

THE ABILITY OF COTTON LEAFWORM, *Spodoptera littoralis* (BOISD.) FIELD STRAIN TO DEVELOP RESISTANCE TOWARD SOME CONVENTIONAL AND BIO-INSECTICIDES

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ABSTRACT

This study aim to investigate the development of resistance in cotton leaf worm (CLW) to spinosad and abamectin comparing with the development of resistance toward cypermethrin and methomyl as conventional insecticides. All tested strains were selected by dipping the whole 4th instar larvae. Spinosad was only the compound which tested by feeding method in addition to the dipping technique. The spinosad feeding resistant strain (SFRS) was built up by supplying the 4th instar larve of *S. littoralis* (Boisduval) with castor leaves treated with spinosad for 23 successive generations continuously in the laboratory. Using dipping technique, spinosad, abamectin, cypermethrin and methomyl resistant strains (SDRS, ADRS, CDRS and MDRS; respectively) were selected by exposing the 4th instar larvae for 25 generations to each of spinosad and abamectin; and for 32 generations to each of cypermethrin and methomyl. Results indicated that the ability of the field strain of CLW to develop resistance toward spinosad by the two tested techniques were very high. Resistance ratio (RR) values for SDRS and SFRS were 108 and 87 fold, respectively. The ability of building up resistance toward abamectin and methomyl were almost like each other and not as high as spinosad (only 19 fold). Developing resistance toward cypermethrin was quiet higher than abamectin and methomyl (31 fold) but still not as high as spinosad.

Keywords: Development of resistance, *Spodoptera littoralis*, Spinosad , abamectin.

INTRODUCTION

More than 540 species became resistant to at least one insecticide (El-Sayed, 2006 & Anonymous, 2006). Insecticide resistance has been reported all over the world to almost of insecticides used against insect pest (Duan *et al.*, 1996; Xu *et al.*, 1996; Gatehouse *et al.*, 1997; and Yeh *et al.*, 1997). In Egypt, the cotton leafworm (CLW), *Spodoptera littoralis* (Boisduval) is a key polyphagus cotton pest. Its larvae feed not only on cotton but also attack more than 29 hosts from other crops and vegetables, and more than 60 different cultivated and wild plants (Gordon, 1961). Farmers often use large quantities of insecticides and spray cocktails of chemical to control this insect, in addition to the life cycle of this insect without hibernation period, it has destructive feeding habits and its demonstrated ability to develop resistance to chemical insecticides. One of recommended strategies to manage resistance problem is using insecticides with novel modes of action. Abamectin and spinosad are two of the most promising insecticides from microbial origin for controlling lepidopterous pests (El-Malla *et al.*, 2003). Shono and Scott, 2003 stated that with new insecticide we have to answer: how rapidly could resistance develop? and what level of resistance? To

answer these questions, in our studying, we selected the 4th instar larvae of field cotton leafworm strain by spinosad and abamectin in the laboratory comparing them with selection by cypermethrin and methomyl as conventional insecticides.

MATERIALS AND METHODS

1- Insecticides:

A- Bioinsecticides

a- Spinosyns

Spinosad (SC 24 %, Dow AgroSciences Co.)

b-Avermectins

Abamectin (EC 1.8 %, Roan Agrochemicals Co.)

B- Synthetic Insecticides:

Cypermethrin (EC 20 %, Dow AgroSciences Co.)

(*RS*)- α -cyano-3-phenoxybenzyl(1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate.

Methomyl (SP 90 %, DuPont Agricultural Co.)

S-methyl-*N*-(methyl carbamoyloxy)thioacetimidate

Chemicals used as surfactant

Triton X₁₀₀ (100 % purity, BDH Chem, Ltd. Poole England)

2- Insects

A- Parent field strain (PS)

The parent field strain of cotton leafworm, *S. littoralis* was brought as eggs and new hatches larvae from Alexandria university laboratory and kept away from insecticidal contamination in Plant Protection laboratory at Assiut University for two years to be stable. The strain was then divided into sub-strains to start the present study.

