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تاثیرات حشره کش‌های پیرتروییدی مصنوعی و نیونیکوتینوییدی و سینرژیست‌ها روی جمعیت *Spodoptera littoralis* (Lepidoptera: Noctuidae)

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چکیده

Spodoptera littoralis (Lepidoptera: Noctuidae) تحت عنوان کرم برگخوار پنبه مصری شناخته شده و یکی از متداولترین آفات پنبه در ناحیه آدانای ترکیه به حساب می‌آید. هدف از این تحقیق توصیف تاثیرات سموم پیرتروییدی مصنوعی (Gamma-cyhalthrin) و نیونیکوتینوییدی (Imidacloprid) روی این آفت به منظور یافتن روند القای مقاومت طی چندین نسل متوالی بوده‌است. مقاومت ایجاد شده به سم gamma-cyhalothrin تا ۱۲ نسل و به سم Imidacloprid تا ۷ نسل به وسیله محاسبه نرخ مقاومت (RR) مربوط به LC_{50} و LC_{90} در بازه‌ی خطای ۹۵٪ انجام شد. میزان مقاومت (RR) برای LC_{50} و LC_{90} سم gamma-cyhalothrin ۱۶/۸۲ و برای سم Imidacloprid به‌ترتیب ۹/۷۶ و ۸/۶۸ بدست آمد. به‌منظور بررسی تاثیر سینرژیست‌ها روی سموم، سه سینرژیست مختلف شامل S,S,S-tributilfosforotritioat (DEF), piperonyl butoxide (PBO) و maleate (DEM diethyl)استفاده و به‌وسیله محاسبه نرخ سینرژیستی (SR) نسبت به شاهد به‌دست آمد. در مورد هر دو سم، بیش‌ترین مقدار SR به سینرژیست DEM تعلق یافت که می‌توان نتیجه گرفت، استفاده و کاربرد این سینرژیست همراه با سموم gamma-cyhalothrin و Imidacloprid می‌تواند به جلوگیری و یا به تاخیر انداختن مقاومت به این سموم مفید واقع شود.

واژه‌های کلیدی: *Spodoptera littoralis*، سنجش زیستی، مقاومت، حشره‌کش، سینرژیست

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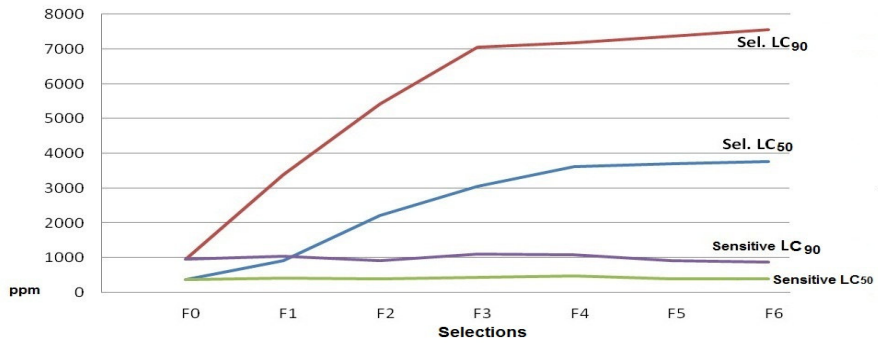

Fig. 2- The trend of changes in LC_{50} and LC_{90} values of gamma-cyhalothrin on selected and sensitive populations of *S.lithuralis* larvae

Discussion

In this study, all three synergists were effective on *S. litturalis* exposed to frequent application of gamma-cyhalothrin. Therefore, all the detoxification enzymes have efficiency to raise the resistance to both insecticide. However, DEM had the highest value of SR that it probably is an agent to inhibit the glutation *S*-transferase which has the major role in inducing resistance into *S. litturalis* (Van Leeuwen *et al* .2004).

Pyrethroids are axonic excitoxins which the toxic effects of them are mediate through preventing the closure of the voltage-gated sodium channels in the axonal membranes. Gamma-cyhalothrin acts as a voltage-dependent sodium channel blocker belonging to the oxadiazine insecticide group .Resistance to this insecticide probably is a major cause of changes in activity or structure of this part. The resistance to synthetic pyrethroids is also due to an increase in the amount or activity of cytochrome P-450 monooxygenase enzyme. However, sometimes carboxylesterase enzyme is also effective on reducing the sensitivity of individuals to the insecticide (Delorme *et al* ,.1988 ,Ahmad *et al* ,.2007 .)

