RESULTS AND DISCUSSION

IV. RESULTS AND DISCUSSION

1-Rate of resistance.development

Studies were conducted to determine rate of resistance development in a laboratory strain of ***Phthorimaea operculella*** **(Zeller) Lepidoptera: Gelechiidae**. to three insecticides namely : fenitrothion , azadirachtin and profenofos at egg stage and Dipel 2x (***Bacillus thuringiensis*** . *subsp* ***kurastaki***) B.t. at ***1st*** instar larvae .

The susceptibility of larvae to tested insecticides was assessed every generation during the period of selection to determine the effect of selection on the resulting generations. The results are given in Table (1) , (2) , (3) and (4)

1.1-Rate of resistance development to fenitrothion:

The rate resistance of development to fenitrothion in eggs of ***P. operculella*** during 20 generations of selection with fenitrothion at the LC_{50} level is shown in Table (1) . The results clearly showed that the resistance ratios increased

Table (1) : Toxicity of Fenitrothion on the eggs of ***Phthorimaea operculella*** (Zeller), during different generation of selection

Generations	Lethal concentrations and their 95 % confidence limits (g/l)			Slope ± SE	R.R*
	LC 50	LC 90	LC 95		
P	1.84 (1.41 – 2.36)	8.6 (5.69 – 18.14)	13.35 (18 – 34)	1.97 ± 0.3	----
G1	2.96 (2.24 – 4.34)	11.63 (6.99 – 30.7)	85.9 (50.4 – 112.7)	2.16 ± 0.37	1.6
G2	16.13 (10.78 – 31.66)	33.4 (10.9 – 46.35)	107.4 (24.3 – 360.7)	0.97 ± 0.19	8.77
G3	26.6 (16.46 – 37.32)	78.59 (50.1 – 453.71)	106.8 (61 – 1029.6)	2.73 ± 0.6	14.48
G4	37.95 (27.5 – 39.6)	92.37 (65.5 – 561.2)	314.7 (256 – 998.5)	1.66 ± 0.2	20.6
G5	24.5 (18.76 – 33.3)	307.2 (159.66 – 964.23)	629.23 (281.3 – 2609.3)	1.17 ± 0.17	13..3
G6	-------------	-------------	-------	---	-----
G7	44.16 (31.31 – 72.3)	411.81 (31.31 – 72.26)	775.68 (303.4 – 5434.3)	1.3 ± 0.3	24
G8	44.31 (33.97 – 59.5)	273.79 (164.4 – 672.61)	458.91 (247.3 – 1390.8)	1.6 ± 0.26	24.1
G9	--------------	-------------	------------	-------	---
G10	52.8 (41.1 – 70.4)	378.9 (223.73 – 932.5)	622.54 (349.4 – 2009.7)	1.49 ± 0.22	28.7
G11	60.2 (43.57 – 87.8)	794.4 (358.3 – 3986)	16514.5 (6214 – 9439.8)	1.14 ± 0.2	32.7
G12	--------------	-------------	------------	-------	---
G13	62.2 (39.13 – 121.71)	1497 (447.1 – 43082)	3690.8 (831.3 – 24424)	0.93 ± 0.23	33.8
G14	66.8 (50.6 – 93.1)	794 (400.4 – 27613)	1602.6 (691.8 – 75142)	1.19 ± 0.18	36..3
G15	79.6 (54 – 136.9)	1105.66 (430.35 – 10252)	2331.6 (736 – 36718)	1.12 ± 0.2	43..3
G16	--------------	-------------	------------	-------	---
G17	90.5 (64.25 – 143.35)	1071.2 (470.57 – 6041.5)	2158.7 (792.68 – 18224.8)	1.19 ± 0.23	49..3
G18	--------------	-------------	------------	-------	---
G19	95.48 (66.18 – 163.5)	1588.4 (606.4 – 11255)	3525.5 (1092.3 – 44755.2)	1.05 ± 0.2	51..9
G20	119.8 (82.7 – 194.5)	2777.6 (9219 – 21912)	5848.6 (1729 – 88381)	0.97 ± 0.19	65..3

*R.R = Resistance ratio.

sharply after the first generation exposed to fenitrothion from 1.6 - fold to 8.77 – fold in G_2 then to 20.6 - fold in G_4 reflecting the action of such insecticide as a powerfull sieve for concentrating resistant mutants that were present in low frequencies (Crow, 1957) .G_5 exhibited lower resistance ratio of13.3-fold then increased sharply again at G_7 there after. Resistance ratios were slightly increased to reach 65.2-fold at the end of selection in G_{20} .

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 may suggest a very slow grouping of the genetic material which contributes to resistance (**Hoskins** and **Gordon** , 1956). Development of resistance to fenitrothion was studied by many researchers . **Georghiou** and **Calman** (1969) found that selection of ***Culex pipiens fatigans*** and ***Anopheles albianus*** by fenitrothion for 30 and 25 generations resulted in only 2.2 - fold and 1.1 - fold increase in tolerance, respectively . **Salem** (1970) studied the development of resistance to fenitrothion in

Spodoptera littoralis . A 2- fold increase inresistance was obtained after the end of selection in generation 15. Howerver , **Madi** (1976) showed that the tolerance of ***S . littoralis*** to fenitrothion increased from 1.26 to 12.9 - fold after 8 generations of selection .

The present results could be discussed in the light of the findings obtained with other organophosphate compound .**EL-Guindy *et al*** (1975 b) showed that selection of ***S . littoralis*** withchlorpyrifos for 16 generations induced only 5.2-fold increase in tolerance as compared to the susceptible strain . **EL-Guindy *et al*** (1975c) ndicated that selection of ***S . littoralis*** for 13 generations with *Cyolane* had resulted in only 5-fold increase in tolerance . Te same authors (1975 a) had showed however that selection of same pest with phosvel had resulted in 118 - fold increase in resistance. **Guriguis *et al*** (1985) found that eggs of a chlorpyrifos selected strain of ***S . littoralis*** showed 6.7 - fold increase in resistance to chlorpyrifos after 10 generations of selection . **Khalid** (1997) found that the field strain of ***S . littoralis*** when exposed to chlorpyrifos about 19 generations had reached 6.6 - fold increase in resistance .

