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Toxicity and development of resistance in *Tribolium castaneum* and *Sitophilus oryzae* to certain selected insecticides

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Abstract:

The rust red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and the rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) are considered the most important insect species which destruct the stored grain. Application of synthetic insecticides for stored grain protection has been created resistance and cross-resistance in storage insects. Laboratory bioassays were undertaken to assess the toxicity, resistance and cross-resistance of insecticides, chlorpyrifos, chlorpyrifos-methyl, malathion, pirimiphos-methyl, cyfluthrin, cypermethrin and methomyl, to *T. castaneum* and *S. oryzae.* Two methods, exposure to treated surface and mixing with wheat grain as well as selective pressure and cross-resistance studies using LC_{50} were used. Results obtained after 24h by thin film method revealed that pirimiphos-methyl was the premier insecticide to *T. castaneum* with LC_{50} of 0.007 $\mu g/cm^2$. While methomyl showed low toxicity. On the other hand, chlorpyrifos-methyl was the most toxic compound to *S. oryzae* with LC_{50} of 0.007 $\mu g/cm^2$. For mixing with medium the order of toxicity obtained in this technique was different from that in thin film residue method. The development of resistance in *T. castaneum* indicated that methomyl failed to build up resistance after 4 generations since its resistance factor was 5 fold. The highest level of resistance was induced by malathion followed by pirimipho-methyl with resistance factor, R.F., of 214.4 and 67.4-fold, respectively. Selective pressure with chlorpyrifos for 4 generations did not succeed in building up more than 10.9 fold. Data also, presented that all of the tested Op-resistant strains of *T. castaneum* show only low level of cross-resistance to the pyrethroid insecticides cyfluthrin and cypermethrin at the LC_{50} level (R.F. values ranged from 2.55 to 6.4 fold). These resistant strains were more susceptible to the insecticide, chlorpyrifos-methyl than the susceptible strain (RF values ranged from 0.29 to 0.66). It is concluded that the common insecticides, malathion and pirimipho-methyl have created high level of resistance compared to methomyl which was rarely used in grain protection.

Introduction

Stored products are subjected to attack by numerous of insects which reduce weight and quality. These are the rust red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and the rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae, which cause misfortune wastages to wheat grains and their products. Rice weevil has lately become the number one pest of whole grains in modern commercial storage system (Kljajić *et al*., 2006). Conventional pesticides have been used as the major tools for stored grain and food protection. Actually, whatever the efficiency of the chemical insecticides for protecting stored grains, but it has created some problems involving pesticide resistance, toxic residues, health hazards and pest resurgence. Pesticide resistance is an increased tolerance to a pesticide that has a genetic basis. As a heritable trait, the development and spread of resistance will be influenced by the selective pressures of pesticide use, fitness costs associated with individuals carrying resistance gene, and movement of pests on geographical scales. Since the mid-20th century insecticides have been drawn mainly from the organophosphates (OPS) (malathion, chlorpyrifos, methomyl and dichlorvos), the pyrethroids (bioresmethrin, deltamethrin, and beta-cyfluthrin) and the Juvenil hormone analogues (JHAs) (methoprene and hydroprene) (Opit *et al*. ,2012). Cross - resistance is when resistance to a given pesticide causes resistance to another pesticide without the insect having been exposed to the latter pesticide (Scott *et al*., 1990). For example, *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) that is resistant to one organophosphate has a tendency to be resistant to other organophosphates. A similar situation occurs with pyrethroid resistant *T. castaneum* and *R. dominica* (Collins, 1990; Guedes *et al.,* 1996 and Daglish *et al.,* 2003). Many authors established many cases of insect resistance against more registered insecticides which belong to different groups over worldwide. Resistance to malathion in *S. oryzae* has been reported from Egypt (Toppozada *et al*., 1969), Asutralia (Rimes and Moulden, 1979) and USA (Halisack and Beeman, 1987 and Irshad and Jillani, 1992). The development of resistance became ordinary phenomenon especially in grain stored because of the suitable conditions. To prevent or minimize the resistance and cross-resistance the current study dealt with their extent notably with *T. castaneum* and *S. oryzae* to some chemical insecticides (chlorpyrifos, chlorpyrifos-methyl, cyfluthrin, cypermethrin, malathion, methomyl and pirimiphos-methyl) through the selective pressure bioassay pattern and how to suppress it.

