EFFICACY OF CERTAIN PESTICIDES AGAINST *RHYZOPERTHA DOMINICA* (F.) AND *SITOPHILUS GRANARIUS* (L.) ADULTS AND THEIR RESIDUE LEVELS IN TREATED WHEAT GRAINS

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Abstract

The effectiveness of Malatox (1%D), Chess (25%WP), Rizolex (10%D) and Ecotech Bio (10%WP) against the lesser grain borer, *Rhyzopertha dominica* (F .) and the grain weevil, *Sitophilus granarius* (L .) adults were evaluated in the laboratory. Direct treatments of wheat grains with the tested pesticides was effective on insects than sack treatments. Toxicity of the examined pesticides could be arranged in the following descending order : Malatox > Chess > Rizolex > Ecotech.Bio . The grain weevil *S. granarius* was more susceptible to the effect of four tested pesticides than the lesser grain borer *R. domainica*. All the tested pesticides reduced the (F_1) progeny of the treated insects. High significant reductions in total carbohydrates and lipids obtained in body tissues of the two pests feed on LC_{50} treated grains with four tested pesticides. The reduction in total proteins was insignificant in weevils only. The activity of transaminases (GOT and GPT) was highly reduced in *R.dominica* than *S. granarius* but this reduction in acetyl cholinesterase and amylase was insignificant in the two pests.

Quantitative analysis of Malatox (1% D Malathion), Rizolex (10 % D Tolclofos-methyl) and Chess (25% WP Pymetrozine) as active ingredient for residues in treated wheat grains were performed by GC and HPLC systems. High residues of pesticides were presented in unwashed grains while the washed grains contain very small residues of tested pesticides

Key words : *Rhyzopertha dominica* , *Sitophilus granarius* , Proteins, Carbohydrates, Lipids , enzymes, residue of pesticides in grains.

INTRODUCTION

The granary weevil, *Sitophilus granarius* (L.) and the lesser grain borer, *Rhyzopertha dominica* (F.) are the most common pests of many kinds of stored grains. The two pests cause extensive damage to stored product materials and contaminated it with insect fragments, faeces, webbing, ill-smelling metabolic products and abundance of fungi growth (Barney *et al.*, 1995, Schnur, 1999 and Pajom, 2000).

A large proportion of stored cereal grain is protected against insect attack by insecticide that one good potential residues. Contact insecticide admixture with grain entering stores is widely used especially for stored wheat protection. Active substances with a long persistence of insecticidal activity, such as the organophosphate pesticides are the most commonly used for this purpose (Fleurat-Lessard *et al.*, 1998 and Cox *et al.*, 2000). Malathion is particularly valuable for the protection of stored products due to it's low acute toxicity to human and other warm blooded animals (Williame *et al.*, 1978 and Patoural and Joyeb, 1988).

The control on pesticide residues in grain is generally based on maximum residue limit (MRL) expected if the registered dosages are applied according to good agricultural practice. However, for wheat the distribution of insecticide residues in the milled products or the fate of residues during storage of treated grain remain largely unknown (Feillet, 2000).

The most promising alternative to residual pesticides in stored grain is the use of microbial control, particularly the use of *Bacillus thuringiensis* (B.t.). Keever (1994) reported that the ABG-6206 formulation of thuringiensin (*Bacillus thuringiensis* subsp. *Thuringiensis*) reduced adult emergence of the stored product pests *Sitophilus zeamais, R. dominica* and *Ephestia elutella* by 82, 99.3 and 100% resp. when added to diet medium. Price *et al.*, (1997) mentioned that seven novel B.t. isolates were effective against foreign grain beetle adults and nineteen isolates were toxic to the same insect larvae.

In the current study, we evaluated the toxicity of four pesticides; Malatox (1% D), Chess (25% WP), Rizolex (10% D) and Ecotech Bio (10% WP) against *S. granarius, (L.) and R. dominica* (F.) by two methods of treatments; sack treatment and wheat grain treatment. Effect of LC_{50} grain treatments with four tested pesticides on total carbohydrates, lipids and proteins was carried out, also, the activity of amylase, acetylcholine esterase and transaminases (GOT and GPT) enzymes of whole body tissue of two insect adults were determined. Residues of three chemical pesticides in treated wheat grains were detected.

MATERIALS AND METHODS

1- Insects:

The two insect species *S. granarius,* and *R. dominica* were reared on clean and sterilized wheat grains under constant conditions of 25±2 °C, 60±5% R.H. and a photoperiod of 16:8 hrs. L:D (Singh and Wilbur, 1966) for five years in Centeral Agricultural pesticides laboratory, Agriculture Research Center, without any exposure to insecticides.

2- Pesticides:

- Malatox [1% D Malathion, organophosphorus insecticide] used at concentrations 56, 42, 28, 14, 7, 3.5 and 1.75 ppm.
- Chess [25% WP Pymetrozine, selective feeding blocker insecticide] used at concentrations 2143, 1071.5, 535.75, 267.88, 133.94, 66.96 and 33.48 ppm.
- Rizolex [10% D Tolclofos-methyl, aromatic hydrocarbon fungicide] used at concentrations 3571.43, 1785.71, 892.86, 446.43, 223.21, 111.61 and 55.80 ppm.
- Ecotech Bio [10% WP 16000 iu/mg Bacillus thuringiensis subsp. Kurstaki (EC 2371) Ecogen bioinsecticide] used at concentrations 3571.43, 1785.71, 892.86, 446.43, 223.21, 111.61 and 55.80 ppm.

