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CO-TOXICITY EVALUATION OF LAMBDA-CYHALOTHRIN AND ITS SYNERGIST PBO FOR SUSCEPTIBILITY OF *ALPHITOBIUS DIAPERINUS* (COLEOPTERA: TENEBRIONIDAE)

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ABSTRACT

To investigate the co-toxicity and co-efficient activity of λ-cyhalothrin (karate 2.5EC), a pyrethroid and piperonyl butoxide (PBO) against *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae). Dose mortality test was done by the residual film assay technique. Statistically the dose mortality relationship was expressed as a median lethal dose (LD_{50}) by the probit analysis. The regression lines and isoboles were drawn using the Fig-P (Biosoft) package. The Co-efficient values showed that all ratios of λ-cyhalothrin and piperonyl butoxide offered synergistic action to both larvae and adult. We observed that the toxicity of the λ-cyhalothrin was decreased as the ratio (amount) of PBO was increased. The individual LD_{50} value of λ-cyhalothrin for adult is 0.0292μgcm^{-2}. But in the mixture, the share of λ-cyhalothrin are 0.0125, 0.0145, 0.0182 and 0.0233μgcm^{-2} at ratios of 1:1, 1:3, 1:5; 1:10 when PBO causes reduction of dose level of 78.76%**,** 79.10%, 89.72% and 92.80% respectively. In case of larvae the individual LD_{50} value of λ-cyhalothrin is 0.0813μgcm^{-2}. But in the mixture, the share of fenitrothion are 0.0155, 0.0250, 0.0269 and 0.0287μgcm^{-2} at ratios of 1:1, 1:3, 1:5; 1:10 when PBO causes reduction of dose level of 93.23%, 94.34%, 98.40% and 98.40% respectively. The study

suggests that the mortality rate of lesser mealworm is increase with the increase of insecticide dose. The LD_{50} values of the insecticides are inversely related to the toxicity of the insecticides i.e. higher the LD_{50} value lower the toxicity of the insecticide.

KEYWORDS: Pyrethroid, Metabolic inhibitor, Neurotoxin, Acetone, Darkling beetle, Integration of pest management, Residual film assay.

INTRODUCTION

Insects infesting grain after harvest cause economic loss to producers and the grain and food industry. In this investigation the lesser mealworm *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) is used. *A. diaperinus* commonly called darkling beetle is a notorious pest of the stored grains and cereals. It is one of the key insect pests in the poultry industry. The adults are general feeders, while the larvae are adapted for feeding on cemented food substances from linseed, cottonseed, oilseed products, tobacco and drugs.[1-2] A report was found in the state of Georgia, with estimated cost of damage and control equating to around $12,000,00 million in 2006.[3] Estimation for Bangladesh shows that the annual crop loss due to insect pest alone is 16% for rice, 11% for wheat, 20% for sugarcane, 25% for vegetables, 15% for jute and 25% for pulse.[4] However, loss of 20% or more may occur in the tropical countries through insect attack after harvest.[5] Because the climate and storage conditions in the tropical countries are highly favorable for insect growth and development.

However, the importance of this species as a pest with in poultry facilities is not limited to structural damage. *A. diaperinus* is a known reservoir for many human and poultry pathogens. Several genera of bacteria have been isolated from *A. diaperinus* including *Micrococcus, Streptococcus, Staphylococcus, Serratia, Klebsiella, Pseudomonas,* and *Salmonell*.[6] It has been reported to be competent reservoirs of tapeworms, avian leucosis virus (ALVs) and turkey enterovirus, *Salmonella typhimurium*[7], *Escherichia coli*[8], *Campylobacter jejuni* [9-10], infectious bursal disease virus (IDBV).[11]

