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Orijinal araştırma (Original article)

Analysis of hexythiazox resistance mechanisms in a laboratory selected predatory mite *Neoseiulus californicus* (Acari: Phytoseiidae)[1]

Laboratuvarda seleksiyona maruz bırakılan avcı akar *Neoseiulus californicus* (Acari: Phytoseiidae)'da hexythiazox direnç mekanizmasının incelenmesi

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Summary

Hexythiazox resistance in a *Neoseiulus californicus* population selected in laboratory conditions was analyzed in the present study . LC_{50} and LC_{60} values of *N. californicus* were determined via the leaf disk method using a spray tower. Resistance ratios were determined by dividing the LC_{50} values of selected populations to the LC_{50} value of the laboratory population. Resistance ratio was increased to 64.04 -fold in selected population (named HEX14) after 14 selection cycles with hexythiazox. The effects of PBO, IBP and DEM were examined on hexythiazox resistance. Furthermore, glutathione S-transferase, cytochrome P450 monooxygenases and acetylcholinesterase enzymes were determined using kinetic methods and esterase enzyme was determined using electrophoretic and kinetic methods in laboratory and HEX14 populations. The study conducted with PBO, IBP and DEM in the HEX14 population showed that synergistic effect rates were 1.71, 3.25 and 1.98 -fold, respectively. The cross resistance ratios of the HEX14 population to spirodiclofen, etoxazole, spiromesifen, propargite, clofentezine and milbectin were 8.12, 14.41, 17.96, 17.48, 12.67 and 11.22 -fold, respectively. The hexythiazox resistance of the HEX14 population was incompletely dominant and monogenic. A 3.27 -fold esterase, 2.35 -fold GST and 2.02 -fold cytochrome P450 monooxygenase and 3.34 -fold AChE enzyme activity were detected in the HEX14 population in biochemical assays.

Key words: *Neoseiulus californicus*, hexythiazox, synergist, inheritance, detoxification enzymes

Özet

Bu çalışmada laboratuar ortamında seleksiyona maruz bırakılaran *N. californicus* popülasyonu üzerinde hexythiazox direnci incelenmiştir. *N. californicus* popülasyonlarının LC_{50} ve LC_{60} değerleri ilaçlama kulesi kullanılarak yaprak disk metodu ile belirlenmiştir. Direnç oranları seleksiyona maruz bırakılan popülasyonların LC_{50} değerlerinin hassas popülasyonunun LC_{50} değerine bölünmesiyle belirlenmiştir. Seleksiyon çalışmasında, 14 defa hexythiazox uygulaması yapılan populasyonda hexythiazox'a karşı 64.04 kat direnç geliştiği tespit edilmiştir ve bu populasyon HEX14 olarak adlandırılmıştır. Sinerjistler,PBO, IBP ve DEM'in hexythiazox direnci üzerine olan etkileri incelenmiştir. Ayrıca laboratuar ve HEX14 popülasyonlarında glutathion S-transferaz, sitokrom P450 monooksigenaz ve asetilkolinesteraz enzimleri kinetik yöntemle, esteraz enzimi elektroforetik ve kinetik yöntemlerle belirlenmiştir. HEX14 popülasyonunda PBO, IBP ve DEM ile yapılan çalışma sonucunda sırasıyla 1.71, 3.25, 1.98 kat sinerjistik etki oranları belirlenmiştir. HEX14 popülasyonunun spirodiclofen, etoxazole, spiromesifen, propargite, clofentezine ve milbectin'e karşı gösterdiği çapraz direnç oranları sırasıyla, 8.12, 14.41, 17.96, 17.48, 12.67 ve 11.22 kat olarak bulunmuştur. HEX14 popülasyonunda hexhythiazox direncinin eksik baskın ve monogenik özellikte olduğu belirlenmiştir. Biyokimyasal testlerde,HEX14 popülasyonunda 3.27 kat esteraz, 2.35 kat GST, 2.02 kat sitokrom P450 monoksigenaz ve 3.34 kat AChE enzim aktivitesi bulunmuştur.

Anahtar sözcükler: *Neoseiulus californicus,* hexythiazox, sinerjist, kalıtım, detoksifikasyon enzimleri

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Introduction

Mite species of the family Phytoseiidae are predators on plant parasitic mite populations in greenhouses, fruit orchards and vineyards (Hoy, 1985). *Neoseiulus californicus* (Acari: Phytoseiidae) is an effective and common predatory mite species used in the control of pest mites (Castagnoli & Simoni, 1999). Wide-spectrum pesticides used in agriculture affect the predatory mites of the Phytoseiidae family, as well asother natural enemies. Therefore, *N. californicus* is affected by the pesticides used, and thus cannot maintain its natural populations. As a result, predatory pressure upon spider mites decreases, which then reproduce rapidly, creating large populations that cause great damage (Castognoli et al., 1999). In addition to the side effects of pesticides on natural enemies, predators and parasitoids gain chemical resistance. The reason for this is that natural enemies are indirectly affected by the intensive use of chemicals in the area, although they are not the target. Bonafos et al. (2007) reported that *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) and *Amblyseius andersoni* (Chant) (Acari: Phytoseiidae) populations collected from vineyards had medium to high-level resistance to deltamethrin, lambda-cyhalothrin and chlorpyrifos-ethyl. Mugo et al. (2011) determined a 1.0–10.0 -fold resistance of the field populations of *Euseius kenyae* (Swirski & Ragusa) (Acari: Phytoseiidae) to chlorpyrifos, and Tirello et al. (2012) found that four different *Kampimodromus aberrans* (Oudemans) (Acari: Phytoseiidae) populations collected from vineyards and apple orchardshad 1.85–6.83 -fold resistance to chlorpyrifos.

Pest mites quickly develop resistance to pesticides and natural enemy populations decrease due to the pesticides used, which is one of the main problems obstructing integrated pest management (IPM) (Sato et al., 2006). Natural enemies resistant to pesticides may be a solution for this problem. Sato et al. (2000) reported that the resistance of *Amblyseius womersleyi* Schicha (Acari: Phytoseiidae) to methidathion increased 311 -fold after 4 selection cycles. Auger et al. (2005) found that the resistance of *T. pyri* to mancozeb increased 73 -fold after 10 selection cycles. Sayyed et al. (2010) found that the resistance of *Chrysoperla carnae* (Stephens) (Neuroptera: Chrysopidae) increased 896 -fold after selection with deltamethrin.

Hexythiazox is a non-systemic acaricide with contact and stomach action from the Thiazolidine group which has ovicidal, larvicidal and nymphicidal activity. It is not active against adults, but eggs laid by treated females are non-viable. This compound is a selective miticide which is active against various pest mites of agricultural importance, and has no or few effects on their natural enemies (Boller et al.,,, 2005; Sanatgar et al., 2011). The miticide interferes with mite growth and reproduction. Thus, hexythiazox has been used for controlling mites on various crops under the integrated pest management system (Yamamoto et al., 1996).

