RESULTS AND DISCUSSION

IV. RESULTS AND DISCUSSION

Of central importance in both toxicology and biochemistry is the relationship between the nature and degree of consequent toxic effects of toxicant and the defense mechanism which an insect exhibits to tolerate such effects. In view of these characteristic considerations, the experimentation of the present investigation is dealing with the toxicological and biochemical evaluation of resistance phenomena to insecticides in insects.

4.1. Toxicolgical studies:

Data shown in Tables (1, 2, 3, 4, 5& 6) and graphically presented in Figs. (1, 2, 3, 4, 5& 6) revealed the susceptibility of four different strains in response to the three tested insecticides.

Based on LC_{50} values, the field strain (F-) tolerated moderately the tested insecticides, recording LC_{50} of 42.02, 82.12, and 79.61 ppm for Spinosad (Sps). Chlorpyrifos (Cpf) and Methoxyfenoziode (Mfz) respectively compared with 11.88, 17.48 and 36.33ppm against the standard laboratory strain, and exhibiting tolerance ratios of 3.53, 2.26 and 4.52, respectively.

On the other hand, both strains(R and S) distinguished through discriminative doses exhibited responses to the tested insecticides more variable than that of either field or laboratory strains. In this respect the R genotype strains obtained from the isolated egg-masses (families) exhibited LC_{50} of 111.79, 233.68 and 271.01 for Sps, Cpf and Mfz, respectively and results in resistance ratios of 2.66, 3.30 and 2.93 fold relative to field strain versus 9.4, 13.36, 7.45 and fold when compared to laboratory strain, for the same compounds respectively.

As for S genotype strains obtained from the isolated egg-masses (families), the obtained LC_{50} values were remarkably lower than those of field strain, recording 17.01, 64.43 and 45.05 ppm for the former versus 42.02, 79.61, and 82.12 ppm for the later and resulted in resistance ratios of 0.40, 0.81 and 0.55 folds relative to field strain versus 1.43, 3.62 and1.24 folds when compared to laboratory strain for Sps, Cpf and Mfz, respectively.

Summarizing the forementioned results, it could be concluded that, using the discriminating doses through such approach of rearing isolated egg-masses (families) could save time and costs in easily producing resistant or/and susceptible strains from any field population. However, such procedure can help in carrying biochemical studies for the already resistance genotypes distributed in a field population and thus can be accordingly linking the enzymatic activity with the genotype specific for resistance to an insecticide early in the season before build up of resistance to such insecticide.

Table(1): Susceptibility of different strains in response to spinosad after 24 hr.

Line name	LC50	Lower limit	Upper limit	RR	Slope	LC90
L-Strain	40.477			1	0.867	1218.591
S-Strain	173.618.			4.289	1.237	1885.728
R-Strain	126.855	51.032	214.726	3.134	1.183	1537.425

Resistance Ratio (RR) compared with L-Strain

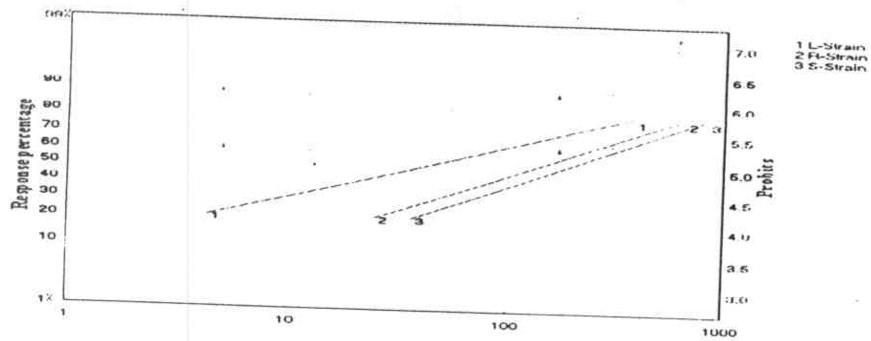

Fig.(1): Ldp-lines of different strains in response to spinosad after 24hr.

Table(2): Susceptibility of different strains in response to spinosad after 48 hr.

Line name	LC50	Lower limit	Upper limit	RR	Slope	LC90
L-Strain	11.876	6.458	19.011	1	1.524	82.328
S-Strain	17.01			1.4323	0.223	9.71E+03
F-Strain	42.02			3.538228	0.348	1.04E+06
R-Strain	111.79			9.413102	1.096	1030.706

Resistance Ratio (RR) compared with L-Strain

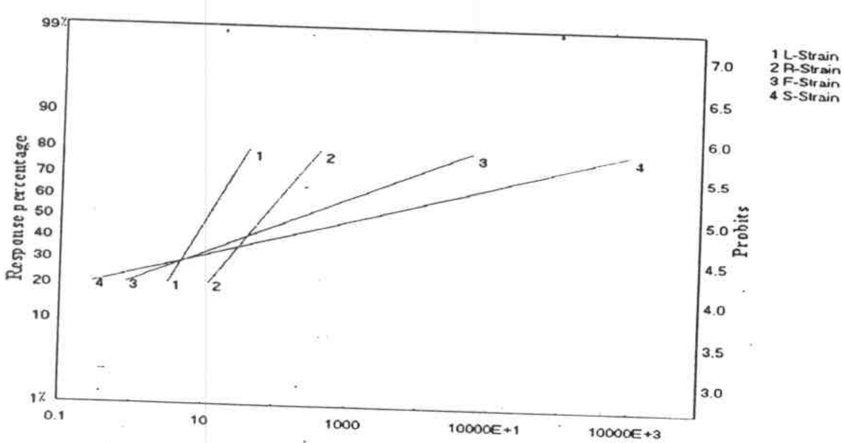

Fig.(2): Ldp-lines of different strains in response to spinosad after 48hr.

Table(5): Susceptibility of different strains in response to methoxyfenozide after 24 hr.

Line name	LC50	Lower limit	Upper limit	RR	Slope	LC90
L-Strain	52.413			1	1.673	305.826
S-Strain	67.898			1.295	0.801	2704.979
F-Strain	234.349	138.656	1266.196	4.471	1.645	1408.968
R-Strain	706.757			13.48438	0.685	52438.85

Resistance Ratio (RR) compared with S-Strain

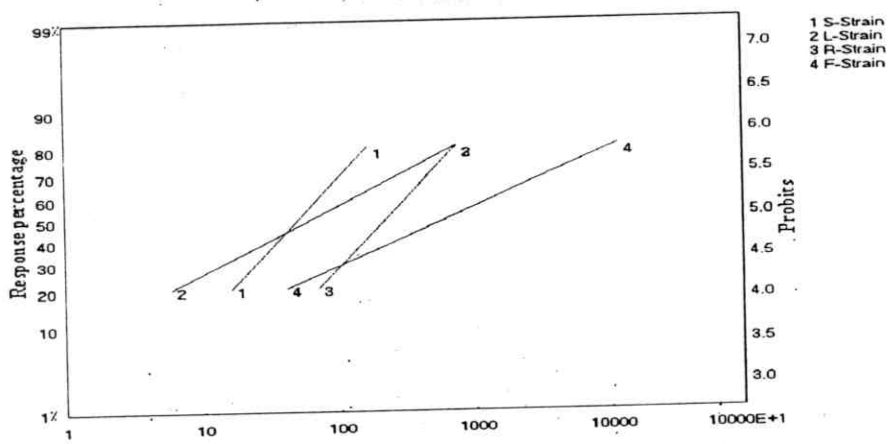

Fig.(5): Ldp-lines of different strains in response to methoxyfenozide after 24hr.

Table(6): Susceptibility of different strains in response to methoxyfenozide after 48 hr.

Line name	LC50	Lower limit	Upper limit	RR	Slope	LC90
L-Strain	36.33	2.883	20.459	1	0.642	693.704
S-Strain	45.045			1.239884	0.804	1162.929
F-Strain	82.12			2.260391	0.826	2448.584
R-Strain	271.01	130.6	725.689	7.459675	1.285	1004.943

Resistance Ratio (RR) compared with L-Strain

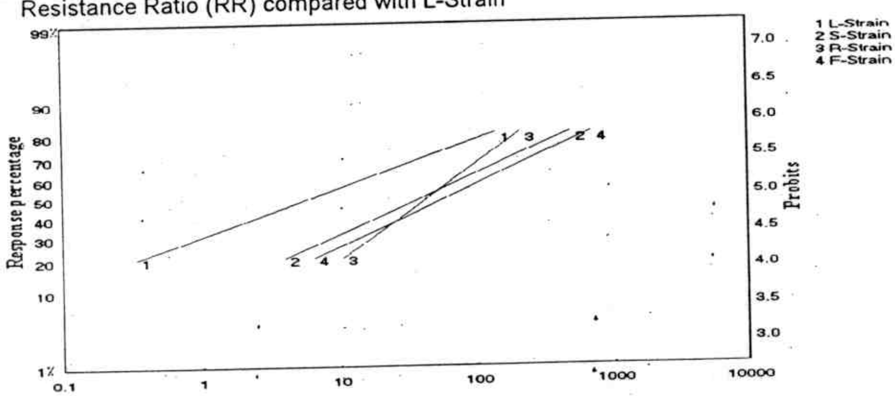

Fig.(6): Ldp-lines of different strains in response to methoxyfenozide after 48hr.

Table(3): Susceptibility of different strains in response to chlorpyrifos after 24 hr.

Line name	LC50	Lower limit	Upper limit	RR	Slope	LC90
L-Strain	24.083	19.45	29.263	1	4.363	47.367
S-Strain	64.535			2.679691	1.129	881.719
F-Strain	403.267	317.961	749.754	16.74488	3.353	972.279
R-Strain	1367.635			56.7884	0.8	54660.56

Resistance Ratio (RR) compared with L-Strain

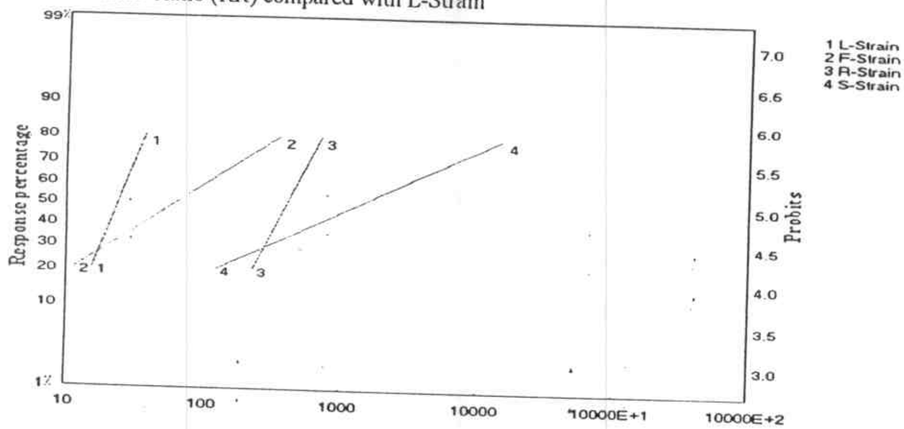

Fig.(3): Ldp-lines of different strains in response to chorpyrifos after 24hr.

Table(4): Susceptibility of different strains in response to chlorpyrifos after 48 hr.

Line name	LC50	Lower limit	Upper limit	RR	Slope	LC90
L-Strain	17.479	12.643	23.655	1	2.208	66.53
S-Strain	64.43			3.686138	0.362	488.681
F-Strain	79.61	19.394	63.646	4.554608	1.094	917.961
R-Strain	233.68	195.195	287.943	13.36919	2.409	1.53E+06

Resistance Ratio (RR) compared with L-Strain

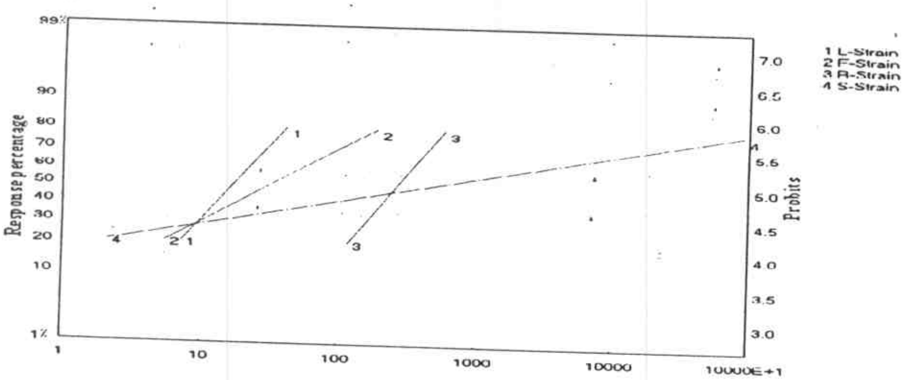

Fig.(4): Ldp-lines of different strains in response to chorpyrifos after 48hr.

