J. Entomol. Res. Soc., 15(1): 37-45, 2013 *ISSN:1302-0250*

Determination of Susceptibility Levels of *Helicoverpa armigera* (Hübner) (Noctuidae: Lepidoptera) Strains Collected from Different Regions to Some Insecticides in Turkey

Sakine UĞURLU KARAAĞAÇ[1] Metin KONUŞ[2] Musa BÜYÜK[3]

[1]Ankara Plant Protection Central Research Institute, Gayret Mah. FSM Boulevard, No: 66-68, 06172 Yenimahalle-Ankara, TURKEY
[2]Biochemistry Joint Graduate Program, Middle East Technical University, Ankara, TURKEY , and Yüzüncü Yil University, Institute of Science, Van, TURKEY.
[3]Diyarbakir Vocational School, Dicle University, Yenişehir- Diyarbakır, TURKEY.
*Corresponding author: sugurlu@hotmail.com

ABSTRACT

Cotton bollworm, *Helicoverpa armigera*, is the main insect pest of cotton plant in Turkey and most part of the world. The aims of this study was to determine susceptibility levels of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) strains collected from cotton fields in Adana and Mardin provinces to registered seven insecticides in Turkey. Third instar larvae of *H. armigera* were used to determine the LD_{50} values by using topical application method with active substances of commonly used insecticides for controlling *H. armigera* in cotton fields. The active substances were azinphos-methyl, bifenthrin, beta-cyfluthrin, esfenvalerate, indoxacarb, lambda-cyhalothrin and methomyl. Insects were treated on the region of the body between the head and the abdomen, thorax, with 1 µl aliquots of insecticides in acetone for all treatments and acetone alone for control. Results showed that Adana and Mardin strains had high resistance ratios to pyrethroid insecticides such as beta-cyfluthrin, bifenthrin and esfenvalerate, whereas they did not have significant resistance for indoxacarb and methomyl. These results can be used in the resistance management programs for the control of *H. armigera* in the region.

Key words: Helicoverpa armigera, Lethal Doses (LD_{50}), insecticides, insecticide resistance.

INTRODUCTION

The cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), is the most important insect pest of cotton in the world (Vaissayre and Cauquil, 2000). The larvae of *H. armigera* are mainly responsible for the damages to cotton. It causes significant loss of cotton production by damaging the generative organs of cotton. In Turkey, the life cycle of this insect includes two or three generations in a year in cotton fields. In greenhouses, vegetables such as okra, pepper, tomatoes and legumes remain the main host plants. If vegetable host crops are grown during the long term of year in greenhouse, the life cycle of *H. armigera* is more than three generations in a year (Koclu and Karsavuran, 2000). Generally, insecticides have been used extensively to control of *H. armigera*. Cotton is generally protected from the damage by applications of insecticides such as carbamates, organophosphates and synthetic pyrethroids. Compared to carbamates and organophosphates, synthetic pyrethroids have low toxicity to mammals and breakdowns easily. The low toxicity to mammals of synthetic pyrethroid insecticides has encouraged their use in intensive agriculture. Furthermore, they are effective at low dosages, too (Moore and Waring, 2001). As a result, they have been preferred by the farmers for the economically important crops such as cotton.

Synthetic pyrethroids have been widely used in different countries worldwide for controlling *H. armigera*. This resulted in development of resistance against synthetic pyrethroid insecticides. Resistance developments have been reported from different parts of the world against pyrethroid insecticides commonly used to control *H. armigera*.

The first report related with pyrethroid resistance in *H. armigera* reported from Australia in 1980s (Forrester *et al*., 1983). Later, Gunning *et al*. 1984, 1991, 1997 and Daly (1988) reported pyrethroid resistance in *H. armigera* from Australia, too. It was also reported from other countries such as Thailand and Colombia in 1984-85 (Ahmad and McCaffery, 1988), Pakistan (Ahmad *et al.*, 1995) and South India and Andra Pradesh in India (Armes *et al*., 1992, 1996), Tamil Nadu (Pasupathy and Regupathy, 1994), Delhi, Penjab and Hayrana (Mehrotra and Phokela, 1992), Israel (Horowitz *et al*., 1993), China and India in 1987 (McCaffery, 1998), Tamil Nadu in South India (Duraimurugan and Regupathy, 2005), West Africa (Martin *et al*., 2002), France (Bues *et al*., 2005), Turkey (Ernst and Dittrich, 1992, Ugurlu *et al*., 2007, Ugurlu and Gurkan, 2007), and Spain (Avilla *et al*., 2010).

In addition, from years 2003 and 2004 in some cotton fields in southern part of Turkey, insecticide applications of synthetic pyrethroids were not successful in controlling larvae of *H. armigera.* Therefore, Republic of Turkey Ministry of Agriculture and Rural Affairs restricted to use some of synthetic pyrethroids in cotton fields in the southern part of Turkey in 2004. In Turkey, 15 registered insecticides were actively used against *H. armigera* in 2007 and 2008 years. However, insecticide spray history of these insecticides was unknown in cotton growing areas.

