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CROSS- RESISTANCE OF SOME COMPOUNDS ON *Rhizoglyphus robini* (CLAPAREDE) INFESTING STORED ONION

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ABSTRACT

Studies were conducted to determine rate of resistance development in laboratory strain of *Rhizoglyphus robini* to five compounds namely; Damaseisa, Vertmic, Bio – Fly, Neemix and Endo. The susceptibility of the mites to tested compounds was assessed every generation during the period of selection to determine the effect of selection on the resulting generations. The rates of resistance development of *Rhizoglyphus robini* during nine generations of selection with Damaseisa; Vertmic; Bio-Fly; Neemix and Endo. The resistance ratios of the *R. robini* individual's during the generation of selection varied in their tolerant against the different compounds under laboratory condition.

INTRODUCTION

Storage onions are available throughout the fall and winter. They are stronger in taste, usually smaller, have the traditional onion heat and store well. Both of these types are grown either on mineral soils or on high organic 'muck' soils, depending on the region. The total area cultivated with onion in 2004/2005 season in Egypt reached 100.540 hek, which produced 1302130 tons (Abo-Dahab2006).

The two most important and widespread bulb mite species are *Rhizoglyphus echinopus* and *R. robini*. Both species apparently are distributed virtually worldwide. One of the reasons that the mites are so widespread is that they can survive on numerous food sources and are often shipped long distances on bulbs, corms and tubers (Diaz *et al.* 2000). Besides their role in transmitting the pathogens of different plant diseases (Ragunathan *et al.* 1974; Abdel- Sater and Eraky, 2002). *Rhizoglyphus robini* (Claparede) is the most common and harmful mite species occurring on bulbs plants throughout the growing season as well as of storage period.

MATERIALS AND METHODS

1- Plant extract:
Petroleum either of Damaseisa leaves (*Ambrosia maritime*)
Fam.: Compositae.
Preparing of the plant extracts:

500 gm from each material were ground in electric mill into fine powder. The ground plant material was soaked in solvent (petroleum ether) in a large flask for 72 hours, then the flask shacked for half hour in shaker and

its content was filtered. The solvent was evaporated at 45c in an evaporator as described by Su (1985). The extracts which in the form of acrude gum were weighed and redissolved in the solvent to give 10% (w/v) stock solution. Concentration of 20; 10 and 5 % (w/v) were prepared from the stock solution for the extract of Damaseisa leaves for conducting the experiment.

2- Bioacaricides:

1- Vertmic 1.8% E.C. at rate 40 cc. /100 liter water.

Chemical name: Abamectin, a mixture containing a minimum 80% Avermectin B,a (5-0- demethyl- Avermectin A, a) and a maximum of 20% Avermectin B,b ~~(5-0- demethyl-~~ 25- de- (1- methylpropyl-25-(1-methyl) Avermectin A,a).

2- Neemix 4.5 EC AGROLINK for Agribusiness

3- Bio - Fly 3×10^7 unit / ml. Spores of the fungus; *Beauveria bassiana.*

3- Acaricides:

1-ENDO 50% EC O- tetraethyle S, s- methylene bis (phosphorodithioate)

4- Control: used water (without any material)

Rearing cell:

Mites were reared as individuals using small hemispherical plastic cells of 1/2 inch in diameter and less 1/4 inch in depth. Bottom of each cell was covered with plaster of mixed of paris charcoal and top of each cell covered with small slide glass.

For the preparation of pure culture of astigmatid and mesostigmatid mite were placed singly in rearing cells. Each mite was supplied daily with food.

Selection procedure:

The parent strain after being reared in the laboratory for 9 generation free from insecticidal contamination was divided into five sub groups as follows:

1- The 1st group was selected by Endo 50% EC for 9 successive generations.
2- The 2nd group was selected by Vertmic 1.8 % EC for 9 successive generations.
3- The 3rd group was selected by Neemix 4.5 for 9 successive generations.
4- The 4th group was selected by Bio - Fly 3×10^7 unit / ml for 9 successive generations.
5- The 5th group was selected by Damaseisa for 9 successive generations.