1.2-Rate of resistance development to profenofos:

The rate of resistance development to profenofos during 15 generations of selection is shown in Table (2). As indicated from values of the resistance ratio (R.R), resistance gradually increased starting from G_1 (3.06 - fold) to reach 25.1 - fold in G_7 then remained nearly constant until generation G_{10} . Starting from generation 11 , resistance ratio increased to reach 54.99 - fold in G_{15}.

The Slope values of the mortality regression lines decreased along the course of selection indicating considerable degree of heterogeneity . The regression lines were characterized by a gradual increase in LC_{50} values and a decrease in slopes . The response 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 of individuals possessing the resistance mechanism is increasing , although there are still many susceptible individuals present (**Hoskins** and **Gordon,** 1956). Selections of ***S . littoralis*** larvae by profenofos revealed 16.9-fold resistance after 19 generations of selection (**Rofael** , 1990) .

Table (2) : Toicity of profenofos on the eggs of ***Phthorimaea operculella*** (Zeller), during different generatins of selection.

Generations	Lethal concentrations and their 95 % confidence limits (g/l)			Slope ± SE	R.R*
	LC 50	LC 90	LC 95		
P	0.77 (0.52 – 1.01)	3.39 (2.43 – 6.07)	5.17 (3.4 – 11.17)	1.99 ± 0.34	
G1	3.37 (1.91 – 2.91)	13.64 (9.66 – 22.59)	22.39 (14.69 – 42.04)	1.69 ± 0.19	3.06
G2	7.24 (5.6 – 10.05)	38.3 (22.95 – 93.05)	61.47 (33.41 – 179.17)	1.77 ± 0.2	9.4
G3	----------	----------	----------	----------	--
G4	6.51 (3.44 – 10.01)	30.43 (17.35 – 80.19)	45.35 (16.4 – 131.34)	1.22 ± 0.18	8.45
G5	8.85 (6.86 – 11.98)	54.4 (32.44 – 133.02)	91.13 (48.87 – 271.43)	1.62 ± 0.24	11.4
G6	11.2 (8.4 – 15.83)	110 (57.28 – 364.45)	210.14 (94.77 – 924.89)	1.29 ± 0.21	14.5
G7	19.4 (13.86 – 31.5)	236.8 (102.6 – 1277.46)	312.2 (134.14 – 1551.8)	1.17 ± 0.2	25.1
G8	---------------	--------------	-------------	-------	---
G9	19.34 (14.9 – 26.12)	159.87 (89.93 – 440.2)	291 (144.23 – 1017.66)	1.14 ± 0.2	25
G10	19.72 (13.2 – 33.3)	295.9 (114.9 – 271.63)	637.87 (199.96 – 10049.2)	1.08 ± 0.23	25..61
G11	21.7 (15.61 – 31.82)	236.45 (115.6 – 966.64)	465.4 (193.43 – 159.26)	1.24 ± 0.22	28.16
G12	---------------	--------------	-------------	-------	---
G13	29.09 (22.19 – 39.36)	277.4 (152.87 – 788.99)	525.92 (253.4 – 1922.75)	1.3 ± 0.19	37.6
G14	40.7 (28.97 – 64.05)	581.12 (247.59 – 3335.6)	1234.79 (435.6 – 10687.5)	1.1 ± 0.23	52.7
G15	42.51 (32.5 – 58.43)	412.32 (223.04 – 1196.36)	785.4 (371.7 – 2917.5)	1.291 ± 0.22	54..99

*R.R = Resistance ratio.

1.3 – Rate of resistance development to (azadirachtin) :

The Rate of development of resistance to the (azadirachtin) in ***P. operculella*** during 20 generations of selections is given in Table (3) .

As indicated in Table (3) , the resistance ratios remained relatively constant through the first 6 generations of selections . A slight increase was then observed till the end of selection in G_{20} (8.9 - fold). This low level of tolerance was considered as a manifestation of vigor tolerance .

The slope values of the mortality regression lines were shallow and decreased till G_6 then became steady from G_{11} to G_{20}

This pattern of changes in slopes is a typical response of mortality regression lines undergoing true resistance .

Table (3) : Toxicity of azadircatin on the eggs of ***Phthorimaea operculell*** (Zeller), during different generations of selection.

Generations	Lethal concentrations and their 95 % confidence limits (g/l)			Slope ± SE	R.R *
	LC 50	LC 90	LC 95		
P	0.19 (0.16 – 0.24)	0.48 (0.54 – 0.85)	0.75 (0.49– 1.51)	2.14 ± 0.5	----
G1	0.18 (0.16 – 0.23)	0.67 (0.51 – 0.98)	0.96 (0.69 – 1.52)	2.3 ± 0.22	0.97
G2	0.29 (0.23 – 0.4)	2.65 (1.45 – 7.3)	4.96 (2.39 – 17.02)	1.39 ± 0.18	1.6
G3	0.31 (0.25 – 0.41)	2.88 (1.65 – 7.09)	5.4 (2.76 – 16.38)	1.3 ± 0.17	1.7
G4	---------------	--------------	-------------	-------	---
G5	0.34 (0.27 – 0.43)	2.42 (1.56 – 4.88)	4.25 (2.46 – 10.16)	1.5 ± 0.18	1.8
G6	0.28 (0.19 – 0.36)	2.23 (0.19 – 3.41)	3.98 (2.23 – 11.45)	1.82 ± 0.18	1.5
G7	0.65 (0.45 – 1.15)	17.76 (5.77 – 21.93)	45.36 (11.42 – 101.42)	0.89 ± 0.19	3.4
G8	---------------	--------------	-------------	-------	---
G9	0.62 (0.46 – 0.91)	4.42 (2.34 – 14.17)	7.7 (3.65 – 31.94)	1.51 ± 0.25	3.2
G10	0.69 (0.48 – 1.11)	9.33 (3.92 – 62.61)	19.47 (6.74 – 207.16)	1.12 ± 0.23	3.6
G11	0.75 (0.54 – 1.13)	8.52 (3.99 – 39.24)	16.97 (6.72 – 112.7)	1.12 ± 0.22	3.9
G12	0.81 (0.5 – 1.51)	31.09 (8.32 – 118.98)	87.56 (16.94 – 859.22)	0.81 ± 0.19	4.2
G13	---------------	--------------	-------------	-------	---
G14	0.59 (0.42 – 0.83)	5.17 (2.79 – 8.25)	9.58 (4.49 – 42.77)	1.35 ± 0.28	3.1
G15	1.02 (0.73 – 1.48)	11.6 (5.74 – 45.66)	23.21 (9.73 – 128.01)	1.27 ± 0.28	5.3
G16	1.19 (0.87 – 1.69)	13.11 (6.69 – 45.3)	25.89 (11.36 – 121.07)	1.23 ± 0.19	6.2
G17	---------------	--------------	-------------	-------	---
G18	1.39 (1.01 – 1.94)	14.59 (7.66 – 47.4)	28.07 (12.73 – 121.99)	1.25 ± 0.19	7.3
G19	1.56 (1.11 – 2.23)	19.15 (9.35 – 77.59)	34.57 (14.81 – 180.91)	1.25 ± 0.19	8.2
G20	1.7 (1.23 – 2.39)	19.35 (10.01 – 66.48)	38.48 (17.04 – 181.74)	1.2 ± 0.2	8.9