The objective of this work was to determine the resistance ratios of Adana and Mardin field strains of *H. armigera* to registered seven insecticides, azinphos-methyl, bifenthrin, beta-cyfluthrin, esfenvalerate, indoxacarb, lambda-cyhalothrin and methomyl in Turkey.

MATERIALS AND METHODS

Insects

A susceptible strain of *H. armigera* was obtained from Bayer CropScience (Monheim, Germany) and reared in the laboratory for the experiments. Field strains of *H. armigera* were collected from cotton crops cultivated in Kızıltepe, Mardin province (South eastern Anatolia Region) in 2007 and Adana province (Mediterranean Region) in 2008. The location of the collection sites is marked with dot in Fig. 1. In order to feed

H. armigera strains in laboratory, multiple species insect diets were used, obtained from Southland Products Inc. (USA). The diet was prepared by mixing 162 g diet and 930 ml hot water. Then, this mixture was placed on 5.5 cm sterile disposable Petri dishes. Larvae were fed on these artificial diets in laboratory and allowed to pupate. Then, emerging adults were allowed to breed. After two or three days, they laid eggs. The eggs were hatched in four or five days. Finally, when the larvae succeeded to reach third instar larval stage, they were used for the bioassays.

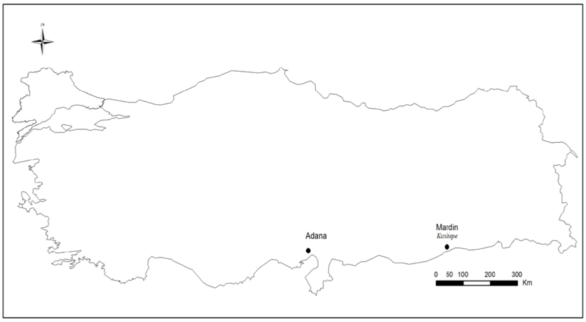

Fig. 1. Exact location of the collection sites of *Helicoverpa armigera* populations.

Chemicals

Active substances of insecticides used in the topical bioassays were obtained as follows: Indoxacarb (99.6%) and methomyl (99.8%) were obtained from DuPont Agricultural Products. Bifenthrin (97%), azinphos-methyl (99%) and esfenvalerate (98%) were obtained from Dr. Ehrenstorfor GmbH, producer of organic reference materials for residue analysis and research purposes worldwide. Beta-cyfluthrin (98.8%) was obtained from Bayer CropScience, which is innovative crop science companie in the areas of seeds, crop protection and non-agricultural pest control in Germany. Lambda-cyhalothrin (98.7%) was obtained from Syngenta Crop Protection UK Limited. All insecticides (technical grade samples) were used as received from the manufacturers.

Insecticide bioassays

Serial dilutions of technical grade insecticides in acetone were prepared such that each was one-half of the previous concentration. Insecticide solutions were prepared in different doses for each insecticide. Dose rates varied from 0.0002 to 0.95 µg/µl for synthetic pyrethroid insecticides, from 0.20 to 1.48 µg/µl for azinphos-methyl, from 0.023 to 0.188 µg/µl for methomyl and from 0.006 to 0.198 µg/µl for indoxacarb. One micro litre of insecticide solutions was applied topically to the region of the body between the head and the abdomen, thoraxic dorsum of the third instar larvae (30-40 mg larvae) using micro-applicator in five to six concentrations causing >0% and <100% mortality (Anonymous, 1970). Control insects were treated with acetone alone. At least 20 third instar larvae for each dose were used to estimate the Lethal Dose (LD_{50}) values. After treatments, the larvae were individually placed on the diet in a 5.5 cm diameters plastic Petri dishes in a controlled room at 25 ±1°C and under a photoperiod of 16:10 (L:D) during the post-treatment observation period. Mortality was recorded 48 h after treatment. Larvae were considered as dead when they were not able to move when prodded with a blunt probe or brush. LD_{50} values were calculated using POLO-PC probit analysis on computer (LeOra Software, 1994).

Resistance ratios (RRs) were calculated by dividing the LD_{50} value of each field strain by the LD_{50} value of the susceptible strain. Confidence limits of RRs were calculated according to Robertson and Preisler (1992). According to these RRs that level of insecticide resistance was classified as reported by Torres-Vila *et al.* (2002). Susceptible (RR=0-1), low resistance (RR=2-10), moderate resistance (RR=11-30), high resistance (RR=31-100).