The selection in experimental groups was carried out on the adult mites with LC50 level of five materials. Some 100 adults were selected in each generation. Based on the resistance ratio, the LC50 values resulting from the strain exposed to the different selections were compared with those of parent strain as follows:

$$\text{Resistance ratio (R.R)} = \frac{\text{LC50 of selected strain}}{\text{LC50 of parent strain}}$$

Statistical analysis:

Mortality data were corrected by (Abbot 1925) and probit analysis (Finney 1971) to calculate the lethal concentration as mentioned above.

RESULTS AND DISCUSSION

1- Rate of resistance development:

Studies were conducted to determine the rate of resistance development in laboratory strain of *Rhizoglyphus robini* to five compounds and acaricides namely; Damaseisa, Vertmic, Bio – Fly, Neemix and Endo.

The susceptibility of the mites to tested compounds was assessed every generation during the period of selection to determine the effect of selection on the resulting generations.

1.1- Rate of resistance development to plant extracts (Damaseisa):

The results clearly showed that, the resistance ratios relatively constant through the first four generations of selections from 1.09- fold in G_1 to 1.75- fold in G_4. Then a slight increase was observed from 2.21- fold in G_5 to 3.14- fold in G_6 to 3.27- fold in G_7. the resistance ratios increased in last two generations from 6.2- fold in G_8 to 12.15- fold in G_9 reflecting the action of such plant extract as powerful sieve for concentrating resistant mutants that were present in high frequencies.

The slope values of the mortality regression lines decreased generally along the course of selection, indicating a considerable degree of heterogeneity, in spite of the chemical pressure applied, reflects the reorganization of genetic factors contributing to resistance, or many suggest a very slow grouping of the genetic material which contributes to resistance (Table1).

Table (1): Toxicity of plant extract (Damaseisa) on the adult of *Rhizoglyphus robini* during different generations of selection

Generation	Lethal Concentrations and Their 95% Confidence Limits	Lethal Concentrations and Their 95% Confidence Limits	Lethal Concentrations and Their 95% Confidence Limits	Slope ± SE	R.R*
	LC 50	LC 90	LC 95		
P	3.58	9.19	12.01	3.13 ± 0.364	-
G1	3.93	20.61	32.96	1.78 ± 0.31	1.098
G2	4.27	42.36	81.19	1.29 ± 0.3	1.193
G3	5.0	167.41	452.91	0.84 ± 0.3	1.397
G4	6.27	30.9	48.55	1.85 ± 0.32	1.751
G5	7.94	9097.83	67009.26	0.42 ± 0.3	2.218
G6	11.25	87.86	157.34	1.44 ± 0.32	3.142
G7	11.71	116.27	222.87	1.28 ± 0.3	3.271
G8	22.23	461.08	891.44	0.86 ± 0.3	6.209
G9	43.5	1840.3	3565.76	0.79 ± 0.3	12.151

*R.R = resistance ratio

1.2- Rate of resistance development to Vertmic:

The resistance ratios (R.R.), resistance gradually increased starting from G_1 (4.95- fold) to reach 15.46- fold in G_6 reflecting the action of such Vertmic as powerful sieve for concentrating resistant mutants that were present in low frequencies. Then, the resistance ratios increased sharply from G_7 (31.01- fold) to reach 359.42- fold in G_8.

The slope values of the mortality regression lines decreased generally along the course of selection, indicating a considerable degree of heterogeneity. The regression lines were characterized by a gradual increase in LC_{50} values and a decrease in slopes. The responses of these generations typify those of populations beginning the development of true resistance. The shifting to the right and the decrease in the slope values of the regression lines during early selections indicate that the percentage individuals possessing the resistance mechanism is increasing, although there are still many susceptible individuals present (Table2).