*R.R = Resistance ratio.

1.4 – Rate of resistance development to Dipel 2x (*B.t*):

The rate of resistance development to a microbial insecticide derived from ***Bacillus thuringiensis*** (Dipel 2x) in the*1st* instar larvae **of** ***Phthorimaea operculella*** during 15 generations of selection is given in Table (4) . The results clearly showed that the resistance ratios increased very slowly during the first ten generations (from 1.6 - fold in G_1 to 5.3 - fold in G_{10}) . RR values the elevation of was higherstarting from G_{11} (6.7 - fold) to reach 11.9 – fold at the end of the selection course in G_{15}.

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

The regression line of the response of ***P. operculella*** larvae in the selections 1-10 were characterized by slight increases in LC_{50} values and had nearly similar but low slope values . Results of this investigation provide evidence that the mechanism of ***Bacillus thuringiensis*** resistance is an inheritable one **.**

Table (4) : Toxicity of (***Bacillus thuringiensis***) Subsp ***kurastki*** on the 1*st* instar larvaeof ***Phthorimaea operculella*** (Zeller),during different generations of selection .

Generations	Lethal concentrations and their 95 % confidence limits (IU)			Slope ± SE	R.R*
	LC 50	LC 90	LC 95		
P	1.5×10^6 (0.8×10^6 - 2.2×10^6)	20.3×10^6 (12.04×10^6 - 54.9×10^6)	42×10^6 (2.12×10^6 - 166.2×10^6)	1.14 ± 0.2	----
G1	1.8×10^6 (0.9×10^6 - 2.9×10^6)	22.3×10^6 (12.5×10^6 - 63×10^6)	43×10^6 (3.55×10^6 - 123×10^6)	1.3 ± 0.2	1.2
G2	2.1×10^6 (1.6×10^6 - 3.3×10^6)	25.7×10^6 (15.2×10^6 - 51.3×10^6)	45.8×10^6 (23.9×10^6 - 113×10^6)	1.53 ± 0.29	1.4
G3	3.8×10^6 (2.9×10^6 - 4.9×10^6)	28.7×10^6 (18.4×10^6 - 60×10^6)	50×10^6 (28.9×10^6 - 127.9×10^6)	1.49 ± 0.19	2.53
G4	5.7×10^6 (4.9×10^6 - 7.7×10^6)	72.5×10^6 (38.4×10^6 - 215.6×10^6)	142×10^6 (66×10^6 - 572×10^6)	1.16 ± 0.18	3.8
G5	----------------------------	----------------------------	----------------------------	-------	---
G6	6.2×10^6 (4.2×10^6 - 7.7×10^6)	120×10^6 (55×10^6 - 573×10^6)	278×10^6 (104×10^6 - 2093×10^6)	0.99 ± 0.18	4.13
G7	6.1×10^6 (4.7×10^6 - 7.8×10^6)	41.8×10^6 (28.6×10^6 - 75.5×10^6)	71.8×10^6 (44.8×10^6 - 153×10^6)	1.58 ± 0.19	4
G8	6.3×10^6 (4.5×10^6 - 8.2×10^6)	25.2×10^6 (17.6×10^6 - 44.15×10^6)	37.3×10^6 (24.53×10^6 - 76×10^6)	2.13 ± 0.3	4.08
G9	----------------------------	----------------------------	----------------------------	-------	---
G10	8.16×10^6 (5.2×10^6 - 9.3×10^6)	147.4×10^6 (68.1×10^6 - 165×10^6)	334×10^6 (124.37×10^6 - 3320×10^6)	1.02 ± 0.2	5.3
G11	10.3×10^6 (7.3×10^6 - 14.2×10^6)	139×10^6 (76.5×10^6 - 211×10^6)	293×10^6 (135.9×10^6 - 1236×10^6)	1.13 ± 0.18	6.9
G12	14.7×10^6 (10.5×10^6 - 20.3×10^6)	87.2×10^6 (53×10^6 - 215×10^6)	144×10^6 (79.3×10^6 - 751.9×10^6)	1.56 ± 0.27	9.8
G13	----------------------------	----------------------------	----------------------------	-------	---
G14	11.5×10^6 (8.3×10^6 - 14.3×10^6)	119×10^6 (71×10^6 - 292×10^6)	232×10^6 (121×10^6 - 736×10^6)	1.25 ± 0.18	7.7
G15	18.5×10^6 (13.2×10^6 - 25.9×10^6)	109×10^6 (66.8×10^6 - 271×10^6)	181×10^6 (99×10^6 - 568×10^6)	1.65 ± 0.23	12.33

*R.R = Resistance ratio.

The stability of the slope values in the first selected generations , in spite of the chemical pressure applied , reflects the reoryamzation of genetic factors contributing to resistance ,or may suggest a very slow grouping of genetic material contributes to resistance (**Hoskins and Gordon** , 1956) .

Results of generations 10 –15 showed that the slope of the regression lines increased steadily indicating a degree of homogeneity. **Haskins** and **Gordon** (1956) mentioned that once the slope of the regression lines had decreased , then increased again, the population is approaching the limit of resistance characteristic for the material used in selection .

