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Toxicity of newer insecticide molecules against lesser grain borer, *Rhyzopertha dominica* (Fabricius) (Bostrichidae: Coleoptera)

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Abstract

To find out the insecticidal activity of commercial formulations of newer insecticides with novel mode of action *viz.*, emamectin benzoate, lufenuron, chlorfenapyr, chlorantranilprole are compared with check malathion and deltamethrin were evaluated for their toxicity against *Rhyzopertha dominica* by jute cloth disc impregnation method under laboratory conditions during the year 2015-16 at the Department of Entomology. Among the new insecticides tested for the management of *R. dominica*, chlorfenapyr was found to be the best at LC_{99.9} level with the highest relative toxicity of 7.45 times than malathion and 7.34 times than deltamethrin. Emamectin benzoate was found to be the best at LC_{99.9} level with the relative toxicity of 1.82 times than malathion and 1.79 times than deltamethrin. The relative toxicity at LC₅₀ level of chlorantranilprole 2.67 times than malathion and 4.25 times than deltamethrin. Lufenuron expressed relative toxicity at LC₅₀ level 0.63 times than malathion and 0.68 times than deltamethrin and at LC₉₉ level 1.00 times than malathion and 0.67 times than deltamethrin.

Keywords: *Rhyzopertha dominica*, malathion, deltamethrin, newer insecticide molecules

1. Introduction

Storage of the grains and seeds is very important in agriculture to start a new life and also in food security point of view. The quality grains and seeds during storage depends on various factors such as crop or variety, initial seed quality, storage conditions, seed moisture content, insect pests, bacteria and fungi^[1]. Among these factors insect pests are problematic throughout the world, because they decrease the quantity and quality of grain. Food grain accounts for 20-25% damage by storage insect pests^[2] which is really a brainstorming matter. Among various storage insect pests lesser grain borer, *Rhyzopertha dominica* is a severe insect pest of wheat and other cereal grains under storage condition. It is an internal feeder and cause significant loss to cereals affecting the quantity as well as the quality of the grains. The use of chemical agents to prevent or control insect infestations has been the main method of grain protection, since it is the simplest and most cost-effective means of dealing with stored product pests^[3]. Insect pests may behave differently when in contact with pesticides and these changes in behaviour may contribute to their tolerance of pesticide. Resistance of insect pests to pesticides is an example of evolution of the species showing how they can survive and change physiologically under pressure by chemicals. Therefore, there is a great need to find out suitable insecticides which prove effective against the insecticide resistant insect species.

2. Materials and methods**2.1. Beetle culture and maintenance**

The population of the test insect, *R. dominica*, collected from the Rice Research Unit (Bapatla) were maintained on wheat grains in the laboratory. Initially the wheat grains were disinfested by fumigating with aluminium phosphide tablets @ one per 30 kg grains for seven days to ensure that they were free from insects and mites. About 250 g of wheat grains were taken in a plastic jar measuring 45 × 15 cm and about 20 adults were released into it for oviposition. Adults were removed after 20 days and released into another jar containing wheat grains, thus a succession of the insects was maintained for utilizing the eggs laid staggeredly to ensure constant supply of test insects of known age. The newly emerged adults were transferred into new containers with fresh wheat grains and used for the multiplication of culture and also for conducting the experiments.

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The pest population was maintained at an optimum temperature of $32 \pm 1^{\circ}\text{C}$ and 75 per cent relative humidity throughout the period of investigation^[4].

2.2 Test insecticides

S. No	Common Name and Strength of the insecticide	Trade Name
1	Emamectin benzoate 5% SG	Proclaim
2	Lufenuron 5.4% SC	Cigna
3	Chlorfenapyr 10% SC	Intrepid
4	Chlorantraniliprole 18.5% SC	Rynaxypyr
5	Malathion (Check) 50% EC	Meltox
6	Deltamethrin (Check) 2.8% EC	Decis

2.3 Test insect population

The adult beetles of *R. dominica* (seven days old) of the F1 generation, reared in the laboratory from the initial culture obtained from the Rice Research Unit (RRU) - Bapatla, Andhra Pradesh were subjected to bioassay.