Therefore, the use of integrated pest management and chemical control of the darkling beetle is recommended, providing benefits, such as lowering costs and health risks, and maximizing productivity. In Australia, four insecticides, fenitrothion, cyfluthrin, β-cyfluthrin, and spinosad, are currently registered for use against the pest in broiler houses, and another compound, γ-cyhalothrin, has been laboratory tested [12], Although scientific studies advocate different chemical groups for control of darkling beetles, such as pyrethroids and organophosphates[13], macrocyclic lactones, organochlorines, and carbamates[14], Lambkin and Furlong[15] studied the synergistic interaction between piperonyl butoxide and several pyrethroids in cyfluthrin–fenitrothion-resistant populations of *A. diaperinus* from southeastern Queensland. Lambkin and Furlong[16] further reported that spinosad affected the susceptibility of insecticide-resistant beetles, not only to pyrethroids but also to another organophosphate, fenitrothion. Throughout the world an estimation of 4.1 thousand million pounds of pesticides is being applied annually of these 50% are used only for the protection of agricultural commodities. According to statistics from the government of Bangladesh consumption of pesticides has become more than double since 1992 rising from 7,350 metric tons in 2001.[17]

However, Lambda cyhalothrin, an insecticide, belongs to the pyrethroid chemical class of pesticides. It was registered with the environmental protection agency (EPA) in 1988. It is used as contact insecticide, effective against a wide range of pests on rice, cereals, fruits, vegetables, stored grains, cotton and forests and also in public health programs as a vector control agent for malaria, flies, mosquitoes and cockroaches. It is also classified as an anti acetyl cholinesterase neurotoxin, but it is remarkably less toxic to mammals than other organophosphorus insecticides such as parathion (O,O-diethyl O-(4-nitrophenyl) phosphorothioate) and paraoxon (diethyl 4-nitrophenyl phosphate), the reason for which it is used widely [18]. Germano et al.[19] demonstrated on row and fruit crops. Despite the broad spectrum activity that includes natural enemies.[20]

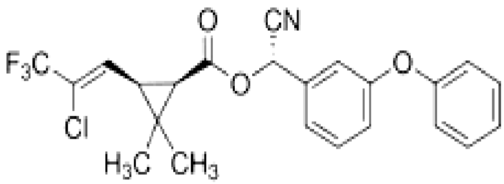
The susceptibility of *A. diaperinus* as the test organism to evaluate the toxicity of commercially formulated insecticides, λ-cyhalothrin and its synergistic effect in combination with a reference synergist Piperonyl butoxide (PBO) were examined through exposure on treated plywood panels. In addition to effectiveness and biological security, the absence of residues in meat and/or eggs, and the low interference in poultry metabolism are very important aspects to consider when recommending new alternatives for beetle control. λ-cyhalothrin is easily degraded on soil and plants but can be effective for weeks when applied to indoor inert surfaces. Exposure to sunlight, water and oxygen will accelerate its decomposition. Cypermethrin a pyrethroid can induce impairments of the structure of seminiferous tubules and spermatogenesis in male rats at high doses.[21] Fenitrothion is also caused deleterious effects on the sperm and testes of Sprague-Dawley rats.[22] The mode of action is the inhibition of insect acetylcholinesterase, interfering in neuromuscular transmission with consequent parasite death. There are no published data on the effects of piperonyl-butoxide (PBO) in combination with λ-cyhalothrin on mortality of adults and larvae of *A. diaperinus*.

Although the resistance mechanisms in several herbivorous insect species are relatively well known, the nature of pyrethroid resistance in predators such as lesser mealworm has not yet been clarified. Features of the physiology of lambda-cyhalothrin metabolism appear to be associated with differential survival of *A. diaperinus* populations after exposure to higher doses of this insecticide in the field. This hypothesis was tested in both susceptible and resistant *A. diaperinus* populations through the use of metabolic inhibitors (synergists) and biochemical assays in vitro.

MATERIAL AND METHODS

A. diaperinus were collected from the storehouse of the flour mills of different local markets under Rajshashi City Corporation, Rajshahi, Bangladesh. Cultures were maintained in an incubator at 30º ± 0.5º C in jars (1L) and subcultures in beakers (500 ml) containing food medium. A standard mixture of wheat meal, corn meal and yeast (10:10:1.5) were used as the food medium in this experiment and treated with the following chemicals.