B-Spinosad dipping resistant strain (SDRS)

This strain was obtained by selecting a part of the parent field strain with spinosad (SC, 24%) solution. Dipping of 4th instar larvae was followed for 25 generations.

C-Spinosad feeding resistant strain (SFRS)

This strain was obtained by selecting a part of the parent field strain with spinosad (SC, 24%) solution. Feeding method to 4th instar larvae was followed for 23 generations.

D-Abamectin dipping resistant strain (ADRS)

This strain was obtained by selecting a part of the parent field strain with abamectin (EC, 1.8 %) solution. Dipping of the 4th instar larvae was followed for 25 generations.

E-Cypermethrin dipping resistant strain (CDRS)

This strain was obtained by selecting a part of the parent field strain with cypermethrin (EC, 20 %) solution. Dipping of the 4th instar larvae was followed for 32 generations.

F-Methomyl dipping resistant strain (MDRS)

This strain was obtained by selecting a part of the parent field strain with methomyl (SP, 90 %) solution. Dipping of the 4th instar larvae was followed for 32 generations.

Field populations

Five field populations collected from El-Behera, El-Minia, Assuit and El-Badary Egyptian cotton field as egg masses and new hatched larvae during June and July 2005 season. The populations transferred to the laboratory and reared on fresh castor bean leaves to reach 4th instar larvae under optimum conditions (26 ± 2 C° and 65 ± 5 % RH).

Rearing and insecticide pressuring of *S. littoralis* strains

Rearing procedur

Egg masses were put separately in plastic plate. Then, the egg masses kept in glass jars (2 liter in volume) and covered with muslin and perforated polyethylene, the new hatched larvae were transferred to clean glass jars and supplied daily with fresh castor bean leaves. At the beginning of 5th instar larval stage, every 40 larvae were kept in separate clean jar (2 Kg) containing one inch of sawdust for pupation. The jars were supplied with castor bean leaves and covered with muslin cloth till pupation. Pupae were separated and transferred with sawdust in plastic plate and kept in wood cages until adults emergence. The cages were then supplied with 10% sugar solution and oleander (Tafia), *Nerium oleander* (L.) leaves for egg laying. Egg masses were transferred to glass jars as previously described to start a new generation. All insects used were maintained at 26 ± 2 C° and 65 ± 5 % RH, under the normal daily light and dark.

Selection methods

Whole- larval dipping method

The larval dip technique (Babu and Santharam, 2002; Nayak and Chhibber, 2002 and Young *et al.*, 2000) was carried out to build up the spinosad dipping resistant strain (SDRS), abamectin dipping resistant strain (ADRS), cypermethrin dipping resistant strain (CDRS) and methomyl dipping resistant strain (MDRS). Selection was carried out by using the 4th instar larvae (the mean weight of larvae = 40 ± 5 mg). At each generation, aqueous solution of the selected insecticide concentration which used in selection pressure plus 0.1 % triton \times_{100} as a surfactant was prepared. This concentration equals the LC₅₀ value of the previous selected generation. The larvae were dipped in the selection concentration for 5 seconds by metal net. The treated larvae were put in a large dry container that contained filter paper to dry the larvae. Then the dipped larvae were supplied with a fresh castor leaves and put under the optimum conditions. After 24 hrs., dead larvae were separated and removed. However, the lived ones were distributed in clean jars (2 Kg), supplied with fresh untreated castor bean leaves and cared to get a new generation. Selection was carried out continuously through 25 generations for SDRS and ADRS. While For CDRS and MDRS, the selection was carried out for 32 generations.

Leaf dipping method

Leaf dip technique (Moulton *et al.* 1999 & 2000 and Young *et al.*, 2000) was used to build up the SFRS. Selection were carried out by the same technique mentioned above, except that the fresh castor leaves (instead of larvae) were dipped in the spinosad concentration for 5 seconds. Dipped leaves were put in a container with filter paper for 20-30 minutes to dry. After drying, the 4th instar larvae were supplied with the treated leaves for

24 hrs. The lived larvae were separated and cared, then supplied with fresh untreated castor bean leaves to get a new generation. Selection was carried out continuously for 23 generations. In both selection methods, about 15000-20000 larvae in each generation were selected.