2.4 Bioassay

The adult beetles of *R. dominica* of one week old were subjected to the bioassay with the test insecticides by following jute cloth disc impregnation method^[5]. Stock solution of one per cent concentration of respective test insecticides was prepared by weighing the required quantities of insecticides, by using acetone as solvent. The graded concentrations of the test insecticides were prepared with acetone as solvent by following serial dilution technique. The quantity of insecticidal solution required to impregnate the jute cloth of nine centimeters diameter was determined. After impregnation, the jute cloth discs are air dried. One day old beetles were collected from the culture and kept under starvation for two hours. The starved beetles are transferred to the petriplates containing insecticide impregnated jute cloth disc @ 20 beetles/petriplate in three replications for each test insecticide. The insects were confined to the treated surface for 24 hours, 48 hours and 72 hours. Simultaneously, a control was also maintained with jute cloth disc impregnated in acetone only.

2.5 Data collection

Mortality was recorded at 24, 48 and 72 hours after treatment (HAT). A preliminary experiment was conducted with a wide range of concentrations followed by a narrow range to get mortality in the range of 5-90%. There was no occasion of the use of Abbott's formula since mortality was not recorded in control, because of the use of almost same age beetles and the experiment conducted under controlled ambient conditions of 32°C temperature and 75 per cent relative humidity. Moreover, a separate set of new petriplates were utilized for control avoiding the use of petriplates that were used for insecticidal treatments. In order to know the immediate toxicity of the chemical, the mortalities were recorded at 24 HAT and subsequently at 48 HAT. Mortality at 72 HAT was also recorded to know the mortality end point.

2.6 Assessment of relative toxicity of newer insecticide molecules:

The relative toxicity of newer insecticide molecules over malathion and deltamethrin is calculated by following formula

$$\text{Relative toxicity of newer insecticide} = \frac{\text{LC}_{50} \text{ of malathion or deltamethrin (check)}}{\text{LC}_{50} \text{ of newer insecticide molecules}}$$

2.7 Statistical Analysis

Data was analyzed by using Probit analysis in SPSS 16.0 v. (Statistical Package for Social Sciences) software to calculate LC_{50} , $\text{LC}_{99.9}$, heterogeneity (χ^2), intercept : (a), slope of the regression line (b), regression equation and fiducial limits. The degree of resistance acquired by *R. dominica* were calculated for the data at 72 HAT. The resistance factor was calculated by dividing the $\text{LC}_{50} / \text{LC}_{99.9}$ value of each population with the $\text{LC}_{50} / \text{LC}_{99.9}$ value of susceptible populations. The log concentration probit (lcp) lines were drawn by plotting log concentrations on X-axis and probits on Y-axis and the response of test insect populations was studied at different concentrations of the test insecticides^[6].

3. Results

3.1 Toxicity of insecticides against the *R. dominica*

3.1.1 Malathion

R. dominica recorded (Table 1) the LC_{50} and $\text{LC}_{99.9}$ values as 0.0578 and 8.1402 per cent, respectively to malathion at 24 HAT. The corresponding values slightly decreased with increased mortality at 48 HAT (0.0387% and 6.9915%) and at 72 HAT (0.0217% and 3.4883%). The slope (b) values of log concentration probit (lcp) lines of malathion were 1.082, 1.030 and 1.054 at 24, 48 and 72 HAT, respectively.

3.1.2 Deltamethrin

The data (Table 1) revealed that LC_{50} and $\text{LC}_{99.9}$ values of deltamethrin with population of *R. dominica* at 24 HAT were 0.0752 and 6.3903 per cent, respectively. At 48 and 72 HAT there was a slight increase in the mortality with consequent decrease in the LC_{50} (0.0533 and 0.0345%) and $\text{LC}_{99.9}$ (5.2900% and 3.4338%) values, respectively. The slope (b) values of log concentration probit (lcp) lines were 1.205, 1.165 and 1.164 at 24, 48 and 72 HAT, respectively.

