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Synergistic effects of chlorpyrifos with piperonyl butoxide (pbo) against the lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae)

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ABSTRACT

Objective: To investigate the co–toxicity and co–efficient activity of Chlorpyrifos (Dursban 20EC), an organophosphate and Pyperonyl butoxide (PBO) against the lesser meal worm Alphitobius diaperinus (Panzer) (Coleoptera: Tenebrionidae) (*A. diaperinus*). **Methods:** The repellent activity was carried out 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. **Results:** The Co–efficient values showed that all ratios of chlorpyrifos and piperonyl butoxide offered synergistic action to both larvae and adult. We observed that the toxicity of the chlorpyrifos was decreased as the ratio (amount) of PBO was increased. The individual LD_{50} value of chlorpyrifos for adult is 0.1241 μg/cm^2. But in the mixture, the share of chlorpyrifos are 0.0298, 0.0366, 0.0246 and 0.0108 μg/cm^2 at ratios of 1:1, 1:3, 1:5, 1:10 when PBO causes reduction of dose level of 75.84%, 70.53 %, 80.19% and 91.30% respectively. In case of larvae the individual LD_{50} value of chlorpyrifos is 0.2943 μg/cm^2. But in the mixture, the share of chlorpyrifos are 0.05, 0.019, 0.015 and 0.010 μg/cm at ratios of 1:1, 1:3, 1:5;1:10 when PBO causes reduction of dose level of 80.01%, 93.54%, 94.90% and 96.60% respectively. **Conclusions:** The study suggests that the mortality rate of lesser meal worm 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.

1. Introduction

Insects infesting grain after harvest cause economic loss to producers and the grain and food industry. In this investigation the lesser meal worm *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) (*A. diaperinus*) 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. This beetle was originally a pest of dried meats and stored grains[1]. The adults are general feeders, while the larvae are adapted for feeding on cemented food substances from linseed, cottonseed, oilseed products, tobacco and drugs[2–3]. A report was found in the state of Georgia, with estimated cost of damage and control equating to around $1 200 000 million in 2006[4]. 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[5]. However, loss of 20% or more may occur in the tropical countries through insect attack after harvest[6]. Because the climate

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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 *Salmonella*[7]. It has been reported to be competent reservoirs of tapeworms, avian leucosis virus (ALVs) and turkey enterovirus, *Salmonella typhimurium*[8], *Escherichia coli*[9], *Campylobacter jejuni*[10-11], infectious bursal disease virus (IDBV)[12].

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. Although scientific studies advocate different chemical groups for control of darkling beetles, such as pyrethroids and organophosphates[13], macrocycliclactones, organochlorines, and carbamates[14], resistance to some groups of chemicals, including fenitrothion and ciflutrin, have been recorded[15]. 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[16].

The susceptibility of *A. diaperinus* as the test organism to evaluate the toxicity of two commercially formulated insecticides, an organophospate; chlorpyrifos 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. Chlorpyrifos is an organophosphate, which 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 chlorpyrifos on mortality of adults and larvae of *A. diaperinus*. This led to the present work.

2. 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 (30 ± 0.5) ℃ 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: Dursban 10 EC, Common name: Chlorpyrifos (IUPAC name: O,O-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate), Chemical class: a crystalline organophosphate insecticide (Figure 1A).

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 1B).

3.Acetone: The solvent has been chosen following the guideline or it is a rather generalist solvent.

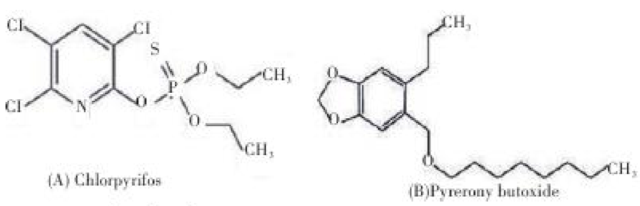

Figure 1. Chemical structure.

The repellent activity carried out by the residual film assay technique with the adapted method[17]. The label rate for each insecticide was prepared with acetone. For each insecticide, 1 mL of the label rate for floor and wall treatment was applied to each of three 9 cm^2 filter papers.

2.1. 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[18]. 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 μg/cm^2. Selected doses were prepared prior to the experiment. According to the results obtained from the pilot experiment doses were prepared of which 1 mL of each of the doses was poured down on the Petri dish (9 cm; *r* =4.5 cm) 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 (30–0.5) ℃ for 24 hours. Mortality of the beetles was recorded after 24 hours of treatment.