3- Bioassay techniques:

3.1 Sack treatment:

Aqueous emulsion of seven serial concentrations of Malatox (1% D), Chess (25% WP), Rizolex (10% D) and Ecotech Bio (10% WP) pesticides were prepared. Cotton cloth sacks (15X20 cm) were dipped (2 min.) in each concentration of four tested pesticides then left for a suitable time (3 hrs.) until dryness. Three replicates for each concentration and control (dipped in water) were used. Clean wheat grains were sterilized by drying at 50 °C for 6 hrs. To kill any prior insect infestation. Each Control and treated sack was filled with wheat grains (50 grams) and thirty adults (7-14 days old) from two insect species were added to each sack. The sacks were binded tightly to prevent the insects escaping and kept under laboratory conditions for one week. Mortality counts were recorded and corrected with Abbott's formula (1925). Results were statistically analyzed by Finney (1971) and the toxicity index of each pesticide was determined according to Sun (1950).

3.2 Grain treatment:

Wheat grains (50 grams) were mixed with each concentrations of four tested pesticides in glass jars and thirty (1-2 weeks old) of two insect species were added to each jars (three replicates for control and each concentration). The percentages of mortality were recorded 7 days after treatment and the data were analyzed.

The effect of tested pesticides on F_1 Progeny of two insect species was evaluated when LC_{50} concentration of each pesticide was mixed with hundred grams of wheat grains in glass jars, then fifteen females and fifteen males of two insects were added. The observed mortality was recorded after seven and fourteen days, the remaining living adults were removed to prevent their mixing with first generation (F_1) off spring. The jars were kept under laboratory condition until the emergence of adults (after 45 days of treatment). Percentage of reduction in adult emergence of F_1 Progeny was calculated as the following equation:

Percent reduction of progeny = (Cn – Tn)/ Cn X 100

Where:

Cn is the mean number of emerged adults in control

Tn is the mean number of emerged adults in treatment

To study the bio-residual activity of tested pesticides, the grains were treated with recommended concentration of the three chemical and LC_{90} of Ecotech-Bio pesticides and exposed to two insect species after different periods of storage; 1,3 and 5 months post treatment. The percentages of mortalities were recorded after 7 and 14 days of treatment then the parent adults were removed and the reduction in F_1 Progeny were determined after 45 day of insect treatment. Germination of treated grains was examined after initial treatment and different storage periods (according to El-Nahal *et al.,* 1969).

4- Biochemical determination:

About 100 mg of the control and LC_{50} treated adults (Feed on treated grains for 14 days) of *S. granarius,* and *R. dominica* were crushed in 5 ml of 0.15M NaCl solution at 4 °C with the help of the mortar driven Teflon-glass homogenizer. The homogenate of insect whole body tissues was centrifuged at 4000 rpm for 10 min at 4 °C. The supernatant obtained was used for determination of total proteins, lipids and carbohydrates in total body homogenate of two insect species adults according to Bradford (1976) Singh and Sinha (1977) and Knight *et al.*, (1972) respectively. The activity of acetylcholinestrase (AChE) in body homogenate of insects was measured according to method of Simpson *et al.*, (1964), by using acetylcholine bromide (AChBr). The ´activity of Glutamic oxaloacetic transaminase and Glutamic pyruvie transaminase (GOT anf GPT) were determined according to method of Reitman and Frank (1959). Determination of amylase activity was described by Ishaaya and Swirisiki (1976) by using 3-5 dinitro salicylic acid reagent. The data statistically analyzed according to the equation of Dixon and Matssay (1957).

5- Residues analysis:

Hundred grams of wheat grains were treated with the recommended concentration of Malatox 1% D Malathion (80 mg/kg^{-1}) and Rizolex 10% D Tolclofos-methyl (300 mg/kg^{-1}) and 80 mg/kg^{-1} of Chess 25% WP Pymetrozine. Three replicates of each treatment were prepared then one of them was frigerate (-80 °C) and the other two replicates were stored at laboratory temperature (25±2 °C) for five months. After the storage period, one replicate of each treatment was unwashed and the other one was washed with run water for five minutes then left (6 hrs.) to dryness. The unwashed and washed samples were frozen.

5.1 Extraction:

The method described by (Storherr and Watts, 1968) was followed for extraction Malathion, Tolclofos-methyl and Pymetrozine residues from initial treated and stored (unwashed and washed) wheat grains. Twenty grams of each wheat sample were transferred into a glass flask with 50 gm activated anhydrous sodium sulfate (Na_2SO_4) and 200 ml ethyl acetate (HPLC grade) and shaken for 16 hrs. on high speed. The samples were filtered and evaporated to dryness using rotary evaporator at 35 °C.

5.2- Cleaning up:

The florisil column clean up procedure of (Mills et al., 1972) was used in cleaning the sample extract for Tolclofos-methyl and Malathion. The sample extract was transferred quantitvely to the column and then eluted with 200 ml eluant (50% dichloromethane, 48.5% n-hexane and 1.5% acetonitrill) at rate of 5 ml/min the eluant was evaporated to dryness by rotary evaporator at 35 °C and the residues were ready for chromatographic determination. No clean up was done for Pymetrozine samples.