3.1.3 Lufenuron

The toxicity of lufenuron to the adults of *R. dominica* revealed (Table 1) the LC_{50} and $\text{LC}_{99.9}$ values of 0.0826 and 8.1678; 0.0641 and 7.5409; 0.0342 and 5.1004 per cent at 24, 48 and 72 HAT, respectively. The slope (b) values of log concentration probit (lcp) lines for lufenuron were 1.166, 1.123 and 1.070 at 24, 48 and 72 HAT, respectively. Lufenuron was 0.99, 0.92 and 0.68 times more toxic than malathion at 24, 48 and 72 HAT, respectively at $\text{LC}_{99.9}$ level and while at LC_{50} level, it showed relatively less toxic in comparison with malathion. (Table 2). At LC_{50} it showed 0.91, 0.83 and 1.00 times more toxic than deltamethrin (Table 3), whereas at $\text{LC}_{99.9}$ it showed less relative toxicity.

3.1.4 Emamectin Benzoate

The toxicity of emamectin benzoate to the adults of *R. dominica* revealed (Table 1) that the LC_{50} and $\text{LC}_{99.9}$ values were 0.0256 and 6.8105; 0.0164 and 3.2302 ; 0.0085 and 1.9150 per cent at 24, 48 and 72 HAT respectively. The slope (b) values of log concentration probit (lcp) lines for emamectin benzoate were 0.959, 1.013 and 0.988 at 24, 48 and 72 HAT, respectively. At LC_{50} emamectin benzoate was 2.25, 2.35 and 2.55 times more toxic than malathion (Table 2), whereas same insecticide was found to be 2.93, 3.25 and 4.05 times more toxic than deltamethrin (Table 3) at 24, 48 and 72 HAT, respectively while at $\text{LC}_{99.9}$ level it showed less relative toxicity.

3.1.5 Chlorfenapyr

The toxicity of chlorfenapyr to the adults of *R. dominica* revealed the LC₅₀ and LC_{99.9} values of 0.0837 and 2.5970; 0.0616 and 1.5367; 0.0555 and 0.4678 per cent at 24, 48 and 72 HAT, respectively (Table 1). The slope (b) values of lcp lines for deltamethrin were 1.559, 1.665 and 2.512 at 24, 48 and 72 HAT, respectively. The chi-square test revealed that the population used in the study was homogenous ($P < 0.05\%$). Chlorfenapyr was 3.13, 4.54 and 7.45 times more toxic than malathion (Table 2) and 2.46, 3.44 and 7.34 times more toxic than deltamethrin (Table 3) at 24, 48 and 72 HAT, respectively at LC_{99.9}. While at LC₅₀ level it did not show relative toxicity in comparison with malathion and deltamethrin.

3.1.6 Chlorantraniliprole

Chlorantraniliprole showed LC₅₀ and LC_{99.9} values of 0.0214 and 8.8799; 0.0113 and 4.4143; 0.0081 and 3.1491 per cent at 24, 48 and 72 HAT, respectively to the adults of *R. dominica* (Table 1). The slope (b) values of lcp lines for deltamethrin were 0.888, 0.898 and 0.897 at 24, 48 and 72 HAT, respectively. The chi-square test revealed that the population used in the study was homogenous ($P < 0.05\%$). At LC₅₀ chlorfenapyr was recorded as 2.70, 3.42 and 2.67 times more toxic than malathion (Table 2) and 3.51, 4.71 and 4.25 times more toxic than deltamethrin (Table 3) at 24, 48 and 72 HAT, respectively. While at LC_{99.9} level 0.91, 1.58 and 1.10 times more toxic than malathion and 0.71, 1.19 and 1.09 times more toxic than deltamethrin.

A comparison of the LC₅₀ and LC_{99.9} values of the newer insecticides with those of malathion revealed the relative toxicity in decreasing order as chlorantraniliprole (2.67) > emamectin benzoate (2.55) > lufenuron (0.63) > chlorfenapyr (0.39) at LC₅₀ level and chlorfenapyr (7.45) > emamectin benzoate (1.82) > chlorantraniliprole (1.10) > lufenuron (0.68) at LC_{99.9} level at 72 HAT. The LC₅₀ and LC_{99.9} values of the newer insecticides with those of deltamethrin revealed the relative toxicity in decreasing order as chlorantraniliprole (4.25) > emamectin benzoate (4.05) > lufenuron (1.00) > chlorfenapyr (0.62) at LC₅₀ level and chlorfenapyr (7.34) > emamectin benzoate (1.79) > chlorantraniliprole (1.09) > lufenuron (0.67) at LC_{99.9} level at 72 HAT.