Bioassay experiments

The same methods used in the selection pressure with some modification were used to determine the toxicity of insecticides.

Larval-dip bioassay

Fourth instar larvae of *S. littoralis* at an average weight of 38-40 mg / larva were selected. Serial water aqueous solution of concentration of the tested insecticide prepared+ triton \times_{100} (0.1 %) were used for bioassay tests. Three replicates at least were used for each concentration using 10 larvae/ replicate. Larvae of each replicate were dipped in the tested concentration for 5 seconds and then transferred to Petri-dishes containing filter papers to dry. Same number of larvae for each replicate were similarly dipped in distilled water plus the surfactant as a control treatment.

The treated larvae were supplied with fresh castor leaves and incubated at 26 ± 2 temperature and 12:12 L:D and 65 ± 5 RH until recording the results. Mortality was counted 48 hrs after treatment. The larva was considered dead if no movement was detected when it was touched with a small brush. Results corrected by Abbott's formula (Abbott, 1925) and LC_{50} and slope values were determined by a computerized probit analysis program. The toxicity of each insecticide was replicated 2 to 3 times.

Leaf -dip bioassay

The same steps of the above mentioned bioassay except that the 4th instar larvae of CLW were fed on dried insecticide treated castor bean leaves for 24 hrs. The larvae were allowed to feed on untreated fresh castor bean leaves for another 24 hrs, then mortality was counted. Mortality percentages were corrected by Abbott's formula (Abbott, 1925) and LC_{50} and slope values were determined by a computerized probit analysis program. Each experiment was replicated 2 to 3 times.

RESULTS AND DISCUSSION

Development of resistance to spinosad

The spinosad dipping resistant strain (SDRS) was selected by Exposing the 4th instar larvae of parent field strain to spinosad for 25 successive generations in the laboratory by larval dipping method. Nineteen generations were tested with series of spinosad concentrations to measure their building up resistance to spinosad. Table 3 and figures 5& 6 show the LC_{50} values of the tested generations. These data revealed that resistance ratio (RR) values increased gradually with slight fluctuations, from the first generation till the 22 nd generation (see figure 6). Then, increased suddenly from 20 fold in G22 to more than 85 fold in G23. Then continue increasing to reach 108 fold in G25. The slope values of the regression lines obtained decreased during all tested generations (Table 3). The lowest slope value was (1.16) in G5 and the highest one was (3.36) in G1. The data indicates

that the insect population was relatively heterogenous in their susceptibility toward spinosad using larval dip method. The spinosad feeding resistant strain (SFRS) (Table 4, figures 7 & 8) was built by supplying part of parent field strain larvae of *S. littoralis* to castor leaves treated with spinosad for 23 successive generations continuously in the laboratory. Out of 23 generations, 17 were tested with a series of spinosad concentrations for measuring their relative resistance to spinosad. Table 4 and figure 7 shows the LC50 values in tested generations while figure 8 shows RR values during selected generations. RR values were increased gradually from G1 to G13 to reach about 10-fold. Then increased in one generation (from G13 to G14) about 28-fold. The ratio was slightly stable from G14 to G19 with some fluctuations. From G19 to G20, the ratio increased suddenly to 66-fold resistance and increased again to reach 86.85-fold in G 23. The slope values of regression lines shown in figure 7 showed almost the same fluctuations as observed in SDRS (Table 3). This result indicates that the insect population was relatively heterogenous in their responsibility toward spinosad using feeding method.

The present results indicate that the ability of field strains of cotton leafworm to develop resistance toward the biorational spinosad insecticide by the two methods of selection was very high. After one generation of selection by larval-dip method and by leaf-dip method, selected strain had 4.08 and 4.34 fold by the two methods, respectively. After 23 generations, the RR were 85.24 and 86.85-fold, respectively. The same trend of building up resistance was also found in some lepidopteran species.