5.3- Determination:

Quantitative analysis of Tolclofos-methyl and Malathion residues were performed by the Gas Chromatograph (GC) Hp6890 serial equipped with flame photometric detector (FPD) operated in the phosphorus mode (529 nm filter). The extracted samples were dissolved in ethyl acetate and injected under the following conditions; capillary column DB-5MS (30mm 0.32 mm i.d. X 0.25 µm film thickness). Detector temperature was 250 °C, injector temperature was 245 °C and the column temperature was programmed 190°C and hold 2 min. and rises to 240 °C at a rate of 6 °C/min and hold 5 min. Nitrogen carrier gas flow was 3 ml/min, hydrogen flow was 75 ml/min. and air flow was 100 ml/min. Using splitless mode of injection. The retention time for Malathion and Tolclofos-methyl was 5.97 and 3.52 min respectively.

Quantitative analysis of Pymetrozine residues were performed by Knauer High Performance Liquid Chromatotergraph (HPLC) system equipped with knauer variable wave length detector. 100 A reversed phase HPLC column was used isocratically with mobile phase of acetonitrile, water at the ratio of 60:40 v/v with flow rate of 1 ml/min and detector wavelength were 230 nm. Pymetrozine retention time under these condition was 3.89 min.

Quantification of the pesticides was performed by comparing the peak area of samples to that of a calibration curve of standards.

RESULTS AND DISCUSSION

1- Effect of tested pesticides on *S. granarius,* and *R. dominica* adults:

Toxic effect of four pesticides; Malatox (1% D), Chess (25% WP), Rizolex (10% D) and Ecotech-Bio (10% WP) against adults of the grain weevil, *Sitophilus granarius* (L.) and the lesser grain borer, *Rhyzopertha dominica* (F.) was evaluated by two methods; sack treatment and grain treatment. Table 1 show the LC_{50} (ppm), slope value and toxicity index (%) of four tested pesticides against the two insect species adults with sack treatment method. Malatox (1% D) had the strongest action on *S. granarius* and *R. dominica* adults (LC_{50} = 21.69 & 28.57 ppm and toxicity index 100%, respectively) followed by Rizolex 10% D (LC_{50} = 895.27 & 1232.86 ppm and 2.42 & 2.32% toxicity index, resp.) and Chess 25% WP (LC_{50} =1423.86 & 1746.71 ppm and 1.52 & 1.64% toxicity index, resp.). Ecotech-Bio 10% WP, however was the lowest toxicant (LC_{50} = 3133.41 & 7142.86 ppm and 0.62 & 0.4 % toxicity index, resp.) for the two insect species. The slope value of four tested pesticides regression lines means, the high homogenous response of two insect species adults to the tested pesticides. This result agree with those of Bhuiyah (1993) who reported that Fenitrothion (as Edthion, Sovathion and Sumithion) and Malathion (as Syfanon and Fyfanon) were effective against *S. oryzae* as sack treatments of stored wheat. All insecticides at 1.5, 2.0 and 2.5 ml/liter gave significant reductions in numbers of the pest.

The toxicity of four pesticides on the two insect adults by grain treatment method was shown in table 2. The LC_{50} values of tested pesticides were highly decreased than that of sac treatments which correlated with the effect of pesticides as contact and stomach toxicants. Malatox also is the superior toxicant (LC_{50} = 2.37 & 7.19 ppm and 100 % toxicity index) followed by Chess (82.11 & 128.17 ppm and 2.82 & 5.61%), Rizolex (97.23 & 140.64 ppm and 2.44 & 5.11%) and Ecotech-Bio (377.50 & 518.49 ppm and 0.63 & 1.39%) against *S. granarius* and *R. dominica* adults, resp. The obtained results are in agreement with those done by several researchers. Dakshinamoorthy *et al.*, (1993) reported that the greatest Knock-down effect after 24hrs. was obtained with Malation (4%) at 10g/kg of pearl millet seeds. Significant Knock-down were also observed with Thiram and Thiram + Cabendazim fungicides at 6 g/kg seeds. Price *et al.*, (1997) studied the effect of novel Ecogen and Mycogen strains of B.t. against several beetles. Forty-three isolates were assayed against neonate larvae and young adults of *Ahasverus advena* (foreign grain beetle) *Typhaea stercorea* (hairy fungus beetle) and *Cynaeus angustus* (larger black flour beetle). Foreign grain beetle had mortalities exceeding 20% (maximum 41%) after 14-days of grain treatment with seven isolates, while the larval mortalities were over 20% (maximum 70%) with 19 isolates.

The previous results revealed that the four tested pesticides were highly effective against the two pest species adults when mixed with grains than residual film on treated sacks and the granary weevil, *S. granarius* was more susceptible to the effect of tested pesticides than the lesser grain borer *R. dominica*. Organophosphate Malatox 1% D Malathion was the most effective on two insect species with two methods of treatment. The aromatic hydrocarbon fungicide Rizolex 10% D Tolclofos methyl had a satisfied insecticidal activity which supported the application of it on grains to protect them from insect infestation in stores and fungi when cultivate in field soil. The feeding blocker Chess 25% WP Pymetrozine had a moderate toxic effect on two insects with grain treatment method. Also the Ecotech-Bio [10% WP (EG2371) Ecogen] *Bacillus thuringiensis* subsp. *Kurstaki* with subsp. *aizawi* toxin was effective only with grain treatment method because the spores of *B.t.* when ingested by insect the crystals of endotoxin Cry I and Cry II solubilized; the epithelail cells of insect gut are damaged, insects stop feeding and eventually starved to death.

Table 1. Toxic effect of Malatox, Rizolex, Chess and Ecotech-Bio on *S. granarius,* and *R. dominica* adults by using sack treatment method.