1. **Commercial Name:** Karate 2.5 EC. **Common name:** Lambda-cyhalothrin is a pyrethroid at technical grade (99.5%; Chem Service, West Chester, PA, USA) was used in the bioassays to determine synergism effects and enzymatic activity. Figure 1(A).
2. **Piperonyl butoxide** (PBO): 98% technical grade (Chemical Service). It is a waxy white solid synergist having no pesticidal activity of its own; it enhances the potency of certain pesticides Figure 1(B).
3. **Acetone:** The solvent has been chosen following the guideline or it is a rather generalist solvent.



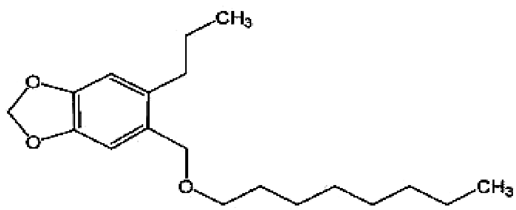


Figure 1. (A) λ-cyhalothrin (B) Piperonyl butoxide

Dose-mortality experiment was done by surface film technique with the adapted method.[23] The label rate for each insecticide was prepared with acetone. For each insecticide, 1ml of the label rate for floor and wall treatment was applied to each of three 9 cm^2 filter papers.

Bioassay of Insecticides. Three replications were maintained for each insecticide. In each replication 60 beetles were used. Insecticide was diluted in acetone and pilot experiments have done according to the indications made by the produces for the users, to obtain doses in which mortality rate was in between 10% to 90% for the beetles.

To carry on tests with the test insecticide residual-film method was used.[24] The actual doses were calculated from the amount of insecticide present in 1 ml of the solution and then the amount of active ingredient was also worked out. Calculated active ingredient of the insecticide was expressed in μgcm^{-2}. Selected doses were prepared prior to the experiment. According to the results obtained from the pilot experiment doses were prepared of which 1ml of each of the doses was poured down on the Petri dish (9cm; r =4.5cm) with a one ml syringe (Hamilton Bonaduz). A control experiment was maintained in which treatment was made only with the solvent. The Petri dish then allowed to dry by evaporation of the solvent 60 insects was released within each Petri dish and kept into the incubator at 30°C ± 0.5°C for 24 hours. Mortality of the beetles was recorded after 24 hours of treatment.

Bioassay of Insecticide and Synergist Mixtures. The insecticide lambda-cyhalothrin and the synergist in technical grade were dissolved in acetone at ratios 1:1, 1: 3, 1: 5 and 1:10 applied as mentioned in section. Initially, bioassays were conducted to determine the sub-lethal synergist dose. The lowest dose of the insecticide was taken proportionate to that of the synergist to make the combined dose. The method used in this experiment was similar to that in bioassay test with insecticidees alone.

Probit Analysis. The percent mortality was subjected to statistical analysis.[25] The dose mortality relationship was expressed as a median lethal dose (LD_{50}), during probit mortality calculation percent mortality of the adult beetles were corrected on the method of Abbott.[26]

$$P = \frac{P_0 - P_c}{100 - P_c} \times 100$$

Where, P_t = Corrected mortality %, P_o = Observed mortality % and P_c = Control mortality %

Probit analysis was done.[24, 27] The median leathal dose (LD_{50}) was calculated by using a Probit analysis program. The LD_{50} values of the insecticides are inversely related to the toxicity of the insecticide i.e. higher the LD_{50} value lower to toxicity of the insecticide.

Determination of Co-toxicity and Co-efficient.[28]

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

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.

Construction of Isobolograms. The regression lines and isoboles were drawn using the Fig-P (Biosoft) package. Isobolograms for the mixtures of insecticides were constructed.[29] This was done as follows: using the LD_{50} values for each ratio, the concentration of each individual compound in the mixture was plotted. Isobole lines below the additive line indicate synergism. Isoboles were drawn by free and curve fitting.