To reveal the effect of enzymatic factor(s), the synergistic tests were conducted in this study. The most commonly used insecticide synergists are metabolic inhibitors that block certain detoxification enzymes so that insecticide detoxification in target insect is abolished or significantly reduced (Capinera, 2008). PBO is a well-known inhibitor of cytochrome P-450 monooxygenases; but recently it has been shown to inhibit esterases as well (Young et al., 2006) . DEF is an established esterase inhibitor, but it also acts as a substrate for cytochrome P-450 monooxygenases (Sanchez *et al* ,.2001) .There is no specific inhibitor available for glutation *S*-transferase) GST), which conjugates with and rapidly depletes glutation, has been commonly used as a synergist to suppress the GST activity (Capinera ,2008) .Yang *et al* .2001) evaluated the susceptibility and possible detoxification mechanisms of *Oligonychus paratensis*) Banks), and two-spotted spider mite to three selected insecticides with three synergists (DEM, DEF and PBO). Their study suggests that esterases, GST and Cytochrome P-450 monooxygenase all play important roles in the detoxification of the pyrethroid, because DEF, DEM and PBO enhanced the toxicity of insecticide by 6.2, 4.1 and 4.5 fold respectively. Pacy *et al* .(2009) showed lower levels of esterase activity with DEF; lower levels of GST activity with DEM and lower levels of cytochrome monooxygenase activity with PBO by the enzyme assays .

Usually, AChE enzyme plays a major role in the resistance that is created as a result of repeated use of imidacloprid insecticide (Pittendrigh ,*et al* ,. 2008) ,while, according to research, the increased activity of cytochrome P-450 monooxygenase enzyme has been involved in creating resistance mechanisms in some cases (Puinean *et al* ,. 2010) .In this research, the PBO synergist had very low effect on increasing the imidacloprid efficiency. This has been approved by many researchers that cytochrome P-450 monooxygenases has no (or little) involvement in resistance ability of the pest .

On the other hand, the homogeneity of the sensitive population is deductible. The use of gamma-cyhalothrin plus three synergists of DEM, DEF and PBO ,synergistic ratios (SR) for LC_5 & .LC_9 .3.6 ,3.9 ;2 ,3.1 and 2.1, 2.7 values were obtained respectively. In the case of use Imidacloprid plus DEM, DEF and PBO, SR were calculated 2.03 ,2.07 ;1.6 ,1.7 and 1.04, 1.25 at the values of LC_5 & .LC_9 .respectively (Table 4).

Table 4- Comparison of the bioassay parameters of 6th generation with the sensitive (control) population of *S littoralis* exposed to Imidacloprid after application of the synergists DEM, DEF and PBO

SR LC_{75}	SR LC_{50}	RR LC_{75}	RR LC_{50}	LC_{90}ppm (Confidence limit 0.95)	LC_{50} ppm (Confidence limit 0.95)	Slope±SE	h	χ^2 (df)	n	
		9.10	9.21	8359.2 (6323.8 - 13640)	3854.4 (3318.8 – 4757.9)	3.81 ±0.57	0.15	2.42 (16)	216	F6
		1.00	1.00	918.1 (716.3 – 1374.6)	418.3 (354.2 – 504.1)	3.76 ±0.54	0.38	4.63 (12)	216	Sensitive
1.0	1.2	9.06	8.49	8056.3 (6199.2 - 12131)	3078.7 (2561.4 – 4016.4)	2,73 ±0.43	0.34	5.1 (15)	216	F6 +PBO
1.1	1.1	1.00	1.00	809 (634 – 1192.2)	362.7 (306.3 – 434.1)	3.67 ±0.51	0.72	8.63 (12)	216	Sensitive +PBO
2.0	2.0	4.99	6.16	4121.9 (3361.3 - 5630)	1862.8 (1634.1 - 2138.2)	3.71 ±0.46	0.74	11.19 (15)	216	F6 +DEM
1.1	1.4	1.00	1.00	825.8 (620.5 – 1283.7)	302.4 (258.3 – 358.6)	2.93 ±0.38	0.77	9.3 (12)	216	Sensitive +DEM
1.6	1.7	6.99	7.73	5114.2 (3490.4 - 13202)	2287.7 (1874.3 - 3268.5)	3.66 ±0.64	1.38	8.5 (12)	216	F6 +DEF
1.3	1.4	1.00	1.00	730.9 (565.7 – 1082)	296.1 (248.8 – 358.9)	3.27 ±0.42	0.73	8.77 (12)	216	Sensitive +DEF