Carlos ***et al*** **.(1997)** stated that eighteen field populations of ***Plutella xylostella*** were tested for susceptibility to ***B.thuringiensis*** . They found that LC_{50} of ***Bacillus thuringiensis*** ranged from 4.3 to 18.3 -9.3 –to 77.2 and 13.3 to 19.5 - fold higher respectively compared with the LC_{50} of susceptible population **Patricia** *et al* . (1999) studied the development of resistance in ***Ostrinia nubilalis*** to -endotoxin of ***Bacillus thuringiensis*** . Resistance reached 162 - fold after 8 generations of selection compared with the susceptible strain .

It seams possible then that ***P. operculella*** posseses low resistance potential to azadirachtin and ***Bacillus thuringiensis*** when used as selective agents.The mechanism of resistance is an inheritable one.

The ***P. operculella*** possess high resistance potential to the organophosphorus compounds (Fenitrothion and Profenofos) where susceptible individuals are still present , so it returns to its susceptibility easily when the population be free of selection . However , with the progress of Azadirachtin selection ,many susceptible individuals are still present Thus the tolerant strain of ***P. operculella*** might rapidly lose its resistance characteristics . While, selection with ***B. thuringiensis*** caused a gradual increase in resistant individuals , there for it might difficult to host its resistance characteristics.

2-Cross - resistance to different pesticides in parent strain and four selected strains of *P. operculella* :

This part of study deals with the pattern of cross – resistance to six pesticides belonging to different chemical groups in the parent strain and four selected strains of ***P. operculella***. The parent strain in selection (P) was originally collected from potato fields of Behera Governorate then reared without insecticide treatment in a conditioned room for nine generations before selection was undertaken . The experiments were carried out by pressuring the parent strain with fenitrothion ,profenofos , (azadirachtin) and ***Bacillus thuringiensis*** for 20 , 15 , 20 and 15 successive generations , respectively.

The impact of selection on the pattern of cross – resistance was studied in generations 5 , 10 , 15 and 20 for fenitrothion and Azadirachtin selected strain . As for the profenofos and ***Bacillus thuringiensis*** selected strains , the pattern of cross - resistance was studied in generations 5 , 10 and 15 . The results obtained are shown in Tables 5 to 8 .

2.1 – Pattern of cross – resistance to pesticides in fenitrothion resistant strain of *P. operculella* :

The results of cross – resistance pattern to several tested insecticides during selection with fenitrothion for 20 generations are shown in Table (5) . As selection

Table (5) : Cross- resistance pattern to some pesticides in fenitrothion ***Phthorimaea operculella*** (Zeller) during certain generatins of selection .

hemical	Parent Strain		Fenitrothion Strain												
	LC 50 and its (95 % Fiducial limits)	Slope± S.E	LC_{50} (G_5) (95 % Fiducial limits)	Slope±S.E	R.R*	LC_{50} (G_{10})(95 % Fiducial limits)	Slope± S. E	R.R*	LC_{50} (G_{15})%(Fiducial limits)	Slope± S.E	R.R*	LC_{50} (G_{20})(95% Fiducial limits)	Slope ±S.E	R.R*
itrothion	1.8(1.4-2.4)	1.97± 0.3	24.5(18.76-33.3)	1.17±0.17	13.3	52.8(41.1-70.4)	1.49± 0.22	28.7	79.6(54-138.9)	1.12± 0.2	43.3	119.8(82.7-194.5)	0.97± 0.19	65.3
ofenofos	0.77(0.5-1)	1.99±0.34	9.8(5.5-26.3)	0.84± 0.1	12.7	4.9(2-6.4)	1.6± 0.2	6.4	7.9(5.2-14.8)	0.8± 0.23	9.2	12.3(9.3-18.3)	1.8± 0.28	15.9
dirachtin	0.19(0.16-0.24)	3.14± 0.5	0.57(0.34-1.6)	1.12± 0.26	3.2	0.6(0.47-0.98)	1.35± 0.2	3.25	0.79(0.5-1.9)	1.24± 0.2	4.2	0.96(0.6-1.8)	0.98± 0.19	5.1
neral oil	1.4(1.3-2.1)	4.3± 1.59	4.4(2.3-7.2)	1.9± 0.2	3.2	2.2(0.3-0.45)	0.69± 0.2	1.6	5.3(3.4-7.5)	1.24± 0.2	3.86	8.9(6.2-12.8)	1.28± 0.24	6.35
oric acid	0.03(0.02-0.044)	1.2± 0.28	0.24(0.008-0.04)	1.88± 0.2	0.8	0.035(0.02-0.07)	1.2± 0.5	1.16	0.04(0.023-0.7)	1.06± 0.26	1.3	0.18(0.13-0.27)	1.37± 0.2	6
ipel 2X	1.5×10^6 (0.8×10^6-2.2×10^6)	1.14± 0.2	1.3×10^6 (0.762×10^6-2.1×10^6)	1.31± 0.22	1.0	1.6×10^6 (0.86×10^6–2.4×10^6)	1.5± 0.3	1.06	3.3×10^6 (1.5×10^6-4.9×10^6)	1.26± 0.28	2.2	85×10^6 (6.6×10^6–10.7×10^6)	1.5± 0.19	5.66

Resistance Ratio (R.R) = LC_{50} of strain / LC_{50} of parent strain.

progressed to G_5 , resistance to fenitrothion reached 13.3-fold , while this for profenofos significantly increased to 11.3 - fold ,for Azadarchtin , Super misrona and Dipel 2x (***B. thuringiensis***) the resistance ratios decreased to 3 , 3.23 and 1-fold , respectively . The resistance ratio to boric acid indicated limited level of negative correlation with resistance to fenitrothion (0.8-fold).

In G_{10} , resistance to fenitrothion increased to 28.7-fold ; low resistance levels to profenofos (6.4-fold) the neem extract, azadirachtin (3.25-fold) and very low levels of resistance to Dipel 2x (***B. thuringiensis***) . Super misrona and boric acid showed (1.06- , 1.6- and 1.16-fold , respectively) .

