Genetic *Techniques To Evaluate Dursban Resistant* And Susceptible Individuals Of *Spodoptera littoralis* (Boisduval) Progeny, And Their Esterases' Activity

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Abstract: The population of any pest consists of three genotypes, (RR, RS, and SS) from the point of view of the pest resistance. Heterozygosity or homozygosity within the individuals of any strain are due to the distribution of its genotype and frequencies. The present work is a trial to isolate and concentrate the highest and lowest susceptible individuals from the progeny of any *Spodoptera littoralis* strain, and save time to obtaining resistant and /or susceptible strains. The conducted technique aimed to evaluate the susceptibility of twenty egg- masses from a laboratory strain of *Spodoptera littoralis* (Boisd.), and the same number from field one (Kaliobeya Governorate, Egypt). Each of them was a base line to susceptible and resistant strain; they were reared in separate containers till 4th instar larvae. Larvae of each container were divided into two portions. The first portion included 50 larvae of (4th instar) which were subjected to treatment with LC_{50} value of the original of each strain. The other portion was left without treatment and label for stress. Another technique was carried out to accelerate and concentrate the two phenomena in the second generation. Traditional LC_{50} dose of the original strains were used as a fixed dose on the first portion 4th instar larvae for accurately discriminate between resistant and susceptible genotypes of each strain**.** Data based on LC_{50} values showed that, developed traditional susceptible strain (dtss.), which resulted from laboratory strain was the highest susceptible strain followed by traditional susceptible strain (Tss). On the other hand, developed traditional resistant strain (individuals of field strain), exhibited remarkably moderate level of resistance of *Dursban*, as compared with traditional resistant strain (Trs), which came next during 4 successive generations. Differences in pseudo and truly esterase activity between various strains were demonstrated. Generally, it was clear that *Dursban* resistance in cotton leaf worm is principally due to increased, Beta esterase, Cholinesterase, followed by Aliphatic esterase, whereas Alpha esterase exhibited the effect slightly in this respect.
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Key words: *Spodoptera littoralis*, Resistant, Susceptible**,** Esterases

1. Introduction:

Much of the new knowledge of resistance monitoring methods resulted from improved knowledge of resistance mechanisms and expression, as well as a change in goals and physiology of monitoring. This placed and increased emphases on relating laboratory to achieve field control (Ball, 1981). previous reviews have investigated improvements in insecticide resistance detection methods (Brown and Brodgon, 1987).

Selection for resistance can occur if a small population of the insects is able to survive although treatment with insecticide. This rare resistant individuals can reproduce and pass on their resistance to offspring. If an insecticide with the same mode of action is repeatedly used against this population, greater production will survive. Ultimately, the once effective product no longer controls the resistant populations (Xin- Ju and Hui- Min 2011 and FOA, 2012). There is a relationship between the increase of insecticide resistance and the activity of detoxification enzymes. (Wolker, and Mackness, 1983). Generally, esterases are a large and diverse groups that hydrolyze numerous substrates. A number of esterases may play a role in detoxification of xenobiotic esters, (Gacer and Tasksn, 2009). Increased esterases' activity is a major mechanism of insecticide insensitivity or resistance in many strains of insect species, (Zhou *et al.*, 2002).

Mohanna, (1998 a, b) succeeded to implement . laboratory techniques valuable in detection of susceptibility in *Spodoptera littoralis* (Boisd) progeny to Cypermethrin in relation to esterases and differentiation of resistant and susceptible individuals.

Behavioristic action o IGR/ insecticide mixtures in control of *Spodoptera littoralis* (Boisd.) and enzymatic activity were investigated. Enzyme activity of, α-E, ß-E, Ali- E, and Ch- E were determined on 4th instar larvae of each of strain (Mohanna *et al*.,1996 and Mohanna and Allam 1999a). Moreover biochemical as well as genetic susceptibility of their progeny to fenvalerate(Mohanna and Allam 1999a) established cumulative data for improved techniques in evaluation and induction of insecticide resistance and susceptibility and resistance. Therefor this study

aimed to isolate and concentrate susceptible and resistant individuals of *Spodoptera littoralis* (Boisd.) to *Dursban beside investigation of esterases 'activity.* .

