38.552	19.276	153.907
1:2	19.272	6.452	461.802
1:5	3.683	0.614	4832.828
1:10	1.114	0.101	29304.508

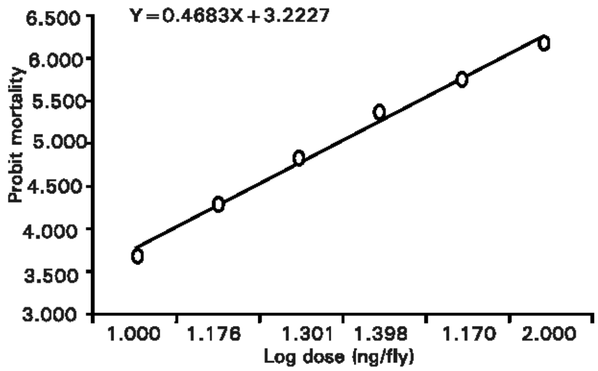

Fig. 1: Regression line of probit mortality of *M. domestica* on log dose of lambda cyhalothrin

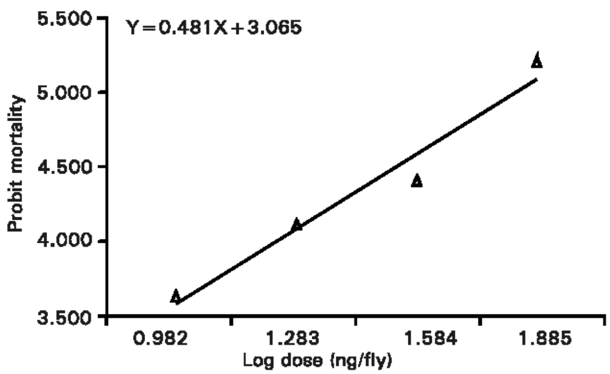

Fig. 2: Regression line of probit mortality of *M. domistica* on log dose of methanol extract of *W. calendulacea*

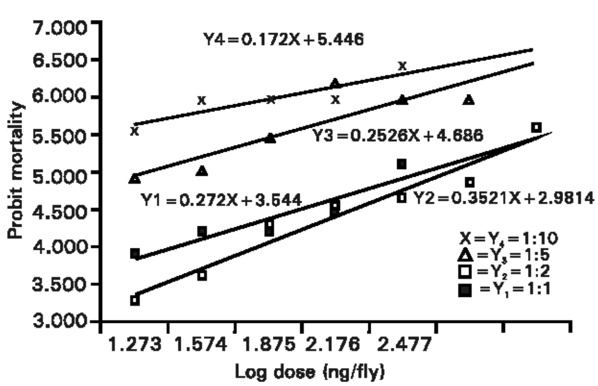

Fig. 3: Probit mortality of log doses of lambda-cyhalothrin and methanolic extract of *W. calendulacea* in the ratio of 1:1, 1:2, 1:5, 1:10

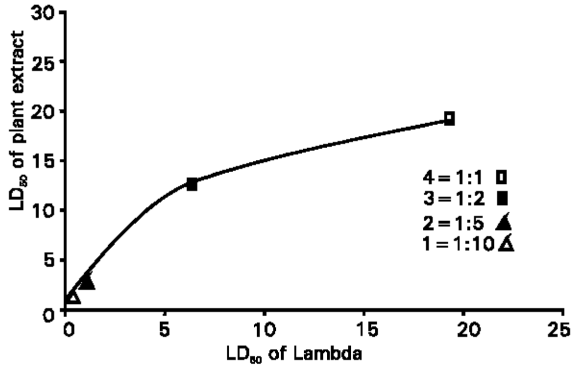

Fig. 4: Isobologram of the LD_{50} of different ratios of lambda-cyhalothrin and *W. calendulacea* plant extract in methanol on *M. domestica* after 24 h of application

The extracts in other solvents were found to be non-toxic to housefly (Table 1) hence methanolic extract was used for further investigation. The dose mortality experiment was done with six different doses of lambda-cyhalothrin (100, 50, 25, 20, 15 and 10 ng/fly) applied topically on 3 days old houseflies. The LD_{50} was calculated as 29.667 ng/fly (Table 1) with 95% confidence limit as 24.08 to 36.541. The regression equation (Fig. 1) showed a good fit in significant χ^2 value (4.861 at 4 df).

Different doses (7.86, 3.84, 1.92 and 0.96 ng/fly) of methanol extract shown wide range of mortality after 24 h. The LD_{50} was calculated as 7.783 ng/fly with 95% confidence limit 4.485 to 13.506. From significant value of the χ^2(0.808 at 2 df) it is evident that the regression line is fitted well (Fig. 2). Ethyl acetate, acetone and petroleum ether extract has shown no toxicity (Table 1).

The combined doses of lambda-cyhalothrin and methanol extract at different ratio (Table 2) were applied to 3 days housefly and the LD_{50} value has been calculated as 38.55, 19.27, 3.68 and 1.11 ng/fly at a ratio of 1:1, 1:2, 1:5 and 1:10 respectively. The regression line is presented in Fig. 3.

Mechanism of synergism in insecticides has been investigated by Hewlett (1968). According to Sun and Johnson (1960a), in the absence of synergist, the insect is able to metabolize some or most of the insecticides to non toxic compounds or to compound less insecticide but synergists reduce this metabolism and thus a greater portion of the insecticides exert it's toxic effect. The sites of detoxification and action appear in general to be separate and thus the synergism does not depend on knowledge of the mode of toxic action of the insecticides. Moreover, the same enzyme complex, within the insect appears to be responsible for oxidative detoxification of insecticides of different chemical structures. According to Hewlett (1960) and Metcalf (1967) synergist inhibit the enzymes responsible for toxicants degradation. Otaki *et al.* (1968) and Otaki and Williums (1970) showed that the insect body contains enzymes for the degradation of hormones like the molting hormone (MH) that may be mode of action of plant extractions. Another possible explanation was advanced by Walker and Thomson (1973) who found that simultaneous application of MH and juvenile hormone (JH) caused an increase in MH activity. A hypothesis for the mode of action of MH and JH when applied together was that the MH activated to synthesis of RNA and JH simultaneously by induced a duplication of DNA. This process causes such a severe disturbance in insect that it leads to its death because the DNA and RNA synthesis are mutually and perhaps exclude other.

In this investigation it was observed that lambda-cyhalothrin when used in conjunction with *W. calendulacea* plant extract offered significant synergism resulting low LD_{50} values for lambda cyhalothrin. From the isobologram (Fig. 4) and co-toxicity coefficient value (Table 3) it may be noticed that the *W.*

calendulacea plant extract in methanol behaves as a synergist from 1:1 to 1:5 ratios and above. The synergistic action of the plant extractions used in this investigation is to some extent similar to the results of Dyte and Rowlands (1967) who reported higher mortality of *T. castaneum* adults in combined doses of insecticides (eg. fenetrithion, bromoxon and maloxon) and synergists (Sesamex, SKF, 525A and PBO-1) in comparison with the mortality due to individual action of chemicals. The result is also similar to that of Ishaaya *et al.* (1983) who reported higher mortality of *T. castaneum* in combined doses of insecticides (eg. trans and cis Cypermethrin) and synergist (Pyperonylbutoxide) in comparison with the mortality due to individual action of the chemicals. Mondal (1990) observed the same results using insecticides (pirimiphosmethyl) and synergist (methylquinone) on *T. castaneum*. Khalequzzaman and Islam (1992) also reported the same results using insecticide (methacrifos) and synergist (leaf and seed extract of dhutura Datura metal) on same insect.

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