Resistance potential to hexythiazox in laboratory-selected *N. californicus* populations was investigated in the present study which compared the resistance rates of hexythiazox resistant and laboratory *N. californicus* populations produced via selection pressure under laboratory conditions. Cross resistance, inheritance and detoxification enzyme activities were also analyzed in the selected and laboratory populations.

Materials and methods

Neoseiulus californicus populations

A collection of *Neoseiulus californicus* from a population in an organic apple orchard at the Isparta Research Institute for Fruit Growing in 2008 was used in the study. The population was then reared in the climate chamber at the Department of Plant Protection, Faculty of Agriculture, Suleyman Demirel University, and was used as the initial (laboratory) population. Two separate populations were obtained from this initial population. One was keet as original without any chemical treatment and the other was used for the selection experiment. *N. californicus* populations were produced in cages with water-filled tubs in climate chambers (temperature 26±1 °C, humidity 60–65%, photoperiod 16:8) so that the populations would not contaminate each other. The food of the predator mite, *Tetranychus urticae* Koch (Acari:Tetranychidae), and kidney beans, were also produced in the climate chambers under the same conditions. The *N. californicus* individuals were immediately frozen and stored at -80 ^{o}C until use for biochemical assays.

Acaricides and chemicals

Acaricides used in this study are as follow: 50 g L^{-1} hexythiazox EC (Hektas, Ltd., Turkey), 240 g L^{-1} spirodiclofen SC (Bayer CropScience, Germany), 240 g L^{-1} spiromesifen SC (Bayer CropScience, Germany), 570 g L^{-1} propargite EW (Hektas, Ltd., Turkey), etoxazole 110 g L^{-1} SC (Sumitomo Chemical, Japan), 500 g L^{-1} clofentezine SC (Irvito Plant Protection, Netherlands) and 9.3 g L^{-1} milbemectin EC (Sankyo Agro Ltd., Japan).

Three synergists, piperonyl butoxide (PBO; purity 90%), S-Benzyl-O,O-diisopropyl phosphorothioate (IBP; 98.2%) and diethyl maleate (DEM; 95%), were obtained from Acros (Belgium). a-Naphthyl acetate (a-NA), 1-chloro-2,4-dinitrobenzene (CDNB) and p-nitroanisole (PNOD) were purchased from Acros (Belgium). NADPH was obtained from Serva (Germany). Acetylcholine iodide (ATChI) and 5.5-dithiobis (2-nitrobenzoic acid) (DTNB) were obtained from Merck (Germany).

Bioassays

Toxicity test to determine the LC value

Hexythiazox was toxic to immature stages of predatory mites so individuals 0–24 hours old were used in all the bioassays. Fifteen Petri dishes (diameter of 9 cm) were prepared to collect same aged eggs. The bottom of each dish was lined with wet cotton. The sides of bean leaves were then coated with Tangle Trap to prevent mites escape from the disk, and the leaves were placed in the Petri dishes. Then, 15 mature females were transferred onto these bean leaf discs. After 24 hours, the eggs that were present on the leaves were transferred to 15 clean Petri dishes. These eggs hatched during the next 24 hours and provided predator mites of the same age for use in the experiments. The LC_{50} and LC_{60} values of the selected and laboratory populations were then determined. To determine these values, 25 *N. californicus* individuals (0–24 hours old) were transferred onto bean leaf discs surrounded with Tangle Trap coating and contained in Petri dishes. The experiments consisted of 1 blank water control and 7 serial concentrations of the acaricide. Three replicates were used for every concentration. The acaricide doses were at 0.5 X intervals. When leaf discs and mites were in the Petri dishes, 2 ml of the appropriate test solution was sprayed onto the surface of each leaf at a pressure of 1 bar by using a spray tower. When the females and males in the Petri dishes were 7 days old, the dead and live mites were counted to determine the LC values.

Selection process

The method of Sato et al. (2000) was modified for the selection studies. *N. californicus* individuals 0–24 hours old were transferred onto bean leaf discs, as described above. The previously established LC_{60} dose was used as the selection dose. Insecticide (LC_{60} concentration) was applied to each leaf in the Petri dishes via the spray tower (2 ml, 1 bar pressure). The Petri dishes were left for 7 days at 26±1°C and 60-65% relative humidity under a 16:8 light:dark photoperiod. Within this 7-day period, *T. urticae* individuals (mixed stage) were transferred to the Petri dishes as a diet for *N. californicus*. The *N. californicus* individuals that survived for 7 days after the treatment were transferred onto bean plants with *T. urticae* as prey. LC_{50} and LC_{60} values of the predatory mite populations were calculated via POLO (LeOra Software, 1994). The LC_{60} dose was re-established for each generation subjected to the selection pressure of the pesticide exposure. The hexythiazox selection of *N. californicus* was completed in 2010–2011.

Synergism test

In order to determine the effects of synergists on hexythiazox resistance, the monooxygenases enzyme inhibitor piperonyl butoxide (PBO) (2000µl/l), esterase enzyme inhibitor S-Benzyl-O,O-diisopropyl phosphorothioate (IBP) (200µl/l) and GST enzyme inhibitor diethylmaleate (DEM) (2000µl/l) were used (Stumpf & Nauen, 2002; Kim et al., 2004; Van Leeuwen et al., 2004; Kang et al., 2006). *Neoseiulus californicus* individuals 0–24 hours old were used in the studies of the synergists in combination with hexythiazox.. The experiments consisted of 1 control and 7 serial concentrations of acaricide. Three replicates were used for every concentration. The synergists were dissolved in acetone and distilled water

at a ratio of 1:1. The spray tower was used to spray the prepared synergist solutions (1 ml at 1 bar pressure) onto leaves placed in Petri dishes lined with wet cotton, as previously described. The Petri dishes were then exposed for 24 hours to 26±1°C, 60-65% humidity and a 16:8 photoperiod. Twenty-four hours after the synergist application, the tower was used to spray the surface of the leaves in the Petri dishes with 2 ml per leaf of pesticide at a concentration of 50%. Dead/live counts of the female and male individuals in the dishes were conducted on the 7^{th} day of exposure to the pesticide-treated leaves. Each day of the exposure, *T. urticae* individuals (mixed stages) were transferred to the dishes to prevent death of the predatory mites from malnutrition. Only the synergist was used in the control. The synergistic effect rate was calculated as the LC_{50} with no synergist/ LC_{50} with synergist (Kim et al., 2004).

Cross resistance

The cross resistance relationship was determined between hexythiazox and six different acaricides in HEX14 and laboratory *N. californicus* populations.. Spiromesifen, spirodiclofen, etoxazole, propargite, clofentezine and milbemectin were used. All the acaricides, except for propargite, were applied to individuals aged 0–24 hours, as in the toxicity tests. As propargite has an effect on the adult stage of the predatory mite, it was applied during that period. 25 female predatory mites were put on the leaf disks, as described previously, and experiments were conducted as stated in the toxicity tests. Dead/alive counts were made 24 hours later. Cross resistance ratios were calculated as the LC_{50} values of HEX14 population/ LC_{50} value of the aboratory population for each acaricide used in the cross resistance studies.