RESULTS

The LD_{50} value of λ-cyhalothrin is 0.0292μgcm^{-2} for the adult, 0.0813μgcm^{-2} for the larvae respectively (Table 1) and the mixture (λ-cyhalothrin: PBO) of different ratios for the adult are 0.0125μgcm^{-2} at 1:1, 0.0145μgcm^{-2} at 1:3, 0.0182μgcm^{-2} at 1:5 and 0.0233μgcm^{-2} at 1:10; and for the larvae are 0.0155μgcm^{-2} at 1:1, 0.0250μgcm^{-2} at 1:3, 0.0269μgcm^{-2} at 1:5, and 0.0287μgcm^{-2} at 1:10 respectively (Table 2). 95% confidence limits, regression equations and chi-squared values have been estimated in the Table 3. Regression lines of different ratios on log probit mortality and the log dose concentrations have been plotted (Figure 2-4).

To compare the LD_{50} values of the mixtures, the LD_{50} values of the insecticide and synergist have been separated as ratios to calculate co-toxicity coefficient activity. The co-toxicity coefficients of mixtures of each ratio are greater than 100 following on the principle of Sun and Johnson.[30] The co-toxicity coefficients were determined as 1543.87, 2129.48, 6861.66 and 8234.00 for adults and 865.94, 1035.36, 2164.86 and 3663.61 for larvae. The present investigation shows that λ- cyhalothrin and PBO produced synergistic action with the adults as well as with the larvae at all ratios. It has been observed that the toxicity of the insecticide was increased when the ratios of PBO were progressive (Table 4).

The free hand curve fitting of isobologram has run the additive line indication synergistic action of the mixture with both adult and larvae indifferently. The individual LD_{50} value of λ-cyhalothrin with adults was 0.0292μgcm^{-2} but in the mixtures, the share of λ-cyhalothrin was 0.0062, 0.0061, 0.0030 and 0.0021μgcm^{-2} at respective ratios when PBO causes reduction of dose level of 78.76%**,** 79.10%, 89.72% and 92.80% respectively (Figure 5). In case of larvae the isobole shows similar in action. The individual LD_{50} value of λ-cyhalothrin with larvae was 0.0813μgcm^{-2} but in the mixtures the share of λ-cyhalothrin was 0.0055, 0.0046, 0.0022 and 0.0013 at respective ratios when PBO causes reduction of dose level of 93.23%, 94.34%, 98.40% and 98.40% respectively (Figure 6).

Reduction of active ingredients in the doses was calculated using the formula as

$$a - s = r \text{ ....(1)}$$

$$\% \text{ or reduced a. i.} = {}^{r}/a \times 100 \text{ ....(2)}$$

Where a = LD_{50} value of the active ingredient alone

s = Share of the active ingredient in the LD_{50} value of the mixture.

r = reduced amount of the a. i. to kill 50% of the test insects.

Table 1. Effect of λ-cyhalothrin on *A. diaperinus* after 24 h of exposure

Dose μg cm^{-2}	Log dose	Num.	Kill	% kill	Cor %	Emp probit	Expt probit	Work probit	Weight	Final probit
Adults										
0.307	1.4871	60	49	81.7	82	5.92	5.8859	5.87	30.18	5.847
0.153	1.1861	60	43	71.7	72	5.58	5.6221	5.58	33.48	5.597
0.076	0.8808	60	38	63.3	63	5.33	5.3545	5.32	36.96	5.344
0.038	0.5798	60	33	55.0	55	5.13	5.0906	5.12	38.22	5.094
0.019	0.2788	60	26	43.3	43	4.82	4.8267	4.84	37.62	4.844
Contr.		60	0							
Y = 4.613322 + 0.8296917 X Log LD_{50} is 0.4660506 LD_{50} is 0.02924494					No significant Heterogeneity Chi-squared is 0.08573 with 3 degrees of freedom 95% Confidence limits are 0.016804 to 0.050893					
Mature larvae										
0.615	1.7888	60	50	83.3	83	5.95	5.8716	5.90	30.18	5.8319
0.307	1.4871	60	42	70.0	69	5.50	5.5734	5.47	34.86	5.5461
0.153	1.1847	60	36	60.0	59	5.23	5.2744	5.25	37.62	5.2596
0.076	0.8808	60	30	50.0	49	4.97	4.9740	4.96	38.04	4.9718
0.038	0.5797	60	24	40.0	39	4.72	4.6764	4.71	36.06	4.6867