4. Discussion

Malathion and deltamethrin has been in use as common

insecticides for postharvest insect control in the United States since 1958^[7]. Its use spread to several countries in the 1960s, and its intensive use against stored grain insect pests resulted in the development of severe resistance in many insect species^(18, 91). Malathion resistance was earlier reported in the United States populations of lesser grain borer by^(18, 10; 111). Several studies in the past have also shown that many OP insecticides including malathion resulted in the development of resistance in many insect species including lesser grain borer^(19; 121). Sublethal doses of malathion greatly suppressed AcP (acid phosphatase) activity in resistant beetles, which might be due to inhibition of this enzyme under insecticidal toxicity and impairing of the lysosomal activity to hydrolyze the macromolecules and in turn limiting the ability of the resistant beetles to use energy rich compounds to obtain energy.

Chlorantraniliprole was found effective at LC₅₀ level for complete control of *R. dominica* followed by emamectin benzoate. The present results are at par with the research findings of^[13] who reported that more than 92 per cent of mortality was observed in *R. dominica* when exposed to chlorantraniliprole about 14 days. Similarly, emamectin benzoate had an adverse effect on stored grain pest *Cryptolestes ferrugineus* and with an increase in concentration the effect also increases^[14]. Emamectin benzoate showed very lethal effects against salmon louse when it is applied to its different stages^[15]. Chlorfenapyr was found to be 7.45 times more toxic than malathion at LC_{99.9} level and 7.34 times more toxic than deltamethrin. But at LC₅₀ level, they did not show any relative higher toxicity *i.e.*, total control of adults of *R. dominica* was not possible.^[16] reported that, no adult of *Tribolium castaneum* survived when exposed on concrete treated with chlorfenapyr at the maximum rate of 1.1 g a.i.m⁻² and no progeny were produced in bioassays conducted at 0-8 weeks post treatment. Chlorfenapyr is an insecticidal pyrrole, and the primary mode of action is to affect oxidative phosphorylation in the mitochondria, which will eventually result in the death of the cell through inhibition of ATP synthesis^[17]. Lufenuron showed less relative toxicity compared to other insecticides. Lufenuron @ 0.5g.kg⁻¹ could completely prevent the adult emergence of rice weevil, *Sitophilus oryzae*^[18]. Similarly,^[19] concluded that at sub-lethal concentrations (LC₁₀, LC₂₀ and LC₄₀) of lufenuron has a very good larvicidal and ovidical activity in *T. castaneum*.

Table 1: Toxicity of newer insecticide molecules against population of lesser grain borer, *R. dominica*

Hours after treatment	LC ₅₀ % (95%FL)	LC _{99.9} % (95%FL) *	Slope b (±SE)	Heterogeneity (χ ²)	Regression Equation (Y=a+bx)
Lufenuron (5.4% EC)					
24	0.0826(0.0650-0.1071)	8.1678(3.5284-28.5061)	1.166 (±0.120)	9.52	Y= 1.62+1.25x
48	0.0641(0.0499-0.0827)	7.5409(3.2542-26.3169)	1.123 (±0.115)	11.88	Y= 2.25+1.50x
72	0.0342(0.0254-0.0444)	5.1004(2.2660-17.0302)	1.070 (±0.110)	7.28	Y= 2.12+1.25x
Emamectin Benzoate (5% Sg)					
24	0.0256(0.0191-0.0351)	6.8105(2.5486-28.4649)	0.959 (±0.093)	2.40	Y= 1.5+1.00x
48	0.0164(0.0123-0.0218)	3.2302(1.3822-10.7993)	1.013 (±0.093)	5.41	Y= 2.1+1.20 x
72	0.0085(0.0061-0.0113)	1.9150(0.8432-6.1848)	0.988 (±0.093)	2.96	Y= 2.6+1.20x
Chlorfenapyr (10% Sc)					
24	0.0837(0.0702-0.1018)	2.5970(2.2531-8.5704)	1.559 (±0.196)	8.18	Y= 2.08+1.66x
48	0.0616(0.0517-0.0727)	1.5367(0.8507-3.8658)	1.665 (±0.190)	11.05	Y= 2.33+1.66x
72	0.0555(0.0471-0.063)	0.4678(0.3263-0.8263)	2.512 (±0.298)	8.51	Y= 3.50+2.50x
Chlorantraniliprole (18.5% Sc)					
24	0.0214(0.0155-0.0299)	8.8799(3.2189-37.9370)	0.888 (±0.083)	3.58	Y= 1.50+1.00x
48	0.0113(0.0081-0.0156)	4.4143(1.7693-15.9536)	0.898 (±0.080)	5.63	Y= 2.00+1.00x
72	0.0081(0.0057-0.0111)	3.1491(1.3063-10.7863)	0.897 (±0.080)	8.56	Y= 2.50+1.00x
Malathion (Check)					