Inheritance experiment

The method of Auger et al. (2005) was used to determine inheritance of resistance. Reciprocal crossing was conducted between the hexythiazox-resistant HEX14 population (R) and laboratory population (S) in order to determine the dominance of the resistance. For this purpose, 20 deutonymph female individuals from the resistant population and 30 male individuals from the laboratory population were put on the leaf disks, as previously described. In addition, this process was reverse implemented. Approximately one day after the fertilization of diploid females by haploid males, the females laid eggs. Male and female individuals were removed from the Petri dish five days later; F1 individuals were put on clean kidney beans and given *T. urticae* as the diet. LC_{50} values of F1 individuals were determined as described in the toxicity test. After the R♀XS♂ and S♀XR♂ crossings, the inheritance of resistance among F1 females was calculated via the formula $D= (2X_2-X_1-X_3)/(X_1-X_3)$ formula (Stone, 1968); whereX_1, X_2 and X_3 represent the logs of the LC_{50} value of the resistant population, F1 females, and laboratory population, respectively. According to this formula, resistance of -1 indicates that inheritance is recessive; for resistance of 0, the sensitivities of the laboratory and resistant populations are the same; and for resistance of 1, inheritance is fully dominant.

In order to test the hypothesis that the inheritance of resistance was monogenic, F1 individuals were back crossed, to calculate the observed and expected death rates. In order to obtain F2 females, F1 females and the laboratory population and resistant male populations were allowed to copulate. LC_{50} values of F2 individuals were determined, as stated in the toxicity tests. Theoretically-expected death rates for F2 females were calculated via the formula c= (0.5)W(F1 females) + (0.5)W (R population). Here, c is the theoretically expected death rate for an applied concentration, and the W value is the observed death rate in females within a population (Georghiou, 1969). This genetic hypothesis was evaluated using values obtained from 1 degree of freedom and x^2 table, where the x^2 values were obtained from the experiments (Winer et al., 1991). If the difference between the observed and expected death rates was significant, the monogenic hypothesis was rejected; if the difference was insignificant, the monogenic hypothesis was accepted.

Biochemical assays

Esterase enzyme activity was determined via gel electrophoresis and microplate reader methods, and glutathione S-transferase (GST), monooxygenase (P450) and acetylcholinesterase (AChE) enzymes were determined via the microplate reader method.

Electrophoresis

The "mini vertical non-denaturing discontinuous polyacrylamide gel electrophoresis" method of Goka & Takafuji (1992) and Ay & Gürkan (2005) was modified to determine esterase enzyme acrylamide concentration and included densities of 7.5% and 3.5%. Five female individuals were homogenized via plastic pestle in a homogenization buffer that was prepared with 50 µl 32% (w v^{-1}) sucrose and 0.1% Triton X-100. Following polymerization, 10 µl homogenate was placed in each gel cell. Electrophoresis was completed at 150 V in about 1.5 hours (BIO-RAD Munchen, Germany). For the esterase activity, the gel was incubated in 0.2 M phosphate buffer (pH 6.5) which included 0.02% α-naphthyl acetate and 1 acetone for 30 minutes. Then, the gel was stained in 0.4% fast blue BB pure dye solution, which was prepared with 0.02% α-naphthyl acetate solution for 1 hour. At the end of this process, the gel was put in 7% acetic acid, and measured with a densitometer after 24 hours. Density of esterase bands was quantified by Lab Works 4.6 in densitometer (UVP ChemiDoc-it imaging system).

Photometric esterase assay

The method of Stumpf & Nauen (2002) was used to determine kinetic esterase activity. Twenty females were homogenized in a plastic pestle in 100 µl sodium phosphate buffer (0.1M, pH 7.5) including 0.1% Triton X-100. This homogenate was centrifuged at 10,000 g and +4 °C for 5 minutes, and then used as the supernatant enzyme source, which was diluted 10 times. 25 µl supernatant and 25 µl 0.2 M, pH 6 phosphate buffer were placed in microplate cells. Only buffer was added to the control cells, so that no enzymatic reaction would occur. The assay was started with the addition of 200 µl substrate solution, and the final concentration included 250 µl overall volume in microplate cells. Substrate solution was obtained by dissolving 30 mg fast blue RR salt in 50 ml 0.2 M sodium phosphate buffer, and the addition of 500 µl 100 Mm α – naphthyl acetate to this mixture. Esterase enzyme activity was measured at 450 nm and 23 °C for 10 minutes via Versamax kinetic microplate reader (Molecular Devices, Sunnyvale, California).

Photometric glutathione S-transferase assay

The method of Stumpf & Nauen (2002) was used for the kinetic determination of GST enzyme. For this purpose, 1-chloro-2,4-dinitrobenzene (CDNB) and reduced glutathione (GSH) were used as the substrate. 30 females were homogenized via plastic pestle in Eppendorf tubes in a 300 µl Tris HCL buffer (0.05M, pH 7.5). Supernatant was centrifuged at 10,000g and +4 °C for 5 minutes. An overall volume of 300 µl, including 100 µl supernatant, 100 µl 1-chloro-2,4-dinitrobenzene (CDNB) and 100 µl reduced glutathione (GSH), was put in microplate cells. Final concentration was 0.4 mM CDNB and 0.4 mM GSH in microplate cells. Absorbance change was measured at 340 nm and 25 °C for 5 minutes via Versamax kinetic microplate reader. Only CDNB and GSH were used in control cells, so that no enzymatic reaction would occur,and then read as non-homogenate..

Photometric P450 monooxygenase assay

The method of Rose et al. (1995) was used for cytochrome P450 monooxygenase enzyme assay, during which *p*-nitroanisole (PNOD) was used as the substrate. Fifty females were homogenized via plastic pestle in 100 µl homogenization buffer (0.05 M Tris-HCl + 1.15% KCl + 1mM EDTA pH (7.7)), and then centrifuged at +4 °C and 10,000 g for 20 minutes. 45 µL buffer + 45 µL supernatant+100 µL 2mM *p*-nitroanisole were added to the microplate cells, and the mixture was incubated at 30 °C for 5 minutes. Reaction was initiated by the addition of 10 µL 9.6 mM NADPH to the microplate cells. P450 enzyme activity was measured at 405 nm and 30 °C for 15 minutes via a Versamax kinetic microplate reader (Molecular Devices).

Photometric acetylcholinesterase assay

The method of Stumpf et al. (2001) was adapted for the acetylcholinesterase enzyme assay. Fifty females were homogenized via plastic pestle in a 0.1 M phosphate buffer (pH 7.5) including 500 µl 0.1% Trion X-100. After the tissues were waited in ice for 20 minutes, the homogenate was centrifuged at 10,000 g and 4 °C for 5 minutes. 100 µl acetylcholine iodide (ATChI), 100 µl 5.5-dithiobis (2-nitrobenzoic

acid) (DTNB) and 100 µl supernatant were added to the microplate cells. ATChI and DTNB were at a volume of 0.5 mM in the final concentration of 300 µL. Absorbance change was measured at 412 nm and 23 °C for 20 minutes via a Versamax kinetic microplate reader (Molecular Devices).