Contr.		60	1							
Y = 4.137499 + .9472412 X Log LD_{50} is 0.91054 LD_{50} is 0.0813842					No significant heterogeneity Chi-squared is 0.36750 with 3 degrees of freedom 95% Confidence limits are 0.05363 to 0.1234826					

Table 2. LD_{50}, 95% confidence limits, regression equation and χ^2 value of Fenitrothion against *A. diaperinus* adult and mature larvae after 24h of exposure.

Insect	LD_{50} (µg cm^{-2})	95% confidence limits		Regression equations	χ^2 value (df=3)
		Upper	Lower		
Adult	0.0292	0.0508	0.0168	Y = 4.613322 + 0.829692 X	0.0860
Larvae	0.0813	0.1234	0.0536	Y = 4.137490 + 0.947241 X	0.3675

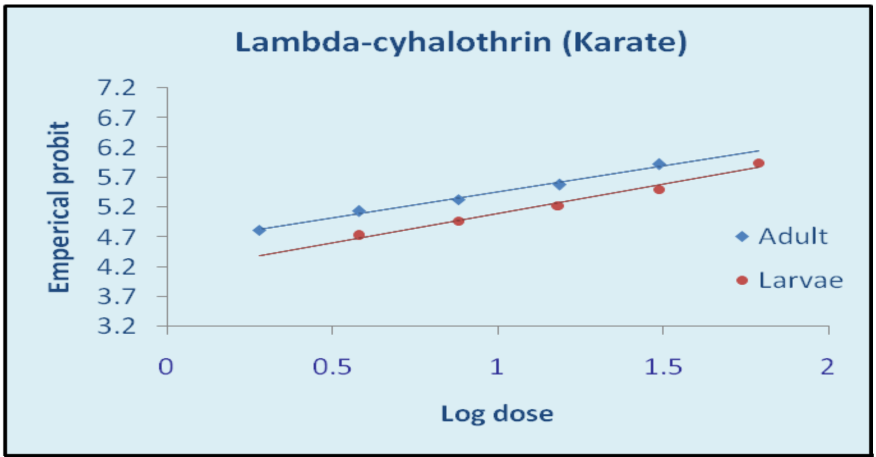


Figure 2. Regression lines of probit mortality on log dose of λ-cyhalothrin against *A. diaperinus* mature adult and larvae after 24h of exposure.

Table 3. LD_{50}, 95% confidence limits, regression equation and χ^2 value of λ-cyhalothrin (Karate) with PBO against *A. diaperinus* with 24h of treatment.

Ratio	LD_{50} (µg cm^{-2})	95% confidence limits		Regression equations	χ^2 value (df=3)
		Upper	Lower		
Adult					
1:1	0.05978	0.017651	0.008884	Y = 3.886656 + 1.014239 X	1.8175
1:3	0.02454	0.031797	0.018939	Y = 3.100861 + 1.366403 X	0.5374
1:5	0.01824	0.023078	0.014418	Y = 2.936690 + 1.636180 X	2.7455
1:10	0.02330	0.029481	0.018425	Y = 4.322266 + 1.844256 X	1.0589
Mature larvae					
1:1	0.01559	0.020244	0.012019	Y = 3.388412 + 1.350753 X	2.6883
1:3	0.02502	0.033350	0.018770	Y = 3.208694 + 1.281016 X	1.7164
1:5	0.02695	0.037040	0.019606	Y = 4.434860 + 1.312466 X	0.2750
1:10	0.02871	0.042280	0.019506	Y = 4.332924 + 1.455939 X	0.4643

Table 4: Co-toxicity coefficient of piperonyl butoxide (PBO) with λ-cyhalothrin applied in different ratios on *A. diaperinus* after 24 h of application.