4.2. Biochemical studies:

4.2.1. The main components:

The main metabolites (total proteins, total lipids and total carbohydrates) are major biochemical components necessary for an organism to develop, grow and perform its vital activities. Thus it may be of interest to study the differences in the contents of main metabolites among susceptible, resistant, field and laboratory strains, thus we can answer the question whether there is a relation between main metabolites and resistance.

4.2.1.1. Total protein:

Tables (7, 8 and 9) & fig. (7) revealed the relation between susceptibility level (LC_{50}) to three insecticides, Sps. Cpf & Mfz and total protein content in the homogenate of 4*th* instar larvae of four different strains of *S. littoralis*.

From data obtained for the mean values of total protein in the four strains with different resistance levels to Spinosad, the total protein of Sps- R, F-.and S-strains were significantly lower than that of L- Strain. , the lowest value was significantly recorded in Sps-R strain (40.84% of L-strain).

A negative correlation (-0.9476) was obtained between resistance levels to Sps and total protein. The same trend was observed in the case of Cpf and Mfz-resistant strains, where the percentages values were 51.03 % and 48.24% of L-strains while the correlation coefficients were -0.9135 and -0.8476 respectively.

Although the reports about the relation between protein content and resistance to insecticides are rare, the findings could be supported and interpreted in the light of many previous works. **Saha *et al.*, (1986)** reported that total protein decreased significantly in *Chrysocoris stollii* following treatment with a juvenile hormone analogue or ecdysterone.

Table (7): Relation between total protein and Spinosad resistance level in four different strains of *S.littolaris.*

	LC_{50}	RR (folds)	Mean ± SD *	% of L-Strain	Cor.Coef
L-strain	11.88	1	4.767±0.035a	100	-0.9476
S-strain	17.01	1.43	3.864±0.01b	81.0573	
F-strain	42.02	3.53	3.233±0.011c	67.8204	
R-strain	111.79	9.4	1.947±0.1d	40.8433	

*Total proteins = mg/larva
LSD.05 = 0.1007

Table (8): Relation between total protein and Chlorpyrifos resistance level in four different strains of *S.littolaris.*

	LC_{50}	RR (fold)	Mean ± SD *	% of L-Strain	Cor.Coef
L-strain	17.48	1	4.767±0.035a	100	-0.9135
S-strain	64.43	3.68	3.803±0.1b	79.7776	
F-strain	79.61	4.55	3.233±0.011c	67.8204	
R-strain	233.68	13.6	2.433±0.10d	51.0384	

*Total proteins = mg/larva
LSD.05 = 0.176812

Table (9): Relation between protein content and Methoxyfenoziode resistance level in four different strains of *S.littolaris.*

	LC_{50}	RR (folds)	Mean ± SD *	% of L-Strain	Cor.Coef
L-strain	36.33	1	4.767±0.035a	100	-0.8476
S-strain	45.05	1.24	3.597±0.1b	75.4563	
F-strain	82.12	2.26	3.233±0.011c	67.8204	
R-strain	271.01	7.54	2.3±0.1112d	48.2484	

*Total proteins = mg/larva
LSD.05 = 0.176812

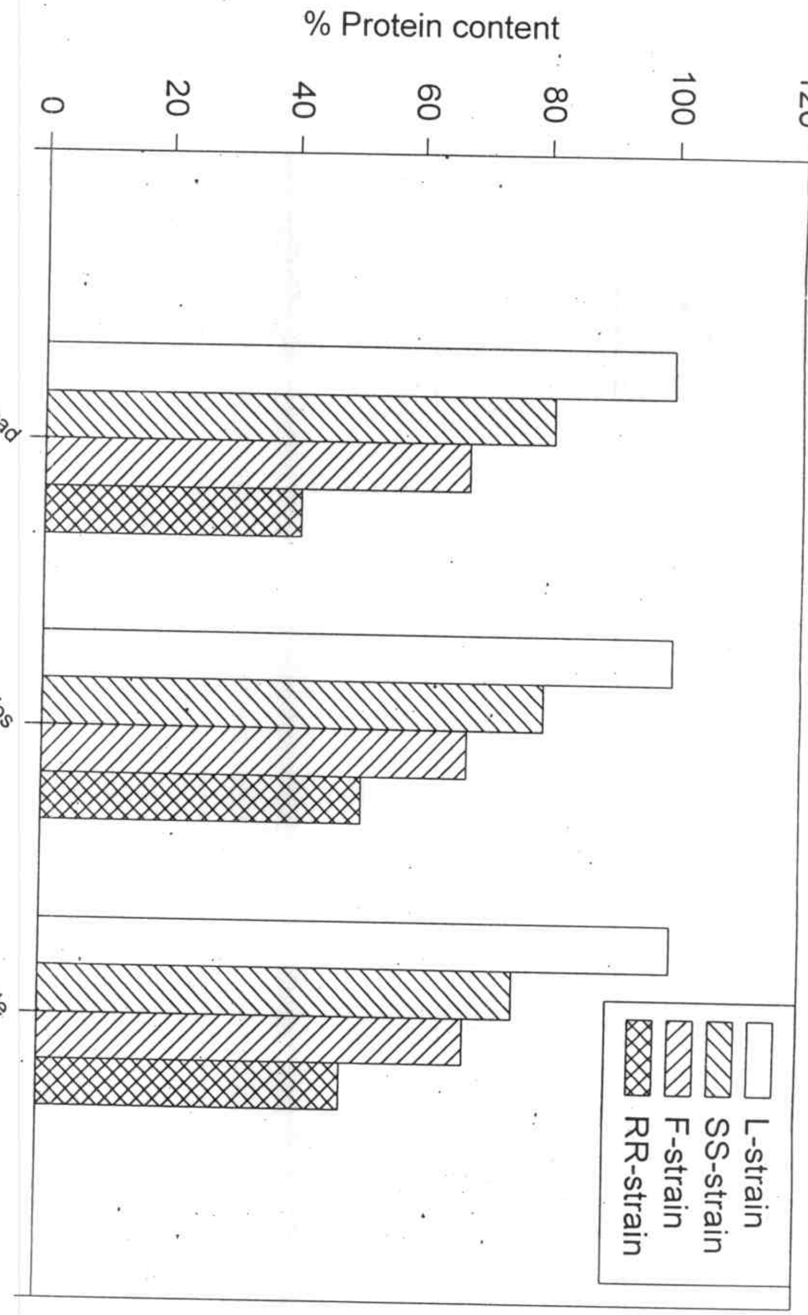

Fig.7): Relation between total protein and resistance levels to three insecticides in four different strains of *S.littoralis*

Ahmed and Mostafa (1989) also found that two benzoylphenyl urea derivatives; reduced the total protein in *S.littoralis*

The reduction in protein content may also be due to the production of amino acid through the protein metabolism. this hypothesis could be supported by the finding of **Nath *et al.*, 1997** that *Bombyx mori* exposed to sublethal doses of fenitrothion and ethion The content indicated a total protein depletion followed by a concomitant increase in accumulation of free amino acids. The activity of proteinases also increased at the same time

The inhibition of total proteins synthesis as a result of IGR's and abamectin treatment may be due to the effect of these compounds on the enzyme of DNA synthesis (**Mitlin *et al.*, 1977** and **Deloach *et al.*, 1981**). **Ferkovich *et al.* (1981)** supported the concept that, in *G. mellonella*, 20-hydroxyecdysone stimulated chitin production requires the synthesis of RNA and protein. They added that there were new proteins synthesized by imaginal wing discs incubated with 20- hydroxyecdysone. The function of which is unknown but they could include cuticle structural proteins, chitin synthetase or the activator of that enzyme. Thus, the overall picture gives the inhibition of proteins synthesis after treatment a significant value.

The above mentioned reports in addition to the present results could led to the conclusion that the depletion of protein content in the resistant and field strains may be due to the lack or inhibition of enzymes responsible of protein synthesis.

4.2.1.2. Total lipids:

Tables (10, 11 and 12) & fig. (8) showed the relation between resistance level (LC_{50}) to three insecticides, Sps, Cpf & Mfz and total lipids content in the homogenate of 4*th* instar larvae of four different strains of *S. littoralis*.

From data obtained for the mean values of total lipids in the four strains with different resistance levels to Spinosad, the Sps- R, F-.and S-strains were higher than that of L-strain. The highest value was recorded in Sps-R strain (217.56 % of L-strain).

When a correlation coefficient was carried out between resistance level to Sps and total lipids, a positive correlation was obtained (0.8263). The same trend was observed in the case of Cpf and Mfz resistant strains, where the highest values were recorded in Cpf-R and Mfz-R strains with percentages 224.89 % and 302.92 % of L-strains, while the correlation coefficients were 0.7740 and 0.8830 respectively.

The observed positive correlation coefficient, is consistent with those reported by **Arjumand (1988); Babu *et al.* (1994) Barwal and Kalra; (1988) Patel *et al.* (1996).** According to **Patel *et al.* (1996)**, the selected monocrotophos-resistant strain of (*Chrysopa scelestes*) showed greater amounts of total lipids, polar lipids and non-polar lipids as compared to susceptible strain. **Barwal and Kalra (1988)** also mentioned that the lindane-resistant strain had a higher total lipid and mono-di-glyceride content than susceptible strains. It was concluded that lindane selection results in alterations in the nature of phospholipids fatty acids.

Table (10): Relation between total lipids and Spinosad resistance level in four different strains of *S.littolaris.*

	LC_{50}	RR (folds)	Mean ± SD *	% of L-Strain	Cor.Coef
L-strain	11.88	1	0.683±0.02b	100	0.8263
S-strain	17.01	1.43	0.769±0.01b	112.5915	
F-strain	42.02	3.53	1.414±0.126a	207.0278	
R-strain	111.79	9.4	1.486±0.1a	217.5695	

*Total lipids = mg/larva
LSD.05 = 0.2289

Table (11): Relation between total lipids and Chlorpyrifos resistance level in four different strains of *S:littolaris.*

	LC_{50}	RR (folds)	Mean ± SD *	% of L-Strain	Cor.Coef
L-strain	17.48	1	0.683±0.02c	100	0.7740
S-strain	64.43	3.68	1.236±0.1b	180.9663	
F-strain	79.61	4.55	1.414±0.126ab	207.0278	
R-strain	233.68	13.36	1.536±0.0320a	224.8902	

*Total lipids = mg/larva
LSD.05 = 0.176812

Table (12):Relation between total lipids and Methoxyfenoziode resistance level in four different strains of *S.littolaris.*

	LC_{50}	RR (folds)	Mean ± SD *	% of L-Strain	Cor.Coef
L-strain	36.33	1	0.683±0.02c	100	0.8830
S-strain	45.05	1.24	1.317±0.08b	192.8258	
F-strain	82.12	2.26	1.414±0.126b	207.0278	
R-strain	271.01	7.45	2.069±0.126a	302.9283	