Data analysis

Bioassay data, including LC_{50} values and their 95% confidence limits, were calculated from probit regressions using the POLO-PC computer program (LeOra Software, Berkeley, CA). LC_{50} values of specific acaracides against *N. californicus* were considered significantly different (P <0.05) if their 95% confidential limits did not overlap. All enzyme activities wereconducted with at least 4 repetitions. The absence of homogenate in the well served as a control. All the enzyme activities were analyzed using Softmax PRO software, and the results are given in mOD min^{-1} mg^{-1}. The method of "total protein determination" of Bradford (1976) was used to determine the total protein contents of the samples, and Bovine Serum Albumin (BSA) was used as the standard. Data was analyzed via one-way analysis of variance (ANOVA), and the differences between the populations were determined via Tukey test (Winer et al., 1991).

Results

Selection for resistance

The laboratory population was used in a selection study for hexythiazox resistance under laboratory conditions. *Neoseiulus californicus* was exposed to 14 selection cycles with hexythiazox in order to determine the development of hexythiazox resistance. The resistance ratio to hexythiazox in the selected population (HEX14) increased from 0.00 to 64.04 (Table 1) .

Table 1. Resistance ratio and LC levels determined after selection with hexythiazox from the *Neoseiulus californicus* populations

Population	n[a]	Slope+SE (0.95% CI[b])	LC_{50} (mg a.i l^{-1}) (0.95% CI[b])	LC_{60} (mg a.i l^{-1}) (0.95% CI[b])	RR[c]
Laboratory population	604	1.601±0.135	1.64 1.31-2.01	2.23 1.93-2.88	-
Select-1	604	1.403±0.123	6.10 4.85-7.60	9.25 7.43-11.68	**3.71**
Select-2	600	1.319±0.122	7.66 5.92-9.73	11.93 9.40-15.31	**4.65**
Select-3	601	1.139±0.106	10.87 7.80-15.00	18.14 13.21-26.35	**6.60**
Select-4	600	1.085±0.110	15.57 9.43-24.76	26.67 16.97-46.20	**9.46**
Select-5	600	1.161±0.115	20.89 11.49-36.08	34.54 20.39-66.64	**12.70**
Select-6	600	1.073±0.104	22.91 12.35-42.86	39.45 22.57-88.65	**13.92**
Select-7	600	1.164±0.119	27.76 17.70-41.50	45.83 30.65-71.84	**16.87**
Select-8	600	1.343±0.130	31.54 20.85-45.07	48.70 33.80-71.15	**19.17**
Select-9	600	1.330±0.139	39.33 20.77-63.93	60.99 35.96-102.56	**23.90**
Select-10	600	1.284±0.124	41.08 27.50-58.40	62.22 45.29-94.28	**24.97**
Select-11	600	1.284±0.117	41.14 27.60-58.82	64.81 45.26-95.89	**25.01**
Select-12	600	1.334±0.128	64.01 38.78-98.34	99.11 63.94-158.88	**38.91**
Select-13	600	1.339±0.121	72.88 45.06-112.43	112.66 73.25-184.04	**44.30**
HEX14	600	1.413±0.141	105.04 59.24-167.26	156.41 112.28-266.06	**64.04**

[a] Total number of mites used
[b] Confidence Limit
[c] Resistance ratio = LC_{50} value of resistance population/LC_{50} value of the laboratory population.

Cross resistance

The toxicity of six different acaricides was tested on the laboratory and HEX14 populations of *N. californicus* using a spray tower via the leaf disk method. Cross resistances to spirodiclofen, etoxazole, spiromesifen, propargite, clofentezine and milbectin were observed in the HEX14 population. Compared to the HEX14 population, the laboratory population exhibited moderate levels of cross-resistance to spirodiclofen (8.12-fold), etoxazole (14.41-fold), spiromesifen (17.96-fold), propargite (17.48-fold), clofentezine (12.67-fold) and milbectin (11.22-fold), respectively (Table 2).

Table 2. Cross resistances spectrum of *Neoseiulus californicus* HEX14 population against to six acaricides

Acaricides	Population	n[a]	Slope ±SE	LC_{50} (mg a.i l^{-1}) (95% CL)	R[b]
Spirodiclofen	HEX14	600	1.288±0.111	44.47 35.54-55.03	8.12
	Laboratory population	600	1.572±0.133	5.47 4.36-6.69	-
Etoxazole	HEX14	602	1.316±0.117	19.18 13.62-26.48	14.41
	Laboratory population	600	1.739±0.141	1.33 1.08-1.59	-
Spiromesifen	HEX14	601	1.371±0.123	121.60 94.8-152.9	17.96
	Laboratory population	604	1.603±0.133	6.76 4.75-9.14	-
Propargite	HEX14	602	1.408±0.138	381.72 251.8-568.6	17.48
	Laboratory population	600	1.517±0.122	21.83 15.33-29.98	-
Clofentezine	HEX14	600	1.657±0.149	103.30 67.00-147.35	12.67
	Laboratory population	602	1.671±0.135	8.15 5.90-10.90	-
Milbemectin	HEX14	602	1.525±0.160	8.01 6.01-10.26	11.22
	Laboratory population	600	1.798±0.144	0.71 0.54-0.90	-

[a] Total number of predatory mites used
[b] Resistance ratio = LC_{50} value of resistance population / LC_{50} value of the laboratory population.

Synergistic effects

Synergistic effects of PBO, IBP and DEM synergists on hexythiazox were used to understand the hexythiazox resistance mechanism. The synergistic effects of PBO, IBP and DEM with hexythiazox against the HEX14 and laboratory populations are shown in Table 3. The synergistic effects of PBO, IBP and DEM synergists were 1.71, 3.25 and 1.98 -fold in the HEX14 population,compared with 2.15, 2.30 and 1.94 -fold in the laboratory population.

Mode of inheritance of hexythiazox resistance

In all cases, reciprocal crosses between laboratory and HEX14 populations were successful and yielded female progeny. Results of the crosses are given in Table 4. Reciprocal crossings were made between the HEX14 and laboratory populations to determine the inheritance of hexythiazox resistance and back-crossing. Degree of dominance levels (0.80 and 0.51 -fold) of the F1 individuals obtained from the HEX14♀xS♂ and S♀xHEX14♂ crossings between the HEX14 and laboratory populations were found to incompletely fairly dominant. Chi square values for F2 individuals obtained via RS♀XR♂ crossing showed that hexythiazox resistance was monogenic (Table 4). Figure 1 shows the expected (on the basis of monogenic control) and observed concentration-mortality lines for backcross F2 females. The response of F2 females from the reciprocal crosses suggests that the resistance to hexythiazox was under monogenic control.