2.2. Development of resistance to abamectin

The abamectin dipping resistant strain (ADRS) was built by exposing the 4th instar larvae of parent field strain to abamectin for 25 successive generations in the laboratory using larval dipping method. Out of 25 generations, 17 were tested with series of abamectin concentrations for measuring their relative resistance to abamectin. Table (3) shows the LC50 , slope and RR values of tested generations. In table (3), the RR values were slightly increased gradually from G2 to G 7 to reach 2.09-fold, then increased in one generation to reach 8.78- fold in G11, then were stable for about three generations then jumped in G 16 to reach 12.60-fold resistance. RR became nearly stable till G 22. A gradual increase with slight fluctuations was observed from G23 to G25 to reach 18-fold resistance. The slope values of regression lines obtained in Table (3) were generally higher in the late generations than in early ones except some fluctuations. This indicates that the populations took a trend to be resistant generation by another under selection pressure.

The present results indicate that the ability of cotton leafworm strain employed to develop resistance toward abamectin in selection was not as high as spinosad. After 7 selected generations by larval-dip method, the abamectin strain had only 2.0 -fold resistance and after 23 selected generations the strain had 15-fold resistance. The present result was similar to that of some studies.

Table (1): Toxicity of spinosad to 4th instar larvae of *S. littoralis* in relatively successive selected generations for detecting resistance to spinosad using larval-dip method.

| Generation | LC50a | 95% Confidence limits | | Slope ± SE b | RR c |
|------------|----------|-----------------------|----------|--------------|--------|
| | | Lower | Upper | | |
| Pd | 162.03* | 39.99 | 275.29 | 1.42±0.49 | 1.00 |
| 1 | 660.49 | 508.89 | 795.80 | 3.36±0.71 | 4.08 |
| 2 | 969.33 | 741.56 | 1309.70 | 2.24±0.37 | 5.98 |
| 3 | 1041.86 | 799.91 | 1367.68 | 2.93±0.49 | 6.43 |
| 4 | 761.96 | 574.36 | 1001.15 | 2.79±0.49 | 4.07 |
| 5 | 626.75 | 332.04 | 2720.20 | 1.16± 0.43 | 3.87 |
| 7 | 627.32 | 437.52 | 1034.17 | 2.59 ± 0.75 | 3.87 |
| 8 | 723.93 | 498.60 | 1730.66 | 2.11± 0.73 | 4.47 |
| 12 | 892.10 | 663.73 | 1289.84 | 2.37± 0.43 | 5.47 |
| 13 | 993.33 | 680.23 | 1843.35 | 2.01± 0.49 | 6.09 |
| 14 | 2066.73 | 785.18 | 3133.49 | 1.88± 0.46 | 12.68 |
| 15 | 4044.76 | 2346.51 | 5981.65 | 2.20± 0.54 | 24.81 |
| 16 | 2456.27 | 1304.86 | 3870.58 | 1.43± 0.37 | 15.07 |
| 17 | 3144.11 | 2339.37 | 4183.68 | 2.11± 0.37 | 19.29 |
| 18 | 2455.17 | 1268.20 | 3752.34 | 1.34 ±0.29 | 15.06 |
| 21 | 4208.41 | 2416.78 | 9075.46 | 1.21± 0.36 | 25.81 |
| 22 | 3261.60 | 2208.41 | 4424.26 | 1.84± 0.38 | 20.01 |
| 23 | 13897.89 | 10760.77 | 20389.30 | 2.32 ±0.46 | 85.24 |
| 24 | 16081.21 | 10975.91 | 24778.05 | 1.79 ±0.39 | 98.64 |
| 25 | 17627.75 | 13935.27 | 22232.53 | 2.85±0.47 | 108.13 |

a, a.i. : active ingredient, µg ml⁻¹

b, SE : standard error

c, RR : resistance ratio = LC50 of the selected generation/ LC50 of the parent field strain

d, P : parent field strain

* : No significant difference in LC50 values of selected insecticides against parent fieldstrain in the beginning and in the end of selection pressure under laboratory condition.