*Total lipids = mg/larva
LSD.05 = 0.1630

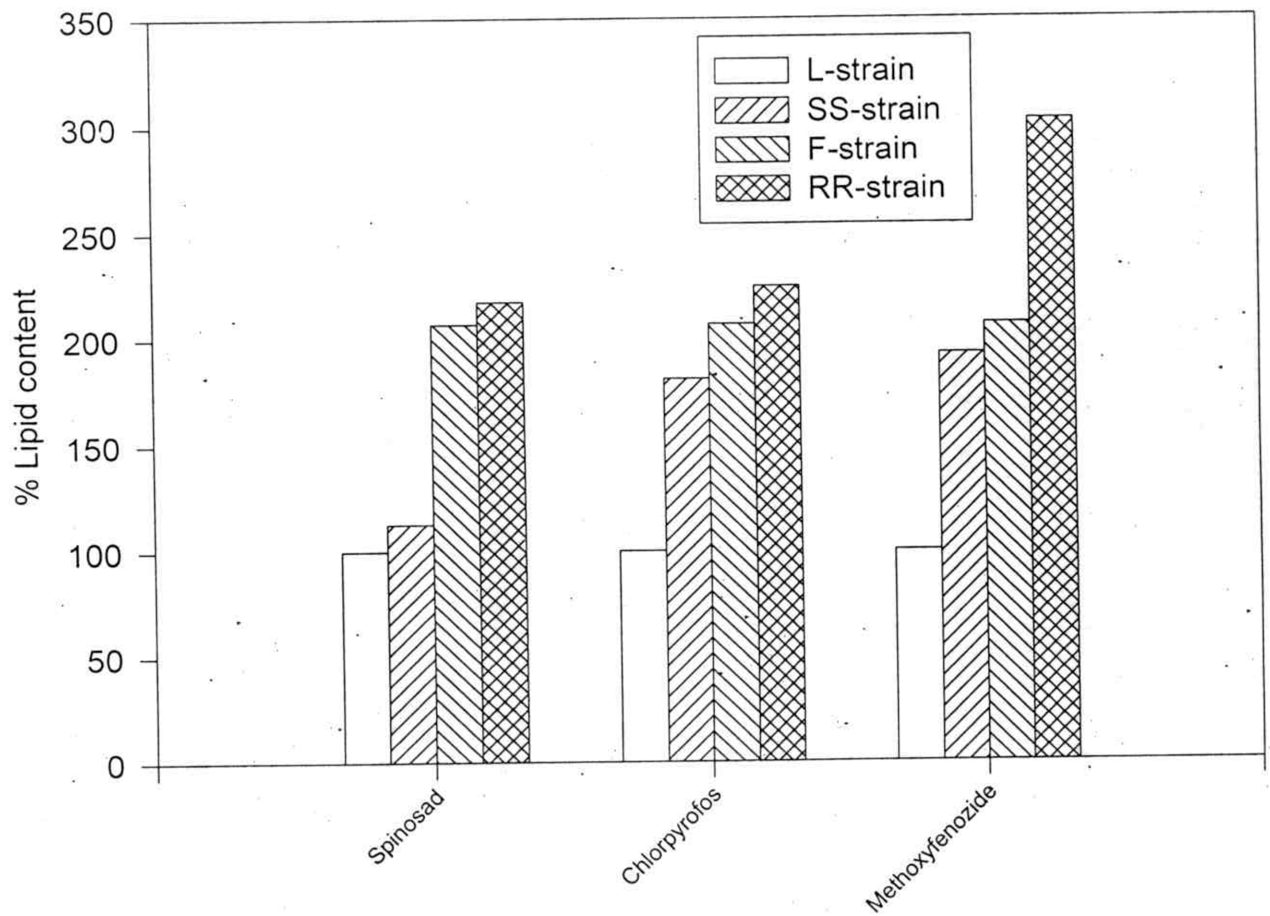

Fig.(2): Relation between total lipids and resistance levels to three insecticides in four different strains of *S.littoralis*

4.2.1.3. Total Carbohydrates:

Tables (13, 14 and 15) & fig. (9) showed the relation between resistance level (LC_{50}) to three insecticides; Sps; Cpf& Mfz and total carbohydrates content in the homogenate of 4*th* instar larvae of four different strains of *S. littoralis*.

It is clear from the data obtained that the carbohydrate content was significantly higher in the Sps-R(157.87%) and F- strains(142.77%) than that in S-strain (104.03%) and L-strain (100%) but the difference is non significant between S- and L-strain. The same trend was observed in the case of Cpf and Mfz, where the maximum carbohydrate titre was recorded in R-strains (160.67 and 176.62% of L-strains) respectively. It should be noticed also that there were significant differences between the four strains in both of the two compounds.

When a Cor.Coef. was carried out between resistance level. and total carbohydrates, positive correlations were obtained (0.9002, 0.8887 and 0.8972) for Sps, Cpf and Mfz respectively. The positive Cor.Coef. observed in the present study could be explained on the light of several results obtained by **Lohar & Wright (1990) and Saha *et al.* (1986). Lohar and Wright (1990)** found that sub-lethal doses of malathion significantly increased the total carbohydrates in the haemolymph of *Tenebrio molito* – R.

Table (13): Relation between total carbohydrates and Spinosad resistance level in four different strains of *S.littolaris*.

	LC_{50}	RR (folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	11.88	1	1.536 ± 0.0335c	100.0000	0.9002
S-strain	17.01	1.43	1.598 ± 0.0191c	104.0365	
F-strain	42.02	3.53	2.193 ±0.0639 b	142.7734	
R-strain	111.79	9.4	2.425 ±0.0065 a	157.8776	

*Total carbohydrates = mg/larva
LSD.05 = 0.0705

Table (14): Relation between total carbohydrates and Chlorpyrifos resistance level in four different strains of *S.littolaris*.

	LC_{50}	RR (folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	17.48	1	1.536 ± 0.0335d	100.0000	0.8887
S-strain	64.43	3.68	1.956 ± 0.074 c	127.3438	
F-strain	79.61	4.55	2.193 ± 0.0639 b	142.7734	
R-strain	233.68	13.36	2.468 ± 0.058 a	160.6771	

*Total carbohydrates = mg/larva
LSD.05 = 0.0395

Table (15): Relation between total carbohydrates and Methoxyfenoziode resistance level in four different strains of *S.littolaris**

	LC_{50}	RR (folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	36.33	1	1.536 ± 0.0335 d	100.0000	0.8972
S-strain	45.05	1.24	1.997 ± 0.0051c	130.0130	
F-strain	82.12	2.26	2.193 ± 0.0639 b	142.7734	
R-strain	271.01	7.45	2.713 ± 0.0152 a	176.6276	

Total carbohydrates = mg/larva
LSD.05 = 0.2679

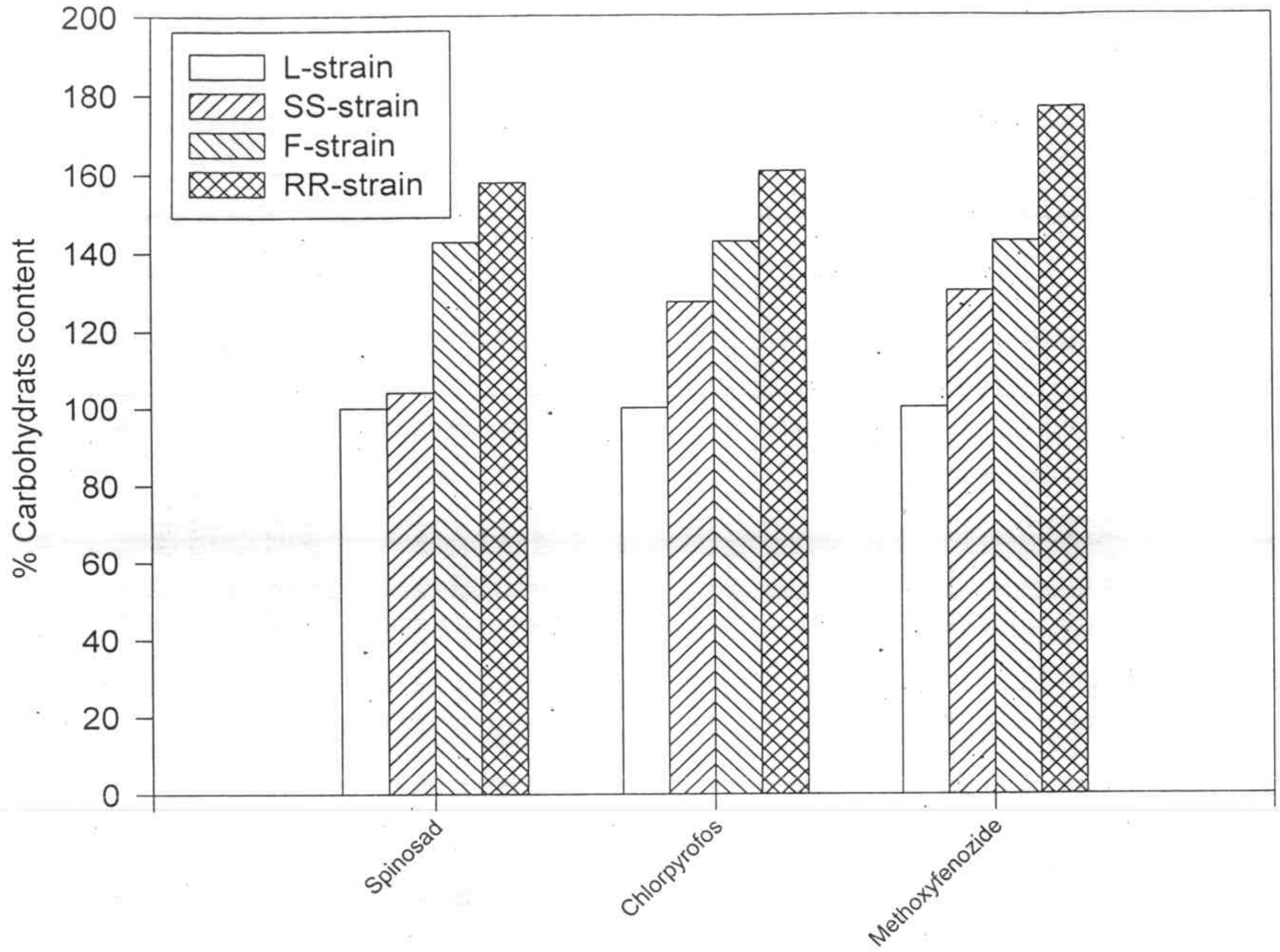

Fig.(3): Relation between total carbohydrates and resistance levels to three insecticides in four different strains of *S.littoralis*.

The increased level of carbohydrates was correlated with a release of hormones which control carbohydrate metabolism in the insect. Also **Saha *et al.* (1986)** found that juvenile hormone analogue and ecdysterone at a selective dosage, total carbohydrate, increased significantly in *Chrysocoris stollii*. The increase in carbohydrates level after treatment of nymphs of desert locust with CFA was also found by **El-Gammal *et al.* (1993)**. This can be interpreted as chitin synthesis in treated larvae was lower than control. It is known that carbohydrates provides glucose and trehalose for chitin synthesis, and since chitin synthesis consumes large amount of carbohydrates viz glycogen as have already been mentioned, one would expect lower level of carbohydrates in control larvae, where their chitin synthesis goes on without affection..

So, the high titre of carbohydrate recorded in the R- and F-strains could be explained by the release of hormones which control carbohydrate metabolism in the insect.

4.2.2. Carbohydrate hydrolyzing enzymes :

Carbohydrates are very efficiently utilized by insects and most species derive the main part of their nourishment from these nutrients. The utilization of these nutrients depends on the digestive enzymes; amylase, trehalase, invertase.This work concerned with studies on invertase, trehalase and amylase, activities of *S. littoralis* Laboratory, field, susceptible and resistant strains for three different insecticides.

4.2.2.1.Invertase enzyme activity:

Tables (16, 17 and 18) & fig. (10) showed the relation between the susceptibility level (LC_{50}) to three insecticides, Sps, Cpf & Mfz and invertase activities in the homogenate of 4*th* instar larvae of four different strains of *S. littoralis*.

From data obtained for the mean values of invertase activities in the four strains with different resistance levels to Spinosad, the enzyme activity of R, F-.and S-strains were lower than that of L-strain. , the lowest activity was recorded in Sps-R strain as 65.47 % of L-strain.

When a correlation coefficient was carried out between resistance level to Sps and invertase activities, a negative correlation was obtained (- 0.8654). The same trend was observed in the case of Mfz resistant strains, where the lowest activity was recorded in Mfz-R strains with percentage 56.15 % of L-strains, while the correlation coefficients was - 0.6437. On the other hand a different pattern was observed in the case of Chlorpyrifos, where a low positive correlation coefficient was recorded (0.5243) and the invertase activity in Cps-R was significantly higher than L-strain with a percentage 119.44 5 of L-strain.

Table (16):Relation between invertase activity and Spinosad resistance level in four different strains of *S.littolaris*

	LC_{50}	RR (folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	11.88	1	4.6667 ± 0.3326 d	100	-0.8654
S-strain	17.01	1.43	4.25 ± 0.3354 c	91.0708	
F-strain	42.02	3.53	3.3611 ±0.0421 b	72.0231	
R-strain	111.79	9.4	3.0556 ± 0.0245a	65.4767	

Activity=μg. glucose liberated/larva/min
LSD.05 = 0.0939

Table (17):Relation between invertase activity and Chlorpyrifos resistance level in four different strains of *S.littolaris*

	LC_{50}	RR (folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	17.48	1	4.6667 ± 0.3326 c	100	0.5194
S-strain	64.43	3.68	4.9815 ±0.0137 b	106.7457	
F-strain	79.61	4.55	3.3611 ±0.0421 b	72.0231	
R-strain	233.68	13.36	5.5741± 0.01816 a	119.4441	

Activity=μg. glucose liberated/larva/min
LSD.05 = 0.0412.