Pesticide	*S. granarius*			*R. dominica*		
	LC_{50}±S.E ppm	Slope	TI %	LC_{50}±S.E. ppm	Slope	TI %
Malatox (1%D)	21.69 ±2.25	1.21	100	28.57 ±3.12	1.76	100
Rizolex (10%D)	895.27 ±43.63	1.11	2.42	1232.86 ±111.15	1.37	2.32
Chess (25%WP)	1423.86 ±92.28	1.81	1.52	1746.71 ±288.37	2.52	1.64
Ecotech-Bio (10% WP)	3133.41 ±308.62	1.15	0.62	7142.86 ±611.23	1.45	0.40

S.E.=Standard error

Table 2. Toxic effect of Malatox, Rizolex, Chess and Ecotech-Bio on S. granarius, and R. dominica adults by using grain treatment method.

Pesticide	*S. granarius*			*R. dominica*		
	LC_{50}±S.E. ppm	Slope	TI %	LC_{50}±S.E. ppm	Slope	TI %
Malatox (1%D)	2.37 ±0.54	2.25	100	7.19 ±0.96	2.03	100
Chess (25%WP)	82.11 ±13.71	2.15	2.89	128.17 ±29.99	1.98	5.61
Rizolex (10%D)	97.23 ±11.86	2.11	2.44	140.64 ±14.08	1.55	5.11
Ecotech-Bio (10% WP)	377.50 ±68.32	1.12	0.63	518.49 ±107.72	1.43	1.39

Data summarized in Table 3 revealed that the treatment of wheat grains with LC_{50} of four tested pesticides produced high mortality of adults of two insect species after 14 days and reduced the (F_1) progeny of these adults. The mortality percentage of *S. granarius* and *R. dominica* after two weeks of treatment reached to 83.6 and 79.4% for Malatox, 75.4 and 73.3% for Chess, 62.7 and 70.2% for Rizolex and 56.3 and 57.7% for Ecotech-Bio, resp. The decreasing in number of emerged adults (F_1) progeny reached to 78.6 and 73.6% with Malatox, 59.5 and 57.8% for Chess, 46.9 and 51.4% for Rizolex and 65.3 and 69.2% for Ecotech-Bio treatments of *S. granarius* and *R. Dominica,* resp. compared with the control of untreated insects. Malatox was the most effective pesticides on reduction of (F_1) progeny of two insect species followed by Echotech-Bio, Chess and Rizolex. These results agree with those of Bandyopadhyay *et al.*, (1996) which reported that the effect of sublethal concentration (0.003%) of Dichlorvos, Malathion, Fenvalerate, Permethrin, Cypermethrin and Etrimfos on *S. oryzae* reproduction are transmitted at least to the next generation by surviving insects. Chander and Ahmed (1989) mentioned that the seed treatment of Quinone fungicides Chloranil and Dichlone at 0.05% and 0.0125% concentration produced high mortality of treated adult after 14 days and significantly reduced the production of progeny by *S. oryzae* and *R. dominica.*

Table 4 show the percentages of mortality and reduction in (F_1) progeny of *S. granarius* adults when exposed to wheat grains prior treatment with recommended concentration of Malatox and Rizolex (8 and 300 ppm, resp.), 200 ppm of Chess and

LC_{90} of Ecotech-Bio (1237.77 ppm) and stored for 5 months at laboratory conditions. Malatox produced 100% & 100%, 80.3% & 96% and 71.6 & 83.3% mortality of adult after 7 and 14 days of exposure for 1, 3 and 5 months of storage, resp. These percentage of mortalities were decreased to 65.6 & 82.6 and 57.0 & 68.3 and 38.2 & 51.6% for Chess, 74.2 & 90.6 and 59.2 & 66.0 and 37.8 & 45.3 % for Rizolex and 56.3 & 61.3 and 31.8 & 33.3 and 9.9 & 10.3% for Ecotech-Bio after 7 and 14 days of exposure for 1, 3 and 5 months of storage, resp. The reduction percentages of (F_1) progeny reached to 100,93.8 and 74.5 with Malatox 80.8, 65.9 and 45.2 with Chess, 86.8, 68.5 and 36.4 with Rizolex and 76.9, 59.7 and 29.3 with Ecotech-Bio after 1,3 and 5 months storage periods.

Table 5 show that the treatment with recommended concentration (8 ppm) of Malatox produced 59.3 & 82.6 and 42.9 & 56.3 and 20.0 & 29.6% of mortality in *R. dominica* adults after 7 and 14 days of exposure for 1,3 and 5months of storage, resp. The treatment with 200 ppm of Chess produced 66.6 & 78.3 and 39.3 & 48.6 and 11.6 & 20.3 %, Rizolex (300 ppm) produced 69.3 & 86.0 and 43.6 & 53.3 and 21.3 & 27 % , while LC_{90} (1785.86 ppm) Ecotech-Bio produced 53.6 & 59.3 and 25.3 & 29.6 and 0.0 & 10.6 % of adult mortality after 7 and 14 days of exposure for 1,3 and 5 months storage periods, resp. The reduction percentages in progeny reached to 76.0, 69.7 and 56.0% for Malatox, 73.3, 61.2 and 39.5% for Chess, 75.5, 63.0 and 42.3% for Rizolex and 79.2, 56.1 and 33.0% for Ecotech-Bio after 1, 3 and 5 months of storage, resp.

The germination test of treated grains revealed that no effect of the four tested pesticides on germination of wheat grains in different treatment periods was observed. This result agree with that of Abdel-Aziz and Abdel-Gawad (2005).

