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Synergistic Action of *Wedelia calendulacea* Less. Plant Extracts with Lamda Cyhalothrin on Adult Red Flour Beetle *Tribolium casta*neum Herbst

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Abstract: Synergistic effect of *W. calendulacea* plant extract combined with *Lamda cyhalothrin* were demonstrated against red flour beetle *T. castaneum* in methanol extract. *W. calendulacea* plant extract offered synergistic action when used *Lamda cyhalothrin*. It was noted that plant extract indicates synergistic action from 1:1 to 1:5 ratio and above.

Key words: Synergistic action, *Wedelia calendulacea, Lamda cyhalothrin, Tribolium castaneum*

INTRODUCTION

Since 1940s increasingly large amount of insecticides and herbicides have been developed and marketed for insect pest and weed control. Resistance within or between whole classes of insecticides is a serious threat to control and management of many important insect pests. Pest control with pesticides is now a very important issue with different synthetic chemicals possessed several side effects. 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[1].

Resistant strains of any 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[2-4].

Development of insecticide resistance in stored grain pests is a global problem[5]. Resistance among population of *T. castaneum* is widespread throughout the world[6-11]. *T. castaneum* has been reported to develop great resistance against different chlorinated hydrocarbon (e.g. DDT, BHC/lindane) and organophosphates insecticides (e.g. malathion, fenetrothion, pirimiphos-methyl, dichliovos), carbamate (e.g. carbaryl and certain fumigants (e.g. methyl bromide, phosphine)[5, 9,12-17].

Synergists could play an important role in combating resistant population of *T. castaneum* and other insect pests, as well as reduction of insecticides application rates, in potential environmental contamination and improvement in the performance of integrated pest management programs. The presence of pesticides 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 pesticides or plant extract as synergist could be great beneficial, both economically and ecologically, especially since tests have shown that synergism increases in toxicity of insecticide is only towards insects and not mammals[18].

Wodelia calendulacea Less. (Asteraceae) is a traditional ayurvedic herbe which grow abundantly in the tropical and sub-tropical parts of the world. In ayurvedic 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 insecticide or synergistic effect against red flour beetle *T. castaneum* Herbst.

MATERIALS AND METHODS

Local strain of the red flour beetle *T. castaneum* Herbst. were used in the present study which obtained from the stock culture maintained in the IPM Laboratory, Institute of Biological Sciences, University of Rajshahi, Bangladesh.

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

Technical grade lambda-cyhalothrin 25 EC or 250 g L^{-1}[(RS)-a-cyno-3-phenozybenyl(Z)-(1RS)-(2-Chloro-3,3,3-trifluropropenyl)-2-dimehyl-cyelopropene carboxylate], commercial available as karate[A product of ACI Pharmaceutical Company, Bangladesh Limited was used].

Bioassay procedures: Lamda 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 *T. castaneum* using surface film contact method. Four concentrations were used in which six replications of beetles were treated. One batch of control was maintained in which only methanol was applied. Mortality of treated beetles was recorded after 24 h.

Analysis of data: The mortality data was corrected using Abbott's formula[19], p_r-p_o-p_c, where, p_r = corrected mortality%, p_o = observed, p_c = control. Probit analyses were carried out as described by Busvine[20], using apple computer. Co-toxicity co-efficient were calculated as described by Sun and Johnson[21].

Abbott's formula: $p_r = p_o - p_c / 100 - p_c$

Co-toxicity coefficient = (LD_{50} of toxicant alone/ LD_{50} of toxicant in mixture)x100

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

RESULTS AND DISCUSSION

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

Table 1: Effect of Lamda cyhalothrin and *Wedelia calendulacea* plant extract against *Tribolium castaneum* (Herbst.) after 24 h of exposure

Name of toxicant	Dose ($\mu g\ cm^{-2}$)	LD_{50} ($\mu g\ cm^{-2}$)	95% Confidence limit Lower - Upper	χ^2-value
Lamda cyhalothrin	1800.00	219.0014	147.224-325.7723	0.7294(3)
	900.00			
	450.00			
	225.00			
	112.00			
	Control			
Wedelia calendulacea (Methanol extract)	750.00	89.9280	62.3025-129.3870	0.73206(3)
	375.00			
	187.00			
	93.95			
	46.875			
	Control			
E.A. extract	1500.00	-	-	-
	750.00			
	375.00			
	187.00			
	93.95			
	Control			
Acetone extract	2000.00	-	-	-
	1000.00			
	500.00			
	250.00			
	125.00			
	Control			
Petrolium extract	2000.00	-	-	-
	1000.00			
	500.00			
	250.00			
	125.00			
	Control			

(-) indicates no sensitivity

Extracts in other solvents were found to be non-toxic to *T. castaneum* (Table 1) though methanolic extract was used for further investigation. Dose mortality experiment was done with five different doses of lambda-cyhalothrin (1800, 900, 450, 225 and 112.50 $\mu g\ cm^{-2}$) applied on five days old beetles. The LD_{50} was calculated as 219.0014 $\mu g\ cm^{-2}$) (Table 1) with 95% confidence limit as 147.224 to 325.7723 $\mu g\ cm^{-2}$. The regression equation (Fig. 1) showed a good fit in significant χ^2-value (0.729 at 3 df).