Table (18): Relation between invertase activity and Methoxyfenoziode resistance level in four different strains of *S.littolaris*

	LC_{50}	RR (folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	36.33	1	4.6667 ± 0.3326 a	100	-0.6436
S-strain	45.05	1.24	2.9722 ± 0.0273 b	63.6895	
F-strain	82.12	2.26	3.3611 ± 0.0421 c	72.0231	
R-strain	271.01	7.54	2.6204 ± 0.0185d	56.1510	

Activity=μg. glucose liberated/larva/min
LSD.05 = 0.0501

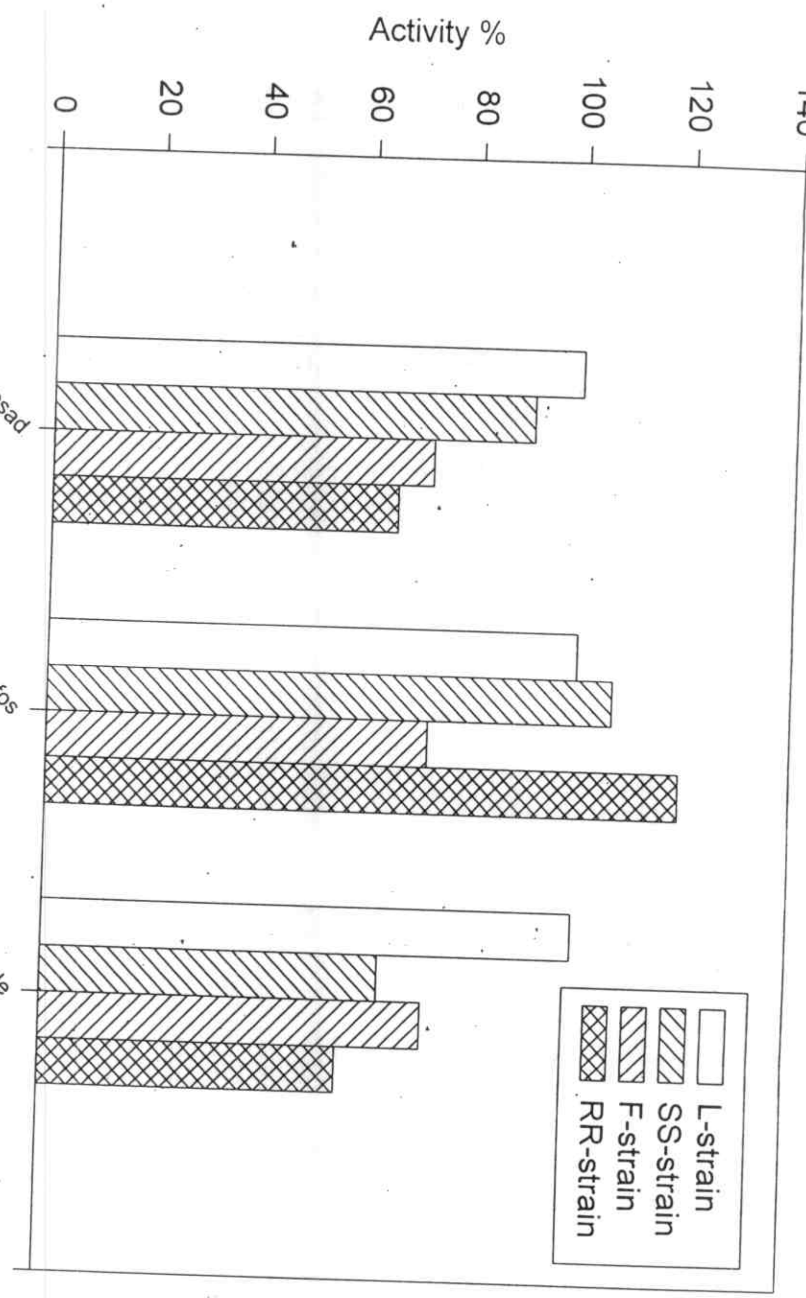

Fig.(4): Relation between invertase activity and resistance levels to three insecticides in four different strains of *S.littoralis*.

4.2.2.2. Trehalase enzyme activity:

Data in Table (19, 20 & 21) and Fig. (11) showed the pattern of relationship between resistance level (LC_{50}) to three insecticides, Sps, Cpf & Mfz and trehalase activities in the homogenate of 4*th* instar larvae of four different strains of *S. littoralis*.

The obtained results revealed high significant levels of enzymes in resistant strains when compared with L- strain as reference, the activities percentages were 141.76, 212.91 and 146.03. for Sps- R, Cpf-R and Mfz R, respectively.

The correlation coefficient between resistance level and trehalase activities, showed a positive correlation for the three compounds (0.7064, 0.8928 and 0.8060) for Sps, Cpf and Mfz respectively. However it could be easily observed that the highest correlation coefficient was recorded in the case of Cpf, suggesting a high relationship between resistance level to organophosphates and trehalase activity.

4.2.2.3. Amylase enzyme activity:

Tables (22, 23 and 24) & figs. (12) showed the relation between the susceptibility (LC_{50}) to three insecticides, Sps, Cpf & Mfz and amylase activities in the homogenate of 4*th* instar larvae of four different strains of *S. littoralis*. The obtained results revealed low significant levels of enzymes activities in R, F and S-strains when compared with L-strain as reference, the lowest activity was recorded in Sps, Cpf and Mfz-R strains as 53.49, 40.57 and 26.84% of L-strain.

When a correlation coefficient was carried out between resistance level to Sps and amylase activity, a negative correlation was obtained (-0.8419). The same rend was observed in the case of Cpf and Mfz resistant strains recording correlation coefficient of -0.8863 and 0.9352, respectively.

El-Saidy and Degheele (1990) studied the effect of diflubenzuron (DFB) on the digestive enzymes in *S.littoralis*.

Auda and Hedaya (1997) studied the effect of diflubenzuron (DFB) on growth. development and digestive enzymes. They found that DFB reduced amylase activity *in vivo* and the reduction was positively correlated with the DFB concentration. Whereas invertase, trehalase, and protease were not affected by the DFB treatment. The high activities of trehalase observed in the resistant strains in the present study, could be explained by the report of **Wyatt 1967**, they found that trehalase played a significant role in the supply of energy to the insect and the activity of trehalase might serve as an indicator of energy reserves resulting from availability of carbohydrate nutrients During moulting cycles, the trehalose-trehalase system is activated to generate glucose needed, probably, for chitin build-up in the newly synthesized cuticle **(Candy and Kilby, 1962)**. It is well known that in insects, trehalase degrades the disaccharide trehalose to glucose for internal energy supply and generates. (during moulting) glucose needed for chitin build-up.

Table (19): Relation between trehalase activity and Spinosad resistance level in four different strains of *S.littolaris*.

	LC_{50}	RR (folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	11.88	1	2.2222 ±0.0121 c	100	0.7064
S-strain	17.01	1.43	2.9071 ± 0.0567b	130.8208	
F-strain	42.02	3.53	2.9259 ± 0.0545 b	131.6668	
R-strain	111.79	9.4	3.1502 ± 0.0305 a	141.7604	

Activity=µg. glucose liberated/larva/min
LSD.05 = 0.0803

Table(20):Relation between trehalase activity and Chlorpyrifos resistance level in four different strains of *S.littolaris*.

	LC_{50}	RR (folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	17.48	1	2.2222 ±0.0121 c	100	0.8928
S-strain	64.43	3.68	2.463 ±0.04729 c	110.8361	
F-strain	79.61	4.55	2.9259 ± 0.0545 b	131.6668	
R-strain	233.68	13.36	4.7315 ± 0.0280 a	212. 9196	

Activity=µg. glucose liberated/larva/min
LSD.05 = 0.0645

Table (21): Relation between trehalase activity and Methoxyfenoziode resistance level in four different strains of *S.littolaris*.

	LC_{50}	RR (folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	36.33	1	2.2222 ±0.0121 c	100	0.8060
S-strain	45.05	1.24	2.75 ± 0351c	123.7512	
F-strain	82.12	2.26	2.9259 ± 0.0545 b	131.6668	
R-strain	271.01	7.54	3.2451 ± 0.0328 a	146.0310	

Activity=µg. glucose liberated/larva/min
LSD.05 = 0.0555

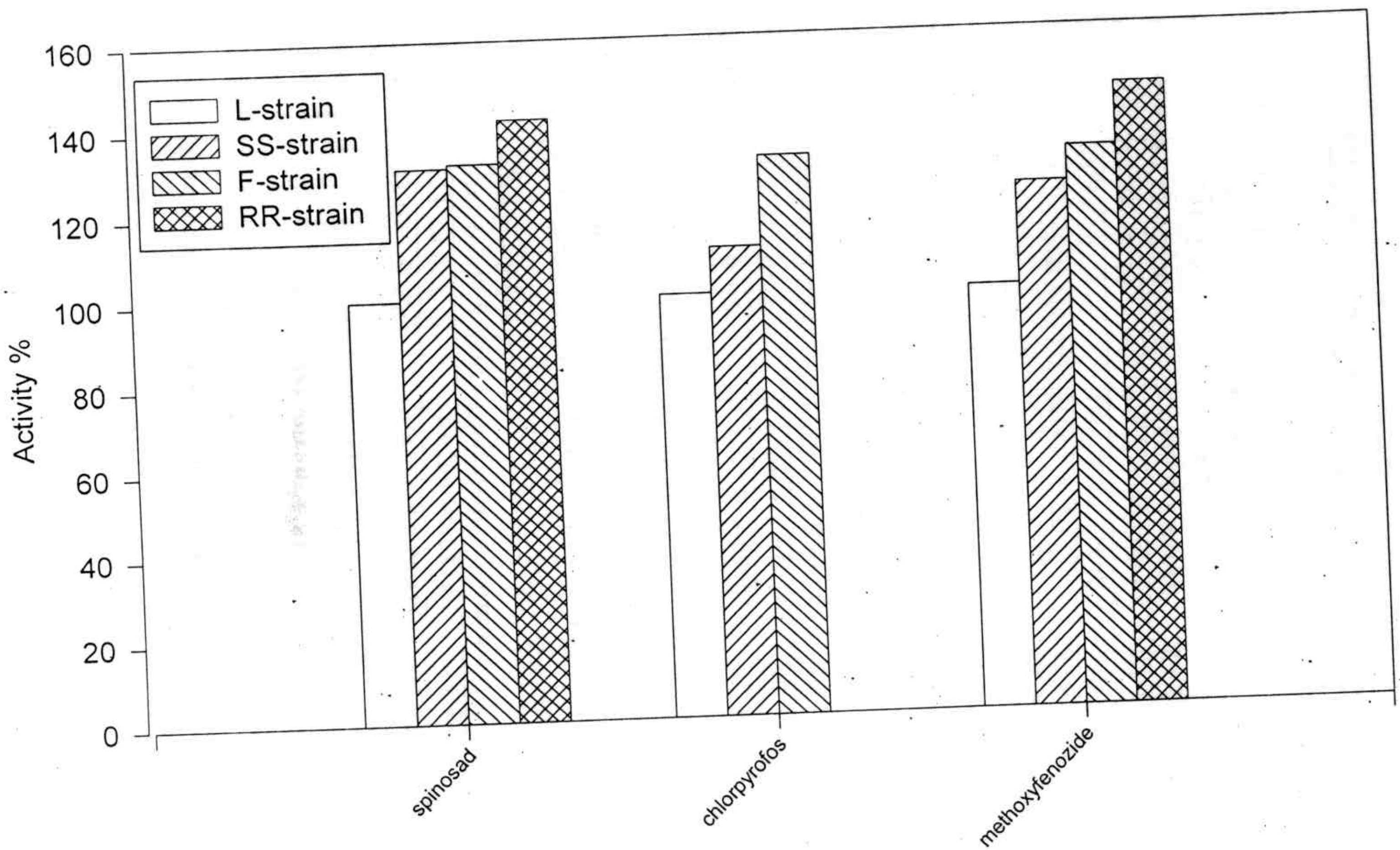

Fig.(5): Relation between trehalase activity and resistance levels to three insecticides in four different strains of *S.littolaris*