n =Number of larvae used
h =Heterogeneous value
RR =resistance ratio (LC ۵۰or LC ۹۵values of resistance population / LC ۵۰or LC ۹۵values of sensitive population(
RS =Synergistic ratio (LC50 or LC90 of the insecticide / LC50 or LC90 of the insecticide plus synergist(

synergistic tests

According to the results, all three synergists increased the effect of the insecticides. The greatest impact was observed in DEM synergist, so that in the case of the simultaneous use of gamma-cyhalothrin in the LC_9 .level, the impact of pesticide increased by 3.9 times (Table 3). At the same time, the simultaneous use of synergist and insecticide in sensitive population has no significant impact on the effectiveness of insecticides.

Table 3- Comparison of bioassay parameters Synergism of gamma-cyhalothrin by DEM, DEF and PBO

SR LC_{90}	SR LC_{50}	RR LC_{90}	RR LC_{50}	LC_{90}ppm (Confidence limit 0.95)	LC_{50} ppm (Confidence limit 0.95)	Slope ±SE	h	$\chi 2$ (df)	n	
		18.82	16.85	24340.0 (16427.0 - 47492.0)	11194.0 (9341.2 – 16705.0)	3.79 ±0.88	0.25	3,31 (13)	216	F12
		1.00	1.00	1105.0 (912.0 – 1458.1)	664.1 (558.1 – 791.9)	4.93 ±0.86	0.92	4,52 (11)	216	control
1.7	2.1	13.92	11.5	14062.0 (10848.0 - 22080.0)	5248.6 (4444.9 – 6150.8)	2.99 ±0.46	0.62	8,13 (13)	216	F12 +PBO
1.1	1.4	1.00	1.00	1010.2 (897.7 – 1318.8)	456.1 (382.6 – 535.7)	3.32 ±0.42	0.44	5.37 (12)	216	control +PBO
3.9	3.6	6.00	6.58	6177.8 (4966.0 – 8733.5)	3092.1 (2649.4 – 3663.0)	4.26 ±0.59	0.46	8,34 (18)	216	F12 +DEM
1.1	1.4	1.00	1.00	1028.9 (851.3 – 1350.6)	469.9 (400.2 – 545.2)	3.76 ±0.46	0.75	8,98 (12)	216	control +DEM
2.0	3.1	11.51	7.19	12029 (9061.5 – 20279.0)	3553.9 (2677.5- 4322.9)	2.42 ±0.42	0.21	2,84 (13)	216	F12 +DEF
1.1	1.3	1.00	1.00	1045.1 (994.9 – 1442.9)	494.1 (414.2 – 582.6)	3.19 ±0.41	0.48	5.74 (12)	216	control +DEF

n =Number of larvae used
h =Heterogeneous value
RR =resistance ratio (LC_{50}or LC_{90}values of resistance population / LC_{50}or LC_{90}values of sensitive population(
SR =Synergistic ratio (LC50 or LC90 of the insecticide / LC50 or LC90 of the insecticide plus synergist(