The obtained results revealed that the recommended concentration of Malatox produced high percentage of reduction in progeny of *S. granarius* after different period of grain storage followed by Rizolex (except 5 months of storage), Chess and Ecotech-Bio. The high reduction effect in *R. dominica* progeny was recorded with Ecotech-Bio followed by Malatox, Rizolex and Chess after 1 month of grain storage, but this effect was noticed with Malatox, Rizolex and Chess than Ecotech-Bio after 3 and 5 months of storage . These results agree with Keever (1994) who mentioned that the ABG-6206 formulation of thuringiensin when added to diet medium reduced adult emergence of stored products pests, *S. zeamais, R. dominica,* and *E. elutella* by 82.0, 99.3 and 100%, respectively. Abd el-Aziz and Abdel-Gawad (2005) reported that Malathion at 16mg/kg concentration reduced the population of *S. oryzae* than *R. dominica* population in treated wheat and maize grains after 12 months of field storage in Qalubia and Sharkia governorates.

Table 3. Effect of LC_{50} grain treatment of four tested pesticides on adult mortality and reduction of F_1 progeny of *S. granarius,* and *R. dominica* adults.

Pesticide	*S. granarius*					*R. dominica*				
	LC_{50} ppm	% of mortality±S.E		Mean No. of Emerged adults±S.E	Reduction %	LC_{50} ppm	% of mortality±S.E		Mean No. of Emerged adults±S.E	Reduction %
		7-days	14-days				7-days	14-days		
Malatox (1%D)	2.37	51.2±2.3	83.6±4.6	18.7±1.2	78.6	7.19	50.2±1.1	79.4±2.9	16.5±2.4	73.6
Chess (25%wp)	82.11	49.6±3.1	75.4±5.2	35.4±3.4	59.5	128.17	51.4±2.3	73.3±6.4	26.4±4.2	57.8
Rizolex (10%D)	97.23	50.6±1.4	62.7±2.1	46.4±2.8	46.9	140.64	49.8±3.8	70.2±4.4	30.4±2.2	51.4
Ecotech-Bio (10% wp)	377.50	48.0±3.0	156.3±4.2	30.3±3.6	65.3	518.49	49.3±4.6	57.7±5.9	19.3±2.3	69.2
Control	0.0	0.0	0.0	87.3±1.6	0.0	0.0	0.0	0.0	62.6±3.3	0.0

Table 4. Effect of recommended concentration of three chemical pesticides and LC90 of Ecotech-Bio on S. granarius adults exposed to treated grains and their F1 progeny after different storage periods.

Pesticide	Recommended Concentration ppm	Storage period post treatment											
		1-Month				3-Months				5-Months			
		% of mortality±S.E		Mean No. of Emerged adults±S.E	Reduction %	% of mortality±S.E		Mean No. of Emerged adults±S.E	Reduction %	% of mortality±S.E		Mean No. of Emerged adults±S.E	Reduction %
		7-days	14-days			7-days	14-days			7-days	14-days		
Malatox (1%D)	8	100	100	0.0	100	80.3 ±3.6	96.0 ±1.0	6.0 ±0.0	93.8	71.6 ±4.3	83.3 ±1.6	20.3 ±2.2	74.5
Chess (25%wp)	200	65.6 ±5.2	82.6 ±3.3	16.3 ±1.9	80.8	57.0 ±6.0	68.3 ±4.2	32.8 ±4.2	65.9	38.2 ±5.2	51.6 ±5.2	43.6 ±3.3	45.2
Rizolex (10%D)	300	74.2 ±4.3	90.6 ±4.8	11.2 ±2.3	86.8	59.2 ±3.0	66.0 ±4.0	30.3 ±5.6	68.5	37.8 ±2.6	45.3 ±5.1	50.6 ±3.0	36.4
Ecotech-Bio (10% wp)	LC_{90} 1237.77	56.3 ±1.6	61.3 ±2.9	19.6 ±1.2	76.9	31.8 ±3.6	33.3 ±2.1	38.8 ±4.3	59.7	9.9 ±1.6	10.3 ±2.6	56.3	29.3
Control	0.0	0.0	0.0	84.8 ±4.3	0.0	0.0	0.0	96.2 ±7.2	0.0	0.0	0.0	79.6 ±3.6	0.0

Table 5. Effect of recommended concentration of three chemical pesticides and LC90 of Ecotech-Bio on R. dominica adults exposed to treated grains and their F1 progeny after different storage periods.

Pesticide	Recommended Concentration ppm	Storage period post treatment											
		1-Month				3-Months				5-Months			
		% of mortality±S.E		Mean No. of Emerged adults±S.E	Reduction %	% of mortality±S.E		Mean No. of Emerged adults±S.E	Reduction %	% of mortality±S.E		Mean No. of Emerged adults±S.E	Reduction %
		7-days	14-days			7-days	14-days			7-days	14-days		
Malatox (1%D)	8	59.3 ±3.6	82.6 ±2.2	19.6 ±2.6	76.0	42.9 ±4.3	56.3 ±2.1	21.3 ±1.6	69.7	20.0 ±2.0	29.6 ±3.3	29.0 ±5.9	56.0
Chess (25%wp)	200	66.6 ±1.3	78.3 ±4.2	21.8 ±1.3	73.3	39.3 ±4.6	48.6 ±3.9	27.3 ±2.6	61.2	11.6 ±1.9	20.3 ±2.6	39.9 ±4.6	39.5
Rizolex (10%D)	300	69.3 ±3.6	86.0 ±0.0	20.0 ±2.0	75.5	43.6 ±4.3	53.3 ±2.6	26.0 ±0.0	63.0	21.3 ±2.6	27.0 ±0.0	38.0 ±1.3	42.3
Ecotech-Bio (10% p)	LC90 1785.86	53.6 ±3.3	59.3 ±2.1	17.0 ±1.3	79.2	25.3 ±2.6	29.6 ±3.3	30.9 ±0.0	56.0	0.0 ±0.0	5.6 ±1.3	44.3 ±2.6	33.0
Control	0.0	0.0	0.0	81.6 ±5.3	0.0	0.0	0.0	70.3 ±4.9	0.0	0.0	0.0	65.9 ±6.6	0.0