Materials and methods

1. Insecticides:

2. Chlorpyrifos:

Chemical name: O. O-diethyl-O-3, 5, 6-trichloro-2 pyridyl phosphorothioate.

48% E.C. produced by Dow Chemical Co.

Trade marks: Dursban, Brodan and Dowco 179.

1.2. Chlorpyrifos-methyl:

Chemical name: O,O, dimethyl O- (3, 5, 6-trichloro-2 – pyridyl) phosphorothioate.

E.C. 50% Dow Chemical Company.

Trade marks: Dowco 214, Reldan and Zertell.

3. Malathion:

Chemical name: O.O-dimethyl phosphorodithioate ester of diethyl mercaptosuccinate.

E.C. 57% produced by Sumitomo Chemical Company.

Trade marks: Charbophos, Chemathion and Malaspray.

1.4. Pirimiphos-methyl:

Chemical name: 2-diethyl-amino –6 – methyl pyrimidin – 4 – yl O.O-dimethyl phosphorothioate.

Trade marks: Actellic, Blex and Silosan

1.5. Cyfluthrin:

Chemical name: Cyano (4-fluoro- 3 – phenoxyphenyl) methyl 3- (2,2 –

dichloroethenyl) -2, 2 – dimethyl – cyclopropanecarboxylate.
5% E.C. Bayer AG of West Germany. Being developed in the U.S. by Mobay chemical corp.
Trade marks: Baythroid and Bay-FCR-1272.

1.6. Cypermethrin:
Chemical name: (±) alpha-cyano-3-phenoxybenzyl (±) cis. trans 3-(2,2-dichlorovinyl)-2, 2-dimethyl cyclopropane-carboxylate.
E.C. 30% ICI, FMC, Sumitomo and Shell Chemical Co.
Trade marks: Ripcord, Ammo and Arrivo.

1.7.Methomyl:
Chemical name: S- methyl – N – (methyl carbamoyl oxy) thioacetimidate.
Oxime carbamate produced by Shell Chemical Co.
(90% soluble liquid).
Trade marks: Lannate, Nu. Bait and Nudrin.

1. Insects:

2.1. The rust red flour beetle, *Tribolium castaneum*:

The adults of rust red flour beetle, *T. castaneum* were collected from Kafr El-Sheikh rice and wheat mills companies. Insects were reared in a mixture of wheat seeds and wheat flour under laboratory conditions of 26±1°C and 65±5% R.H. The media contained also 5% dried yeast. Insects were reared for two years with the same above conditions. Adults of 2-3 weeks old were selected for toxicity evaluation tests, resistance and cross-resistance

2.2. The rice weevil, *Sitophilus oryzae*:

The adults of the rice weevil, *S. oryzae* were collected from Kafr El-Sheikh rice mills Co. they were reared on wheat grains, under laboratory conditions of 26±1°C, 65±5% R.H. insects were reared for two years in the Department of stored product pest Research, Sakha Agricultural Research station, adults of 16-21 days old were used for toxicity evaluation tests.

3. Bioassay procedures:

3.1. Exposure to treated surface (thin film technique):

Stock dilution (w/v) of each compound was prepared by dissolving the desired quantity in acetone and series of dilutions were prepared. Toxicity tests were carried out on films achieved by spreading aliquot of one ml of each concentration at the bottom of a Petri dish of 9 cm in diameter and left to dry. After complete dryness of the insecticide film, ten adult beetles from each of the tested insects were placed in each of the treated Petri dishes. The same number of insects also was confined on Petri dishes treated with acetone only and served as control. Mortality was recorded after 24 h of exposure and corrected by **Abbott's formula (1925)**. The LC_{50} values for all insecticides were calculated by the method of **Litchfield and Wilcoxon (1949)**.