Table (2): Toxicity of Vertamic on the adult of *Rhizoglyphus robini* during different generations of selection

Generation	Lethal Concentrations and Their 95% Confidence Limits	Lethal Concentrations and Their 95% Confidence Limits	Lethal Concentrations and Their 95% Confidence Limits	Slope ± SE	R.R*
	LC 50	LC 90	LC 95		
P	0.63	3.09	4.86	1.85 ± 0.32	-
G1	3.12	14.95	23.32	1.88 ± 0.37	4.952
G2	3.96	24.31	40.66	1.63 ± 0.37	6.286
G3	5.6	48.84	90.23	1.36 ± 0.37	8.889
G4	6.9	61.77	114.96	1.35 ± 0.39	10.952
G5	8.16	58.41	102.07	1.49 ± 0.45	12.952
G6	9.74	147.95	319.94	1.09 ± 0.38	15.460
G7	19.54	278.87	592.46	1.11 ± 0.45	31.016
G8	226.44	21990.87	80461.49	0.65 ± 0.46	359.429
G9	--	--	--	---	0.000

*R.R = resistance ratio

1.3- Rate of resistance development to Bio – Fly:

The rate of resistance development to Bio – Fly during nine generations of selection showed in Table (3). The resistance ratios through the nine generations of selections was slightly increased, observed from G_1 (5.25- fold) to reach 17.13- fold till the end of selection in G_9. This low level of tolerance considered as a manifestation of vigor tolerance.

The slope values of the mortality regression lines increased in the first generation G_1 and second generation G_2 then decreased until the end of selection in G_9. This pattern of change in slopes is a typical response of mortality regression lines undergoing true resistance.

Table (3): Toxicity of Biofly on the adult of *Rhizoglyphus robini* during different generations of selection

Generation	Lethal Concentrations and Their 95% Confidence Limits	Lethal Concentrations and Their 95% Confidence Limits	Lethal Concentrations and Their 95% Confidence Limits	Slope ± SE	R.R*
	LC 50	LC 90	LC 95		
P	2.77	12.45	19.07	1.96 ± 0.46	-
G1	14.55	36.14	46.77	3.24 ± 0.34	5.253
G2	19.3	29.17	32.79	7.15 ± 0.72	6.968
G3	22.79	40.52	47.69	5.13 ± 0.56	8.227
G4	25.08	48.62	58.66	4.46 ± 0.53	9.054
G5	28.14	60.31	74.86	3.87 ± 0.49	10.159
G6	32.67	79.27	101.91	3.33 ± 0.48	11.794
G7	40.3	115.87	156.31	2.79 ± 0.46	14.549
G8	46.59	141.67	194.18	2.65 ± 0.49	16.819
G9	47.46	126.08	166.32	3.02 ± 0.58	17.134

*R.R = resistance ratio

1.4- Rate of resistance development to Neemix:

The results clearly showed that, the resistance ratios increased very slowly during first three generations (from 4.99- fold in G_1 to 7.3- fold in G_3). The resistance ratios become relatively constant through five generations from G_3 of selections to 8.27- fold in G_7. Then, the resistance ratios increased from G_8 (10.98- fold) to reach 24.6- fold in G_9.

The slope values of the mortality regression lines inconstant from the parent strain (P) until the end of selection in G_9.

The low slope value of the regression line of the parent strain (P) depicts the laboratory strain as slightly heterogeneous to the action of the mites (Table4).

Table (4): Toxicity of Neemix on the adult of *Rhizoglyphus robini* during different generations of selection

Generation	Lethal Concentrations and Their 95% Confidence Limits	Lethal Concentrations and Their 95% Confidence Limits	Lethal Concentrations and Their 95% Confidence Limits	Slope ± SE	R.R*
	LC 50	LC 90	LC 95		
P	3.46	33.74	64.33	1.29 ± 0.38	-
G1	17.29	52.07	71.19	2.68 ± 0.36	4.997
G2	19.85	590.53	1545.07	0.87 ± 0.3	5.737
G3	25.29	94.35	137.02	2.24 ± 0.38	7.309
G4	26.09	309.08	622.86	1.19 ± 0.32	7.540
G5	26.68	86.16	120.12	2.52 ± 0.42	7.711
G6	28.15	318.25	632.91	1.22 ± 0.32	8.136
G7	28.62	2527.59	9002.66	0.66 ± 0.3	8.272
G8	37.99	3044.23	10547.51	0.67 ± 0.31	10.980
G9	85.13	757.53	1407.7	1.35 ± 0.42	24.604