2- Biochemical effects of LC_{50} treatment with the four tested pesticides on *S. granarius* and *R. dominica* adults

The effects of LC_{50} treatment with four tested pesticides on total carbohydrate, lipid and protein contents of *S. granarius* and *R. dominica* adult whole body tissues were described in Tables 6 and 7. All treatments adversely affected carbohydrate levels in two insect species. The percentage of decrease in carbohydrate levels comparing with the untreated control reached to 63.63, 55.79, 73.56 and 29.08 % in *S. granarius* adults and 55.03, 60.62, 73.74 and 59.78 % in *R. dominica* adults treated with LC_{50} of Malatox, Chess, Rizolex and Ecotech-Bio, resp.

The reduction in lipid contents reached 23.26, 78.81, 53.59 and 88.11 % in *S. granarius* adults and 51.74, 40.28, 49.18 and 28.71% in *R. dominica* adults for Malatox, Chess, Rizolex and Ecotech-Bio treatments, resp. The reduction in total protein content of *S. granarius* adults was 22.28, 7.25 % and 3.99 % and of *R. dominica* was 49.58, 68.87 and 77.37 % for Malatox, Chess and Rizolex treatments resp. Ecotech-Bio treatment produced a slight increase (4.91 %) in protein of *S. granarius* tissues, and a significant decrease (49.51) in *R. dominica* tissues. The high significant decrease in carbohydrates was recorded with Rizolex treatment of two insect species, followed by Malatox and Chess treatments of *S. granarius* and Chess, Ecotech-Bio and Malatox treatments of *R. dominica* . The high significant difference in total lipids recorded with Ecotech-Bio, Chess and Rizolex treatments of *S. granarius* but the treatment with Malatox, Rizolex and Chess produced significant reduction in lipids of *R. dominica*. The highly significant reduction in protein level recorded in *R. dominica* tissues with Rizolex and Chess treatments. The depletion of carbohydrate in body homogenate may be due to their increased utilization in response to hyper activity caused by pesticide treatments, hence these results are in accordance with the finding of Singh (1986). Saleem, *et al.*, (1998), found that the adult of *Tribolium castaneum* which was treated with the LC_{50} of Ripcord showed utilization of carbohydrates, proteins and lipids in the given order, perhaps to produce extra energy to combat insecticidal stress.

The effects of LC_{50} of four tested pesticides on Acetylcholinestrerase, Amylase and Transaminases (GOT and GPT) of *S. granarius* and *R. dominica* adult body tissues are shown in Tables 8 and 9. Data clearly indicated that all tested pesticides decreased the activity of Acetylcholine estrerase (Ach-E) by insignificant values in two insect species compared with the untreated control. The decreasing percentages reached to 33.93, 10.21, 8.20 and 2.00 % in *S. granarius* tissues and reached to 24.94, 24.11, 13.24 and 7.36 % in *R. dominica* tissues with Rizolex, Chess, Ecotech-Bio and Malatox treatments, resp. The insignificant reduction in Amylase activity was detected in all treatment, which reached to 25.48, 23.72, 20.13 and 15.7% for Rizolex, Malatox, Chess and Ecotech-Bio treatment ,resp. of *S. granarius* tissues and reached to 24.14, 14.32, 12.35 and 9.26 % for Rizolex, Ecotech-Bio, Malatox and Chess treatments, resp. of *R. dominica* tissues. The highly significant reduction (83.38%) in activity of GOT was detected in tissues of *S. granarius* with Rizolex treatment, but the other pesticides produced insignificant decrease. A significant decrease in GOT activity was presented in *R. dominica* tissues as 56.16, 50.69, 45.76 and 38.36 % with Chess, Ecotech-Bio, Rizolex and Malatox treatments, resp. The same trend of significant decrease was presented in GPT activity of body tissues of *R. dominica* which reached to 46.25, 40.23, 39.43 and 33.52 % with Ecotech-Bio, Malatox, Chess and Rizolex treatments resp. In adult tissues of *S. granarius* the insignificant decrease (21.85%) in GPT activity was detected with Chess treatment, while Malatox, Rizolex and Ecotech-Bio produced insignificant increase (28.8, 12.00 and 9.85 % resp.) in activity of this enzyme. From the previous results we can conclude that the fungicide Rizolex has a high effect on enzymes disturbance in *S. granarius* adult tissues, the same effect was produced by Chess and Ecotech-Bio in *R. dominica* adult tissues. The effects of pesticides on the insect enzymes were reported by many investigators. Hirashima *et al.*, (1989) mentioned that Fenitrothion treatments (5, 10 ppm) of *T. castaueum* larvae reduced 14 and 60% of larval weight gain and suppressed the activity of the Trehalase, Invertase, Amylase and Acetylcholine esterase. Naveeda *et al.*, (1995) reported that the treatment with Fluvalinate insecticide at 250 and 500ppm disturbed the main metabolite contents and decreased the activity of two Aminotransferases (GOT and GPT) in adult of *T. castaueum.*