3.2. Exposure to treated wheat grains (mixing with feeding medium):

Batches of uninfected wheat grain (of moisture content 9%) were weighed and placed in wide-mouth glass jars. The insecticides were diluted in water and added to the grains at rates which give the required concentrations. Jars were mechanically shaken for adequate and fixed time to ensure complete mixing process. Serial concentrations were made. The treated grains were allowed to dry at room temperature. For each concentration, twenty grams of treated grains were placed in Petri dishes (9-cm diameter) and this was replicated four times. Ten adults of the tested insects (2-3 weeks old) were transferred to each dish. The same numbers of insects were transferred to Petri dishes containing non-treated grains. Mortality counts were recorded after 24 h and corrected by **Abbott's formula (1925)**. LC_{50} values were calculated by the method of **Litchfield and Wilcoxon (1949)**.

3.3. Selective pressure and cross - resistance studies:

Adults of the laboratory strain *T. castaneum* were exposed to the LC_{50} of each of the tested insecticides pirimiphos-methyl, malathion, chlorpyrifos and methomyl for 24 h using thin film technique. The survival insects were transferred into clean Jars containing clean and sterilized medium which was preheated in an oven at 70°C for 2 h. After 15 days the parent adult individuals were eliminated. After 60 days the adults of the new generation were treated in the same manner as mentioned before and so on up to four generations. At every generation, LC_{50}'s of the tested insecticides were determined using thin film technique. To determine the cross -resistance patterns, concentrations response tests for each resistant strain to each insecticide were carried out and compared with these of susceptible strain.

Results and Discussion

The toxic potency of tested insecticides was evaluated against *T. castaneum* and *S. oryzae* adults using to methods of bioassay, thin film residue and mixing with feeding medium.

1.Thin film residue:

Log-dosage probit regression lines were drawn and statistically analyzed according to the method of Litchfield and Wilcoxon (1949). Values of LC_{50}, confidence limits and slopes were calculated and recorded in Table (1). Based on the LC_{50} values, pirimiphos methyl was the most toxic compound to *T. castaneum* with LC_{50} value of 0.007 μg/cm^2 followed by chlorpyrifos, malathion, chlorpyrifos-methyl, cyfluthrin and cypermethrin. Insecticide, methomyl showed low toxicity (LC_{50} 1.1 μg/cm^2). On the other hand, chlorpyrifos- methyl was the premier insecticide to *S. oryzae* with LC_{50} of 0.007 μg/cm^2 followed by pirimiphos-methyl, chlorpyrifos, malathion, cypermethrin, cyfluthrin and methomyl with LC_{50} values of 0.02, 0.031, 0.132, 0.236, 0.283 and 0.472 μg/cm^2, respectively.

Tablel (1): Toxicity of insecticides against *Tribolium castaneum* and *Sitophilus oryzae* after 24h exposure to treated surface.

Insecticides	*Tribolium castaneum*			*Sitophilus oryzae*		
	LC50 (μg/cm2)	Confidence limits	slope	LC50 (μg/cm2)	Confidence limits	slope
Chlorpyrifos	0.036	0.05-0.025	1.39	0.031	0.04-0.028	5.6
Chlorpyrifos- methyl	0.088	0.12-0.036	1.5	0.007	0.009-0.006	1.7
Cyfluthrin	0.197	0.28-0.140	1.43	0.283	0.65-0.123	0.83
Cypermethrin	0.220	0.39-0.130	1.8	0.236	0.34-0.16	1.45
Malathion	0.066	0.08-0.060	2.6	0.132	0.15-0.12	5.26
Methomyl	1.100	1.77-0.680	1.23	0.472	0.66-0.34	2.2
Pirimiphos-methyl	0.007	0.01-0.005	1.6	0.020	0.03-0.015	2.6

The red flour beetle, *T. castaneum* is one of the most damage insect species invading warehouses and mills around the world (Rees, 2004; Almaši, 2008; Mahroof and Hagstrum, 2012). Regarding the control of that and some other stored product pests, a variety of factors decide the effectiveness of contact insecticides, the most important of which is insect resistance (Subramanyam and Hagstrum, 1996; Kljajić and Perić, 2005, 2006; Kljajić *et al.*, 2009; Boyer *et al.*, 2012 and Opit *et al.*, 2012).