Table (2) Toxicity of spinosad to 4th instar larvae of *S. littoralis* in relatively successive selected generations for detecting resistance to spinosad using leaf-dip method.

| Generation | LC50a | 95 % Confidence limits | | Slope ± SE b | RR c |
|------------|---------|------------------------|----------|--------------|-------|
| | | Lower | Upper | | |
| Pd | 101.87* | 30.51 | 194.17 | 1.27±0.31 | 1.00 |
| 1 | 442.62 | 317.86 | 602.71 | 2.33±0.34 | 4.34 |
| 2 | 342.72 | 230.20 | 486.67 | 1.73±0.29 | 3.36 |
| 3 | 452.40 | 30.79 | 869.38 | 1.27±0.48 | 4.40 |
| 5 | 730.30 | 524.51 | 956.75 | 2.44±0.38 | 7.17 |
| 6 | 795.36 | 470.54 | 5468.99 | 1.24±0.62 | 7.81 |
| 10 | 800.26 | 634.59 | 970.90 | 3.62±0.68 | 7.86 |
| 12 | 1274.79 | 914.22 | 1829.20 | 2.13±0.40 | 12.51 |
| 13 | 1029.29 | 154.59 | 1865.68 | 1.22±0.39 | 10.10 |
| 14 | 2855.27 | 154.22 | 15422.52 | 2.77±0.43 | 28.03 |
| 15 | 3107.04 | 2198.79 | 4308.15 | 2.17±0.41 | 30.50 |
| 17 | 3124.00 | 2574.94 | 3939.56 | 3.62±0.68 | 30.67 |
| 18 | 3314.34 | 1877.12 | 5883.82 | 1.31±0.36 | 32.53 |
| 19 | 2836.90 | 2062.45 | 3745.22 | 2.63±0.49 | 27.85 |
| 20 | 6733.70 | 4705.35 | 9592.38 | 2.03±0.40 | 66.10 |
| 21 | 6473.76 | 4497.36 | 9182.86 | 2.03±0.40 | 63.55 |
| 22 | 5004.29 | 3958.03 | 7890.53 | 2.42±0.49 | 56.98 |
| 23 | 6847.36 | 6215.02 | 15119.35 | 1.95±0.42 | 66.85 |

a, a.i. : active ingredient, µg ml⁻¹

b, SE : standard error

c, RR : resistance ratio = LC50 of the selected generation/ LC50 of the parent field strain

d, P : parent field strain

* : No significant difference in LC50 values of selected insecticides against parent field strain in the beginning and in the end of selection pressure under laboratory condition.

Development of resistance to cypermethrin

The present study was to compare the speed of building up resistance among cypermethrin, spinosad and abamectin. The cypermethrin dipping resistant strain (CDRS) was built by exposing the 4th instar larvae of parent field strain to cypermethrin for 32 successive generations in the laboratory using larval dipping method. Out of 32 generations, 18 were tested with a series of cypermethrin concentrations for measuring their relative resistance to cypermethrin. Table (4) shows the LC50 values of tested generations. Figure (12) shows the relationship between tested generations and resistance ratios. RR values increased rapidly from G1 to G6 to reach 8.6 fold, then increased to be 10.23 in G7. In G8 generation, RR value jumped to 15.6 fold. From G8 to G15, RR values were fluctuated. From G15 to G20, RR increased to 26.35-fold then increased gradually to reach 36-fold resistance to cypermethrin in G 32. The slope values of CDRS (Table 4) indicate that the earlier selected generations were generally higher than those of the rest generations except of some fluctuations. In other words, the selected generations became more heterogeneous after selecting the strain by cypermethrin for about 18 generations. These observations suggest that cypermethrin selected generations may be able to become higher resistant and more homogenous in the case of selecting it for more than 32 generations. The present results indicate that the ability of tested strain of cotton leafworm employed to develop resistance toward cypermethrin by selection is quiet higher than abamectin but not as high as spinosad.