Insecticide LD_{50} (μgcm^{-2})	Ratio Insecticide :PBO	Combined LD_{50} (μgcm^{-2})	Insecticide LD_{50} (μgcm^{-2})	PBO LD_{50} (μgcm^{-2})	Co-toxicity coefficient
Adult					
0.0292	1:1	0.05978	0.0062	0.0062	470.96
	1:3	0.02454	0.0061	0.0184	478.68
	1:5	0.01824	0.0030	0.0152	973.33
	1:10	0.0233	0.0021	0.0211	1390.47
Mature larvae					
0.0813	1:1	0.01559	0.0077	0.0077	1055.84
	1:3	0.02502	0.0062	0.0187	1311.29
	1:5	0.02695	0.0044	0.0224	1847.72
	1:10	0.02871	0.0026	0.0261	3126.92

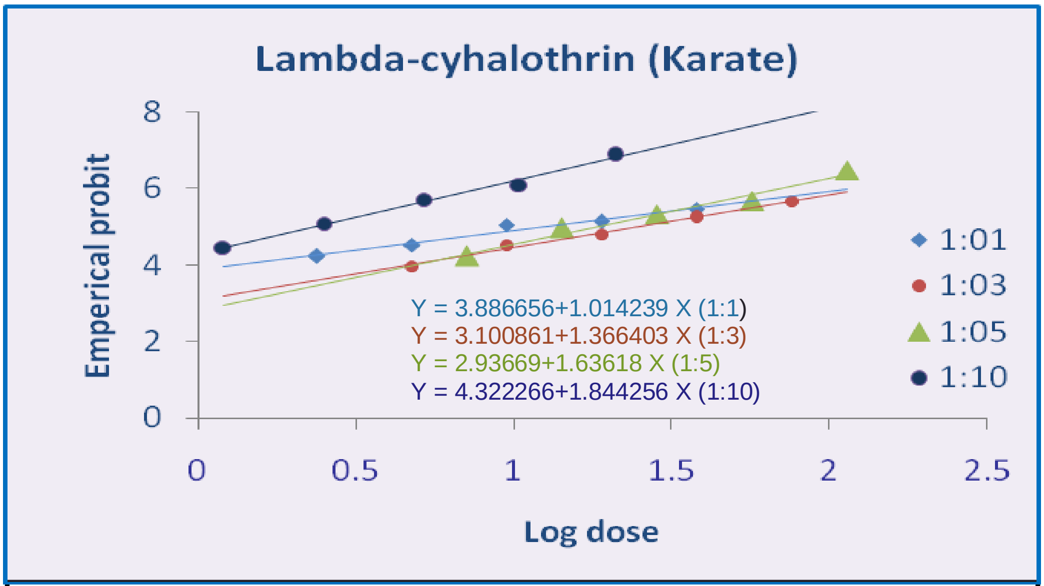


Figure 3. Regression lines of probit mortality on log dose of λ-cyhalothrin and piperonyl butoxide at the ratio of 1:1, 1:3, 1:5 and 1:10 against *A. diaperinus* adult after 24h of exposure.