*R.R = resistance ratio

1.5- Rate of resistance development to Endo:

The rate of resistance development to Endo during nine generations of selection showed in Table (5). The results clearly showed that, the resistance ratios relatively constant through the first four generations of selections from 1.29- fold in G_1 to 2.91- fold in G_4. Then a slight increase observed from 3.26- fold in G_5 to 7.7- fold in G_8 then to 38.52- fold in G_9.

Table (5): Toxicity of Endo on the adult of *Rhizoglyphus robini* during different generations of selection

Generation	Lethal Concentrations and Their 95% Confidence Limits	Lethal Concentrations and Their 95% Confidence Limits	Lethal Concentrations and Their 95% Confidence Limits	Slope ± SE	R.R*
	LC 50	LC 90	LC 95		
P	0.34	1.17	1.65	2.41 ± 0.38	-
G1	0.44	2.41	3.89	1.74 ± 0.31	1.294
G2	0.68	6.66	12.69	1.29 ± 0.31	2.000
G3	0.98	8.19	14.95	1.39 ± 0.31	2.882
G4	0.99	29.53	77.25	0.87 ± 0.3	2.912
G5	1.11	32.01	83.07	0.88 ± 0.3	3.265
G6	1.96	38.17	88.57	0.99 ± 0.32	5.765
G7	2.14	39.09	89.07	1.02 ± 0.32	6.294
G8	2.62	15.29	25.21	1.67 ± 0.42	7.706
G9	13.1	2621.88	11774.74	0.56 ± 0.33	38.529

*R.R = resistance ratio

The slope values of the mortality regression lines decreased until G_4 then become steady in the next two generations G_5 and G_6. Then the slope values decreased until the end of generations.