Table 3. Toxicity of hexythiazox with and without synergist to laboratory and HEX14 populations of *Neoseiulus californicus*

Population	Treatment	n[a]	Slope ±SE	LC_{50} (mg a.i l^{-1}) (95% CL)	SR[b]
HEX14	Hexythiazox only	600	1.297±0.128	73.80 39.10-125.00	-
	Hexythiazox+PBO	600	1.513±0.126	42.96 34.68-52.63	1.71
	Hexythiazox+IBP	600	1.865±0.159	22.70 18.25-27.58	3.25
	Hexythiazox+DEM	600	1.611±0.157	37.17 24.56-51.97	1.98
Laboratory population	Hexythiazox only	604	1.601±0.135	1.64 1.31-2.01	-
	Hexythiazox+PBO	600	1.380±0.189	0.76 0.46-1.03	2.15
	Hexythiazox+IBP	600	1.495±0.205	0.71 0.24-1.22	2.30
	Hexythiazox+DEM	600	1.558±0.252	0.84 0.54-1.12	1.94

[a] Total number of predatory mites used
[b] Synergistic ratio:LC_{50} for hexythiazox alone/LC_{50} for hexythiazox with synergist.

Table 4. LC_{50} values resistance ratios and degree of dominance for hexythiazox tested in adult females from laboratory and hexythiazox-resistant (HEX14) populations and from progeny of reciprocal crosses between the populations

Genotype	n[a]	x^2(df)	Slope ±SE	LC_{50} (mg a.i l^{-1}) (95% CL)	RR[b]	D[c]
S	604	3.91 (5)	1.601±0.135	1.64 1.31-2.00	-	
R	600	10.14(5)	1.413±0.141	105.04 59.24-167.26	64.04	
R♀XS♂	602	8.98(5)	1.357±0.121	69.47 55.12-87.05	42.35	0.80
S♀XR♂	603	6.12(5)	1.834±0.153	37.94 30.72-45.89	23.13	0.51

[a] Total number of predatory mites used
[b] Resistance ratio, [c]Degree of dominance.

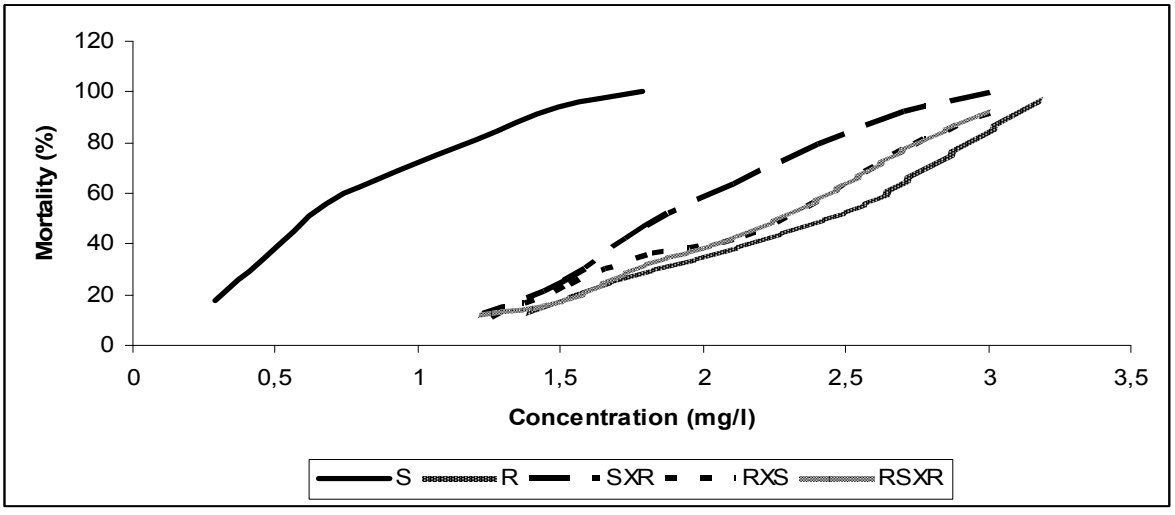

Figure 1. Concentration-mortality linear curves for hexythiazox in HEX14, laboratory population, the reciprocal crosses, the (laboratory population X HEX14) F1♀XHEX14♂ backcross and the theoretical backcross on the basis of monogenic inheritance.

Detoxifying enzyme activity

In order to evaluate the role of metabolic detoxification mechanisms in hexythiazox resistance, activities of esterases, glutathione S-transferases, cytochrome P450 monooxygenases, and acetylcholinesterase were measured in both strains (Table 5). All enzyme activities increased in the hexythiazox-resistant HEX14 population compared to the laboratory population ($P<0.05$). Electrophoretic examination showed that the esterase enzymes of *N. californicus* individuals consisted of a single band. The esterase enzymes band consisted of a much thicker band in the HEX14 individuals than in the laboratory population (Fig. 2). Moreover, the densities of the esterase enzyme bands of the HEX14 population were higher than that of the laboratory population in electrophoresis gels (Table 6).

Table 5. Esterase, GST, P450 and AChE activities in laboratory and HEX14 of *Neoseiulus californicus* populations ($P < 0.05$)[a]

Population	Esterase		GST		P450		AChE	
	mOD min^{-1} mg^{-1} proteins±SE	Ratio[b]	mOD min^{-1} mg^{-1} proteins±SE	Ratio[b]	mOD min^{-1} mg^{-1} proteins±SE	Ratio[b]	mOD min^{-1} mg^{-1} proteins±SE	Ratio[b]
Laboratory population	7,62±0.161 B	-	2,25±0.017 B	-	0,0075± 0.03B	-	0,0151±0.001 B	-
HEX14	24,54±0.410 A	3.22	5,29±0.021 A	2.35	0,0152± 0.05A	2.02	0,0505±0.007 A	3.34

[a] Means with different letters in column for each enzyme are significantly different ($P < 0.05$)
[b] enzyme activity HEX14/ enzyme activity laboratory population.

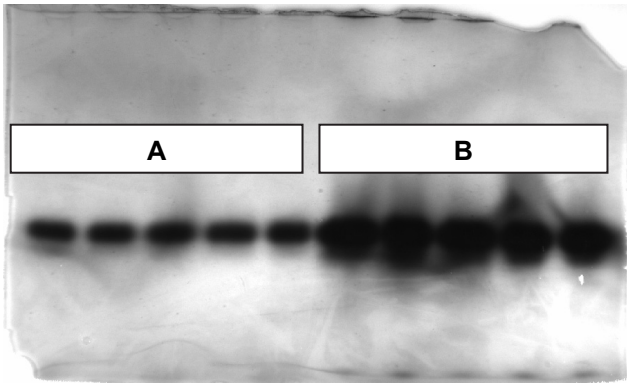

Figure 2. Esterase zones in the different strains of the *Neoseiulus californicus* individuals (A, esterase zones of the laboratory population and B, esterase zones of the HEX14 population).

Table 6. Density of esterase bands from non-denaturing gel in densitometer ($P< 0.05$)[a]

Population	Total density±SE	Ratio[b]
Laboratory population	3410.01±1.191 B	-
HEX14	6449.31±1.99 A	1.89

[a] Means with different letters in column for each enzyme are significantly different ($P < 0.05$)
[b] enzyme activity HEX14/ enzyme activity laboratory population.