2. Material and Methods

Strains:

Two strains of *Spodoptera littoralis* (Boisd.) were used, parent strain collected from Kaliobeya Governorate, Egypt, during two seasons of 2013 / 2014 of cotton growing, for extracting a strong strain. After selection, strain was reared to one generation free from any insecticide contamination for more purification. Twenty egg- masses, completely fit in the shape and size were chosen by eye, fitted from the production of parent strain. Each egg mass was reared alone in a separate container labeled . A group of 50 larvae of 4^{th} instar for each were treated with *Dursban* at a level of 50% mortality of the parent strain. Mortality percent was calculated in each of 20 treatments. All egg masses which exhibited low mortality (20%) were collected together to become a base line of resistant strain. On the other hand, laboratory strain supplied by Central Laboratory of Pesticides (C.L.P.), Research Center of Agricultural, Giza, Egypt was subdued to a modified technique of the previously mentioned by Mohanna (1998a) .

1- Laboratory strain, traditional susceptible strain (Tss) originated from Lab. C.L.P. It has been reared free from insecticide contamination

2- Untreated traditional susceptible strain (uTss) originated from lab. Strain and left to 4 generations without any treatments.

3- Developed traditional susceptible strain (dTss) resulted from lab. strain after developing techniques for 4 untreated generations.

4- Parent strain (original) collected from Kaliobeya governorate field.

5- Traditional resistant strain (Trs) obtained from parent strain after selection with selected agent for 4 successive generations.

6- Developed traditional resistant strain (dTrs) originated from parent strain after concentrating the resistant individuals, and subjected to selection pressure for 4 successive generations.

Rearing Programs:

Rearing programs were carried out according to El- Defrawe *et al*., (1964).

In a condition rearing room. All strains were reared at, (25± 5ºC) and (65±5% RH), with continuous care and cleanup in all steps.

Toxicological Studies:

Tested strains were subjected to selected agent for 4 generations. A castor bean leaves were dipped for 15 seconds in each aqueous concentrations of the tested compound then left to dry. The treated leaves offered to newly molted 4^{th} instar larvae for 24 hr. The average of mortality percentage was corrected according to Abbott (1925) formula. The corrected mortality percentage was statistically computed according to Finny (1971).

Enzyme assays:

In these measurements 4^{th} instar larvae (average weight 37- 45 mg.) were collected from each of experimented strain. Larvae of all samples were starved for 4 hr. before being homogenized in distilled water (5 larvae /ml.). The homogenates were centrifuged for15 min. at 1000 r.p.m. at 2 ºC and the supernatant fraction was used for enzyme assay.

The activity of α- E, and ß- E were determined according to the method described by Van Asprin (1962), using alphanaphthol acetate as substrates, diazoblue sodium lauryl sulphate reagent was used for estimation of naphthol produced. Alpha naphthol gives a dark blue colour (maximum absorption at 600 mu.), and beta naphthol gives a deep red colour (maximum absorption at 550 mu.) when this reagent is used.

The reagent used for colorimetric assay of cholinesterase, (Ch-E), and aliphaticesterase (Ali-E), activity were the same as those described by Simpson *et al*. (1964) except that 0.5 ml. of acetyl choline bromide at 2.5×10^{-3} M. and also 0.5 ml. of methyl n butyrate at 4×10^{-3} M. was used as a substrates, respectively. The hydroxyl amine, sodium hydroxide, hydrochloric acid, and ferric chloride reagents were used as the same molar concentrations.

The colorimetric determinations were based on a minimum of 4 replications for each sample. All homogenates were incubated with the substrates at 37 ºC for half an hour.

Results and Discussion:

The target of this work is to concentrate the highest susceptible individuals from laboratory strain and low susceptible from field strain.

The technique was conducted to evaluate a sufficient number of pairs (♀ and ♂) from fit moths of *Sipodotera littoralis (*Boisd.) of G1, after the first technique was done. Each pair (♀ and ♂) were left copulated in a separate lantern glass. Fifty pairs of normal moths emerging from G1 of field strain were prepared for this work. Each of them was labeled for stress. On the other hand, twenty five pairs of G1 from lab. strain were prepared for the same technique., Healthy and fit egg-masses of the two strains, were transferred carefully to another containers for rearing. The level of susceptibility were estimated. Twenty two treatments of parent strain were exhibited a fluctuation ranged between a moderate to a highest level of resistance (Table 1). On the other hand, twelve of laboratory strain were exhibited a moderate

level of susceptibility towards the selective agent. The fluctuated groups of the two experimented strains were investigated. The groups of two strains which exhibited a moderate to high level of susceptibility were taken to accomplish to the target of this study.