These obtained results are in agreement with some authors Carroll *et al.* (1983) the result obtained based on LD50s and comparisons with a susceptible strain, adults of the onion pest *Delia antiqua* (Mg.) of a 24-fold parathion-resistant strain showed 15-, 9.3- and 7.7-fold levels of cross-resistance to chlorpyrifos, naled and chlorfenvinphos, respectively, when applied by direct contact in the laboratory in Canada. Resistance to pyrethroids was lower, being 3.4-, 2.9-, 1.6- and 1.3-fold for deltamethrin, permethrin, cypermethrin and fenvalerate, respectively. The pyrethroids were 17-137 times as toxic as parathion. Chen and Lo (1990) stated that some field strains of *Rhizoglyphus robini* collected from green onion fields in Taiwan were found to have developed high multi-resistance to organophosphorus insecticides including diazinon, dimethoate, fenitrothion, malathion, methyl parathion [parathion-methyl], omethoate, parathion and prothiofos. The organophosphorus resistance of the mite remained stable when pesticide pressure was relaxed in the laboratory. Changes in susceptibility to several organophosphorus insecticides for a strain after relaxation of selection pressure for 3 and 27 generations were compared. Since susceptible mites could not be found in the field, a reverse selection for susceptibility to diazinon was designed and conducted. Although heterogeneity was observed in the progeny of the mixed populations, the susceptibility of these reversely-selected mites increased slowly. A stable and susceptible strain was obtained after 26 generations of selection. Katundu and Aliniazee (1990) investigated that the development of resistance in the filbert aphit, *Myzocallis coryli* (Goetze), to commonly used insecticides. Data suggest that *M. coryli* populations have become resistant to many insecticides. Resistance to endosulfan, carbaryl, diazinon, phosalone, and oxydemetonmethyl was detected. Resistance levels varied from 1.5 to 4,090-fold for carbaryl, 1.2-to 288-fold for diazinon, 1.8-to 50-fold against endosulfan, 1.7-to 49,069-fold against phosalone, and 3.4-to 112.4-fold against oxydemetonmethyl. Some seasonal variations were noticed. With some exceptions, summer and fall populations were generally more resistant to pesticides than the spring populations. Although development of resistance on a regional basis was not evident, a more localized (orchard-by-orchard) resistance was observed. This pattern suggests limited aphid movement and gene flow. Clark *et al.* (1994) stated that the avermectins represent a group of natural compounds with potent pesticidal activities. Because of their novel mode of action, they represent an important resource for pest control and resistance management. In the Colorado potato beetle, the house fly, and the two-spotted spider mite, resistance to abamectin is usually autosomal, recessive, and polygenic. Although these aspects are beneficial in resistance management, the fact that resistance could be readily selected for suggests that abamectin needs to be used in moderation. Goka (1998) stated that the genetics of resistance to three new types of acaricide tebufenpyrad, fenpyroximate and pyridaben was studied by crossing a resistant (R) with a susceptible (S) strain of *Tetranychus kanzawai* Kishida. The resistance ratios calculated frcm the LC50s of the R and S strains were 97, 1265 and 134 for tebufenpyrad, fenpyroximate and pyridaben, respectively. The responses to the three acaricides in F1 females from reciprocal crosses between the R and S strains showed that the modes of inheritance of resistance to tebufenpyrad, fenpyroximate and pyridaben were intermediate, incompletely dominant and completely recessive, respectively. Furthermore, the responses of F2 females from the reciprocal crosses indicated that the resistance to the three acaricides was under monogenic control. Uesugi *et al.* (2002) studied the genetic basis of resistance to two new acaricides, chlorfenapyr and etoxazole, which have different chemical structures and modes of action in the two-spotted spider mite, *Tetranychus urticae* Koch. The resistance ratios calculated from the LC_{50}s of resistant and susceptible strains were 483 for chlorfenapyr and >100,000 for etoxazole. Mortality caused by the two acaricides in F_1 progeny from reciprocal crosses between the resistant and susceptible strains indicated that the modes of inheritance of resistance to chlorfenapyr and etoxazole were completely dominant and completely recessive, respectively. Mortality in F_2 progeny indicated that for both acaricides, the resistance was under monogenic control. Repeated backcross experiments indicated a linkage relationship among the two acaricide resistances and malate dehydrogenase, although phosphoglucoisomerase was not linked with them. The recombination ratio between the resistances was 14.8%. From this result, we suggest that heavy spraying of the two acaricides will lead to apparent cross-resistance as a consequence of crossing over; the two resistance genes are so close to each other that it would be difficult to segregate them once they came together on the same chromosome. Kim *et al.* (2004) stated that a field colony of the Two-spotted spider mite, *Tetranychus urticae* (Koch), resistant to fenpyroximate was further selected with fenpyroximate 5SC for 20 generations at a selection pressure of 30-50% mortality (designated as FR-20 strain). Resistance and cross-resistance levels of the FR-20 strain to 18 acaricides were determined using a spray method. The FR-20 strain was extremely resistant to fenpyroximate [resistance ratio (RR) 252]. The strain exhibited extremely strong positive cross-resistance to acrinathrin (RR 196), and high levels of resistance to benzoximate (RR 55) and propargite (RR 64). Moderate levels of cross-resistance (RR 11-40) to abamectin, fenbutatin oxide, fenpropathrin, pyridaben, pyridaben + bifenthrin and tebufenpyrad were observed. The FR-20 strain showed low levels of resistance (RR < 10) to azocyclotin, bromopropylate, chlorfenapyr, chlorfenapyr + bifenthrin, chlorfenapyr + pyridaben, dicofol, fenazaquin and milbemectin. Synergist experiments with different metabolic inhibitors revealed that piperonyl butoxide had the greatest effect on the efficacy of fenpyroximate, followed by iprobenfos and triphenyl phosphate. In a comparative assay with detoxifying enzymes, the FR-20 strain showed 2.5-fold higher activity in *p*-nitroanisole-*O*-demethylation, and 2.5- and 2.2-fold higher activities in α- and β-naphthyl acetate hydrolysis, respectively. These results suggested that enhanced activities of both mixed-function oxidases and esterases likely contribute to the fenpyroximate resistance of the FR-20 strain of *T urticae* Sato *et al.* (2004) performed that the artificial laboratory selections for resistance and susceptibility to fenpyroximate in a population of the two-spotted spider mite, *Tetranychus urticae* Koch (green-form), collected from a commercial strawberry field in the State of São Paulo, Brazil. After five selections for resistance and three selections for susceptibility, the resistance ratios (R/S) at the LC_{50} and LC_{95} reached 2,910 and 2,280, respectively. Cross-resistance relationships between fenpyroximate and eight other acaricides were evaluated using the selected susceptible and resistant strains of *T. urticae*. The results indicate positive cross-resistance between fenpyroximate and acaricides, pyridaben and dimethoate. No cross-resistance was detected for acaricides, propargite, abamectin, milbemectin, fenpropathrin and cyhexatin. The studies on genetics of fenpyroximate resistance indicate that the fenpyroximate resistance is controlled by one major, incompletely dominant factor. Frequencies of fenpyroximate resistance decline significantly in the absence of selection pressure, under laboratory conditions, however, the rate of decline is not high.