The present results are in agreement with El-Sayed *et al.* (1985) who published that the cotton leafworm, *S. littoralis* selected with cypermethrin for 15 generations has reached 13.89-fold of resistance. Ishaaya and Klein (1990) collected *S. littoralis* larvae from Israel cotton fields that had been heavily sprayed with conventional insecticides. They found that these insects have more than 102- times resistance to cypermethrin than susceptible strain.

2.4. Development of resistance to methomyl

This study was carried out to compare the speed of building up resistance of *S. littoralis* larvae to methomyl with that to spinosad and abamectin selection using larval dipping method. The methomyl dipping resistant strain (MDRS) was built up by exposing the 4th instar larvae of parent field strain to methomyl for 32 successive generations in the laboratory using larval dipping method. Out of 32 generations, 18 generations were tested with series of methomyl concentrations for measuring their relative resistance to methomyl. Table (5) shows the LC50, slope and RR values of tested generations. Figure (14) shows the relationship between tested generations and the resistance ratios. The RR values increased slowly for 7 generations to become 2.4 fold resistance, then the RR increased slightly from G6 to G12 to reach 3.91-fold resistance. Then RR increased from G12 to G17 to reach 12.58 fold. The tested generations were slightly fluctuated in RR values from G18 to G23. Resistance level was increased again from G23 to G24 to reach

Table (3). Toxicity of abamectin to 4th instar larvae of *S. littoralis* in relatively successive selected generations for detecting resistance to abamectin using larval dipping method.

| Generation | LC50a | 95 % Confidence limits | | Slope \pm SE b | RR c |
|------------|---------|------------------------|---------|------------------|-------|
| | | Lower | Upper | | |
| Pd | 84.46* | 34.15 | 203.67 | 1.25 \pm 0.38 | 1.00 |
| 2 | 126.03 | 89.13 | 210.91 | 2.00 \pm 0.42 | 1.49 |
| 3 | 160.69 | 128.15 | 210.23 | 3.07 \pm 0.51 | 1.90 |
| 4 | 126.49 | 105.50 | 156.56 | 4.76 \pm 0.79 | 1.50 |
| 5 | 151.17 | 105.30 | 265.79 | 1.8 \pm 0.43 | 1.79 |
| 6 | 154.77 | 129.58 | 181.88 | 4.82 \pm 0.85 | 1.83 |
| 7 | 176.69 | 97.02 | 404.51 | 1.39 \pm 0.37 | 2.09 |
| 11 | 741.19 | 563.95 | 981.94 | 3.03 \pm 0.68 | 8.78 |
| 13 | 724.28 | 571.86 | 1040.08 | 2.83 \pm 0.64 | 8.58 |
| 15 | 740.53 | 601.09 | 871.67 | 5.16 \pm 0.84 | 8.77 |
| 16 | 1061.61 | 922.67 | 1225.44 | 6.06 \pm 1.16 | 12.60 |
| 17 | 1064.51 | 930.29 | 1115.98 | 8.97 \pm 1.51 | 12.60 |
| 18 | 1062.71 | 958.62 | 1178.07 | 8.13 \pm 1.54 | 12.58 |
| 20 | 1197.12 | 940.47 | 1526.00 | 3.16 \pm 0.52 | 14.17 |
| 21 | 1068.32 | 808.13 | 1329.92 | 4.21 \pm 1.0 | 12.65 |
| 22 | 1165.46 | 905.17 | 1500.17 | 3.44 \pm 0.86 | 13.80 |
| 23 | 1338.65 | 1140.80 | 1732.38 | 4.50 \pm 1.04 | 15.85 |
| 25 | 1600.80 | 1491.33 | 1993.62 | 4.13 \pm 1.3 | 18.95 |

a, a.i. : active ingredient, $\mu\text{g ml}^{-1}$

b, SE : standard error

c, RR : resistance ratio = LC50 of the selected generation/ LC50 of the parent field strain

d, P : parent field strain

* : No significant difference in LC50 values of selected insecticides against parent field strain in the beginning and in the end of selection pressure under laboratory condition.