As resistance continued to G_{15} , fenitrothion resistance further increased to 43.3-fold and conferred moderate level of resistance to profenofos (10.26-fold). For the other tested chemicals , resistance ratios ranged between 4.2 - fold for azadirachtin, 3.86 - fold for super misrona , 2.2 - fold for ***Bacillus thuringiensis*** and 1.3 - fold for boric acid .

As for G_{20} resistance to fenitrothion increased to 65.3 - fold ; and resistance to profenofos increased to 14.3 – fold . However low levels of resistance to super misrona , boric acid (***Bacillus thuringiensis***) and azadirachtin were found to be 6.35 ,6 ,5.66 and 5.1 - fold respectively.

As shown in Table (5) resistance to OP compounds ranged from 6.4 to - 65.2 -fold . **Allam** *et al* . (1994) found that a chlorpyrifos – selected strain with 9.7 - fold in G_{12} of ***S. littoralis*** showed a high level of resistance to 125 - fold cyanophos . However a slight increase was noticed with the profenofos.

The use of the meniral oil (Super misrona) and boric acid in practice after using fenitrothion to control *P . operculella* would result in a good integrated pest control program .

2.2 – Pattern of cross – resistance to pesticides in profenofos resistant strain of *P. operculella* :

The data of cross – resistance pattern to several insecticides during selection with profenofos for 15 generations are shown in Table (6) . In G_5 resistance to profenofos increased to 14.5 – fold and exhibited very low level of resistance to fenitrothion (1.59 - fold) and 2.6-fold of resistance to azadirachtin . The resistance able (6) : Cross- resistance pattern to some pesticidesin profenfos-resistant strain of ***Phthorimaea operculella*** (Zeller) during certain generations of selection .

Chemical	Parent Strain		Profenofos Strain								
	LC_{50} and its (95 % Fiducial limits)	Slope±S .E	LC_{50} (G_5) (95 % Fiducial limits)	Slope±S.E	R.R*	LC_{50}(G_{10})(95 % Fiducial limits)	Slope±S.E	R.R*	LC_{50} (G_{15})(95% Fiducial limits)	Slope±S.E	R.R*
Profenofos	0.77(0.5-1)	1.97± 0.3	8.85(6.86-11.98)	1.29± 0.2	11.4	19.7(13-33)	1.08± 0.23	25.5	42.5(32.5-58)	1.29± 0.2	54.99
Fenitrothion	1.8(1.4-2.4)	1.99± 0.34	2.9(2-4.2)	1.04± 0.18	1.59	16(10.8-29.9)	0.92± 0.2	8.88	18.3(10-26)	1.04± 0.22	10.16
Azadirachtin	0.19(0.15-0.24)	3.14± 0.5	0.5(0.3-1)	0.99± 0.23	2.6	0.8(0.5-1.7)	1.2± 0.26	4.2	1.2(0.74-2.2)	0.88± 0.19	6.3
Mineral oil	1.4(1.3-2.1)	4.3± 1.59	0.8(0.8-1.7)	0.8± 0.2	0.57	6.8(4.7-9.1)	1.46± 0.24	4.9	8(6-10.3)	1.63± 0.22	5.76
Boric acid	0.031(0.021-0.044)	1.2± 0.28	0.02(0.015-0.03)	1.68± 0.3	0.6	0.025(0.015-0.06)	0.73± 0.2	0.8	0.037(0.19-0.6)	0.84± 0.21	1.2
Dipel 2X	1.5×10^6 (0.8×10^6-2.2×10^6)	1.14± 0.2	1.3×10^6 (0.24×10^6-2.4×10^6)	1.01± 0.3	0.8	4.4×10^6 (2.6×10^6–6.3×10^6)	1.39± 0.28	2.9	9.2×10^6 (6.4×10^6-1.2×10^6)	1.19± 0.18	6.1

Resistance Ratio (R.R) = LC_{50} of strain / LC_{50} of parent strain.

ratio indicated clear negative correlation with boric acid , super misrona and ***Bacillus thuringiensis*** .

As resistance continued to G_{10} , profenofos resistance showed further increase to 25.5-fold , while the resistance level to fonitrothion was only 8.88-fold . Azadirachtin , mineral oil, boric acid and ***B. thuringiensis*** showed lower level of resistance to profenofos –resistance strain. Such level was 4.2-, 4.9-, 0.8- and 2.9- fold for these insecticides respectively .

In G_{15} , resistance to profenofos increased to 54.99 - fold and was accompanied by low resistance levels to fenitrothion(10.16 - fold) , 6.3 - fold to azadirachtin , 5.76 - fold to the mineral oil and 6.1 - fold to ***B. thuringiensis*** , however exihited very low level of resistance to boric acid (1.2-fold) .

These result indicated that selection with profenofos produced slight increase in resistance to the tested chemical . It could be stated therefore that mineral oils boric acid and ***B. thuringiensis*** (Dipel 2x) could be used as promising substitutes for the replacement of the organophosphorus compounds (fenitrothion and profenofos) which are the main compounds in the potato tuber moth control programs after establishment of high levels of resistance to these organophosphates .

2.3 –Pattern of cross-resistance to pesticides in azadirachtin resistant strain of *P . operculella* :

The results of cross-resistance pattern to several pesticides during selection with Azadirachtin for 20 generations are shown in Table (7) .

No significant changes in resistance ratios were observed for Azadirachtin after selection pressure for five generations . This strain exhibited the lowest resistance values to the mineral oil and ***B.thuringiensis*** (1.54 - fold and 1.6 - fold , respectively) . Boric acid, fenitrothion and profenofos showed moderate cross-resistance levels (2.6 -, 6.34- and 8.3-fold , respectively) .

With further selection to G_{10} , resistance to fenitrothion and profenofos decreased from 6.34 - to 3.79 - fold and from 8.3 - to 4.3 - fold respectively . while those for ***B.thuringiensis*** , mineral oil and boric acid remained constant when compared by their corresponding values of the parent strain remained nearly stable and increased lightly.

In G_{15} , resistance to azadirachtin remained nearly stable and increased slightly from 3.6 - fold in G_{10} to 5.3 - fold in G_{15} . Moderate resistance level to the organophosphorus compounds (profenofos and fenitrothion) was also indicated . Boric acid , super misrona and ***B . thuringiensis*** showed lower level of 5.3 - , 3.18 - and 3.1 -fold , respectively .