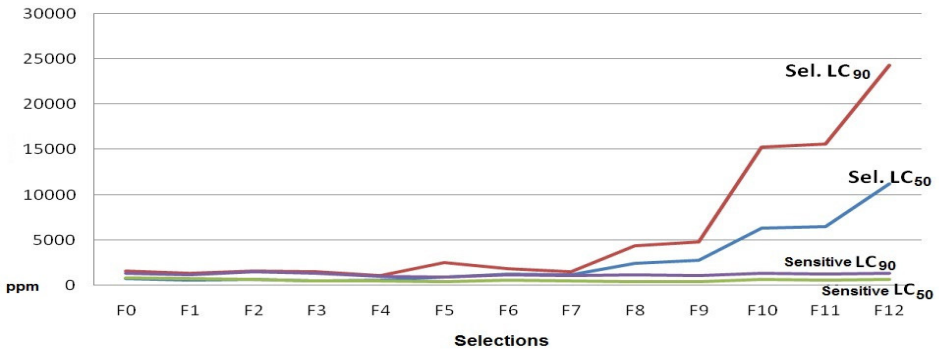

Fig. 1- The trend of changes in LC_{50} and LC_{90} values of imidacloprid on selected and sensitive populations of *S.lithuralis* larvae

The values of LC_5 .and LC_9 .in F0 for imidacloprid were 369.1ppm and 955.2ppm which respectively increased to 3772.1 and 7548.8 in seventh selection (F) (۲Fig.2). This means that the LC_5 .increased tenfold and LC_9 .increased eightfold in the last selection. The RR values reached to 9.76 and 8.68 for LC50 and LC90 respectively (table 2). The heterogeneity ratio (h) in population exposed to imidacloprid decreased from 1.34 to 0.13 in 6th generation. This means that homogeneity to this insecticide increased gradually in selected populations (Table 2).

Table 2- The bioassay parameters of 7 generations of third instar larvae of *S. littoralis* exposed to Imidacloprid

RR LC_{90}	RR LC_{50}	LC_{90}ppm (Confidence limit 0.95)	LC_{50} ppm (Confidence limit 0.95)	Slope ±SE	h	χ^2 (df)	n	Sel.
1.00	1.00	955.2 (722.5 – 1494.0)	369.1 (306.7 - 451.0)	3.10 ±0.35	1.34	29.46 (22)	216	F0
3.23	2.25	3386.9 (2388.9 – 5889.1)	921.1 (766.5 – 1142.5)	2.26 ±0.28	0.66	14.03 (21)	216	F1
5.91	5.65	5416.6 (4466.6 – 7016.7)	2215.8 (1928.7 – 2550.8)	3.30 ±0.31	0.96	20.18 (21)	216	F2
6.38	7.27	7056.4 (5881.5 – 9701.7)	3050.6 (2626.0 – 3708.4)	3.34 ±0.54	0.65	5.71 (12)	216	F3
6.68	7.75	7166.1 (5951.6 – 9653.2)	3624.5 (3250.9 – 4088.4)	4.32 ±0.57	0.47	9.81 (15)	216	F4
8.11	9.64	7371.0 (5504.1 – 12570.0)	3715.1 (2996.5 – 3925.3)	4.06 ±0.64	0.18	2.42 (13)	216	F5
8.68	9.76	7548.8 (5885.4 – 11536.0)	3772.3 (3199.7 – 4418.1)	4.09 ±0.59	0.13	2.2 (16)	216	F6

n =Number of larvae used
h =Heterogeneous value
RR =resistance ratio ($LC_{۵۰}$or $LC_{۹۵}$values of resistance population / $LC_{۵۰}$or $LC_{۹۵}$values of sensitive population(
CL=confidence limit

Table 1- The bioassay parameters of 13 generations of third instar larvae of *S. littoralis* exposed to Gamma-cyhalothrin