Generally, a traditional dose, LC_{50} of the original strains (field & lab.) were used as fixed dose on 4^{th} instar larvae of the first group for accurate discrimination between resistant and susceptible genotypes (i.e. high kill of susceptible, low kill for resistant individuals). It was evident that the collected larvae of the untreated portions exhibited a high susceptibility (ranged between70 – 80 % death). These larvae of untreated were labelled and collected together to start the developed traditional susceptible strain (dTss), without any survived larvae from tested portions. On contrary, the tested larvae of field strain which exhibited a low death (15 – 20 % death), the untreated portion, besides to survive larvae were collected together to become a base line for developed traditional resistant strain, (dTrs). It is worthy to mention that, low susceptibility of individuals may accelerate resistance phenomenon to selection pressure to specific agent due to the accumulation of RR and RS more than SS genotype. On converse, it may increase in susceptible individuals and accelerate the accumulation of SS, RS than RR. genotype in this group.

Similar data were also observed by; Brindley *et al*., (1982), Hemingway *et al*., (1984); Halliday and Georghiou (1985); Mohanna (1998 a, b); Mohanna and Hossain (1999); Mohanna and Allam (1999 a, b) and Mohammed *et al*., (2015). The above mentioned studies and our results defeated the contrary findings of Roush and Miller (1986) who found that the most practical situation perfectly discriminating test is unknown and resistance has, not yet been examined in sufficient detail to facilitate the choice of an appropriate method, particularly if resistance "intensity" is low, in such circumstances.

Susceptibility of 4^{th} instar larvae in Tss and dTss throughout 4 successive generations, without any exposure to insecticide treatments (Table 1) using LC_{50}'s values of the base line of the two strains was, 0.9512. Remarkably dropped in LC_{50} values were observed, Tss (0.951), untreated (uTss), during 4 generations was (0.856). Regarding to, developed traditional susceptible strain (dTss) which exhibited (0.7890), during G2 with continuous relaxation. slight increase in susceptibility was observed till G4 with 0.6072 record. The most susceptible progeny of dTss may be due to the distribution of SS, more than other genotypes RS and RR Mohanna (1998 a). These findings have been supported by several authors, Brindley *et al*. (1982), Hemingway *et al*. (1984) and Halliday, and Georghiou (1985).

Table (1): Susceptibility of traditional susceptible strain (Tss), and developed traditional susceptible strain (dTss), during 4 generations without any exposure to insecticides.

Generation	Laboratory strain(Tss)		Developed strain (dTss)	
	Slope 5% fiducial limits	LC_{50} 5% fiducial limits	Slope 5% fiducial limits	LC_{50} 5% fiducial limits
G0	2.650 (1.911- 3.390)	0.951 (0.770 - 1.158)	2.650 (1.911- 3.390)	0.951 (0.770 - 1.158)
G1	-- -- ---	---- ---- ---	----------	----------
G2	----------	----------	2.278 (1.572- 2.984)	0.789 (0.640 - 1.041)
G3	----------	----------	-- --- ----	-------- -------
G4	2.216 (2.516- 2.921)	0.856 (0.682- 1.141)	2.209 (1.577-2.896)	0.607 (0.480 – 0.771)

The role of development of resistance to *Dursban* on *S. littoralis*, 4^{th} instar larvae with LC_{50} in 4 successive generations on Trs and dTrs were represented in Table (2). LC_{50} of the traditional and developed technique was 1.991 as a base line for resistance. Regarding to classic strain Trs. A slight level of LC_{50} values in G2, and G4; RR, 1.68 and 2.58 fold, respectively. On the other hand, selection for individuals resulted from the developed techniques led to 3.30 and 4.41 fold of tolerance during G3 and G4 respectively.

Table (2). Rate of development of resistance to *Dursban* in *Spodoptera littoralis* (Boisd.), for traditional resistant strain (Trs), and developed (dTrs), after 4 successive generations.

Genera.	Traditional resist. Strain (Trs)			Developed (dTrs)		
	Slope 50% fiducial limits	LC_{50} 50% fiducial limits	RR	Slope 50% fiducial limits	LC_{50} 50% fiducial limits	RR
G0	2.64	1.991 (1.571 – 2.483)	----	2.64	1.991 (1.571 – 2.483)	-----
G1	---	---	-----	2.47	3.775 (3.014- 4.642)	1.90
G2	2.18	3.350 (2.552 -4.209)	1.68	---	---------	-----
G3	----	----------	-----	3.30	6.561 5.457 – 7.918	3.30
G4	2.30	5.128 (3.710 -6.520)	2.58	2.24	8.786 (5.869- 11.254)	4.41