Table (22): Relation between amylase activity and Spinosad resistance level in four different strains of *S.littolaris.*

	LC_{50}	RR (folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	11.88	1	4.8981 ±0.03648 a	100	-0.8419
S-strain	17.01	1.43	4.1481± 0.0227b	84.6879	
F-strain	42.02	3.53	2.9815 ± 0.0101c	60.8705	
R-strain	111.79	9.4	2.6204 ±0.0216d	53.4983	

Activity=µg. glucose liberated/larva/min
LSD.05 = 0.0463

Table (23): Relation between amylase activity and Chlorpyrifos resistance level in four different strains of *S.littolaris.*

	LC_{50}	RR(folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	17.48	1	4.8981 ±0.03648 a	100	-0.8863
S-strain	64.43	3.68	4.713 ± 0.02611b	96.2210	
F-strain	79.61	4.55	2.9815 ± 0.0101c	60.8705	
R-strain	233.68	13.36	1.9875 ± 0.0111	40.5770	

Activity=µg. glucose liberated/larva/min
LSD.05 = 0.0536

Table (24): Relation between amylase activity and Methoxyfenoziode resistance level in four different strains of *S.littolaris.*

	LC_{50}	RR (folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	36.33	1	4.8981 ±0.03648.a	100	-0.9352
S-strain	45.05	1.24	4.752 ± 0.0266 b	97.0172	
F-strain	82.12	2.26	2.9815 ± 0.0101c	60.8705	
R-strain	271.01	7.54	1.3148 ± 0.2105d	26.8431	

Activity=µg. glucose liberated/larva/min
LSD.05 = 0.0113

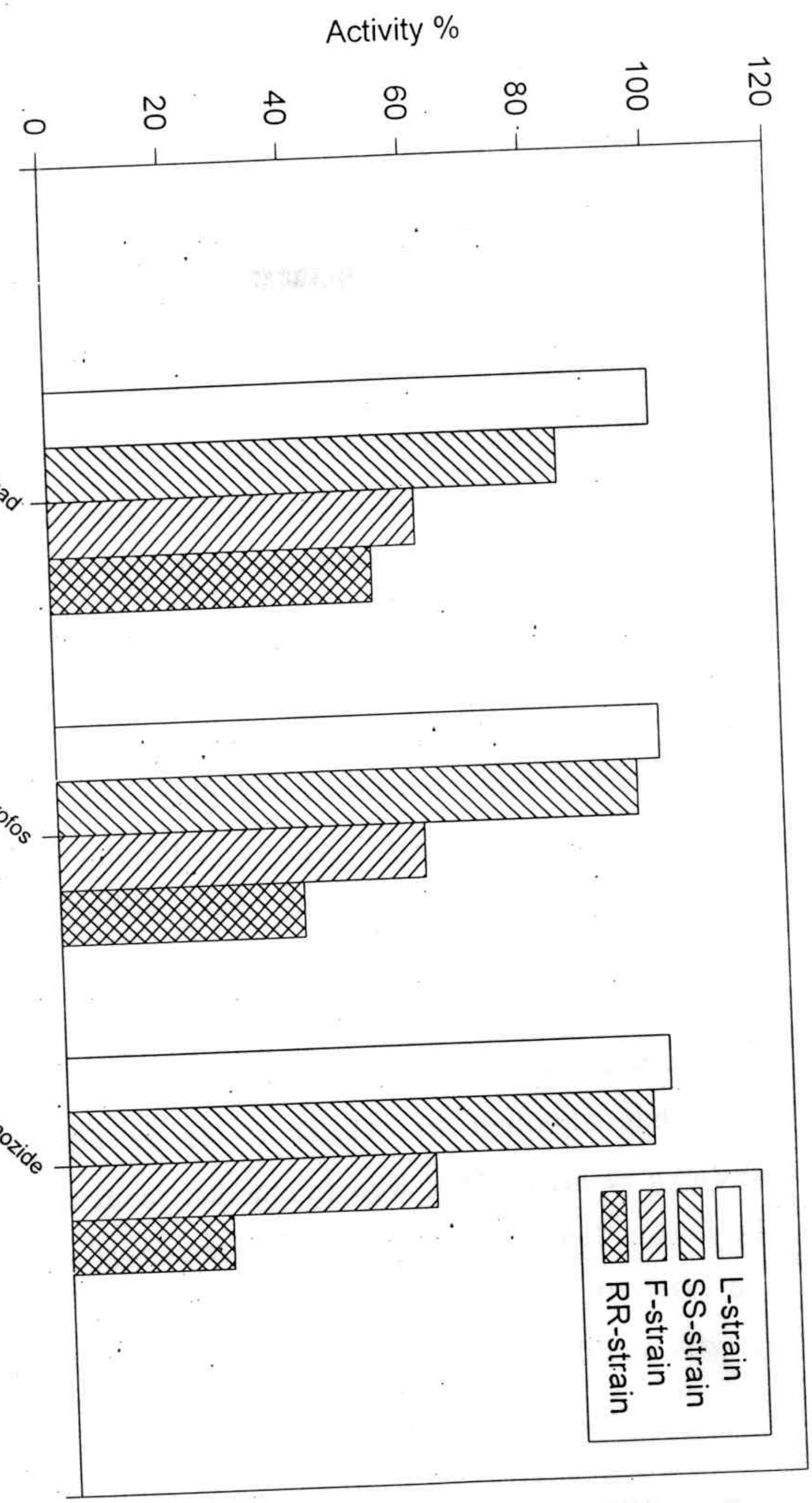

Fig.(6): Relation between amylase activity and resistance levels to three insecticides in four different strains of *S.littoralis.*

Study the mode of action of Dimilin revealed that this compound alter the cuticle composition of insect, especially that of chitin **(Ishaaya and Casida, 1974).** Also **Post and Vincent (1973)** found that the reduced level of chitin in the cuticle is due to the inhibition of biochemical processes leading to chitin formation.

In this concern **Ishaaya and Ascher (1977)** concluded that carbohydrates might be affected due to the reduced levels of amylase, trehalase and invertase of the 4*th* larval instar of *T. castaneum* treated with diflubenzuron. In contrary, **Saleem and Shakoori (1987)** recorded a reduction in trehalase and elevated amylase activity in the 6*th* larval instar of the same insect treated also with diflubenzuron. Similar to our findings **El Saidy and Degheele (1990)** found amylase activity was reduced, but neither invertase nor trehalase activity after treatment with diflubenzuron was affected.

On the other hand, the present data partially agreed with that obtained by **Abdel Hafez *et al.* (1993a)** and **Radwan *et al.* (1985)**, who found that repeated selection of the cotton leafworm larvae with Dentate (Dimilin+ Nudrin) and DC-702 (Dimilin+Dursban) increased the invertase activity and decreased the amylase and trehalase activity.

4.2.3. Transaminases activities:

4.2.3.1. Aspartic aminotransferase (ASAT):

Tables (25, 26 and 27) & fig. (13) showed the relation between susceptibility or and resistance level (RR) to three insecticides, Sps, Cpf & Mfz and ASAT activities in the homogenate of 4*th* instar larvae of four different strains of *S. littoralis*.

From data obtained of ASAT activities in the four strains, the activity in Spinosad- R, F-.and S-strains was lower than that of L-strain.

The lowest activity was recorded in Sps-R strain (30.37 % of L-strain). The same trend was observed in the case of Cpf and Mfz resistant strains, where the lowest activities were recorded in Cpf-R and Mfz-R strains recording percentages of 6.60% and 12.51 % of L-strains, respectively.

When a correlation coefficient was carried out between resistance level to the three insecticides and ASAT activities, a negative correlation was obtained (- 0.8457, - 0.8860 and - 0.7222) for Spinosad, Cpf and Mfz respectively.

The lowest ASAT activity and the highest negative correlation coefficient recorded in the Cpf-R, suggesting a high significant relationship between resistance to Cpf and ASAT activity.

Table (25):Relation between ASAT activity and Spinosad resistance level in four different strains of *S.littolaris*.

	LC_{50}	RR (folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	11.88	1	138.88 ± 2.5151a	100	-0.8457
S-strain	17.01	1.43	124.13 ± 3.7627 b	89.3793	
F-strain	42.02	3.53	55.021 ± 1.50276 c	39.6177	
R-strain	111.79	9.4	42.188 ± 1.2103 d	30.3773	

Activity=µg. pyruvate liberated/larva/min
LSD .05 = 4.6333

Table (26): Relation between ASAT activity and Chlorpyrifos resistance level in four different strains of *S.littolaris*

	LC_{50}	RR (folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	17.48	1	138.88 ± 2.5151a	100	-0.8860
S-strain	64.43	3.68	66 ±0.8074 b	47.5230	
F-strain	79.61	4.55	55.021 ± 1.50276 c	39.6177	
R-strain	233.68	13.36	9.167 ± 0.8568d	6.6007	

Activity=µg. pyruvate liberated/larva/min
LSD .05 = 2.2748

Table (27): Relation between ASAT activity and Methoxyfenoziode resistance level in four different strains of *S.littolaris*

	LC_{50}	RR (folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	36.33	1	138.88 ± 2.5151a	100	-0.7222
S-strain	45.05	1.24	55.917 ± 1.2669 b	40.2628	
F-strain	82.12	2.26	55.021 ± 1.50276 b	39.6177	
R-strain	271.01	7.54	17.375 ± 1.0221 c	12.5108	

Activity=µg. pyruvate liberated/larva/min
LSD .05 = 2.2439

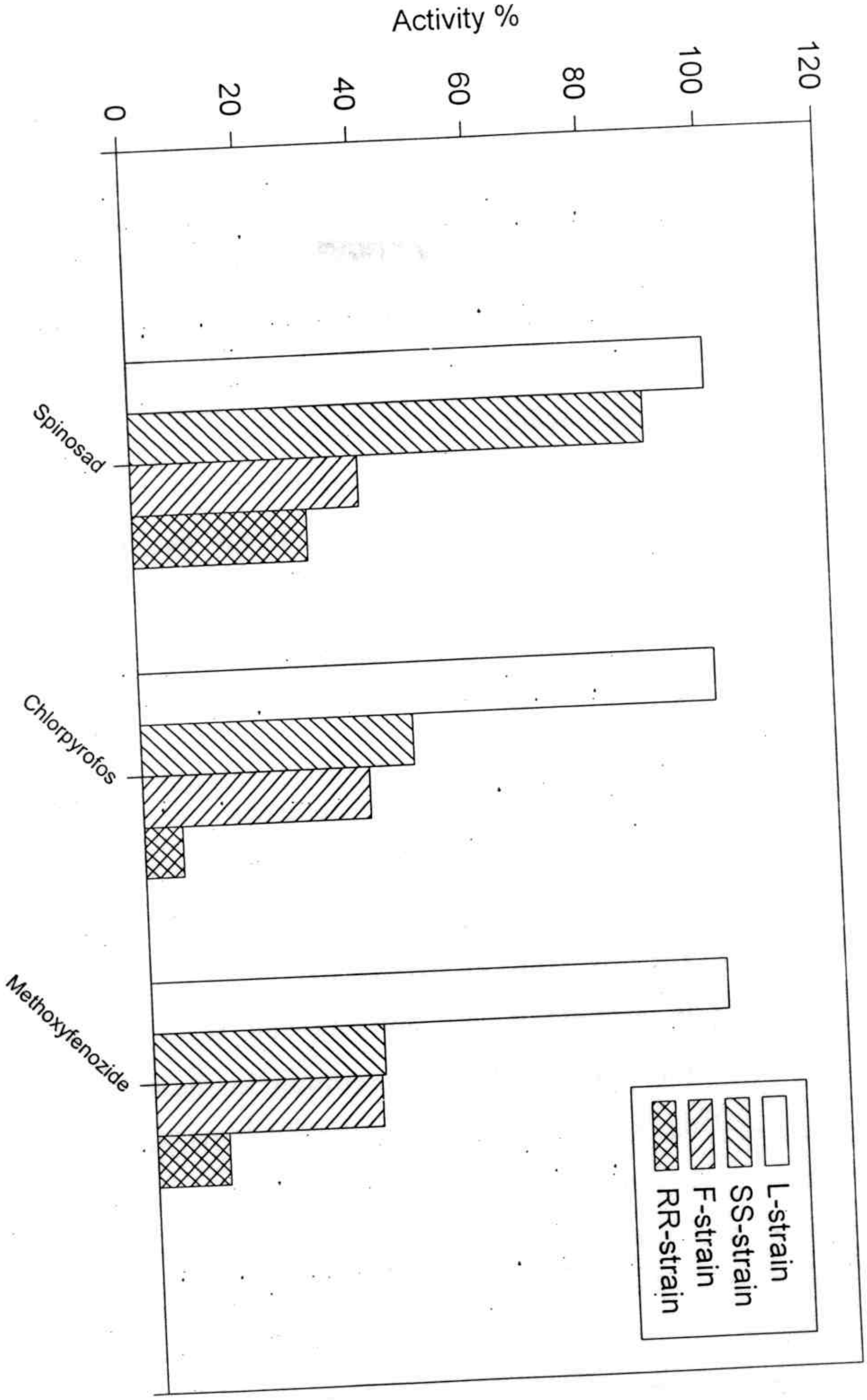

Fig.(7): Relation between AST activity and resistance levels to three insecticides in four different strains of *S.littoralis*

4.2.3.2. Alanine aminotransferase (ALAT):

Tables (28, 29 and 30) & Fig. (14) represented activities of ALAT in the homogenates of 4^{th} larval instar and its relation with resistance level (LC_{50}) to three insecticides, Sps; Cpf & Mfz. From data obtained for of ALAT activities in the four strains with different resistance levels to Spinosad, the Sps- R, F-.and S-strains were higher than that of L-strain.