RR LC_{90}	RR LC_{50}	LC_{90}ppm (CL 0.95)	LC_{50} ppm (CL 0.95)	Slope ±SE	h	χ^2 (df)	n	Sel.
1.00	1.00	1551.5 (1315.2 – 2010.7)	804.1 (441.1 – 734.6)	4.49 ±0.61	2.70	14.82 (21)	216	F0
1.10	0.86	1313.0 (1063.6 – 1917.7)	604.6 (441.1 – 734.6)	3.80 ±0.59	1.32	14.52 (11)	216	F1
1.01	1.01	1629.2 (1443.4 – 3415.4)	651.1 (486.6 -799.5)	2.71 ±0.45	1.07	12.95 (12)	216	F2
1.17	1.03	1510.6 (1059.0 – 4017.6)	517.8 (269.2 – 703.3)	2.75 ±0.47	2.25	27 (12)	216	F3
1.08	1.27	1083.9 (944.5 – 1312.6)	597.9 (515.8 – 675.3)	4.96 ±0.63	1.90	10.83 (12)	216	F4
2.80	2.40	2549.0 (1640.4 – 10571.0)	957.6 (685.5 – 1395.2)	3.01 ±0.47	2.70	32.48 (12)	216	F5
1.56	2.34	1830.0 (1581.1 – 2443.,1)	1264.4 (1096.3 – 1438.4)	7.98 ±0.98	1.43	29.27 (12)	216	F6
1.35	2.28	1501.7 (1397.9 – 1684.5)	1169.8 (1118.9 – 1229.2)	11.81 ±1.60	0.71	8.71 (13)	216	F7
4.22	5.58	4389.1 (3603.4 – 6115.6)	2410.9 (2179.8 – 2768.2)	4,925 ±0.68	0.44	7.49 (18)	216	F8
4.36	6.82	4826.6 (4038.1 – 7263.1)	2746,1 (2451.7 – 3037.9)	5,23 ±0.12	0.41	5.6 (12)	216	F9
11.75	10.10	15318.0 (11796.0 – 23892.0)	6278.1 (4789.7 – 8230.0)	3.24 ±1	0.26	2.41 (15)	216	F10
12.53	11.01	15598,0 (8534.9 – 19803.4)	6483.4 (5669.8 – 7673.5)	3.43 ±0.49	0.26	3.94 (15)	216	F11
18.82	16.85	24340.0 (16427.0 - 47492.0)	11194.0 (9341.2 – 16705.0)	3.79 ±0.88	0.24	3.31 (13)	216	F12

n =Number of larvae used
h =Heterogeneous value
RR =resistance ratio (LC ₅₀or LC ₉₀values of resistance population / LC ₅₀or LC ₉₀values of sensitive population(
CL=confidence limit

Bioassays and selections

A susceptible strain of *S. littoralis*) Boisd.) was collected in 2008 from cotton fields of Adana province in Turkey and reared until now in laboratory without exposure to insecticides. Bioassays were conducted in growth chamber and mortality data were used for susceptibility to insecticides. The newly third-instar larvae of *S. littoralis*) Boisd.) used in bioassay experiments. Serial dilutions of active ingredient were done using distilled water. Lettuce leaves were cut into 9 cm pieces and dipped into the insecticide solution for 10s .These leaves were air dried on tissue paper, under luminar hood. The leaves were dipped in distilled water to use as controls. Leaves treated with insecticides were then transferred to each petri dish lined with moistened filter paper. At least six concentrations and three replications (12 larvae per replication) were used to estimate mortality compare to previous generation. Mortality data were scored 48 h after exposure to insecticides .
Data were analyzed using probit analysis through POLO-PLUS software to estimate LC_5 ,.LC_{75} and LC_9 .values and 95 % confidence limits .The LC ٧٥values for each generation was calculated and used for selection the survived individuals for the next step. All these steps were repeated for 13 and 7 times for gamma-cyhalothrin and imidacloprid respectively .

Synergistic tests

Synergistic bioassay were performed on sensitive population (non-exposed to insecticides (as control and two selected populations of *S. littoralis*) Boisd (.which were resistant to Gamma-cyhalothrin and Imidacloprid. PBO, DEM and DEF were used as synergists to determine the type and amount of the insecticide detoxification. These substances were diluted using acetone solution, and brought to a concentration of 1,000 micrograms per milliliter (Van Leeuwen *et al* .2004) .In each synergistic test, the third instar larvae exposed to different synergists by topical application to the prothorax at the concentration of 1000 µg/ml before performing the bioassay tests. Acetone was applied on individuals instead the synergist in controls. After two hours, the larvae exposed to different doses of insecticide by the leaf dipping method .Finally, the mortality rate was analyzed using Polo-Plus software at 95 % confidence level.