Table 6. Total Carbohydrate, Lipid and Protein contents of *S. granarius* adults treated with LC_{50} of Malatox, Chess, Rizolex and Ecotech-Bio

Pesticide	Total Carbohydrates ±S.E	% of change	Total Lipid ±S.E	% of change	Total Protein ±S.E	% of change
Malatox (1%D)	3.99 ±0.20	(-)63.63**	13.36 ±0.31	(-)23.26	10.50 ±0.45	(-)22.28
Chess (25%WP)	4.85 ±0.22	(-)55.79*	3.69 ±0.12	(-)78.81**	12.53 ±0.36	(-)7.25
Rizolex (10%D)	2.90 ±0.49	(-)73.56**	8.08 ±1.24	(-)53.59*	11.62 ±0.21	(-)13.99
Ecotech-Bio (10% WP)	7.78 ±0.11	(-)29.08	2.07 ±0.89	(-)88.11**	14.17 ±0.42	(+)4.89
Control	10.97 ±0.27	0.0	17.41 ±1.87	0.0	13.51 ±0.22	0.

Table 7. Total Carbohydrate, Lipid and Protein contents of *R. dominica* adults treated with LC50 of Malatox, Chess, Rizolex and Ecotech-Bio

Pesticide	Total Carbohydrates ±S.E	% of change	Total Lipid ±S.E	% of change	Total Protein ±S.E	% of change
Malatox (1%D)	3.22 ±0.37	(-)55.03*	12.64 ±1.11	(-)51.74*	7.24 ±0.45	(-)49.58*
Chess (25%WP)	2.82 ±0.29	(-)60.62**	15.64 ±1.82	(-)40.28*	4.47 ±0.34	(-)68.87**
Rizolex (10%D)	1.88 ±0.45	(-)73.74**	13.31 ±1.27	(-)49.18*	3.25 ±0.52	(-)77.37**
Ecotech-Bio (10% WP)	2.88 ±0.34	(-)59.78**	18.67 ±1.38	(-)28.71	7.25 ±0.61	(-)49.51*
Control	7.16 ±0.21	0.0-	26.19 ±1.60	0.0	14.36 ±0.34	0.0

S.E. = Standard error

(-) = Decrease (+) = Increase

* Significant (P<0.05) ** highly significant (P<0.01)

Total contents = ug /mg insect tissues

Table 8. Activity of Acetyl Colinesterase, Amylase and Transaminases (GOT and GPT) of *S. granarius* adults treated with LC50 of Malatox, Chess, Rizolex and Ecotech-Bio

Pesticide	Acetyl Coline Esterase		Amylase		Transaminases			
					GOT		GPT	
	Activity ±S.E.	% of change	Activity ±S.E.	% of change	Activity ±S.E.	% of change	Activity ±S.E.	% of change
Malatox (1%D)	994.91 ±28.04	(-)10.21	1024.13 ±24.11	(-)23.72	17.95 ±0.82	(-)24.68	4.19 ±0.31	(+)28.92
Chess (25%wp)	1017.23 ±25.23	(-)8.20	1072.45 ±57.71	(-)20.13	17.45 ±0.22	(-)26.77	2.54 ±0.34	(-)21.85
Rizolex (10%D)	732.03 ±29.21	(-)33.93*	1000.61 ±19.71	(-)25.48	3.96 ±0.21	(-)83.38**	3.64 ±1.44	(+)12..00
Ecotech-Bio (10% wp)	1086.24 ±58.76	(-)2.00	1131.93 ±69.35	(-)15.70	20.11 ±0.25	(-)15.61	3.57 ±0.31	(+)9.85
Control	1108.03 ±136.86	0.0	1342.66 ±62.99	0.0	23.83 ±1.82	0.0	3.25 ±0.15	0.0

S.E. = Standard error

(-) = Decrease (+) = Increase

* Significant (P<0.05) ** Highly significant (P<0.01) Activity = ng substrate hydrolyzing / min / mg insect issues

Table 9. Activity of Acetyl Colinesterase, Amylase and Transaminases (GOT and GPT) of *R. dominica* adults treated with LC50 of Malatox, Chess, Rizolex and Ecotech-Bio

Pesticide	Acetyl Esterase Coline		Amylase		Transaminases			
					GOT		GPT	
	Activity ±S.E.	% of change	Activity ±S.E.	% of change	Activity ±S.E.	% of change	Activity ±S.E.	% of change
Malatox (1%D)	1411.89 ±49.18	(-)7.36	1321.67 ±39.20	(-)12.35	5.40 ±0.23	(-)38.36*	5.26 ±0.38	(-)40.23*
Chess (25%wp)	1156.67 ±32.36	(-)24.11	1368.24 ±28.15	(-)9.26	3.84 ±0.28	(-)56.16*	5.33 ±0.19	(-)39.43*
Ecotech-Bio (10% wp)	1322.22 ±26.55	(-)13.24	1291.99 ±30.41	(-)14.32	4.32 ±0.13	(-)50.69*	4.73 ±0.35	(-)46.25*
Rizolex (10%D)	1143.93 ±20.33	(-)24.94	1143.93 ±17.49	(-)24.14	4.78 ±0.19	(-)45.76*	5.85 ±0.22	(-)33.52*
Control	1524.05 ±35.61	0.0	1507.93 ±16.21	0.0	8.76 ±0.11	0.0	8.80 ±0.14	0.0