24	0.0578(0.0432-0.0824)	8.1402(2.8953-40.1016)	1.082 (± 0.120)	3.72	Y=2.33+1.67x
48	0.0387(0.0290-0.0536)	6.9915(2.4898-34.3798)	1.030 (± 0.115)	3.19	Y=1.39+2.11x
72	0.0217(0.0162-0.0288)	3.4883(1.4029-13.8462)	1.054 (± 0.113)	2.40	Y=3.11+1.39x
Deltamethrin (Check)					
24	0.0752(0.0581-0.1059)	6.3903(2.2113-38.5913)	1.205 (± 0.161)	3.65	Y=2.0+1.67x
48	0.0533(0.0415-0.0713)	5.2900(1.9117-28.8660)	1.165 (± 0.151)	4.47	Y=2.5+1.67x
72	0.0345(0.0265-0.0444)	3.4338(1.3685-15.3492)	1.164 (± 0.145)	5.05	Y=3.0+1.67x

*Values in parenthesis indicates fiducial limits (FL)

The chi-square test revealed the homogeneity of the test population ($P < 0.05\%$).

Table 2: Relative toxicity of newer insecticides in comparison with malathion against resistant lesser grain borer, *R. dominica* for its management at 72 HAT

Hours after treatment	Lufenuron (5.4% EC)		Emamectin benzoate (5% SG)		Chlorfenapyr (10% SC)		Chorrantraniliprole (18.5% SC)	
	LC ₅₀	LC _{99.9}	LC ₅₀	LC _{99.9}	LC ₅₀	LC _{99.9}	LC ₅₀	LC _{99.9}
24	0.69	0.99	2.25	1.19	0.69	3.13	2.70	0.91
48	0.60	0.92	2.35	2.16	0.62	4.54	3.42	1.58
72	0.63	0.68	2.55	1.82	0.39	7.45	2.67	1.10

Table 3: Relative toxicity of newer insecticides in comparison with deltamethrin against lesser grain borer, *R. dominica* for its management at 72 HAT

Hours after treatment	Lufenuron (5.4% EC)		Emamectin benzoate (5% SG)		Chlorfenapyr (10% SC)		Chorrantraniliprole (18.5% SC)	
	LC ₅₀	LC _{99.9}	LC ₅₀	LC _{99.9}	LC ₅₀	LC _{99.9}	LC ₅₀	LC _{99.9}
24	0.91	0.78	2.93	0.93	0.89	2.46	3.51	0.71
48	0.83	0.70	3.25	1.63	0.86	3.44	4.71	1.19
72	1.00	0.67	4.05	1.79	0.62	7.34	4.25	1.09

5. Conclusion

Among the new insecticides tested for the management of *R. dominica*, chlorfenapyr (10% SC) was found to be the best treatment at LC_{99.9} level for complete control of *R. dominica* with the highest relative toxicity (7.45 and 7.34 times) than malathion (50% EC) and deltamethrin (2.8% EC) respectively followed by emamectin benzoate (5% SG) (1.82 and 1.79 times). Chlorantraniliprole (18.5% SC) was also found to be effective at LC₅₀ level (2.67; 4.25) compared to malathion and deltamethrin. Lufenuron (5.4% EC) recorded to be toxic at LC₅₀ and LC_{99.9} level (0.63 and 0.68 times) to malathion and (1.00 and 0.67 times) to deltamethrin.

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