Results

In bioassay tests ,the value of LC_5 .in F0 for gamma-cyhalothrin was 8٠4.1ppm. This value increased gently until the seventh generation) F_7 .(However it increased sharply from F_8 to F_{12} .and reached to 9.13-fold of its initial value, i.e .11194 .Similarly, after a sudden rise from the eighth generation, the LC_9 .value reached to15.7-fold compare to initial value ,i.e. 24340 ppm in F_{12} .In Figure 1 shows LC_5 .and LC_9 .values of gamma-cyhalothrin on selected and sensitive populations of *S.lithuralis* larvae .Accordingly, the resistance ratio for LC_5 .and LC_9 .values reached to 16.85 and 18.82-fold in 13th generation, respectively. Consequently, reduced sensitivity or increasing resistance to this insecticide appeared gradually. The heterogeneity ratio (h) in population exposed to gamma-cyhalothrin reduced from 2.7 to 0.24 in 13th generation. This means that homogeneity to gamma-cyhalothrin increased gradually in selected populations (Table 1). The resistance ratios (RR) which is considered as a resistance factor was calculated using LC_5 .or LC_9 .values of resistance population divided by LC_5 .or LC_9 .values of sensitive population increased 16.85 and 18.82 fold compared to sensitive populations (table 1)

Comparison of resistant strains of *S. litura* to organophosphates and pyrethroids collected from five different districts of China with a susceptible strain reared in laboratory, the values of LC_5 . increased 14-229-fold for organophosphates and 12-227-fold for pyrethroids respectively (Tong *et al* ,.2013 .)Application of DDT on *S. littoralis* resulted 43 times more resistant individuals after seventeenth generations; whereas, simultaneous use it with Du-ter (a type of synergists) has induced 11.5 times resistance (Abdallah et *al* ,.1979.) Ahmed and his colleague (2011) using endosulfan on *S. litura* found that the resistance ratio in the 6th generation increased 3.97 fold compare to the 1st generation. Efficiency of pyrethroids and organophosphates on populations of *Spodoptera* significantly increased by using DEF and PBO enzyme inhibitors. It was shown that the resistance to insecticides was associated with esterase and monooxygenase detoxification respectively (Ahmad *et al* ,.2008.) Carboxilesterase is one of the most commonly used enzyme which associated with insecticide resistance. Metabolic resistance to organophosfates increases the esterase activity in insects resistant to carbamates. Some evidence suggest that cytochrome P-450 monooxygenase enzyme involves in synthesis of resistance to carbamates in cases of frequent use of insecticides(Farnsworth *et al* ,.2010.)

Until now ,investigations have indicated that improper use of insecticides with various modes of actions or broad spectrum insecticides cause resistance or reduce susceptibility to a special class of insecticide or cross resistance. The results of these investigations show that esterase, Glutation *S*-transgerase, Cytochrome P-450 monooxygenase induce resistance by increasing metabolism or reducing penetration to cuticle (Riskalla, 1983; Ishaa ,1990 .)

This study aims to find out the effects of two insecticides, Gamma-cyhalothrin (VANTEX (and Imidacloprid (Confidor) on *S. littoralis* using bioassay under laboratory conditions.

Materials and Methods

S. littoralis (Boisd.) was collected from cotton fields of Adana region ,Turkey, in 2008, and since then reared in conditions of 25 ± 2**°C**% ,65 ± 5 relative humidity and 16:8 photoperiod (light: dark) at the Department of Plant Protection, Faculty of Agriculture ,University of Ankara. Third instars larvae (6 days after hatching (were used in all experiments.

Synthetic pyrethroid with the active ingredient of gamma-cyhalothrin (VANTEX, 60 SC, Dow AgroSciences) and Neo-nicotinoid with the active ingredient of imidacloprid (CONFIDOR: 350 SC: Bayer CropScience) were used in this study. We also used the S, S, S-tributilfosforotritioat (DEF) as an inhibitor of esterase enzymes, piperonyl butoxide (PBO) as an inhibitor of the of cytochrome P-450 monooxygenase and carboxylesterase enzymes, and diethyl maleate (DEM) as an inhibitor of Glutation S-transferase enzyme diluted in acetone (1000 g / ml (solution (Van Leeuwen *et al* ,.2004 .)