2. Exposure to treated wheat grain:

The toxicity of the tested insecticides was also determined by exposure of *T. castaneum* and *S. oryzae* to treated wheat grains. The order of toxicity obtained in this technique was different from that in treated surface method. Data recorded in Table (2) showed that, insecticides can be arranged according to their toxicities to *T. castaneum* in the following descending order: Chlorpyrifos- methyl> chlorpyrifos > pirimiphos-methyl > cypermethrin >

cyfluthrin > malathion > methomyl. Against *S. oryzae*, the order of toxicity was slightly changed as follows: chlorpyrifos > chlorpyrifos- methyl > pirimiphos-methyl > cypermethrin > malathion > cyfluthrin > methomyl. Differences in the potencies of insecticides obtained according to the method of application may be explained. In treated surface method, insecticide act as a contact poison whereas in exposure to treated grains, insecticide act as a contact and/or stomach poison. Practically, exposure to treated grain method is largely preferred as a criterion for evaluating insecticides potency. Also, differences in the responses of the tested insects to insecticides may ascribe to the behaviouristical and alimentary habituations.

Generally, from the obtained results, organophosphorus compounds (OP) were the most effective insecticides against both insects. For pyrethroid insecticides, cypermethrin was more potent than cyfluthrin and both exhibited a considerable toxicity to *T. castaneum* and *S. oryzae*. The carbamate insecticide, methomyl showed very low toxicity particularly when mixed with grains and tested against both insects. On the other hand, the rice weevil, *S. oryzae* was more susceptible to the tested insecticides than the red flour beetle, *T. castaneum* when both were exposed to treated grains.

Abbassy *et al.*, 1977 and Masoud *et al.*, 1982 tested various insecticides against *Tribolium confusum* Duval (Coleoptera: Tenebrionidae), *T. castaneum* and *S. oryzae*, they found that, chlorpyrifos and pirimiphos methyl were the most effective compounds followed by certain pyrethroid insecticides e.g. Cypermethrin. Cyfluthrin was reported to be a promising insecticide for the control of pests that attack stored products and packing materials (Behrenz *et al.* 1983). The organophosphorus compound, chlorpyrifos-methyl was reported to have potent effects against many insects of stored products e.g. *S. oryzae, T. castaneum, R. dominica*, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae) (Quinlan *et al.*, 1979, Samson and Parker 1989 a, b; Samson *et al.*, 1989 and Daglish *et al.*, 1993).

Table (2): Toxicity of insecticides against *Tribolium castaneum* and *Sitophilus oryzae* after 24h exposure to treated wheat grain.

Insecticides	*Tribolium castaneum*			*Sitophilus oryzae*		
	LC_{50} (mg/g grain)	Confidence limits	slope	LC_{50} (mg/g grain)	Confidence limits	slope
Chlorpyrifos	0.0034	0.0034-0.0027	2.5	0.00070	0.0084-0.00058	3.3
Chlorpyrifos- methyl	0.003	0.0037-0.0025	2.6	0.0011	0.00136-0.0081	2.3
Cyfluthrin	0.023	0.0304-0.0212	1.9	0.028	0.0384-0.0204	1.67
Cypermethrin	0.0125	0.0178-0.0089	1.4	0.009	0.0124-0.0064	1.7
Pirimiphos-methyl	0.008	0.0099-0.0065	2.92	0.0028	0.00304-0.00257	6.7
Malathion	0.078	0.1053-0.0578	1.75	0.0185	0.0259-0.0132	1.7
Methomyl	>0.50	--- ---	---	0.50	0.5964-0.4167	3.8

3.Resistance and cross -resistance studies: 3.1. Development of resistance to four of the tested insecticides in *Tribolium castaneum*:

The development of resistance in laboratory strain of *T. castaneum* after selective pressure by pirimiphos-methyl, malathion, chlorpyrifos and methomyl for 4 generations was studied. Results are recorded in Table (3). Selective pressure with methomyl failed to build up resistance in the insect after 4 generations since its resistance factor was 5 fold. The highest level of resistance was induced by malathion followed by pirimiphos-methyl with resistance factors, R.F., of 214.4 and 67.4-fold, respectively. Selective pressure with chlorpyrifos for 4 generations did not succeed in building up more than 10.9 fold resistance indicating low level of resistance. This result revealed that, the development of resistance against chlorpyrifos is slower than that of pirimiphos-methyl or malathion.