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دراسة صفة المقاومة لاكاروس البصل *Rhizoglyphus robini* لبعض المركبات الاكاروسية على محصول البصل فى المخزن

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**قسم علم الحيوان- كلية العلوم (بنات)- جامعة الأزهر

أجريت دراسه تطور صفة المقاومة لاكاروس البصل *Rhizoglyphus robini* لبعض المركبات الاكاروسية (المستخلص النباتى الدمسيسه والنيمكس- المبيد الحيوى الفيرتيمك والبيو فلاى – المبيد الكميائى أندو) على محصول البصل فى المخزن.

وأظهرت النتائج الاتى:

١- تطور صفة المقاومة للمستخلص النباتى (Damaseisa):

كان معدل تطور ظاهرة صفة المقاومة ثابت خلال الاربعة أجيال الاولى ثم حدثت زيادة طفيفة فى المعدل من الجيل الخامس الى الجيل السابع ثم ازدادت خلال الجيلين الثامن والتاسع بدرجة كبيرة.

٢- تطور صفة المقلومة للفيرتميك Vertmic:

كان معدل التطور لظاهرة صفة مقاومة الاكاروس للفيرتميك ثابت خلال الأجيال المنتخبة يزداد تدريجيا من الجيل الاول الى الجيل السادس ثم يزداد المعدل بعد ذلك بشدة من الجيل السابع الى الجيل الثامن.

٣- تطور صفة المقلومة للبيوفلاى Bio – Fly:

كان معدل التطور لظاهرة صفة مقاومة الاكاروس للبيوفلاى خلال الأجيال المنتخبة يزداد ببطئ من الجيل الاول الى نهاية الاجيال المنتخبة.

٤- تطور صفة المقاومة للنييمكس Neemix :

كان معدل التطور لظاهرة صفة مقاومة الاكاروس للنييمكس خلال الأجيال المنتخبة تبين زيادة ضعيفة خلال الثلاثة أجيال الاولى ثم يصبح المعدل ثابت تقريبا خلال الخمسة أجيال من الجيل الثالث الى الجيل السابع. ثم يزداد معدل صفة المقاومة فى نهاية الاجيال المنتخبة.

٥- تطور صفة المقاومة للاندو Endo :

معدل التطور للاندو خلال ظاهرة صفة مقلومة الاكاروس خلال الأجيال المنتخبة كانت تقريبا ثابتة خلال الاربعة أجيال الاولى ثم يحدث زيادة ضعيفة فى النسبة من الجيل الخامس الى التاسع.

وبصفة عامة فقد اثبتت أن معدل تطور صفة مقاومة الاكاروس للمبيد خلال الأجيال المنتخبة يختلف باختلاف المبيدات موضع البحث.