Discussion

The present study examined the development of hexythiazox resistance and resistance mechanism in a predatory mite, *N. californicus,* selected under laboratory conditions. Study of the "resistance mechanism" include bioassays, biochemical assay, synergist assay.

The resistance of the laboratory population increased in parallel to the number of selections, with the resistance ratio increasing to 64.04 -fold in the HEX14 population. This result indicated that the predatory mite *N. californicus* has the capability of developing high resistance to hexythiazox under laboratory conditions. Croft et al. (1976) produced an *Amblyseius fallacis* (Garman) (Acari: Phytoseiidae) population that was more than 75 -fold more resistant to azinphos-methyl and 120 -fold more resistant to diazinon following selection with azinphos-methyl and diazinon. Reissig and Hull (1991) reported *Panonychus ulmi* Koch (Acari: Tetranychidae) field and laboratory populations were hexythiazox resistant on apples. Sato et al., (2000) reported that the resistance of *A. womersleyi* increased 311 -fold following four selections with methidathion under laboratory conditions. Campos and Omoto (2002) reported a *Brevipalpus phoenicis* (Geijskes) (Acari: Tenuipalpidae) field population that was 10.000 -fold resistant to hexythiazox. Auger et al. (2005) reported that the resistance of *T. pyri* increased 73 -fold following 10 selections with mancozeb. Sayyed et al. (2010) produced a *C. carnae* population that was 896 -fold resistant to deltamethrin. Similar to the results of the present study, these studies also indicated that natural enemies could develop resistance under laboratory conditions.

In the HEX14 population, moderate levels of cross resistances to spirodiclofen, etoxazole, spiromesifen, propargite, clofentezine and milbectin were 8.12, 14.41, 17.96, 17.48, 12.67 and 11.22 -fold greater than the initial population, respectively. Anber & Overmeer (1988) found that resistance increased 781 -fold for propoxur, 311 -fold for paraoxon, 61 -fold for tetrachlorvinphos, 21 -fold for omethoate and 19 -fold for azinphos-methyl in a field population of *Amblyseius potentillae* (Garman) (Acari:Phytoseiidae). Herron et al. (1993) found cross resistance to hexythiazox >2500-fold in clofentezine resistant *T. urticae*. Sato et al. (2000) reported cross resistances to acephate and malathion of20.4 and 13.1 -fold in *A. womersleyi* with 311 -fold resistance to methidathion. Pree et al. (2002) observed cross resistance to hexythiazox in 2000-fold clofentezine resistant *P. ulmi*. Bonofos et al. (2008) found that sensitivity to deltamethrin and chlorpyrifos decreased in a *Typhlodromus pyri* population under laboratory conditions. Cross-resistance to spiromesifen and spirotetramat in a *T. urticae* strain 680–fold resistant to spirodiclofen) was shown (Demaeght et al., 2013). The development of cross resistance in pest mites is a highly undesirable situation because cross resistance to many pesticides of the same group or of different groups makes integrated pest management more difficult, especially under field conditions. However, cross resistance among natural enemies provides an important advantage in that they survive in fields and become resistant to the chemicals used.

Following reciprocal crossings between the laboratory and selected HEX14 populations, hexythiazox resistance was found to incompletely dominant and monogenic. Chi-square (x^2) goodness-of-fit analysis showed observed mortalities of the F2 females for hexythiazox of x^2: 5.72, df: 5. These results suggest that the resistance to hexythiazox is under monogenic control. The pronounced plateau at about 50 % mortality is a response to the backcross and emphasizes that a single factor confers a high level of resistance. Croft et al. (1976) suggested that resistance was dominant in a 75--fold azinphos-methyl- and 120--fold diazinon-resistant *A. fallacis* population, and could be controlled with only one gene. Herron and Rophail (1993) reported that hexythiazox resistance in *T. urticae* was controlled by one gene. Kostiainen & Hoy (1995) found that the azinphos-methyl resistance was fully dominant in azinphos-methyl-resistant and susceptible populations of *Amblyseius finlandicus* Oud. (Acari:Phytoseiidae). Auger et al. (2005) found that the mancozeb resistance among 73-fold mancozeb resistant and susceptible *T. pyri* populations was not dominant following crossing. Sayyed et al. (2010) reported that resistance among 896-fold deltamethrin-resistant *C. carnae* was dominant, and that the deltamethrin resistance was controlled by more than one gene. Liu et al. (2011) identified 105 and 194 genes related to growth and reproduction, respectively, based on the mode of action of hexythiazox. In phytophagous mites, the management of lack of dominance and monogenic resistance under field conditions is easier than management of polygenic and dominant resistance. For the predator mite *N. californicus*, this situation is undesirable because monogenic spiromesifen resistance with lack of dominance is easily overcome under field conditions.

In the current study, none of the synergists caused hexythiazox resistance to drop to full susceptibility. These results suggest either the existence of an additional resistance mechanism for

hexythiazox, namely the inability of the synergists to fully suppress the enzymatic detoxification mechanisms, or that the amount of synergist may have been too low to fully block detoxification. This lack of synergism, together with the clear monogenic inheritance of hexythiazox resistance, could well point to a target-site based resistance mechanism. A detoxification mechanism that is the same in sensitive and resistant strains can have a much greater impact on degradation if an altered site of action in the resistant strain retards the intoxication The effect of a synergist that blocks the detoxification will then be much larger, which could lead to the false conclusion that the detoxification is the monogenic resistance mechanism (Oppenoorth, 1984; Van Pottelberge et al., 2009).

PBO, IBP and DEM synergists increased the efficiency of hexythiazox in the HEX14 and laboratory populations of *N. californicus* in the present study. These results suggest that the esterase enzyme can be considered important in the development of hexythiazox resistance in the HEX14 population. In addition GST, P450 and AChE enzymes can be effective in the development of hexythiazox resistance. The esterase, P450, GST and AChE enzymes increased by 3.22, 2.02, 2.35 and 3.34 –fold, respectively, in the HEX14 population when compared to the laboratory population. All of the enzyme activities in the HEX14 population were significantly different from the activities in the laboratory population ($P<0.05$). Moreover, electrophoresis bands were denser in the HEX14 population than in the laboratory population. Synergistic effect rates of 3.25 -fold, 1.71 -fold and 1.98 -fold were observed following hexythiazox+IBP, hexythiazox+PBO and hexythiazox+DEM implementations, respectively. Fournier et al. (1987) suggested that the GST enzyme was effective in the development of methidathion resistance in methidathion-resistant *Phytoseiulus persimilis* (Acari: Phytoseiidae). Anber & Overmeer (1988) reported 0.71 -fold and 0.35 -fold increases in the acetylcholinesterase enzyme level in two different populations of *A. potentillae*. Sato et al. (2001) found that the monooxygenase inhibitors piperonyl butoxide and 2-propynyl 2,3,6-trichlorophenyl in *A. womersleyi* had a highly synergistic effect on the methidathion-resistant population. Rauch & Nauen (2003) reported that *T. urticae* populations were 13-fold resistant to spirodiclofen and 1.2 -fold to cerboxylesterase, 1.2-fold to GST and 2.1-fold to P450 in the same (resistant) populations. Sato et al. (2006) reported that monooxygenase activity increased 3.60-fold in a 177--fold methidathion-resistant *A. womersleyi* population compared to the susceptible population. Booth et al. (2007) examined the effects of lambda-cyhalothrin and dimethoate on *Rhopalosiphum padi* (L.) (Hemiptera: Aphidoidea) and the predator *Micromus tasmaniae* Walker (Neuroptera: Hemerobiidae). They found that the cholinesterase enzyme was effective on dimethoate, while the GST enzyme had no effects on lambda-cyhalothrin and dimethoate. Sato et al. (2007) examined the cytochrome P450 gene in methidathion resistant *A. womersleyi*, and found a significant correlation between the CYP4-d gene and monooxygenase activity. Kumral et al. (2011) determined similar carboxylesterase enzyme levels and AChE sensitivity in *P. ulmi* and its predator *Stethorus gilvifrons* (Mulsant) (Coleoptera: Coccinellidae) in terms of parathion-methyl resistance. The synergism studies of Kramer & Nauen (2011) with PBO suggest a possible role of cytochrome-P450-dependent monooxygenases in spirodiclofen detoxification in 7,000.00-fold spirodiclofen resistant *P. ulmi* ().