2.2. Bioassay of insecticide and synergist mixtures

Each insecticide and a synergist are mixed in acetone at ratios 1:1, 1: 3, 1: 5 and 1:10 applied as mentioned in section. 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 tests with insecticides alone.

2.3. Probit analysis

The percent mortality was subjected to statistical analysis[18-19]. 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[20];

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

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

Probit analysis was done[18,21]. 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.

2.4. Determination of co-toxicity and co-efficient[22]

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

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.

2.5. Construction of isobolograms

The regression lines and isoboles were drawn using the Fig-P (Biosoft) package. Isobolograms for the mixtures of insecticides were constructed[23]. 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.

3. Results

The LD50 value of chlorpyrifos is 0.1241 μg/cm^2 for the adults, 0.2943 μg/cm^2 for the larvae respectively (Table 1) and the mixture (Chlorpyrifos: PBO) of different ratios for the adult are 0.0598 μg/cm^2 at 1:1, 0.1465 μg/cm^2 at 1:3, 0.1477 μg/cm^2 at 1:5 and 0.1190 μg/cm^2 at 1:10; and for the larvae are 0.1000 μg/cm^2 at 1:1, 0.0791 μg/cm^2 at 1:3, 0.0944 μg/cm^2 at 1:5, and 0.1163 μg/cm^2 at 1:10 respectively (Table 2). 95% confidence limits, regression equations and *chi*-squared values have been estimated in the Table 2. Regression lines of different ratios on log probit mortality and the log dose concentrations have been plotted (Figure 2, 3).

To compare the LD_{50} values of the mixtures, the LD_{50} values of the insecticide and synergist have been calculated. Having inversely relation between the LD_{50} values of the insecticides and the toxicity of the insecticide then the co-toxicity and co-efficient effects are determined[24]. The co-toxicity and co-efficient were determined as 416.10, 338.79, 504.06 and 1148.14 for adult and 588.60, 1548.00, 1962.00 and 2943.00 for larvae (Table 3).

The co-efficient values showed that all ratios of chlorpyrifos and piperonyl butoxide offered synergistic action to both larvae and adult. It has been observed that the toxicity of the chlorpyrifos has been decreased as the ratio (amount) of PBO is increased. The free hand curve fitting of isobologram has run below the additive line indicating synergistic action of the mixture at all of the ratios of chlorpyrifos: PBO. The individual LD_{50} value of chlorpyrifos for adult was 0.1241 μg/cm^2. But in the mixture, the share of chlorpyrifos was 0.0298, 0.0366, 0.0246 and 0.0108 μg/cm^2 at ratios of 1:1, 1:3, 1:5;1:10 when PBO causes reduction of dose level of 75.84%, 70.53 %, 80.19% and 91.30% respectively (Figure 4). In case of larvae the isobole shows similar in action (Figure 5). The individual LD_{50} value of chlorpyrifos for larvae was 0.2943 μg/cm^2. But in the mixture, the share of chlorpyrifos was 0.05, 0.019, 0.015 and 0.010 μg/cm^2 at ratios of 1:1, 1:3, 1:5;1:10 when PBO causes reduction of dose level of 80.01%, 93.54%, 94.90% and 96.60% respectively.

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

$$a - s = r \quad ......(1)$$

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

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 Chlorpyrifos (Dursban) on *A. diaperinus* after 24 h of exposure.

	Doseμg/cm^2	Log dose	Num.	Kill	% kill	Cor%	Emp probit	Expt probit	Work probit	Weight	Final probit
Adults	0.982	1.9921	60	48	80.0	80	5.85	5.9061	5.87	28.26	5.931
	0.491	1.6911	60	45	75.0	75	5.67	5.6016	5.67	33.48	5.618
	0.246	1.3909	60	38	63.3	63	5.33	5.2983	5.35	37.62	5.306
	0.123	1.0899	60	29	48.3	48	4.95	4.9940	4.94	38.04	4.994
	0.061	0.7888	60	23	38.3	38	4.69	4.6898	4.68	36.06	4.681
	Contr.		60	0							
Mature larvae	1.960	1.2922	60	53	88.3	88	6.18	6.1374	6.18	24.30	6.116
	0.980	0.9912	60	46	76.7	76	5.71	5.7209	5.70	31.92	5.708
	0.490	0.6901	60	36	60.0	59	5.23	5.3044	5.25	37.62	5.300
	0.245	0.3892	60	28	46.7	46	4.90	4.8880	4.92	37.62	4.892
	0.122	0.0864	60	19	31.7	31	4.50	4.4690	4.51	33.48	4.481
	Contr.		60	1							