Based on considering the developed, dTss as the slandered strain, the susceptibility of the 4 thin star larvae of different strains in rearranged forms are shown in Table (3); Summarized data indicate that the Tss exhibited 1.57 fold, when relaxed 4 generations as compared to dTss. Growing through the classic strain Trs, a remarkable increase of 8.45 fold was recorded as compared with dTss. Whereas, parent (field) strain exhibited 3.28 fold after four generations of selections. On the other hand, selection for individuals resulted from developed techniques led to moderate level of resistance. Where developed strain dTrs recorded 14.47 fold relatively to the slandered Tss. Generally, selection pressure during 4 successive generations, in addition to the combined effect of developed technique led to a higher resistance ratios. The same concept also accelerated the susceptibility of dTss more than Tss, which indicated the importance of using developed technique within few generations of the strain maintenance. The same techniques are agreement with those of Mohanna (1998 a & b), Mohanna and Hossain (1999), and, Abu El Ghar *et al.* (2005).

Enzyme activities in different strains for 4th instar larvae of S*podoptera littoralis* (Boisd.), are summarized in table (4).

Table (3); Rearrangement for susceptibility to *Dursban* on *Spodoptera littoralis*(Boisd), in 4 generations, in relation to dTss as a slandered strain.

Strain	Slope (50% fiducial limits)	LC_{50} (50% fiducial limits)	RR
dTss	2.208 (1.577 – 2.891)	0.6072 (0.480 – 0.771)	00
Tss	2.650 (1.911 – 3.390)	0.951 (0.770 – 1.156)	1.57
parent	2.635 (1.911 – 3.369)	1.9910 (1.572 – 2.48)	3.28
Trs	2.301 (1.298 – 3.366)	5.128 (3.710 – 6.520)	8.45
dTrs	2.257 (1.235 – 3.279)	8.786 (5.867 – 11.255)	14.47

Table (4); Activity of Alpha, Beta, Choline, and Aliphatic esterases in 4th instar larvae of *Spodoptera littoralis*(Boisd),in different strains having various levels of susceptibility to *Dursban* as selected agent.

Strain	LC_{50}	A - E	%	B - E	%	Ali-E	%	Ch- E	%
dTss	0.6072	33.67	--	34.09	--	0.015	--	0.013	--
Tss	0.9512	38.22	13.34	22.17	-34.97	0.013	13.33	0.010	23.48
uTss	0.8563	88.22	13.51	41.32	21.21	0.018	20.00	0.014	7.69
parent	1.9910	42.18	25.27	47.14	38.28	0.012	-20	0.019	46.15
Trs	5.1276	46.29	37.48	42.70	25.26	0.021	40.00	0.020	55.49
dTrs	8.7858	51.72	53.61	53. 18	56.13	00.22	46.67	0.021	61.54

The changes in pseudo and truly esterases activity were clear as a result of continuous selections with *Dursban* on different strains. Regarding to the nonspecific esterases, α and ß esterases, *Dursban* caused a moderate levels of synergism for all strains, with the exception of traditional susceptible strain Tss, and untreated (uTss) strain. Developed susceptible strain dTss, which was a slandered or base line for all strains. The lowest activities values in a percentage were; 13.34 and – 34.17 for α and ß esterases. The highest values were recorded, 53.61 and 56.13 for α and ß esterases, in dTrs, respectively. Furthermore, α esterase exhibited slightly active than ß –E in resistant larvae, and both of them was more active in resistant larvae than the susceptible colonies. On the other hand, specific esterase enzymes, Ch-E, exhibited a slightly levels of activity and began to rise in the resistant cases. Activity percentages ranged between 55.49 to 61.54 relative to dTss.

Ali-E, also recorded high level of activity of the resistant larvae to *Dursban* ranged about 40 and 46.67 % relative to dTss. Whereas, in case of parent strain, a low degree of activity, - 20 % had been emphasized.

Data, in general conception, refer to the activity of dTss larvae could be arranged in descending order, as ß –E, Ch-E, Ali-E, and α-E. It is clear that *Dursban* resistance in 4 *th* instar larvae is due to principally to increase Ch-E, followed by ß –E, and α-E. Whereas, Ali-E exhibited a slightly effects. These findings agree with; Abo El Ghar *et al*. (1984), who reported that esterases play an important role in the pyrethroid resistance as well as organophosphate resistant strain of *Spodoptera littoralis* (Boisd); Green *et al.*, (1990) and Byrne *et al.*, (2000) they reported that esterases play a major role in resistance to organophosphates and certain cases might also contribute resistance towards carbamates and certain pyrethroids. Gunning *et al.*, (1995), declared that the larvae of pyrethroids resistance *Helocoverpa armigera* (Hubner) have enhanced esterases activity which is due to increased production of the enzymes. The most resistant individuals have approximately a 50 fold increase in esterase activity.

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