Table (4). Toxicity of cypermethrin to 4th instar larvae of *S. littoralis* in relatively successive selected generations for detecting resistance to cypermethrin using larval dipping method.

| Generation | LC50a | 95 % Confidence limits | | Slope \pm SE b | RR c |
|------------|-------|------------------------|-------|------------------|-------|
| | | Lower | Upper | | |
| Pd | 0.38* | 0.46 | 2.21 | 1.07 \pm 0.28 | 1.00 |
| 6 | 7.58 | 5.71 | 11.60 | 2.50 \pm 0.52 | 8.60 |
| 7 | 8.00 | 5.99 | 16.71 | 1.89 \pm 0.50 | 10.23 |
| 8 | 15.75 | 8.54 | 27.40 | 1.57 \pm 0.32 | 15.60 |
| 9 | 15.32 | 7.72 | 23.55 | 2.70 \pm 0.66 | 15.14 |
| 10 | 17.05 | 12.25 | 26.18 | 2.22 \pm 0.47 | 19.32 |
| 12 | 18.13 | 5.52 | 21.12 | 2.35 \pm 0.43 | 17.16 |
| 13 | 16.84 | 11.54 | 21.92 | 3.11 \pm 0.65 | 19.14 |
| 15 | 14.92 | 6.56 | 20.91 | 3.00 \pm 1.11 | 16.95 |
| 18 | 19.51 | 13.85 | 26.22 | 3.00 \pm 0.69 | 22.28 |
| 19 | 17.58 | 11.24 | 31.58 | 1.78 \pm 0.66 | 19.95 |
| 20 | 23.19 | 13.81 | 37.72 | 1.61 \pm 0.27 | 28.35 |
| 21 | 26.40 | 16.31 | 46.05 | 1.38 \pm 0.24 | 30.00 |
| 22 | 27.58 | 23.31 | 32.89 | 4.47 \pm 0.65 | 31.32 |
| 25 | 28.80 | 20.59 | 39.75 | 2.24 \pm 0.56 | 32.33 |
| 26 | 25.76 | 7.25 | 40.19 | 1.53 \pm 0.45 | 28.63 |
| 27 | 23.43 | 10.13 | 39.76 | 1.22 \pm 0.38 | 28.63 |
| 28 | 28.06 | 30.41 | 34.16 | 5.25 \pm 1.44 | 31.89 |
| 32 | 31.71 | 24.51 | 39.50 | 2.85 \pm 0.48 | 36.03 |

a, a.i. : active ingredient, $\mu\text{g ml}^{-1}$

b, SE : standard error

c, RR : resistance ratio = LC50 of the selected generation/ LC50 of the parent field strain

d, P : parent field strain

* : No significant difference in LC50 values of selected insecticides against parent field strain in the beginning and in the end of selection pressure under laboratory condition.

Table (5). Toxicity of methomyl to 4th instar larvae of *S. littoralis* in relatively successive selected generations for detecting resistance to methomyl using larval-dip method.