For adults: Y = 3.866488 + 1.036074 X, LOG LD_{50} is 1.094046, LD_{50} is 0.12417, No significant heterogeneity, *Chi*-squared is 0.40598 with 3 degrees of freedom, 95% Confidence limits are 0.083991 to 0.183595; For mature larvae: Y = 4.364404 + 1.355468X, Log LD_{50} is 0.4689127, LD_{50} is 0.2943829 No significant heterogeneity, *Chi*-squared is 0.41639 with 3 degrees of freedom, 5% Confidence limits are 0.22173 to 0.390839.

Table 2

LD_{50}, 95% confidence limits, regression equation and χ^2 values of dose mortality experiments of different ratios of Chlorpyrifos (Darsban) with PBO against *A. diaperinus* with 24 h of treatment.

	Ratio	LD_{50} value(μg/cm −2)	95% confidence limits		Regression equations	χ^2 value (*df*=3)
			Upper	Lower		
Adult	1:1	0.05978	0.079181	0.045143	Y = 2.437142 + 1.442558X	2.3727
	1:3	0.14645	0.199346	0.107595	Y = 3.312071 + 1.447993X	0.5632
	1:5	0.14765	0.192832	0.113054	Y = 3.353871 + 1.407869X	0.4063
	1:10	0.11906	0.099477	0.142499	Y = 2.701476 + 2.136632X	1.7241
Mature larvae	1:1	0.10000	0.131540	0.07600	Y = 3.610504 + 1.389269X	2.9184
	1:3	0.07910	0.111270	0.05620	Y = 4.032346 + 1.077065X	2.2875
	1:5	0.09440	0.13250	0.06737	Y = 3.838118 + 1.191158X	0.9401
	1:10	0.11600	0.17390	0.07770	Y = 3.711418 + 1.209154X	0.3704

Table 3

Co-toxicity coefficient of piperonyl butoxide (PBO) with chlorpyrifos applied in different ratios on A. diaperinus after 24 h of application.

	Insecticide LD_{50} (μg/cm^2)	Ratio Insecticid:PBO	Combined LD_{50} (μg/cm^2)	Insecticide LD_{50} (μg/cm^2)	PBO LD_{50} (μg/cm^2)	Co-toxicity coefficient
Adult	0.1242	1:1	0.0597	0.0298	0.0298	416.1
		1:3	0.1464	0.0366	0.1098	338.7
		1:5	0.1476	0.0246	0.1230	504.0
		1:10	0.1190	0.0108	0.1080	1148.0
Mature larvae	0.2943	1:1	0.1000	0.05	0.0500	588.6
		1:3	0.0791	0.019	0.0570	1548.0
		1:5	0.0944	0.015	0.0750	1962.0
		1:10	0.1160	0.010	0.1000	2943.0

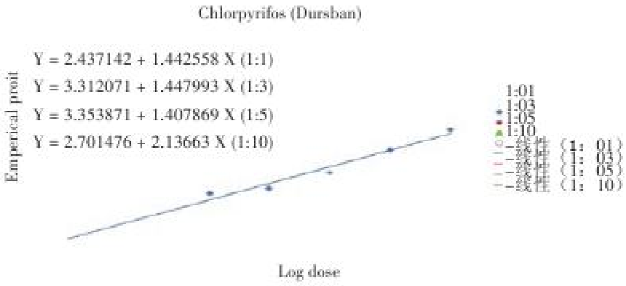

Figure 2. Regression lines of probit mortality on log dose of the mixture of chlorpyrifos and piperonyl- butoxide at the ratio of 1:1, 1:3, 1:5 and 1:10 against *A. diaperinus* adult after 24h of exposure.