With further selection , resistance to azadirachtin reached 8.9-fold in G_{20} . Resistance to profenofos shifted sharply to 25.18-fold . Moderate resistance level to fenitrothion (8.7-fold) and lower levels of resistance to the mineral oil (5.8-fold) , ***B .thuringiensis*** (4.79-fold) and boric acid (4-fold) were also indicated .

It seems therefore that the genes for resistance to those insecticides had not been fixed in the field population . Several authors had emphasized that resistance genes were rare in most cases , of natural population . This means that resistance genes were probably kept at low frequencies by natural selection , and probably the resistant individuals had lower reproductive fitness than the normal genes . An advantage of susceptible individuals would lead to regression toward susceptibility if insecticide selection

Table (7) : Cross- resistance pattern to some insecticides in azadirachtin-resistantstrain of ***Phthorimaea operculella*** (Zeller) during certain generations of selection .

mical	Parent Strain		Azadirachtin (Neem- Azal) Strain											
	LC 50 and its (95 % Fiducial limits)	Slope±S.E	LC 50 (G5) (95 % Fiducial limits)	Slope±S.E	R.R*	LC 50 (G10) (95 % Fiducial limits)	Slope±S.E	R.R*	LC 50 (G15) (95%Fiducial limits)	Slope±S.E	R.R*	LC 50 (G20) (95%Fiducial limits)	Slope±S. E	R.R*
lirachtin	0.19(0.16-0.24)	214± 0.5	0.311(0.25-0.41)	1.3± 0.17	1.8	00.69(0.45-1.13)	1.5± 0.25	3.6	1.02(0.73-1.48)	1.25± 0.24	5.3	1.39(1-1.94)	1.25± 0.19	8.9
itrothion	1.8(1.4-2.4)	1.97± 0.3	11.9(7.6-28.6)	0.97± 0.2	6.34	7.14(2.3-26.5)	1.47± 0.44	3.79	12.19(8.41-21.3)	1.25± 0.24	6.6	16.11(11.32-27.66)	1.3± 0.25	8.7
ofenofos	0.77(0.52-1)	1.99± 0.34	6.4(3.34-9.57)	0.34± 0.19	8.3	3.34(2.33-4.66)	1.14± 0.2	4.3	6.4(4.36-10.7)	1.44± 0.24	8.2	19.47(11.39-51.58)	0.84± 0.16	25.18
neral oll	1.4(1.3-2.12)	4.3± 1.59	2.1(1.7-2.5)	3.25± 0.57	1.54	4.06(1.76-8.53)	2.02± 0.71	2.98	4.34(2.14-6.72)	1± 0.25	3.18	8.04(4.7-12.64)	0.98± 0.25	5.8
ric acid	0.03(0.021-0.04)	1.2± 0.28	0.08(0.04-0.12)	1.33± 0.23	2.6	0.12(0.06-0.49)	0.69± 0.22	3.8	0.16(0.11-0.33)	1.26± 0.27	5.3	0.12(0.089-0.18)	1.58± 0.2	4
pel 2X	1.5 x 106 (0.8 x 106---2.2 x 106)	1.14± 0.2	2.47 x 106 (0.95 x 106-3.5 x 106)	1.35± 0.17	1.6	3.37 x 106 (2.04 x 106 – 5.14x 106)	1.23± 0.27	2	4.92 x 106 (3.1 x 106-6.9 x 106)	1.45± 0.28	3.1	7.68 x 106 (5.8 x 106 – 9.63 x 106)	1.56± 0.19	4.79

Resistance Ratio (R.R) = LC_{50} of strain / LC_{50} of parent strain.

was removed before the improvement of fitness or the attainment of homogeneity of resistance individuals (**Abedi** and Brown , 1960 ; **Keiding ,** 1967)

2.4 - Pattern of cross-resistance to certain pesticides in the *B. thuringiensis* –resistant strain of *P . operculella* :

The data of cross-resistance pattern to the tested insecticides in the 5 ***th*** , 10***th*** and *15* ***th*** generations of selection with ***B.thuringiensis*** is shown in Table (8). As selection progressed to G_5 , resistance to fenitrothion and profenofos increased slightly to 2.8-, and 2.78-fold respectively . The levels of resistance to azadirachtin and boric acid remained nearly constant when compared by their corresponding values of the parent strain (P) .

After 10 generations of selection with ***B . thuringiensis*** resistance to profenofos was apparently established (increased to 12.18- fold) in spite of the slight increasing in resistance ratios to ***B . thuringiensis*** (selection agent) . The other tested insecticides showed resistance level of 4.1-, 6.2-, 3.5- and 3.2- fold for Fenitrothion , boric acid, mineral oil and azadirachtin respectively .

ble (8) : Cross- resistance pattern to some insecticides in ***Bacillus thuringiensis***-resistant strain of ***Phthorimaea operculella*** (Zeller) during certain generations selection .

Chemical	Parent Strain		Dipel 2X Strain								
	LC_{50} and its (95 % Fiducial limits)	Slope±S.E	LC_{50} (G_5) (95 % Fiducial limits)	Slope±S.E	R.R*	LC_{50} (G_{10})(95 % Fiducial limits)	Slope±S.E	R.R*	LC_{50} (G_{15})(95%Fiducial limits)	Slope±S.E	R.R*
Dipel 2X	1.5×10^6 (0.82×10^6-2.2×10^6)	1.14± 0.2	6.2×10^6 (4.17×10^6-8.79×10^6)	0.99± 0.18	4.05	8.17×10^6 (5.25×10^6–10.97×10^6)	1.02± 0.2	5.3	18.5×10^6 (13.2×10^6-25.3×10^6)	1.65± 0.28	12,3
Fenitrothion	1.84(1.4-2.35)	1.97± 0.3	5.12(2.73-8.15)	156± 0.25	2.8	7.66(5.36-10.88)	1.38± 0.24	4.1	16.43(10.39-18.56)	1.45± 0.2	8.9
Profenofos	0.77(0.52-1)	1.99± 0.34	2.15(1.51-2.95)	1.32± 0.22	2.78	9.42(6.63-15.52)	1.35± 0.25	12.18	14.4(10.3-23)	1.17± 0.21	18.6
Mineral oil	1.36(1.32-2.12)	4.3±1.59	4.19(2.82-5.73)	1.45± 0.25	3.1	4.83(3.57-6.24)	1.77± 0.26	3.5	6.95(4.91-9.36)	1.33± 0.22	5
Azadirachtin	0.19(0.16-0.24)	3.14± 0.5	0.24(0.16-0.37)	1.17± 0.21	1.3	0.6(0.44-0.91)	1.19± 0.22	3.2	0.81(0.59-1.16)	1.16± 0.15	4.49
Boric acid	0.03(0.022-0.045)	1.14± 0.2	0.039(0.027-0.063)	1.13± 0.21	1.3	0.2(0.09-0.31)	1.05± 0.25	6.2	0.23(0.16-0.43)	1.36± 0.28	7.6

sistance Ratio (R.R) = LC_{50} of strain / LC_{50} of parent strain.