The highest activity was recorded in Sps-R strain (252.73 % of L-strain). The same trend was observed in the case of Cpf and Mfz resistant strains, where the highest activities were recorded in Cpf-R and Mfz-R strains recording percentages of 283.64 % and 254.55 % of L-strains.

When a correlation coefficient was carried out between resistance level to the three insecticides and ALAT activities. a positive correlation was obtained (0.8502. 0.8235 and 0.6003) for Sps, Cpf and Mfz respectively.

The highest ALAT activity and the highest positive correlation coefficient were recorded in the three R-strains, suggesting a significant relationship between resistance to three compounds and ALAT activity. The reports about the role of transaminases in insecticide resistance are rare, but the low ASAT and high ALAT activity in resistant strains, may be discussed using studies of insecticides effect on transaminases activity. The elevation of transaminases in insects after exposure to insecticides was recorded by many authors, **Anan *et al.* (1993); Mostafa (1993) and Nath *et al.* (1997)** While on the other hand, an opposite trend was reported by other authors, **Abdel-Hafez *et al.* (1988); Ahmed *et al.* (1990); and Saleem and Shakoori (1996).** The elevation of transaminases in insects after exposure to insecticides was recorded by many authors, **Anan *et al.* (1993); Mostafa (1993) and Nath *et al.* (1997).**

While on the other hand, an opposite trend was reported by other authors, **Abdel-Hafez *et al.* (1988); Ahmed *et al.* (1990); Saleem and Shakoori (1996).**

The present findings along with previous reports could led to this explanation; since ASAT and ALAT produce the two amino acids, alanine and aspartic acid respectively, and these amino acids are needed for protein metabolism, then the titre of each enzyme depends on the level and kind of protein (structural or functional) required by the insect to tolerate the insecticide action. An increase in the activities of GPT (ALAT) and GOT (ASAT) paralleled the elevation of glutamate dehydrogenase activity in larvae of *B.mori* following exposure to sublethal concentrations of fenitrothion and ethion (**Nath *et al.*; 1997**). All changes clearly indicated a severe proteolysis and transamination of amino acids. **Abdel-Hafez *et al.* (1988)** also found a reduction in GOT and GPT activities in susceptible and profenofos resistant strain of *S. littoralis* as treated with different concentrations of the IGRs, diflubenzuron and triflumuron .

Table (28): Relation between ALAT activity and Spinosad resistance level in four different strains of *S.littolaris.*

	LC_{50}	RR (folds)	Mean ± SD*	%of L-Strain	Cor.Coef
L-strain	11.88	1	1.1458 ± 0.0268 a	100	0.8502
S-strain	17.01	1.43	1.4275 ± 0.3706 b	124.5854	
F-strain	42.02	3.53	2.6458 ± 0.0591c	230.9129	
R-strain	111.79	9.4	2.8958 ± 0.1070d	252.7317	

Activity=µg. pyruvate liberated/larva/min
LSD .05 = 0.1229

Table (29): Relation between ALAT activity and Chlorpyrifos resistance level in four different strains of *S.littolaris.*

	LC_{50}	RR (folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	17.48	1	1.1458 ± 0.0268 c	100	0.8235
S-strain	64.43	3.68	2.5625 ± 0.1058 b	223.6458	
F-strain	79.61	4.55	2.6458 ± 0.0591b	230.9129	
R-strain	233.68	13.36	3.25 ± 0.0651a	283.6446	

Activity=µg. pyruvate liberated/larva/min
LSD .05 = 0.1198

Table (30): Relation between ALAT activity and Methoxyfenoziode resistance level in four different strains of S.littolaris

	LC_{50}	RR (folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	36.33	1	1.1458 ± 0.0268 c	100	0.6003
S-strain	45.05	1.24	2.5625 ± 0.1179 b	223.6429	
F-strain	82.12	2.26	2.6458 ± 0.0591b	230.9129	
R-strain	271.01	7.54	2.9167 ± 0.0946 a	254.5558	

Activity=µg. pyruvate liberated/larva/min
LSD .05 = 0.1808

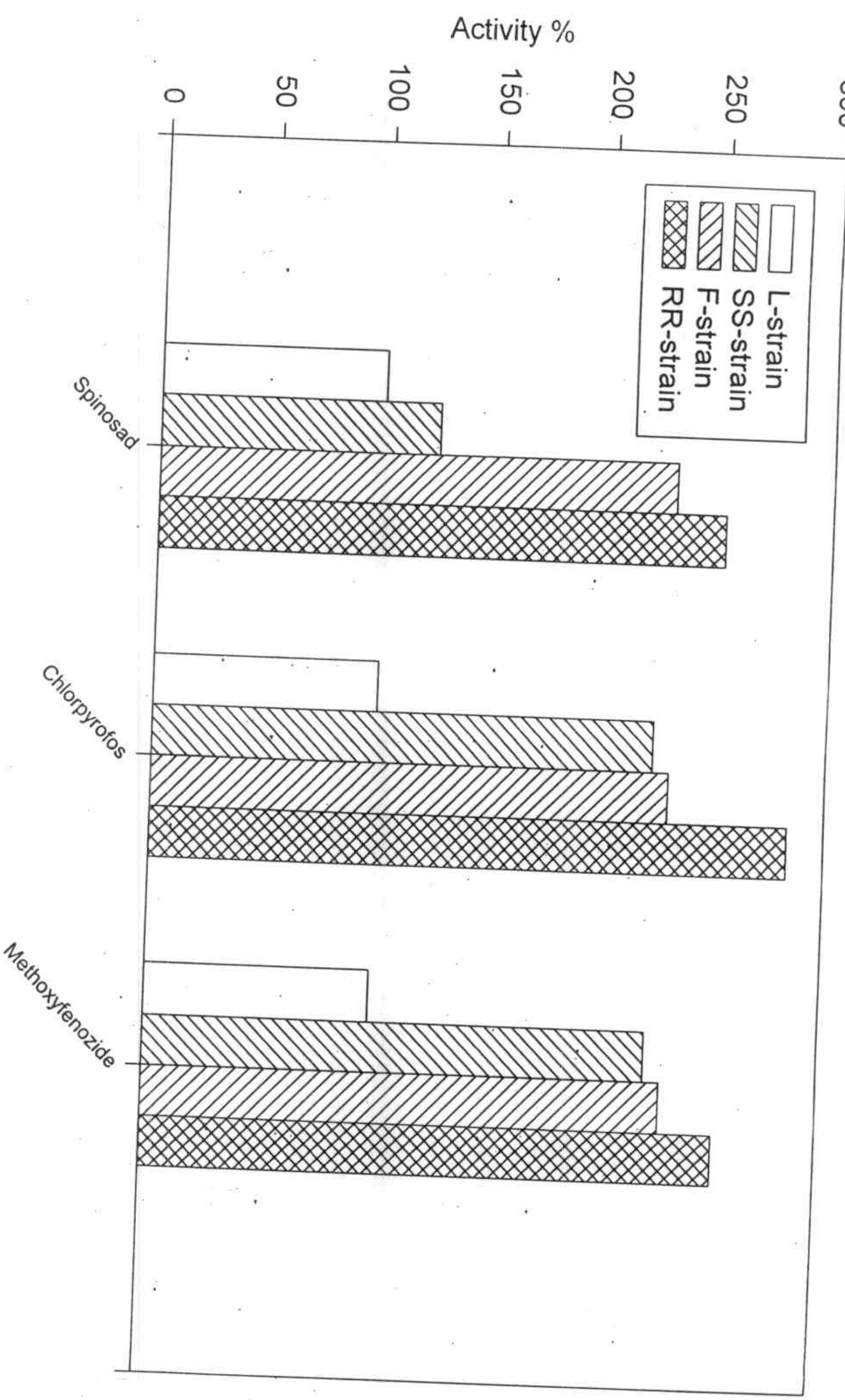

Fig.(8): Relation between ALT activity and resistance levels to three insecticides in four different strains of *S.littoralis*..

Ahmed *et al.* (1990) found a reduction in the activity of the enzyme GOT and GPT in *S. littoralis* larvae that were treated with chlorfluazuron. They suggested that GOT and GPT might be the site of action of chlorfluazuron. On the other hand **Anan *et al.* (1993)** found that treatment of 3rd instar larvae of *P. gossypiella* and *E. insulana* with different concentrations of the juvenoid, pyriproxyfen, increased the specific activities of transaminases. The increase of these enzymes was more pronounced and highly significant at higher concentrations of the juvenoid than at lower concentrations in the two tested species. **Mostafa (1993)** found that the three tested IGRs and also the plant extraction caused a significant increase in GOT activities in *S. littoralis* while GPT showed a different trend in larvae treated with pyriproxyfen, flufenoxuron and diflubenzuron. **Sokar (1995)** reported that hexaflumuron reduced GOT, of *S. littoralis* larvae; whereas the activities of the enzymes GPT were increased. **Saleem and Shakoori (1996)** reported that transaminases viz. alanine aminotransferase and aspartate aminotransferase activities were decreased initially and increased subsequently in *Tribolium castaneum* after treatment with Talcord 10EC.

4.2.4. Non-specific esterases:

4.2.4.1. α-Esterase:

Tables (31, 32 and 33) & Fig. (15) represented activities of α-Esterase in the homogenates of 4th larval instar and its relation with susceptibility (LC_{50}) or/and resistance level (RR) to three insecticides, Sps, Cpf & Mfz.

From data obtained of α-Esterase activities in the four strains with different resistance levels to Spinosad, the enzyme activity in Sps- R, F-.and S-strains were higher than that of L-strain and the highest enzyme activity was recorded in Sps-R strain (501.63 % of L-strain). The same trend was observed in the case of Cpf and Mfz resistant strains, where the highest activities were recorded in Cpf-R and Mfz-R strains with percentages 644.38 % and 490.44 % of L-strains. When a correlation coefficient was carried out between resistance level to the three insecticides and α-Esterase activities, a positive correlation was obtained (0.8771, 0.9285 and 0.7686) for Sps, Cpf and Mfz respectively.