Table (3): Comparative levels of resistance in *Tribolium castaneum* selected by different insecticides for 4 generations.

Insecticides	Generations											
	1			2			3			4		
	LC_{50} (µg /cm^2)	Slope	RF	LC_{50} (µg /cm^2)	Slope	RF	LC_{50} (µg /cm^2)	Slope	RF	LC_{50} (µg /cm^2)	Slope	RF
Chlorpyrifos	0.267	3.30	7.42	0.283	2.11	7.86	0.346	1.92	9.61	0.393	2.00	10.90
Malathion	3.3	2.94	50	6.76	1.50	102.4	12.26	2.80	185.8	14.15	2.30	214.4
Methomyl	1.57	2.14	1.43	1.60	1.48	1.45	2.04	1.32	1.85	5.52	1.23	5.02
Pirimiphos-methyl	0.173	2.00	24.7	0.204	1.56	29.1	0.236	2.10	33.7	0.472	2.13	67.4

The red flour beetle, *T. castaneum* is one of the most abundant and injurious pests of stored grain and flour in warehouses and mills. This insect has been subjected to considerable selection pressure with pesticides as a result of established control practices in the storage environment. As a consequence of the selection pressure, these beetles have developed resistance to many of the commonly used pesticides (Zettler, 1991). Among the insecticides involved in resistance of this insect is malathion. Malathion resistance in *T. castaneum* populations has become common in many regions of the world (Champ and Dyte, 1977) and was frequently reviewed by many authors (Osman and Rejesus, 1981; Navarro *et al.,* 1986; Picollo de Villar *et al.,* 1987; Halliday *et al.,* 1988; Subramanyam *et al.,* 1989; Beeman and Wright, 1990; Collins, 1990; Herron, 1990; Sayaboc and Acda, 1990; Zettler and Cuperus, 1990 and Zettler, 1991).

3.2. Cross-resistance:

Pirimiphos-methyl, chlorpyrifos and malathion resistant strains of *T. castaneum* were tested to determine their susceptibility to chlorpyrifos-methyl, cypermethrin and cyfluthrin. Table (4) revealed that all of the tested OP-resistant strains of *T. castaneum* show only low level of cross -resistance to the pyrethroid insecticides cyfluthrin and cypermethrin at the LC_{50} level (RF values ranged from 2.55 to 6.4 fold). On the other hand, these resistant strains were found to be more susceptible to the insecticide, chlorpyrifos-methyl than the susceptible strain (RF values ranged from 0.29 to 0.66).