Therefore, natural enemies that develop resistance to certain pesticides may be used in integrated pest management programs. Although resistance is not sought in harmful species in pesticide resistance management programs (a part of IPM), natural enemies are required to be resistant to pesticides (Dunley et al., 1991).

Natural enemies that develop resistance to chemicals may survive for longer periods in areas where pesticides are intensely used. Therefore, they are included in integrated pest management programs. However, resistant natural enemies should be tested under field conditions and adaptation studies should be also conducted.

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References

Anber, H.A.I. & W.P.J. Overmeer, 1988. Resistance to organophosphates and carbamates in the predacious mite *Amblyseius potentillae* (Garman) due to insensitive acetylcholinesterase. Pesticide Biochemistry and Physiology, 31(1): 91-98.

Auger, P., R. Bonafos, S. Kreiter, & R., Delorme, 2005. A genetic analysis of moncozeb resistance in *Typhlodromus pyri* (Acari:Phytoseiidae). Experimental and Applied Acarology, 37: 83-91.

Ay, R. & M.O. Gürkan, 2005. Resistance to bifenthrin and resistance mechanisms of different strains of the two-spotted spider mite (*Tetranychus urticae* Koch) from Turkey. Phytoparasitica, 33: 237-244.

Boller, E.F., H. Vogt, P.Ternes, & C. Malavolta, 2005. Working document on selectivity of pesticides (Web sayfası: http://www.iobc wprs.org/ip_ipm/03022_IOBC_pesticide database), (Erişim tarihi: Ağustos 2013).

Bonafos, R., E. Serrano P. , Auger, & S. Kreiter, 2007. Resistance to deltamethrin, lambda-cyhalothrin and chlorpyriphos-ethyl in some populations of *Typhlodromus pyri* Scheuten and *Amblyseius andersoni* (Chant) (Acari:Phytoseiidae) from vineyards in the south-west of france. Crop Protection, 26: 169–172.

Bonafos, R., V. Vigues, E.. Serrano, & P.,Auger, 2008. Resistance monitoring to deltamethrin and chlopyrifos-ethly in 13 populations of *Typhlodromus pyri* Scheuten (Acari:Phytoseiidae) from vineyards in the southwest of france. Crop Protection, 27: 855-858.

Booth, L.H., S.D. Wratten, & P. Kehrli, 2007. Effects of reduced rates of two insecticides on enzyme activity and mortality of an aphid and its lacewing predator. Journal Economic Entomology, 100(1): 11-19.

Bradford, M.M., 1976. A rapid and sensitiv method for the quantitation of microgramm quantities of protein utilizing the principle of protein – dye inding. Analytical Biochemistry, 72: 248-254.

Campos, F.J. & C. Omoto, 2002. Resistance to hexythiazox in *Brevipalpus phoenicis* (Acari: Tenuipalpidae) from Brazilian citrus. Experimental and Applied Acarology, 26: 243–251.

Castagnoli, M., M. Liguori, S. & Simoni, 1999. Effect of two different host plants on biological features of *Neoseiulus californicus* (McGregor). International Journal of Acarology, 25: 145–150.

Castagnoli, M. & S., Simoni, 1999. Effect of long-term feeding history on functional and numerical response of *Neoseiulus californicus* (Acari: Phytoseiidae). Experimental Applied Acarology, 23: 217-234.

Croft, B.A., A.W.A. Brown, & S.A. Hoying, 1976. Organophoshorus-resistance and its inheritance in the predaceous mite *Amblyseius fallacies*. Journal of Economic Entomology, 69: 64-68.

Dekeyser, M.A., 2005. Acaricide mode of action. Pest Management Science, 61: 103–110.

Demaeght, P., W. Dermauw, T. Tsakireli, J. Khajehali, R. Nauen, L. Tirry, J. Vontas, P. Lümmen, & T. Van Leuween, 2013. Molecular analysis resistance to acaricidal spirocylic tetranic acids in *Tetranychus urticae*: CYP392E10 metobolizes spirodiclofen, but not its corresponding enol. Insect Biochemical Molecular Biology, 43: 544-554.

Dunley, J.E., R.H. Messing, & B.A., Croft, 1991. Levels and genetics of organophosphate resistance in Italian and Oregon biotypes of *Amblyseius andersoni* (Acari:Phytoseeidae). Journal of Economic Entomology, 84: 750-755.

Fournier. D., A. Cuany, M. Pralavorio, J.M. Bride, & J.B. Berge, 1987. Analysis of methidathion resistance mechanisms in *Phytoseiulus persimilis* A.H. Pesticide Biochemical and Physiology, 28: 271-278.

Georghiou, G.P., 1969. Genetics of resistance to insecticides in house flies and mosquitoes. Experimental Parasitology, 26: 224–255.

Goka, K.& A. Takafuji, 1992. Enzyme variations among Japanese populations of the two-spotted spider mites, *Tetranychus urticae* Koch. Applied Entomology Zoology, 27: 141–150.

Herron, G.A. & J. Rophail, 1993. Genetics of hexythiazox resistance in two spotted spider mite, *Tetranychus urticae* Koch. Experimental Applied Acarology,17(6): 423-431.

Herron, G.A., V. Edge, & J. Rophail, 1993. Clofentezine and hexythiazox resistance in *Tetranychus urticae* Koch in Australia. Experimental Applied Acarology,17(6): 433-440.

Hoy, M.A., 1985. Recent advances in genetics and genetic improvement of Phytoseiidae. Annual Review Entomoogy, l 30: 345-370.