Introduction

Spodoptera littoralis) Lepidoptera: Noctuidae) (Boisduval) attacks to a wide range host of crops (at least 87 different hosts) in tropical and subtropical regions. It causes economic damages on plants throughout the year. In cotton plant it induces serious damages to leaves ,seeds, buds, and bolls (Salama *et al* .1970).

Despite their efficiencies and being a definitive solution for pest control, pesticides , especially insecticides have endangered human health or have had negative impacts on non-target organisms . They have hazarded the environment, especially when improperly used by farmers during the past fifty years (Topuz, 2005) .Moreover they have induced resistance in target organisms .Besides, overusing the insecticides against pests with several generations a year make them highly resistant in consequence of natural selection (Tiryaki *et al* ,.2010 .)

Extensive investigations on some chemicals have been conducted to inhibit or at least lower the insecticides detoxification by insects .S, S ,S-tributilfosforotritioat (DEF) as an inhibitor of esterase enzymes ,piperonyl butoxide (PBO) as an inhibitor of Cytochrome P-450 monooxygenase enzyme, and Diethyl maleate (DEM (as an inhibitor of Glutation S-transferase enzyme have been used with chemical to enhance the efficiency of insecticides by inhibiting their insecticides detoxification(Van Leeuwen *et al*) .2004 .According to the published reports, changes in content of Cytochrome P-450 monooxygenase enzyme and esterase in the genus *Spodoptera* usually cause an increase the metabolic activities and resistance to insecticide (Armes *et al* ,.1997 ;Zhou and Huang, 2002; Huang and Han, 2007; Ahmad, 2008).

Gamma-cyhalothrin is a contact insecticide which can also be effective through ingestion .It acts as a sodium channel modulator and causes neurotic disorder) Metcalf, 2000). Imidacloprid belongs to neo-nicotinoid insecticides class and is known as an agonist of nicotinic acetylcholine receptor (nAChR) .Acetylcholine is an endogenous agonist as well as a cholinergic signaling of the nervous system which is known as a neurotransmitter (Pittendrigh *et al* ,.2008.)

Imidacloprid is a systemic insecticide which can also be effective through ingestion or contact. It penetrates swiftly into the plant tissues after applying on plants. It has a variety of functions on the nervous system and especially on post-synaptic nicotinic acetylcholine receptor. In class of insects, these receptors are found solely on the central nervous system (CNS). Imidacloprid molecules after attachment to the nicotinic receptor, firstly neurotic signal discharging happens, and then, by any neurotic signal, nerve failure occurs. Reason of receptors non-stop activity is the adhering the insecticide molecules to acetylcholinesterase enzyme irreversibly (Gervais, et al., 2010) .

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Effects of synthetic pyrethroids and neonicotinoids insecticides and synergists on population of *Spodoptera littoralis* (Lepidoptera: Noctuidae)

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Abstract

Spodoptera littoralis (Lepidoptera: Noctuidae) known as the African Cotton Leaf worm or Egyptian Cotton Leaf worm is one of the most common pests of cotton in the region of Adena, Turkey. The aim of this study was to determine the effects of a synthetic pyrethroid) Gamma-cyhalothrin) and neonicotinoids) Imidacloprid) on the pest to find out the resistance inducing process during several generations. Induced resistance to gamma-cyhalothrin up to 12 generations and to Imidacloprid for 7 generations was studied by assessing the resistance ratio (RR) of LC ۵۰ and LC ۹۰with confidence limit of %95. The resistance ratios (RR) for LC ۵۰and LC ۹۰of gamma-cyhalothrin were calculated 16.85 and 18.82 and for Imidacloprid were 9.76 and 8.68, respectively. In order to study the effect of synergists on the insecticides, three different synergists including S, S, S-tributilfosforotritioat (DEF ,(piperonyl butoxide (PBO) and diethyl maleate (DEM) were used and compared the Synergism Ratio (SR) with control. The highest SR for both insecticides belonged to application of DEM synergist. It is concluded that DEM could be recommended to be applied with Gamma-cyhalothrin and Imidacloprid to prevent or postpone the resistance induction into *S .littoralis* .

Key words :*Spodoptera littoralis* ,bioassay, resistance ,insecticide, synergist

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