| Generation | LC50a | 95 % Confidence limits | | Slope \pm SE b | RR c |
|------------|---------|------------------------|---------|------------------|-------|
| | | Lower | Upper | | |
| Pd | 81.14* | 54.51 | 108.41 | 2.88 \pm 0.77 | 1.00 |
| 6 | 194.56 | 149.06 | 239.77 | 2.71 \pm 0.45 | 2.40 |
| 7 | 193.00 | 135.91 | 269.53 | 1.74 \pm 0.28 | 2.38 |
| 8 | 336.68 | 201.94 | 779.16 | 1.27 \pm 0.28 | 4.15 |
| 9 | 326.88 | 205.47 | 636.52 | 1.23 \pm 0.25 | 4.03 |
| 10 | 193.69 | 95.37 | 521.55 | 0.93 \pm 0.26 | 2.39 |
| 12 | 317.61 | 175.44 | 9931.40 | 1.30 \pm 0.57 | 3.91 |
| 17 | 1020.78 | 716.48 | 1348.08 | 2.05 \pm 0.40 | 12.58 |
| 18 | 1166.72 | 738.02 | 1495.29 | 2.82 \pm 0.69 | 14.38 |
| 19 | 1271.46 | 591.98 | 2040.86 | 1.21 \pm 0.26 | 15.67 |
| 20 | 1217.68 | 675.09 | 1921.44 | 1.51 \pm 0.30 | 15.00 |
| 22 | 1108.33 | 839.07 | 1419.06 | 2.49 \pm 0.40 | 13.66 |
| 23 | 966.14 | 694.12 | 1343.52 | 2.09 \pm 0.47 | 11.91 |
| 24 | 1586.50 | 1240.62 | 2042.47 | 3.01 \pm 0.56 | 19.55 |
| 26 | 1536.74 | 1161.33 | 2129.53 | 2.62 \pm 0.48 | 18.94 |
| 27 | 1633.72 | 1307.54 | 2067.08 | 3.52 \pm 0.58 | 20.13 |
| 28 | 1652.39 | 1319.40 | 2008.19 | 3.38 \pm 0.28 | 20.36 |
| 32 | 1516.82 | 1208.32 | 1838.03 | 4.17 \pm 0.90 | 18.69 |

a, a.i. : active ingredient, μ g ml⁻¹

b, SE : standard error

c, RR : resistance ratio = LC50 of the selected generation/ LC50 of the parent field strain

d, P : parent field strain

* : No significant difference in LC50 values of selected insecticides against parent field strain in the beginning and in the end of selection pressure under laboratory condition.

The present results indicate that the ability of selected strain of cotton leafworm to develop resistance toward methomyl was quiet low and less than spinosad.

19-fold resistance, then stayed fluctuated till G32. In table 7 and figure 14, the slope values of regression lines were relatively high in the early generations indicating high homogeneity at susceptibility level. By increasing selection pressure, the percentage of tolerant and resistant individuals became higher than the beginning, leading to lower slope values (from G7 to G12). Beginning with G17, the percentage of resistant individuals became much higher and concomitantly RR values increased and slope values became around 3 (with some fluctuations) until G32. These observations suggest that selected individuals became more homogenous (resistant) compared with the early ones.

The present results conclude that the ability of field strains of CLW to develop resistance toward spinosad by the two used methods of selection was very high. While the ability for developing resistance against abamectin, cypermethrin and methomyl were not as high as spinosad. Its highly recommended to take this ability of building up high level of resistance against spinosad into consideration in IPM programs.

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قابلية دودة ورق القطن لاكتساب مقاومة لبعض المبيدات الحيوية والتقليدية
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تهدف هذه الدراسة لمعرفة قدرة دودة ورق القطن لاكتساب مقاومة ضد مبيدات الاسبينوساد والابامكتين مقارنة بمبيدات السبيرمثرين والميثوميل . و قد تم انتخاب جميع السلالات المقاومة بواسطة عمر يرقات العمر الرابع لمدة ٢٥ جيل في سلالات الاسبينوساد والابامكتين ولمدة ٣٢ جيل في سلالات السبيرمثرين والميثوميل و ذلك بالاضافة الى سلالة الاسبينوساد المنتخبة بالتغذية لمدة ٢٣ جيل متتالي . اوضحت النتائج ان دودة ورق القطن لها القدرة على اكتساب مقاومة لمبيد الاسبينوساد باحدى طريقتي الانتخاب حيث كان معدل المقاومة لسلالة العمر هو ١٠.٨ مرة ومعدل المقاومة في سلالة التغذية هو ١٧ مرة . اظهرت دودة ورق القطن مقاومة لمبيدات الاسبينوساد والميثوميل حيث كان معدل المقاومة متساوي تقريبا في كلتا السلالتين (١٩ مرة) حيث لم تصل المقاومة الى مستوى معدل المقاومة لمبيد السبيرمثرين الذي كان أعلى حوالي ٣١ مرة