Table (31): Relation between α-esterase activity and Spinosad resistance level in four different strains of *S.littolaris.*

	LC_{50}	RR (folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	11.88	1	14.13 ± 0.3277 d	100	0.8771
S-strain	17.01	1.43	18.096 ±0.1211 c	128.0679	
F-strain	42.02	3.53	59.215 ± 0.2766b	419.0729	
R-strain	111.79	9.4	70.881 ±0.1547a	501.6348	

Activity=α-naphthol liberated/larva/min
LSD .05 = 0.4440

Table (32): Relation between α-esterase activity and Chlorpyrifos resistance level in four different strains of *S.littolaris.*

	LC_{50}	RR (folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	17.48	1	14.13 ± 0.3277 d	100	0.9285
S-strain	64.43	3.68	50.581 ± 0.3069 c	357.9689	
F-strain	79.61	4.55	59.215 ± 0.2766b	419.0729	
R-strain	233.68	13.36	91.052 ± 0.7145a	644.3878	

Activity=α-naphthol liberated/larva/min
LSD .05 = 0.8360

Table (33): Relation between α-esterase activity and Methoxyfenoziode resistance level in four different strains of *S.littolaris*

	LC_{50}	RR (folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	36.33	1	14.13 ± 0.3277 d	100	0.7686
S-strain	45.05	1.24	38.993 ± 0.1278 c	275.9590	
F-strain	82.12	2.26	59.215 ± 0.2766b	419.0729	
R-strain	271.01	7.54	69.3 ± 0.1744 a	490.4459	

Activity=α-naphthol liberated/larva/min
LSD .05 = 0.4522

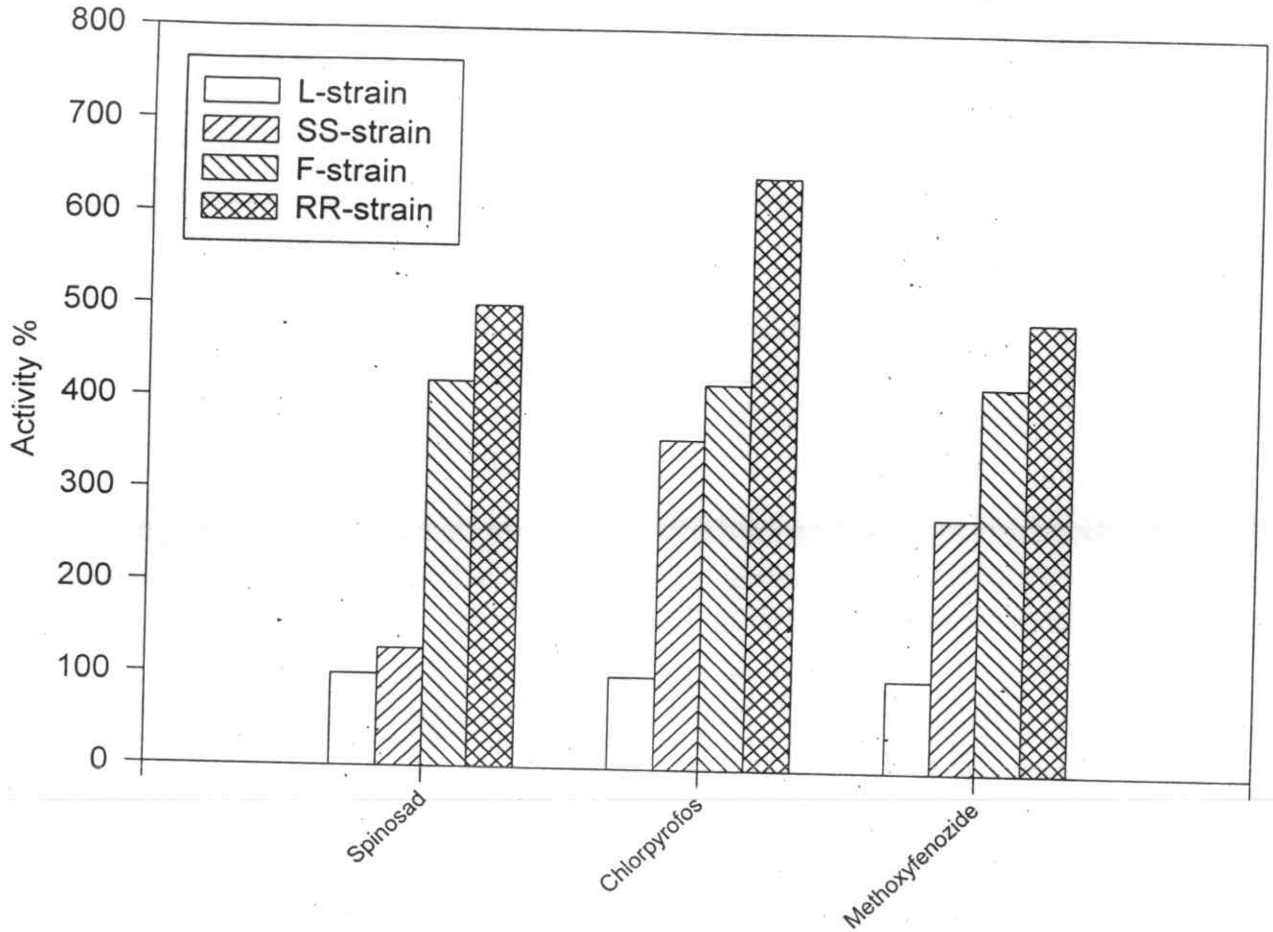

Fig.(9): Relation between Alpha-esterase activity and resistance levels to three insecticides level in four different strains of *S.littoralis*.

4.2.4.2. β-Esterase:

Tables (34, 35 and 36) & Fig. (16) represented activities of β-Esterase in the homogenates of 4th larval instar and its relation with susceptibility (LC_{50}) and/or (RR) resistance level to three insecticides, Sps, Cpf & Mfz.

From data obtained of β-Esterase activities in the four strains with different resistance levels to Spinosad, the activity in Sps- R, F-.and S- strains were higher than that of L-strain and the highest enzyme activity was recorded in Sps-R strain (416.34% of L-strain). The same trend was observed in the case of Cpf and Mfz resistant strains, where the highest activities were recorded in R-strain with percentages 516.89 % and 355.14 % of L-strains, respectively. When a correlation coefficient was carried out between resistance level to the three insecticides and β -Esterase activities, a positive correlation was obtained (0.9172, 0.9399 and 0.7326) for Sps, Cpf and Mfz respectively.

From the results of both α and β-Esterase, it is clearly obvious that the organophsphorus resistant strains had the highest that the organophsphorus resistant strains had the highest activity and in the same time the highest correlation coefficient

Table (34): Relation between β-esterase activity and Spinosad resistance level in four different strains of *S.littolaris.*

	LC_{50}	RR (folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	11.88	1	7.9778 ± 0.1051 d	100	0.9172
S-strain	17.01	1.43	12.726± 0.2725 c	159.5177	
F-strain	42.02	3.53	25.615 ±.2859 b	321.0785	
R-strain	111.79	9.4	33.215 ± 0.2347 a	416.3429	

Activity=α-naphthol liberated/larva/min
LSD .05 = 0.4438

Table (35): Relation between β-esterase activity and Chlorpyrifos resistance level in four different strains of *S.littolaris*

	LC_{50}	RR (folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	17.48	1	7.9778 ± 0.1051 d	100	0.9399
S-strain	64.43	3.68	10.704 ± 0.3703 c	134.1723	
F-strain	79.61	4.55	25.615 ±.2859 b	321.0785	
R-strain	233.68	13.36	41.237 ± 0.2481 a	516.8969	

Activity=β-naphthol liberated/larva/min
LSD .05 = 0.5074

Table (36): Relation between β-esterase activity and Methoxyfenoziode resistance level in four different strains of *S.littolaris.*

	LC_{50}	RR (folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	36.33	1	7.9778 ± 0.1051 d	100	0.7326
S-strain	45.05	1.24	17.881 ± 0.41146 c	224.1345	
F-strain	82.12	2.26	25.615 ±.2859 b	321.0785	
R-strain	271.01	7.54	28.333 ± 0.2733 a	355.1480	

Activity=β-naphthol liberated/larva/min
LSD .05 = 0.54639

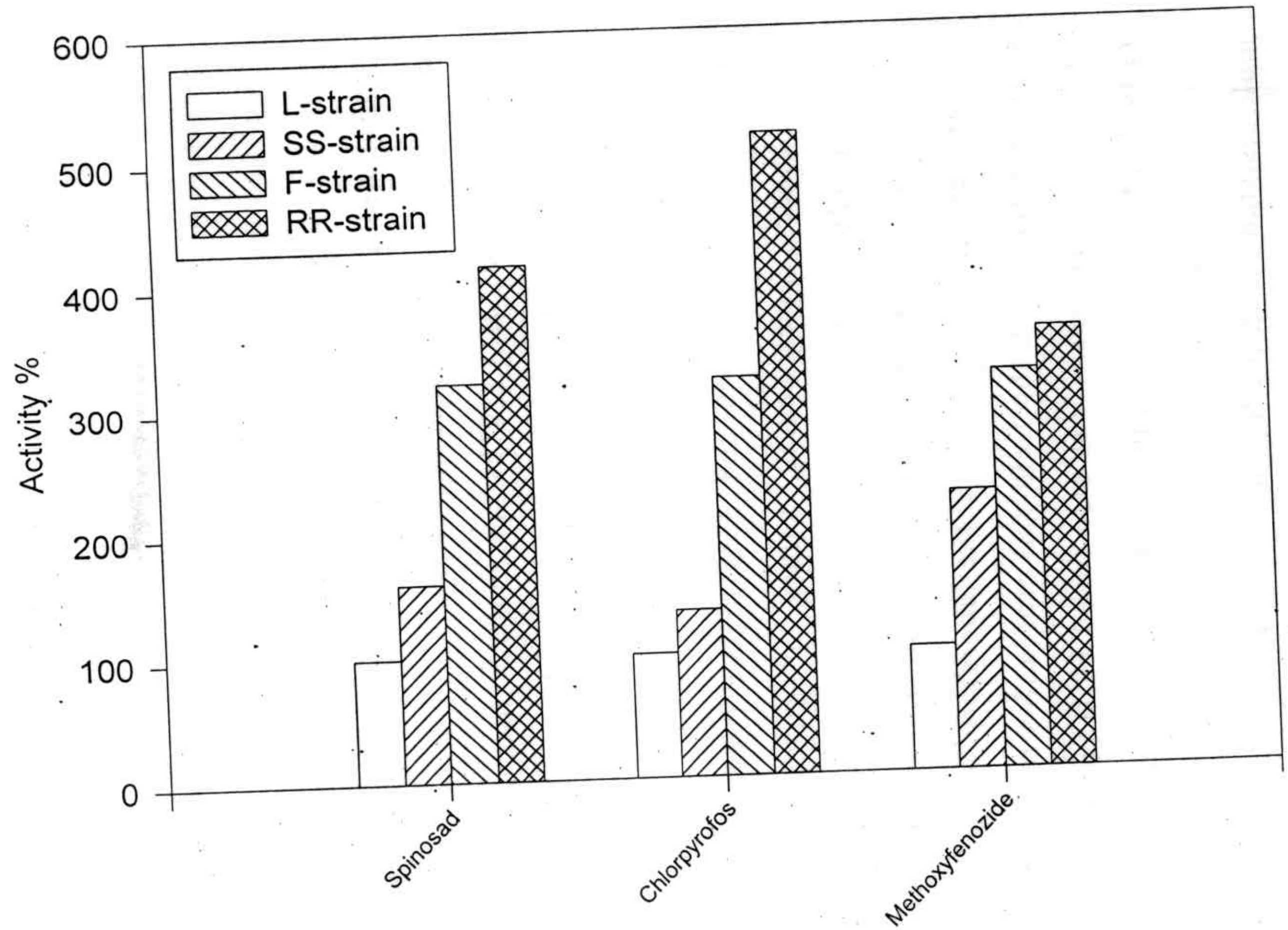

Fig.(10): Relation between Beta-esterase activity and resistance levels to three insecticides in four different strains of *S.littoralis*.

The highly significant α, β-Esterases activity and positive correlation coefficient observed in the three resistant strains, could be supported by and explained on the light of many previous investigations which confirm the close correlation between insecticides resistance, high esterases titre, and consequently the important role may played by the two enzymes in the resistance mechanism to insecticides. **Abdel-Aal and Riskallah (1978). Saleh (1981). Paeporn *et al*. (2003)** and **Harold *et al.* (1999).**

Riskallah *et al.* (1979) concluded that resistance to organophosphorus compounds and high levels of esterase activity were physiologically connected. **Farag (1981).** found that curacron and cypermethrin resistant strains of *S. littoralis* showed 37% and 35% higher β-esterase activity than those corresponding values of susceptible strain. **Hashem (2002)** reported that field and resistant strains larvae of *S. littoralis* were characterized by higher titer of non specific esterases compared to the laboratory strain. The high titer of esterases in the field and profenofos resistant strains of *Heliothis virescens* was also found by **Harold and Ottea (1997).** Moreover, **El-Saidy *et al.* (1989)** found that esterase activity was 2.6 times as high in an organophosphorus-multiresistant as in the S strain of *Spodoptera littoralis*.