With further selection with ***B . thuringiensis*** till G_{15}, resistance to profenofos increased to 18.8 - fold and a ccompanied by moderate to high level of resistance to fenitrothion (8.7 - fold) and boric acid (7.6 - fold) . however,a slight increase was noticed with the mineral oil (5.1 - fold) and the azadirachtin (4.49 - fold) .

In conclusion , certain points can be deduced form the results of cross-resistance pattern observed during selection with the four insecticides which belonge to three different groups of chemical compounds :

1- Selection with fenitrothion produced slight increase in resistance to tested all chemicals , except for boric acid .

2- The organophosphates fenitrothion and profenofos showed highly positive correlation between all selected strains .

3- Azadirachtin was effective against all selected strains.

4- There was a highly positive correlation between azadirachtin , ***B.thuringiensis*** resistance and fenitrothion and profenofos . On the other hand , selection with fenitrothion and profenofos and produced low positive correlation with azadirachtin , and ***B.thuringiensis*** .

5- There was a low positive correlation between ***B.thuringiensis*** and fenitrothion , profenofos and azadirachtin selected strains .

1-Biochemical studies on resistant *P . operculella* strains :

Biochemical mechanisms of resistance was determined in the resistant strains in an attempt to establish a correlation between resistance to pesticides and the detoxifying enzymes esterase and glutathion s-transferase .

3.1- Alpha – esterase activity :

The result in Table (9) showed the activities of alpha esterase which were determined in fenitrothion – resistant strain (Rf – strain) , profenofos strain (R p – strain) azadirachtin – resistant strain (R n– strain) , and ***B . thuringiensis*** - resistant strain (Rd– strain) .

In the Rf-strain , a significant increase in alpha activity was found after 8 generations of selection as compared with the p-strain. The levels of activity were 33.2 and 43.5 M hydrolyzed alph naphthyl /min/mg protein for parent strain and G_8 , respectively (table 9).The elevation of enzyme activity more potent in G_{11}

Table (9) : Specific activity of alpha esterase* in different insecticide-resistant strains of ***Phthorimaea operculella*** (Zeller) during certain generations of selection.

Generations	Rf – Strain	Rp – Strain	Rn – Strain	Rd - Strain
p - Strain	33.2	33.2	33.2	33.2
G1	------------	37 (1.12)	------------	------------
G4	35 (1.01)**	40.9 (1.23)	16.3 (0.49)**	33.4 (1.01)
G6	------------	------------	35.5 (1.06)	------------
G8	43 (1.3)	47.4 (1.43)	------------	------------
G11	53.6 (1.6)	------------	------------	35.5 (1.06)
G13	------------	75.2 (2.3)	41.6 (1.25)	44 (1.3)
G15	------------	------------	39.4 (1.18)	------------
G17	------------	------------	50.1 (1.5)	------------
G20	61.3 (1.8)	------------	49.6 (1.5)	------------

*Alpha esterase activity expressed as μmol hyhrollyzed substrat/ mg protein./min.
** Ratio of esterase activity in resistant strain and parent strain.

and G_{20} sincethe levels of activity were 53.6 and 61.3 M/min/mg protein , respectively .In the Rp-strain alpha estrase activity was found to be 40.9 hydrolysed μmol of alph esterase/ mg/ min giving an increase in the activity by 23.2% as compared to the perent strain .Also, the elevation of enzyme activity was generation – dependent to reach 126.5% of the perent strain

In the Rn – strain , the levels of alpha esterase activity was decreased during the 4 *th* generations of selection with azadirachtin as compared with the parent-strain (33.2- and 16.3 mol/min/mg protein for p-strain and G_4 , respectively) in (Table 9) . But in G_6 it increase to a level 6.9% that achieved in the p-strain . An increase in activity was found in G_{13} (41.6 mole/min/mg protein) . With further selection , the activity slowly increased during the succeeding eight generations . In G_{20} the level of activity reached 49.6 mole/min/mg protein .

Selection with ***Bacillus thuringiensis*** till G_4 resulted 3.6-fold of resistance ,however the alpha esterase activity of the Rd – strain was not affected and remained nearly similar to that of the p-strain . With the further selection no significant difference in alpha esterase activity was detected after 11 generations of selection with ***B . thuringiensis*** as compared with the p-strain. However, an elevation of esterase activity (32.5%) was found in the lost generation of selection as compared to the perent strain.

The level of alpha esterase activity at the end of selection by fenitrothion , profenofos ,Neemazal and Dipel 2x were 0.8 , 1.3 , 0.5 and 0.3 time higher than that of the parent strain (Table 9) . This indicated that alpha esterase may play a role in resistance to these chemicals in ***P . operculella*** .

Abo–EL Ghar *et al* . (1984) reported that esterases play an important role in pyrethroid -resistance as well as in organophosphate–resistance strain of ***S . littoralis*** .Wood *et al* .(1984) found that organophosphate resistance in ***Culex pipiens*** was due to detoxificantion by carboxy esterase whereas carbamate resistance had astrong non-metabolic component , possibly an insensitive AchE . **Dary** *et al* (1990) showed that resistance to organophosphate in mosquito was due to the presence of about 200 times more esterase activity than in the susceptible strain . **Green** *et al* (1990) reported that esterase plays major role in resistance to organophosphate , and certain cases may also contribute resistance toward carbamates and certain pyrethroids . **Sigfried** and **Scott** (1992) studied insecticide resistance mechanisms in the German Cockroach. Resistance to pyrethroids involved a kdr type nerve insensitivity and possibly increased oxidative detoxication .Organophosphate and carbamate resistance mechanisms included increased hydrolytic and oxidative metabolism . **Grant** *et al* . (1989) found that the enzyme activity of glutathione S-transferase and general esterase was more heterogeneous in field population than in the laboratory population . They suggested that this assay will provide data that can be used to evaluate and guide future decisions on pesticide selection .