Similar results were obtained by many authors. Organophosphorus-resistant strains of *Oryzaephillus surinamensis* L. (Coleoptera: Silvanidae), Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae) and *T. castaneum* did not show cross-resistance to chlorpyrifos-methyl (Attia and Frecker, 1984; Summer *et al.*, 1988 and Subramanyam *et al.*, 1989). In malathion-resistant of *T. castaneum*, cross-resistant to the pyrethroid, permethrin was not detected (Zettler and Jones, 1977). Attia and Frecker (1984) reported that, OP-resistant strain of *O. surinamensis* showed low level of resistankce to dichlorovos, bioresmethrin and pyrethrins (<10-fold). Bansode and Campbell (1979) found that, malathion resistant strain of the red flour beetle *T. castaneum*, did not show cross-resistance to 4 other organophosphorus insecticides but exhibited tolerance to these insecticides (0.8-1 fold). Generally, in order to have effective control methods available in the eventuality that resistance in insects increases to the extent that present chemical controls are no longer effective, it is important to test new materials and methods against strains of insects. The tested OP compound, chlorpyrifos-methyl might satisfy the criteria of these materials against the red flour beetle in this respect. No indication of resistance to chlorpyrifos-methyl was found in different strains of *T. castaneum* (Halliday *et al*,. 1988; Zettler and Cuperus 1990 and Zettler, 1991). For pyrethroids, the situation is probably more different. Resistance to α-cyano pyrethroid insecticides e.g. cyfluthrin and cypermethrin in *T. castaneum* has highly significant implications for the grain storage, industry (Collins, 1990). These compounds were regarded as the likely future replacements for the organophosphorus insecticides. However, result of Collins (1990) showed that if the pyrethroids are introduced into the field at currently proposed application rates they will ultimately fail to control *T. castaneum*. How long this will take depends on a combination of factors such as rates of selection and the inherent characteristics of the pyrethroid-resistance gene. On the other hand, this new resistance does not jeopardize current organophosphorus grain protectants nor future materials including methacrifos and methoprene. Andric *et al*. (2015) determined the possible alteration in susceptibility of two field strains of *T. castaneum* in a warehouse to dichlorvos, malathion, chlorpyrifos-methyl, deltamethrin and bifenthrin after previous selection with the LD_{80} of pirpmiphos-methyl and deltamethrin using topical application method. Data obtained showed that chlorpyrifos-methyl was the most toxic insecticide to *T. castaneum* adults, while malathion was the weakest. Also, the selection changed / reduced significantly the toxicity of deltamethrin and bifenthrin, increasing their resistance ratios (RR) at the LD_{50} from 1.1 to 1.8 (bifenthrin) and from 0.9 to 2.2 (deltamethrin). Kljajić and Perić (2007) determined the toxicity of six contact insecticides to local populations of granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) after selection with pirimiphos-methyl and deltamethrin. They found that the population that underwent three selections with the LD_{50} of deltamethrin, the resistant ratios of that insecticide increased significantly, so that the initial 7.0 and 7.2 at the LD_{50} and LD_{95} levels increased to 32.1 and 51.9, respectively. Galleya (1999) studied the deltamethrin resistance in *R. dominica*. Mribeiro *et al.* (2003) surveyed insecticide resistance and synergism in Brazilian population of *S. zeamais* using the discriminating concentrations established from LC_{95}'s estimated for a standard susceptible population against chlorpyrifos-methyl, malathion and pirimiphos-methyl and three pyrethroids (cypermethrin, deltamethrin and permethrin). Collins *et al*. (2003) investigated the resistance that had emerged against fumigants and protectants.

Tablel (4): Cross-resistance patterns in chlorpyrifos, malathion and pirimiphos-methyl resistant strains of *Tribolium castaneum*.

Insecticides	Chlorpyrifos- LC$_{50}$ µg/cm^2			Malathion-resistance LC$_{50}$ µg/cm^2			Pirimiphos-methyl - LC$_{50}$ µg/cm^2		
	S	Rc	R.F	S	Rm	R.F	S	Rp	R.F
Chlorpyrifos-methyl	0.088	0.038	0.43	0.088	0.025	0.290	0.088	0.058	0.660
Cyfluthrin	0.197	1.260	6.40	0.197	0.550	2.800	0.197	0.500	2.550
Cypermethrin	0.220	1.160	5.30	0.220	0.790	3.570	0.220	0.880	4.000

Andric *et al.* (2015) stated in insecticides choices need to be made carefully, considering the target species of stored product insects and their susceptibility to malathion and other insecticides: Attention should also be focused on a crucial role of insecticide selection of different stored grain insect populations in order to enable predictions of resistance evolution in individual populations and, based on such knowledge, sound choices of the most adequate resistance management strategy. Ultimately, a key to the successful management of resistance to insecticides is its early detection and proper characterization. All resistance data are being stored in an integrated database for future reference on trends and frequencies of resistance.

The present study suggests application of sequence of different groups to prevent or delay the resistance and cross-resistance to certain insecticides specially that used in the current study.

It is concluded that the common insecticides, malathion and pirimipho-methyl have created high level of resistance compared to methomyl which was rarely used in grain protection. Furthermore chlorpyrifos-methyl succeeded in covering the resistance in the resistant strains tested.

Conflict of Interest

The present study was performed in absence of any conflict of interest.

Acknowlegement

The author would thank all participants

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