Kang, C.Y., G. Wu, & T., Miyata, 2006. Synergism of enzyme inhibitors and mechanisms of insecticide resistance in *Bemisia tabaci* (Gennadius) (Hom.: Aleyrodidae). Journal Applied Entomology, 130: 377-385.

Kim, Y.J., S.H. Lee, S.W. Lee, & Y.J. Ahn, 2004. Fenpyroximate resistance in *Tetranychus urticae* (Acari: Tetranychidae) cross-resistance and biochemical resistance mechanisms. Pest Management Science, 60: 1001-1006.

Kramer, T. & R. Nauen, 2011. Monitoring of spirodiclofen susceptibility in field populations of European red mites, *Panonychus ulmi* (Koch) (Acari: Tetranychidae), and the cross-resistance pattern of a laboratory-selected strain. Pest Management Science, 67: 1285–1293.

Kostiainen, T. & M.A. Hoy, 1995. Laboratory evaluation of a laboratory-selected organophosphate-resistant strain of *Amblyseius finlandicus* (Acari: Phytoseiidae) for possible use in finnish apple orchards. Biocontrol Science and Technology, 5: 297- 311.

Kumral, N.A., N.S. Gencer, H. Susurluk, C. & Yalcin, 2011. A comparative evaluation of the susceptibility to insecticides and detoxifying enzyme activities ın *Stethorus gılvıfrons* (Coleoptera: Coccinellidae) and *Panonychus ulmi* (Acarina:Tetranychidae). International Journal of Acaroogy,l 37: 255–268.

LeOra Software, 1994. Polo-pc: a user' s guide to probit or logit analysis leora software 28 p Berkeley.

Liu, B., G. Jiang, Y. Zhang J., Li, X. Li, J. Yue, F. Chen, H. Liu, Li, H. S. Zhu, J. Wang, & C. Ran, 2011. Analysis of transcriptome differences between resistant and susceptible strains of the citrus red mite *Panonychus citri* (Acari: Tetranychidae). Plos One, 6(12): doi: e28516. doi:10.1371.

Mugo, H.M., E.M. El-Banhawy, L.W. Irungu, P.N. Ndegwa, & D.N., Mburu, 2011. Resistance of predacious mite, *Euseıus kenyae* (Acari: Phytoseiidae) to chlorpyrifos (Dursban) ın kenyan coffee farms. Jagst Vol 13: 53-64.

Oppenoorth F J, (1984) Biochemistry of insecticide resistance. Pesticide Biochemical and Physiology, 22: 187–193.

Pree, D.J., L.A. Bıttner, & K.J. Whıtty, 2002. Characterization of resistance to clofentezine in populations of european red mite from orchards in Ontario. Experimental and Applied Acarology, 27: 181-193.

Rauch, N. & R. Nauen, 2003. Spirodiclofen resistance risk assessment in *Tetranychus urticae* (Acari: Tetranychidae): a biochemical approach. Pesticide Biochemical and Physiology, 74: 91-101.

Reissig, W.H. & L.A. Hull, 1991. Hexythiazox resistance in a field population of european red mite (Acari: Tetranychidae) on apples. Journal of Economic Entomology, 84(3): 727-735.

Rose, R.L., R. Barbhaiya, G. Rock, & E. Hodgson, 1995. Cytochrome P-450-associated insecticide resistance and the development of biochemical diagnostic assays in *Heliothis virescens*. Pesticide Biochemical Physiology 51: 178–191.

Sanatgar, E., R.V., Shoushtari, A.A., Zamani, M. Arbabi, & E.S. Nejadian, 2011. Effect of frequent application of hexythiazox on predatory mite *Phytoseiulus persimilis* Athias - Henriot (Acari: Phytoseiidae). Acade Journal of Entomology, 4: 94-101.

Sato, E.M., T Miyata,. A. Kawai, & O., Nakano, 2000. Selection for resistance and susceptibility to methidathion and cross resistance in *Amblyseius wormersleyi* Schicha (Acari: Phytoseiidae). Applied Entomology Zoology, 35: 393-399.

Sato, E.M., T. Miyata, A. Kawai, & O., Nakano, 2001. Methidathion resistance mechanisms in *Amblyseius womersleyi* Schicha (Acari: Phytoseiidae). Pesticide Biochemical and Physiology, 69: 1–12.

Sato, E.M., T. Tanaka, & T. Miyata, 2006. Monooxygenase activity in methidathion resistant and susceptible populations of *Amblyseius wormersleyi* Schicha (Acari: Phytoseiidae). Experimental Applied Acarology, 39: 13-24.

Sato, E.M., T. Tanaka, & T.. Miyata, 2007. A cytochrome P450 gene involved in methidathion resistance in *Amblyseius wormersleyi* Schicha (Acari: Phytoseiidae). Pesticide Biochemical and Physiology, 88: 337-345.

Sayyed, A.H., A.K. Pahtan, & U. Faheem, 2010. Cross-resistance, genetics and stability of resistance to deltamethrin in a population of *Chrysoperla carnea* from Multan, Pakistan. Pesticide Biochemical and Physiology, 98: 325–332.

Stone, B.F., 1968. A formula determining degree of dominance in cases of monofactorial inheritance of resistance to chemeicals. Bull World Health Organis 38: 325-326.

Stumpf, N. & R. Nauen, 2002. Biochemical markers linked to abamaectin resistance in *Tetranychus urticae* (Acari: Tetranychidae). Pesticide Biochemical and Physiology, 72: 111-121.

Stumpf, N., P.W. Zebitz, W. Kraus, G.D. Moores, & R.. Nauen, 2001. Resistance to organophosphates and biochemical genotyping of acetylcholinesterases in *Tetranychus urticae* (Acari: Tetranychidae). Pesticide Biochemical and Physiology, 69: 131-142.

Tirello, P., A. Pozzebon, & C. Duso, 2012. Resistance to chlorpyriphos in the predatory mite *Kampimodromus aberrans*. Experimental Applied Acarology, 56: 1–8.

Van Leeuwen, T., V., Stıllatus, & L. Tırry, 2004. Genetic analysis and cross-resistance spectrum of a laboratory-selected chlorfenapyr resistant strain of two-spotted spider mite (Acari: Tetranychidae). Experimental and Applied Acarology, 32: 249–261.

Winer, B.J., Brown, D.R., Michels, K.M., 1991. Statistical Principles in Experimental Design., Universty of New York, New York 552 pp.

Van Pottelberge, S., T. Van Leeuwen, R., Nauen, & L. Tirry, 2009. Resistance mechanisms to mitochondrial electron transport inhibitors in a field-collected strain of Tetranychus urticae Koch (Acari: Tetranychidae). Bulletin of Entomology Research, 99: 23–31.

Yamamoto, A., H. Yoneda, R., Hatano, & M. Asada, 1996. Realized heritability estimates of hexythiazox resistance in the citrus red mite *Panonychus citri* (McGregor). Journal of Pesticide Science, 21: 43-47.

Yu,S.J., 2008. “Insecticide Resistance,65-75” In: The Toxicology and Biochemistry of Insecticide. (Eds. Yu, S.J.) CRC Presd 230 pp.