S.E. = Standard error
(-) = Decrease (+) = Increase
* Significant (P<0.05) ** Highly significant (P<0.01) Activity = ng substrate hydrolyzing / min / mg insect issues

3- Residue analysis of tested chemical pesticides on and in treated wheat grains

The effect of storage on the breakdowon of Malathion (Malatox 1% D), Tolclofos-methyl (Rizolex 10% D) and Pymetrozine (Chess 25%WP) on and in treated wheat grains were examined after 5 months of strage at ambient temperature (25±2°C). The original applied concentration was the recommended for Malathion, (8 ppm/gm) and (300 ppm/gm) Tolclofos-methyl and 200 ppm/gm Pymetrozine. Prior to storage the initial concentration of Malathion, Tolclofos-methyl and Pymetrozine on wheat grain was recorded as 2.04, 82.52 and 38.27 resp. The approved doses of insecticides for stored grain were used, the residue levels exceeded the maximum residue limits (MRLS) at the beginning of storage. The MRL recommended for durable crops for Mālation (2 % D) was 8 mg/kg^{-1} (FAO, 1989). After 5 months of storage the residue of Malathion, Tolclofos-methyl and Pymetrozine decreased to 1.23, 29.66 and 18.25 ppm resp. The degradation of Malathion, Tolclofos-methyl and Pymetrozine in wheat grains was 39.71, 64.06 and 52.31% during the storage period. The results agree with finding of Lalah and Wandiga (2002) for the reduction of Malathion which reach to 47% in maize and 65% in beans after 12 months of storage. Uygun *et al.*, (2007) mentioned that the degradation of Malathion and Isomalathion in barley was observed to be about 65-72% during five and a half months of storage. The difference between the amounts of residues on and in unwashed treated wheat grains and in washed treated wheat grains, gives an idea about the surface residues that could be removed by washing. Nasr (2002) mentioned that washing process was efficient in removing Organophosphorus insecticides from vegetables. Data in Table (10) showed that the washing process apparently reduced the amount of Malathion, Tolclofos-methyl and Pymetrozine to 0.29, 0.84 and 5.74 ppm, resp. The loss in Malathion , Tolclofos-methyl and Pymetrozine residue level in washed wheat grains reached to 85.78, 98.98 and 85.00% resp., when compared with initial concentration of each pesticide. The washing of treated wheat grains and dryness decreased residue level by 46.08, 97.17 and 68.55 % for Malathion , Tolclofos-methyl and Pymetrozine, resp. compared with unwashed treated grains.

Table 10. Residues and loss percentage of Malathion Tolclofos-methyl and Pymetrozine from treated wheat grain

Pesticide	Conc. (ppm)	Initial Residue (ppm)	5 months of storage (unwashed grain)		5 months of storage (washed grain)		Loss (%) Of Unwashed & Washed
			Residue (ppm)	Loss (%)	Residue (ppm)	Loss %	
Malathion	8	2.04	1.23	39.71*	0.29	85.78**	46.08*
Tolclofos-methyl	300	82.52	29.66	64.06**	0.84	98.98**	97.17**
Pymetrozine	200	38.27	18.25	52.31*	5.74	85.0**	68.55*

* Significant (P<0.05) ** Highly significant (P<0.01)

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فاعلية بعض المبيدات ضد الحشرات الكاملة لثاقبة الحبوب الصغري و سوسة الحبوب ومستويات متبقياتها فى حبوب القمح المعاملة

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قدرت كفاءة مبيدات ملاتوكس (١% D)، تشيس (٢٥% WP)، ريزولكس (١٠% D) و إيكوتك-بيو (١٠% WP) ضد الحشرات الكاملة لثاقبة الحبوب الصغرى ريزوبيرثا دومنيكا و سوسة الحبوب سيتوفيليس جرانيريس فى المعمل.

وقد كانت كفاءة المبيدات على الحشرات أعلى فى معاملة الحبوب عن معاملة الأكياس. وترتيب المبيدات المختبرة من حيث سميتها على الحشرات كالتالى:

ملاتوكس > تشيس > ريزولكس > إيكوتك-بيو . و كانت سوسة الحبوب الأكثر حساسية لتأثير المبيدات المختبرة عن ثاقبة الحبوب الصغري كما كان لكل المبيدات المختبرة تأثير كبير فى خفض عدد حشرات الجيل الأول الناتج من الحشرات المعاملة وقد كان لمعاملة الحبوب بالتركيز النصفى المميت للمبيدات LC_{50} تأثيراً معنويا على إنقاص المحتوى الكلى للكربوهيدرات والليبيدات فى أنسجة جسم الحشرات المعاملة وكان هذا النقص فى المحتوى الكلى للبروتينات غير معنوى فى سوسة الحبوب فقط. كما كان التأثير معنويا جداً فى خفض نشاط انزيمات الترانس أمينيز (GOT and GPT) فى ثاقبة الحبوب الصغرى عن سوسة الحبوب فى حين كان الأنخفاض فى نشاط أنزيمي استيل كولين استريز و الأميليز معنويا فى الحشرتين.

وقدرت متبقيات المادة الفعالة لمبيدات ملاتوكس (١% D ملاثيون)، و ريزولكس (١٠% D تلكلفوس ميثيل) و تشيس (٢٥% WP بايمتروزين) فى حبوب القمح المعاملة باستخدام جهازى HPLC, GC وكانت مستويات متبقيات المبيدات فى حبوب القمح المعاملة أعلى بكثير من تلك التى قدرت فى حبوب القمح المعاملة الذى تم غسله و تجفيفه بعد المعاملة.