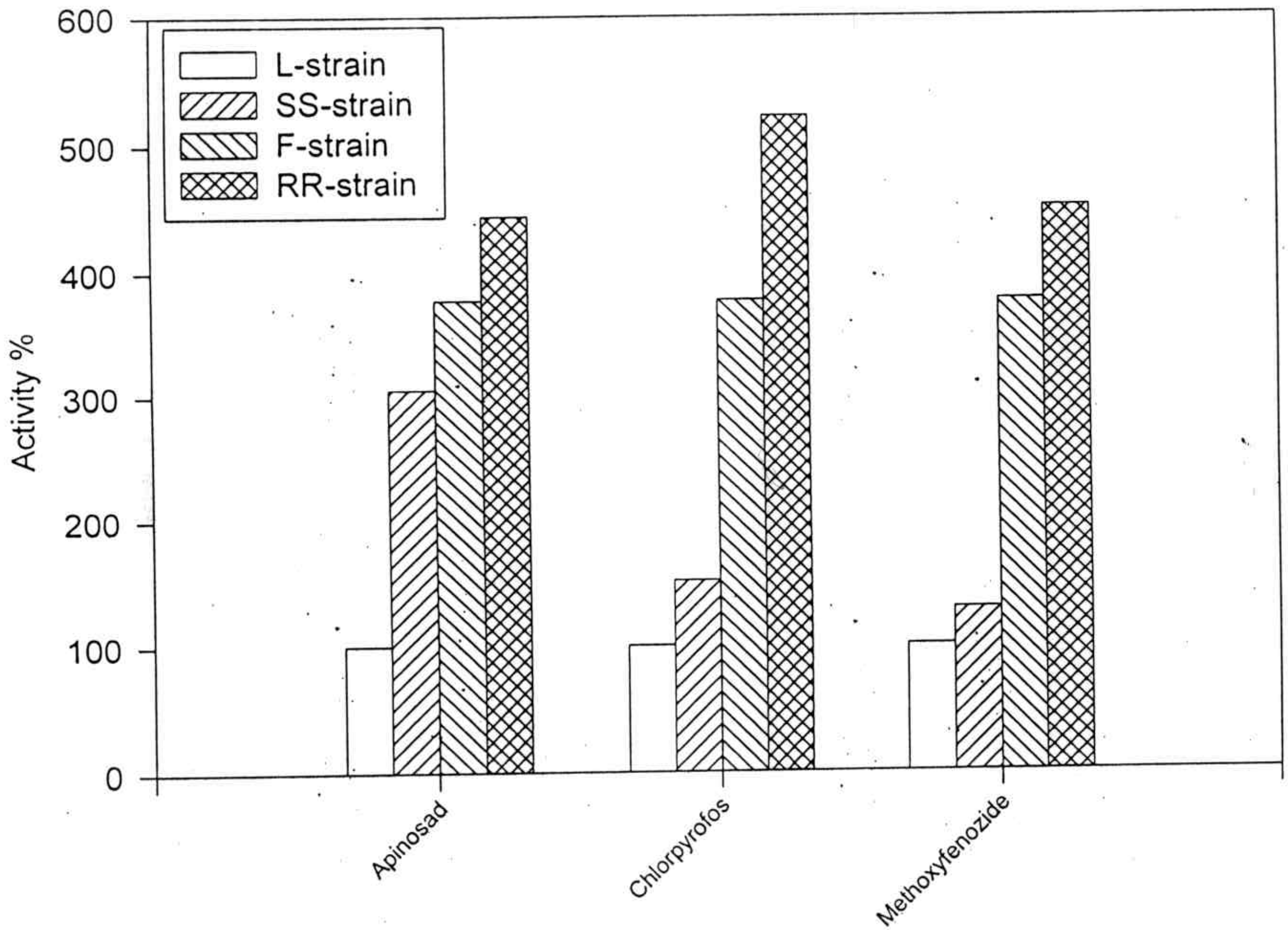

Fig.(11): Relation between alkaline phosphatase activity and resistance levels to three insecticides in four different strains of *S.littoralis*..

4.2.5.2. Acid phosphatase:

Data in tables (40, 41 and 42) & Fig. (18) represented the activities of acid phosphatase in the homogenates of 4th larval instar and its relation with resistance level (LC_{50}) to three insecticides, Sps, Cpf & Mfz.

From data obtained of acid phosphatase activities in the four strains with different resistance levels to Spinosad. the Sps- R, F-.and S-strains were higher than that of L-strain and the highest enzyme activity was recorded in Sps-R strain (172.43 % of L-strain). The same trend was observed in the case of Cpf and Mfz resistant strains, where the highest activities were recorded in Cpf -R and Mfz -R strains with percentages 800.46 % and 231.62 % of L-strains, a positive correlation was obtained between resistance level to the three insecticides and acid phosphatase activities (0.7784, 0.9780 and 0.9111) for Sps, Cpf and Mfz respectively. The highly significant acid phosphatase activity as compared to L- and S- and F- in addition to the positive correlation coefficient observed in the three insecticides, could led to the suggestion that acid phosphatase play an important role in the resistance mechanism to the three tested compounds. It should be mentioned that the highest activity levels of the two enzymes were more pronounced in organophosphate resistant strain (Chlorpyrifos).

Table (40) : Relation between acid phosphatase activity and Spinosad resistance level in four different strains of *S.littolaris*

	LC_{50}	RR (folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	11.88	1	13 ± 0.2647 d	100	0.7784
S-strain	17.01	1.43	17.806 ± 0.1868c	136.9692	
F-strain	42.02	3.53	21.278 ± 0.2780 b	163.6769	
R-strain	111.79	9.4	22.417 ± 0.1963 a	172.4385	

Activity=μg.phenol liberated/larva/min
LSD .05 = 0.4424

Table (41) : Relation between acid phosphatase activity and Chlorpyrifos resistance level in four different strains of *S.littolaris*

	LC_{50}	RR(folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	17.48	1	13 ± 0.2647 a	100	0.9780
S-strain	64.43	3.68	20.056 ± 0.6317b	136.9692	
F-strain	79.61	4.55	21.278 ± 0.2780 b	163.6769	
R-strain	233.68	13.36	104.06 ± 2.7030 C	800.4615	

Activity=μg.phenol liberated/larva/min
LSD .05 = 2.6382

Table (42) : Relation between acid phosphatase activity and Methoxyfnozide resistance level in four different strains of *S.littolaris*.

	LC_{50}	RR (folds)	Mean ± SD*	% of L-Strain	Cor.Coef
L-strain	36.33	1	13 ± 0.2647C	100	0.9111
S-strain	45.05	1.24	9.0833 ±1.0079d	69.8715	
F-strain	82.12	2.26	21.278 ±0.2780b	163.6769	
R-strain	271.01	7.54	30.111 ±1.3786a	231.6231	

Activity=μg.phenol liberated/larva/min
LSD .05 = 1.6478

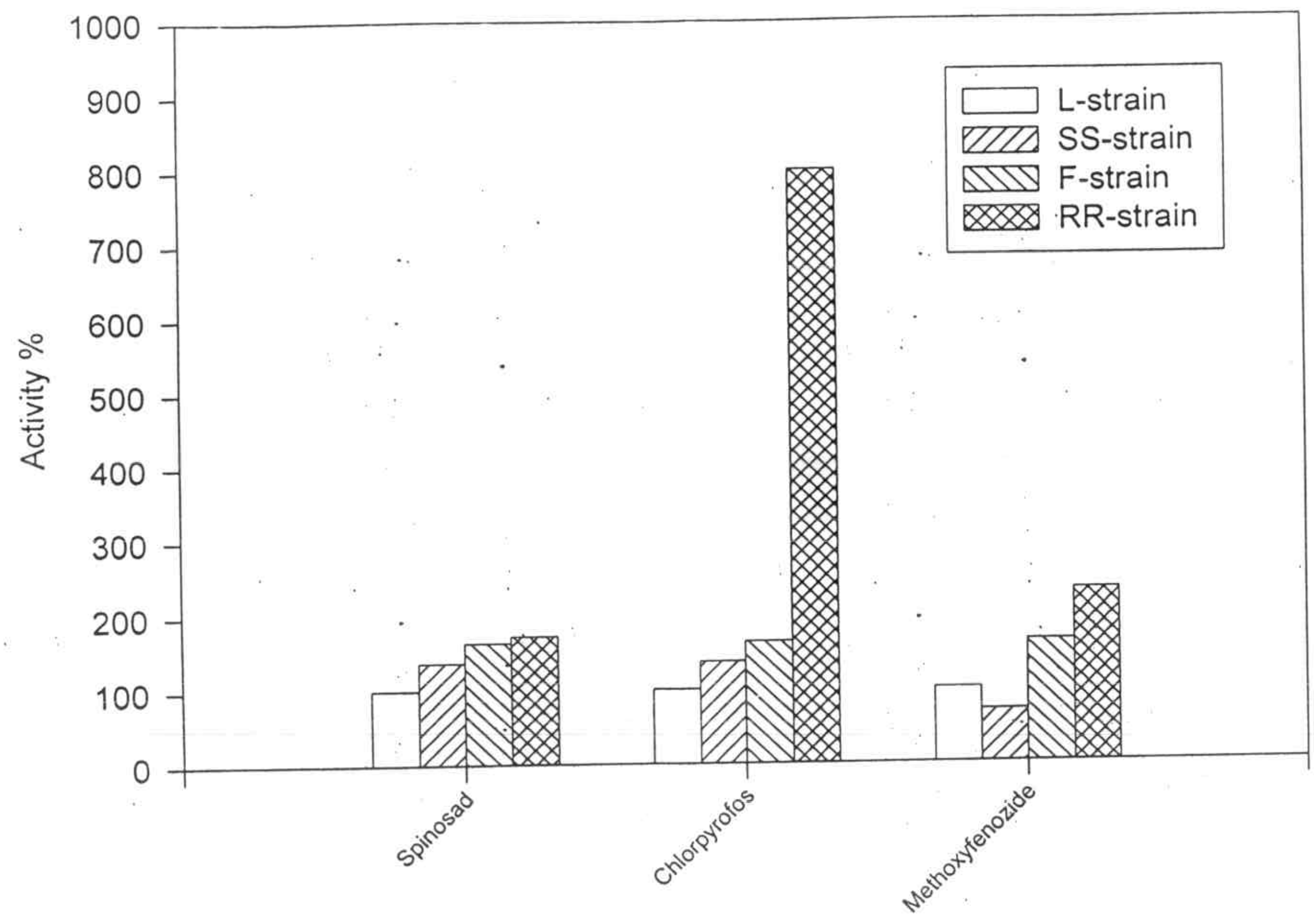

Fig.(12): Relation between acid phosphatase activity and resistance levels to three insecticides in four different strains of *S. littoralis*.

The high folds of alkaline and acid phosphatase activity in Cps resistant strain as compared to L-strain (5 and 8 folds) respectively, indicated that both enzymes are closely correlated to Organophsphorus resistance, this hypothesis could be explained and confirmed by the report of **O'Brien, 1967** who stated that phosphatases are defined as enzymes hydrolyzing any phosphorus ester or anhydride bond, including F-O-C, F-S and others. One generalization can be made safely; all the OP-compounds can be hydrolyzed, in mammals, insects and plants by phosphatases; commonly the major metabolic route. **Kruger and O'Brien (1959)**, also reported that phosphatases activity is predominant in the hydrolysis of OP- insecticides in the houseflies. **Farag (1978)** indicated that acid phosphatase increased with the development of resistance in OP-resistant strain. **Farag (1981)**, found that the acid and alkaline phosphatase activities in cypermethrin and curacron resistant strains of *S. littoralis* were higher than those of the susceptible strains. In the same time the elevation in phosphatases activity in Sps and Mfz R-strains could be also explained by **O'Brien (1967)** who concluded that "a minor increase in phosphatase activity, like other numerous changes in hydrolases, accompanies resistance rather than cause it".

The close relationship between resistance and phosphatases activity are also reported by many several authors, [**Van Asperen and Oppenoorth (1959); Matsumura and Brown (1961); Van Asperen (1964); Oppenoorth (1965); O'Brien (1967); El-Guindy** ***et al.*** **(1985) and Shakoori** ***et al.*** **(1994)**].