3.2 – Glutathione s-trans ferase activity :

Glutathione s-transferase (GST) activities were determined in the fenitrothion-resistant(Rf) strain, the profenofos-resistant (Rp) strain , the azadirachtin-resistant (Rn) strain and ***B. thuringiensis***-resistant (Rd) strain and the results are shown in Table (10) .

Table (10) : Specific activity of GST* in different insecticide-resistant strain of ***Phthorimaea operculella*** (Zeller), during certain generations of selection.

Generation	Rf – Strain	Rp – Strain	Rn – Strain	Rd - Strain
p - Strain	22	22	22	22
G1	------------	27 (1.2) **	------------	------------
G4	25 (1.14)**	31 (1.4)	18 (0.8)**	21 (0.95)**
G6	------------	------------	21 (0.95)	------------
G8	30 (1.4)*	37 (1.68)	------------	------------
G11	37 (1.7)	------------	------------	23 (1.04)
G13	------------	41 (1.8)	17 (0.77)	27 (1.2)
G15	------------	------------	19 (0.86)	------------
G17	47 (2.14)*	------------	23 (1.04)	------------
G20	------------	------------	20 (0.91)	------------

*GST activity expressed as mOD/min/mg protein.
** Ratio of GST activity of resistant strain and parent strain.

In the Rf-strain , a slight increased in the GST activity was observed in G_4 as compared with the parent strain (activity was 25 and 22 mOD / min / mg protein for G_4 and parent strain , respectively).With further selection until G_{11} with fenitrothion , GST activity was increased significantly as compared with G_8 (activity was 1.4 in G_8 and 1.7 in G_{11} , respectively) . At the end of selection in G_{17} the level of activity reached 47 mOD / min / mg protein , having high activity than the parent strain (Ratio of activity was 2.14 - fold) .

In the Rp-strain , GST activity levels steadily increased during the selected generations with profenofos as compared with the p-strain. The levels of activity at the 1 *st* , 4 *th* , 8 *th* and 18 *th* generations were 27 , 31 , 37 and 41 mOD / min / mg protein , respectively , as compared with 22 mOD / min / mg protein for the p-strain .

In the Rn-strain , GST activity in G_4 (18 mOD / min / mg protein) decreased to a level below that of the p-strain (22 mOD / min / mg protein) , then in G_6 increased the level activity was 21 mOD / min / mg protein . Further selection with the Azadirachtin compound until G_{11} did not affect GST activity , and remained nearly similar as that of G_4 .The levels of activity were 23 and 20 mOD / min / mg protein for G_{17} and G_{20} respectively .

During the course of selection until G_{11} with Dipel 2x , GST activity was nearly similar to that of p-strain . The levels activity were 22 , 21 and 23 mOD / min / mg protein for the p-strain , G_4 and G_{11} , respectively . At the end of selection at G_{15} , the activity increased slowly till 27 mOD / min / mg protein .

The results revealed that there was a correlation between GST activity and resistance to the organophosphours compounds (fenitrothion and profenofos) . No correlation however was indicated between GST activity and resistance to Azadirachtin and Dipel 2x (***Bacillus thuringiensis***) .

GST enzymes were found to play a major role for resistance to organophosphours insecticides (Motoyama and Dauterman,1980). The involvement of GST in OP resistance by catalyzing of conjugation of GST to electrophilic sites on these compounds have been found for parathion (**Oppenoorth** et al . , 1972) ; methyl parathion (**Clark** et al., 1973 and 1986) ; tetrachlorvinphos (**Oppenoorth** et al., 1977 and 1979); azinophosmethyl (**Motoyama** and **Dauterman**,1972 and

Wells *et al.*, 1983) ; diazinon (**Hayaoka** and **Dauterman ,** 1983) and cyanophos (**Rofael** *et al.*, 1995).

As for other classes of insecticides such as carbamates , pyrethroids and chlorinated hydrocarbons , glutathione s-transferase was assumed to be relatively an unimportant detoxification mechanism since these compounds were normally metabolized oxidatively via the cytochrome P_{450} system or by esterases(**Matsumura** , 1975) . However , **Grant** and **Matsumura** (1989) found that GST – 2 enzyme activity was substantially high approximately eight-fold in permethrin and DDT-resistant strains of ***Aedes agypti*** compared with susceptible strains . **Pimprikor** and **Georghiou** (1979) concluded that glutathione s-transferase plays a relatively small role in resistance to diflubenzuron in the housefly .

In summary , certain points can be deduced from the aforementioned biochemical results:

1- Selection with OP fenitrothion for 20 generations produced a bout 65.2 - fold . The biochemical assay showed that esterase and GST activities were 1.8 and 2.14 - fold of 84.6% and 113.64% over those of the p-strain , respectively .Also In addition , the activities of esterase and GST activities were closely associated with resistance to fenitrothion .Thus the higher resistance to fenitrothion observed after selection could be due to the combined action of these two resistance mechanisms altogether .

2- The strain selected with the profenofos (OP) showed 54.99 - fold resistance to that insecticide agent in G_{15} . Esterase and GST activities were 126.5 and 86.36% over those of the P-strain .

3-Selection with azadirachtinfor 20 generations produced only about 8.9 - fold . The biochemical assay showed that esterase activity was increased to reach 49.4% over that of p-strain , whereas GST activities decreased by 9.1% of that of the p-stain .

4- The Dipel 2x (***B . thuringensis***) selected strain had developeda 11.9 - fold resistance after 15 generations of selection . This was accompanied by elevation in the activites of both esterase and GST. Such elevation was 32.5 and 22.7% over those of p-strain for there enzymes respectively .There results clearly indicated that there enzymes may play little roles in resistance to ***B . thuringensis*** in ***P.operculella*** .