Different doses (750.00, 375.00, 187.50, 93.95, 46.873 $\mu g\ cm^{-2}$) of methanol extract was shown wide range of mortality after 24 h. The LD_{50} was calculated as 89.928 $\mu g\ cm^{-2}$ with 95% confidence limit 62.3025 to 129.387. From significant value of the χ^2 (0.73206 at 3 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 5 days old *T. castaneum* and the LD_{50} value has been calculated as 179.012, 106.654, 63.0494, 33.5282 $\mu g\ cm^{-2}$ at a ratio of 1:1, 1:2, 1:5 and 1:10, respectively. The regression line is presented in Fig. 3.

Table 2: Toxicity and Co-toxicity Co-efficient of combined doses of Lamda cyhalothrin mixture of *Wedelia calendulacea* plant extract in methanol to adult *Tribolium castaneum* (Herbst.)

Ratio (L. Cyhalothrin extract)	Combined doses ($\mu g\ cm^{-2}$)	Lamda Cyhalothrin	Extract	Mortality	Combined LD_{50} ($\mu g\ cm^{-2}$)	LD_{50} of *Lamda cyhalothrin*	LD_{50} of extract	95% Confidence limits Lower	Upper	Regression Line	χ^2-value	Co-toxicity Co-efficient
1 : 1	450.000	225.000	225.000	70	179.0120	89.506	89.506	117.250	273.300	Y=2.8607+.9495x	4.320(3)	122.338
	225.000	112.500	112.500	50								
	112.500	56.250	56.250	40								
	56.250	28.125	28.125	30								
	28.125	14.060	14.060	26								
1 : 2	675.000	225.000	450.000	74	106.6540	56.170	112.341	61.316	190.000	Y=2.8436+.9684x	0.2961(3)	129.961
	337.500	112.500	225.000	54								
	168.250	56.250	112.000	50								
	84.120	28.125	56.000	36								
	42.185	14.060	28.125	30								
1: 5	1350.000	225.000	1125.000	86	69.0494	11.341	56.707	31.073	953.437	Y=3.4127+.8630x	0.504(2)	317.167
	672.500	112.500	560.000	82								
	286.500	56.250	281.250	74								
	168.740	28.125	140.626	60								
	84.360	14.060	70.300	54								
1 : 10	2475.000	225.000	2250.000	100	33.5282	3.048	30.480	3.975	282.737	Y=3.4740+1.229x	0.11676(1)	653.184
	1237.500	112.500	1125.000	100								
	618.750	56.250	562.500	90								
	309.375	28.125	281.250	84								
	154.660	14.060	140.600	74								

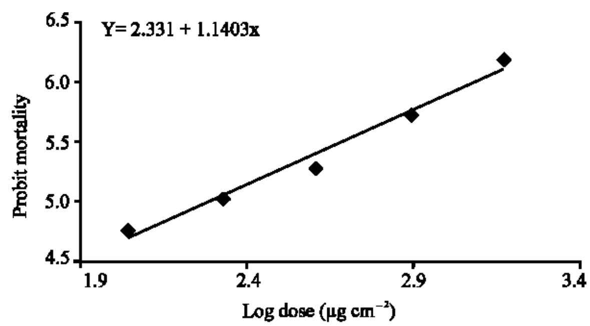

Fig. 1: Regression line of probit mortality of *T. castaneum* on log dose of Lambda cyhalothrin

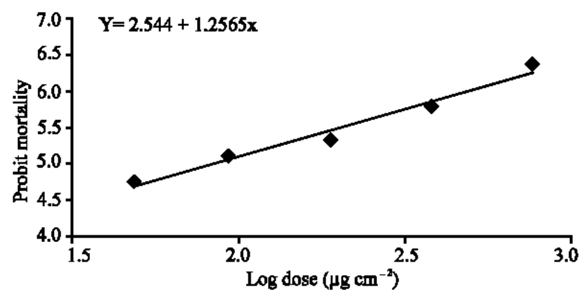

Fig. 2: Regression line of probit mortality of *T. castaneum* on log dose of methanol extract of *W. calendulacea*

Hewlett[22] has been investigated mechanism of synergism in insecticides. Sun and Johnson[23] stated that the insect is able to metabolize some or most of the insecticides to non-toxic compounds or to compound less insecticides in the absence of synergist 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. Synergists inhibit the enzymes responsible for toxicants degradation[24,25]. Otaki *et al.*[26], Otaki and Williums[27] 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[28] 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.

It was observed in this investigation 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 co-toxicity co-efficient value (Table 2) 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

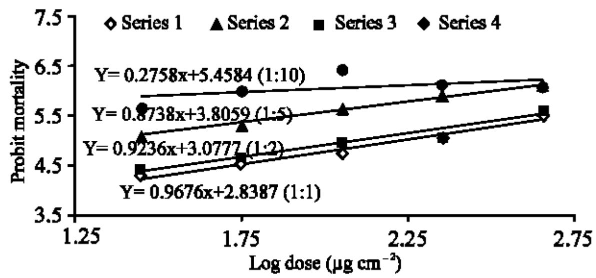

Fig. 3: Probit mortality of log doses of Lambda cyhalothrin and methaolic extract of *W. calendulacea* in the ratio of 1:1, 1:2, 1:5, 1:10

similar to the results of Dyte and Rowlands[29] 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.*[30] who reported higher mortality of *T. castaneum* in combined doses of insecticides (eg. trans and cis Cypermethrin) and synergist (Pyperonyl butoxide) in comparison with the mortality due to individual action of the chemicals. Mondal[31] observed the same results using insecticides (pirimiphos-methyl) and synergist (methyl quinone) on *T. castaneum.* Khalequzzaman and Islam[32] also reported the same results using insecticide (methacrifos) and synergist (leaf and seed extract) of *Datura metal* on same insect.

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