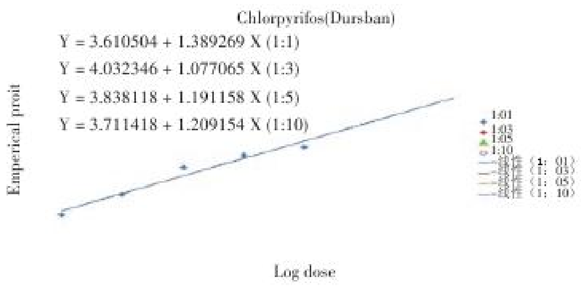

Figure 3. Regression lines of probit mortality on log dose of the mixture of chlorpyrifos and piperonyl- butoxide at the ratio of 1:1, 1:3, 1:5 and 1:10 against *A. diaperinus* mature larvae after 24h of exposure.

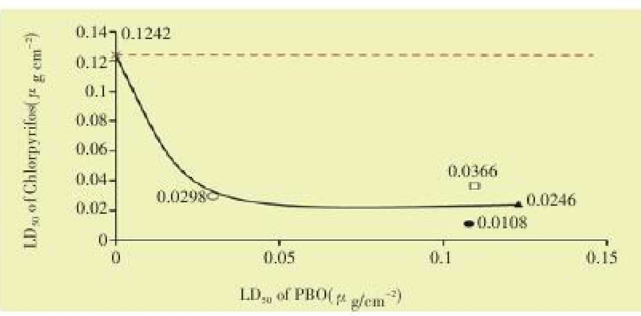

Figure 4. Isobolograms of LD_{50} of Chlorpyrifos and Piperonyl butoxide applied on *A. diaperinus* adults.

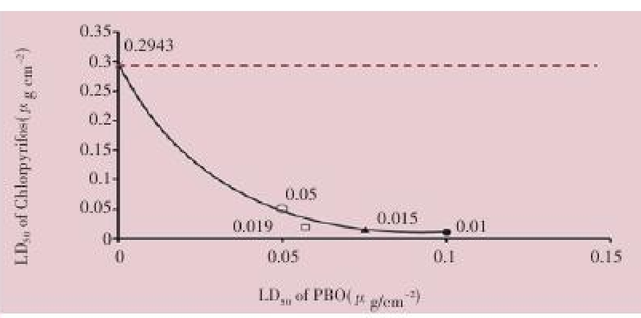

Figure 5. Isobolograms of LD_{50} of Chlorpyrifos and Piperonyl butoxide applied on *A. diaperinus* mature larvae.

4. Discussion

In the present investigation commercial formulation of Chlorpyrifos (Dursban 20EC) was tested against the 7 day old adult and 40 day mature larvae of *A. diaperinus*. The LD_{50} value was recorded 0.1241 μg/cm^2 for adult and 0.2943 μg/cm^2 for larvae respectively after 24 h exposure. It is the general agreement[25] who observed that LD_{50} value of chloropyrifos as 0.896 μg/cm^2. The difference of the result is probably due to the difference in the emulsifiable concentrate formulation as product formulation affect efficacy of insecticide. They used chlorban 20 EC but in this investigation dursban 20 EC was used. Another study[26] were reported that they tested malathion 57 EC against the lesser grain borer Rhizopertha dominica and obtained LD_{50} value as 1.267 μg/cm^2 suggesting that malathion was less toxic than chlorpyrifos. Similar study has been introduced[27] who used tetrachlorvinphos as an organophosphate insecticide against susceptible laboratory reared strain of *A. diaperinus*. At 48h, the LD_{50} values for tetrachlorvinphos were recorded as 0.080 μg/cm^2 for adult and 0.070 μg/cm^2 for larvae. The investigation was conducted at 26.5 ℃. If the temperature would elevate, the same mortality might be obtained at 24 h of exposure, as found in the present investigation.

Other studies concerning investigation have found similar results[28] who tested chlorpyrifos against another stored grain product pest Tribolium castaneum. They obtained the LD_{50} value of chlorpyrifos as 0.0138 μg/cm^2 for adult. The larvae were not included in this bioassay. The results of this investigation are also in general agreement[29] who conducted toxicity trails using chlorpyrifos to compare the susceptibility of resistant and laboratory susceptible population of A. diaperinus to chlorpyrifos by residual film method at (21±2) ℃. He obtained 24h LD_{50} values for the susceptible laboratory population as 0.097 μg/g for adult and 0.07 μg /g for larvae. The variation in the result could be attributed to factor such as he used technical grade of chlorpyrifos but in this investigation commercial formulation was used.

There are no published data on the effects of piperonyl-butoxide (PBO) in combination with chlorpyrifos 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.

Conflict of interest statement

We declare that we have no conflict of interest.

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