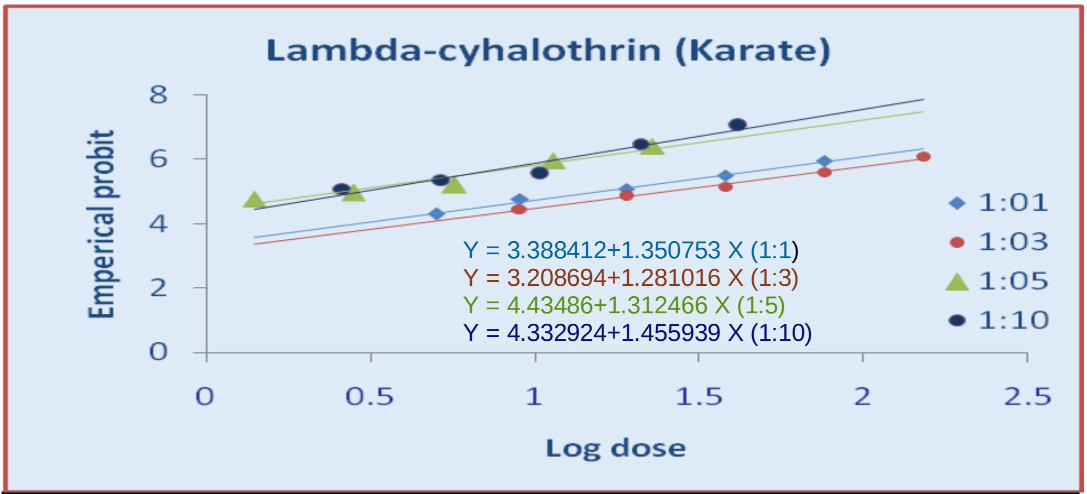


Figure 4. Regression lines of probit mortality on log dose of λ-cyhalothrin and piperonyl butoxide at the ratio of 1:1, 1:3, 1:5 and 1:10 against *A. diaperinus* larvae 24h of exposure.

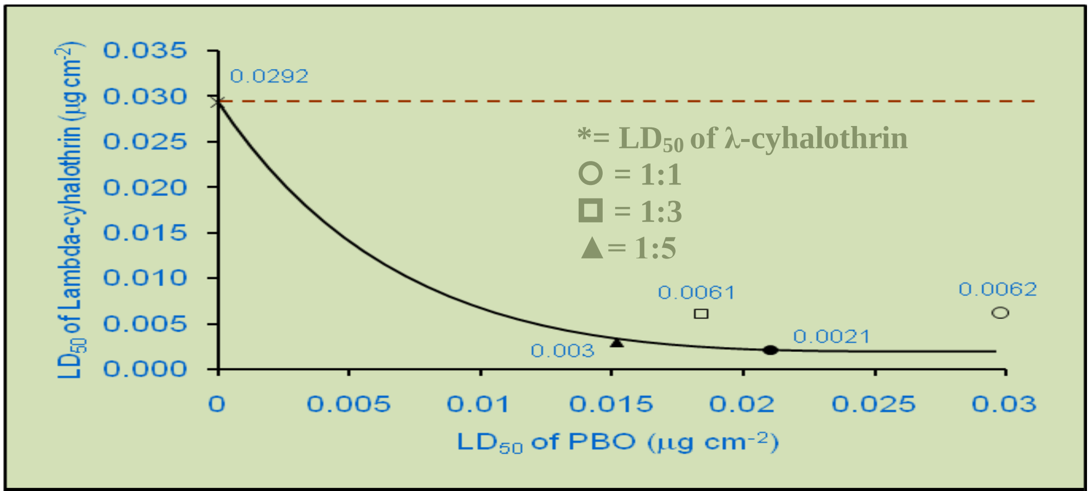


Figure 5. Isobologram of LD_{50} of λ-cyhalothrin and piperonyl butoxide applied on *A. diaperinus* adult.

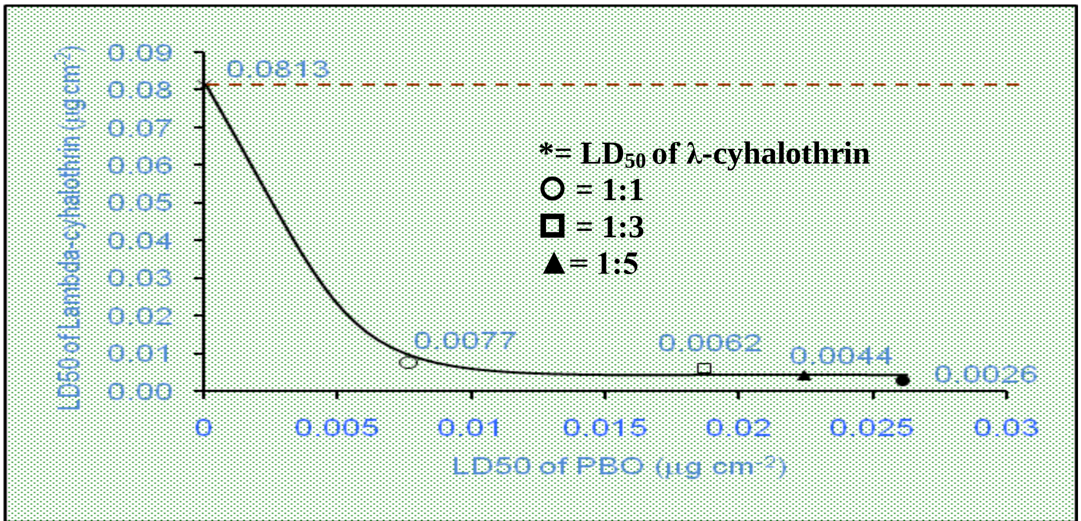


Figure 6. Isobologram of LD_{50} of λ-cyhalothrin and piperonyl butoxide applied on *A. diaperinus* larvae.

DISCUSSION

In the present investigation commercial formulation of pyrethroid insecticide λ-cyhalothrin (karate 2.5EC) was tested against the 7 day old adult and 40 day mature larvae of *A. diaperinus*. The LD_{50} value was recorded 0.0292µgcm^{-2} for adult and 0.0813µgcm^{-2} for larvae respectively after 24h exposure. It is the general agreement of our previous study[31] 0.1235µgcm^{-2} against adult; 0.0476µgcm^{-2} against larvae that the LD_{50} value of another pyrethroid cypermethin and[32] for organophosphate chloropyrifos that the LD_{50} value is 0.8960µgcm^{-2}. In another study, we were obtained that LD_{50} value of chloropyrifos was 0.1241µgcm^{-2} for adult and 0.2943µgcm^{-2} for larvae of *A. diaperinus* respectively after 24h exposure.[33]

In continuation of our study we found that different organophosphate insecticide fenitrothion the LD_{50} value was determined as 0.8737µgcm^{-2} for adult and 0.7328µgcm^{-2} for larvae[34] and the LD_{50} value of a neonicotinoid, imadacloprid is 0.6803µgcm^{-2} for the adult, 0.5168µgcm^{-2} for the larvae.[35] The result is also in general agreement with Steelman[36] who investigated the toxicity of pyrethroid insecticides: cyfluthrin, permethrin and cypermethrin on adult and larvae of the beetle population collected from broiler chicken production firms in Arkansas that having different insecticide application history. Both cypermethrin and λ-cyhalothrin are known to have a very high insecticidal activity either alone or in combination with synergist against various species of insects.[37] Another investigation of us[38] combining PBO both diazinon and cypermethrin indicated a synergistic action at 1:5 ratio followed by 1:2 and 1:1 ratios. PBO increased the toxicity of both diazinon and cypermethrin against *P. americana*. That result suggests that inhibition of oxidative detoxification might be involved to some extent. The results of this investigation also suggest the possibility of PBO as an effective synergist against both adults and larvae of all tested insecticides.

There are no published data on the effects of piperonyl-butoxide (PBO) in combination with λ-cyhalothrin on mortality of adults and larvae of *A. diaperinus*. This led to the present work. The results show that there is an increase in the rate of mortality of the lesser meal worm with the increase of insecticide dose. The median lethal dose (LD_{50}) values of the insecticides are inversely related to the toxicity of the insecticides i.e. higher the LD_{50} value lower the toxicity of the insecticide.

The present data indicates that PBO could be used to restore insecticides susceptibility to *A. diaperinus*. Application of low rates of pyrethroid mixed with PBO will permit growers to attain the benefits of pest management strategy at reduced cost and insecticide input the environment.

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