RESULTS

Results of insecticide bioassays with the Adana and Mardin strains of *H. armigera* are summarized in Table 1. LD_{50} values obtained from Adana and Mardin strains were compared to that of susceptible strains to calculate resistance ratios. The Adana strain was resistant to all tested beta-cyfluthrin, bifenthrin, esfenvalerate and lambda-cyhalothrin (pyrethroid insecticides) and azinphos-methyl (organophosphate). Resistance ratios of tested pyrethroid insecticides in Adana strain ranged from 6- to 67- fold. Resistance ratios of beta-cyfluthrin, bifenthrin, esfenvalerate and lambda-cyhalothrin insecticides were 67-, 59-, 36.6- and 6-fold, respectively. Resistance ratio of azinphos-methyl (organophosphate) was 8.6- fold. However, resistance ratios of indoxacarb (oxadiazine) and methomyl (carbamate) insecticides were 2- and 0.5-fold, respectively, in Adana field strain.

In Mardin strain, resistance ratios of tested pyrethroid insecticides ranged from 7.5- to 62.6- fold. Resistance ratios of esfenvalerate, bifenthrin, beta-cyfluthrin and lambda-cyhalothrin insecticides were 62.6-, 20-, 16- and 7.5- fold, respectively. However, resistance ratios of indoxacarb (oxadiazine), azinphos-methyl (organophosphate) and methomyl (carbamate) insecticides were 0.45-, 2.96- and 0.52-fold, respectively.

DISCUSSION

Chemical insecticides have been widely used in different countries worldwide to control *H. armigera* in agricultural areas. This resulted in the development of resistance of this pest to several classes of insecticides (Tang *et al*., 2000).

In this study, Adana and Mardin *H. armigera* strains showed different resistance ratios to tested seven insecticides. For example, Adana strain showed high resistance levels to synthetic pyrethroids especially for beta-cyfluthrin (67-fold), bifenthrin (59-fold) and esfenvalerate (36.6-fold). Nevertheless, it showed low resistance level to lambda-cyhalothrin (6-fold). In addition, while it showed low resistance level to indoxacarb (oxadiazine, 2-fold) and azinphos-methyl (organophosphate, 8.6-fold), there was no resistance for methomyl (carbamate, 0.5-fold).

Table 1. Toxicity of topically applied insecticides to Susceptible, Adana and Mardin strains of *Helicoverpa armigera.*

Insecticides	Strains	Number of insect[a]	LD_{50} [b] (95% CL)	Slope±SE	Hetero-genity	Resistance Ratio [c]
Lambda-cyhalothrin (Pyrethroid)	HELIAR	100	0.002 (0.001-0.002)	1.96±0.47	0.11	--
	ADANA	140	0.012 (0.007-0.019)	1.23±0.24	0.37	6
	MARDİN	120	0.015 (0.009-0.025)	1.31±0.28	0.17	7.5
Esfenvalerate (Pyrethroid)	HELIAR	100	0.003 (0.002-0.004)	2.44±0.51	0.68	--
	ADANA	100	0.110 (0.074-0.183)	1.80±0.47	0.64	36.6
	MARDİN	120	0.188 (0.126-0.267)	1.848±0.36	0.91	62.6
Beta cyfluthrin (Pyrethroid)	HELIAR	120	0.001 (0.000-0.001)	2.88±0.74	0.29	--
	ADANA	120	0.010 (0.006-0.015)	1.51±0.34	0.33	67
	MARDİN	120	0.016 (0.007-0.026)	1.22±0.32	0.37	16
Bifenthrin (Pyrethroid)	HELIAR	120	0.004 (0.003-0.006)	1.73±0.35	0.75	--
	ADANA	120	0.236 (0.173-0.318)	2.33±0.40	0.43	59
	MARDİN	120	0.081 (0.053-0.023)	1.53±0.34	0.28	20
Methomyl (Carbamate)	HELIAR	230	0.201 (0.112-0.312)	0.98±0.17	0.49	--
	ADANA	140	0.096 (0.059-0.165)	1.19±0.25	0.09	0.5
	MARDİN	110	0.105 (0.079-0.161)	2.380±0.51	0.13	0.52
Indoxacarb (Oxadiazine)	HELIAR	120	0.043 (0.027-0.065)	1.53±0.34	0.41	---
	ADANA	100	0.089 (0.069-0.114)	3.28±0.60	0.02	2
	MARDİN	100	0.020 (0.016-0.027)	2.89±0.55	0.54	0.45
Azinphos-methyl (Organophosphate)	HELIAR	120	0.152 (0.109-0.221)	1.95±0.37	0.05	--
	ADANA	100	1.307 (0.915-1.735)	2.62±0.55	0.20	8.6
	MARDİN	130	0.45 (0.334-0.614)	2.33±0.40	0.23	2.96

a Number of larvae used in the experiment

b mg/larva

c LD50 value of field population / LD 50 value of susceptible population

HELIAR: Susceptible Helicoverpa armigera strain obtained from Germany

ADANA: Field strain of Helicoverpa armigera collected from cotton fields in Adana province

MARDIN: Field strain of Helicoverpa armigera collected from cotton fields in Mardin province

ADANA: Field strain of Helicoverpa armigera collected from cotton fields in Adana province

Bioassays indicate that Mardin strain also displayed different resistance levels to synthetic pyrethroid insecticides. Mardin field strain showed high resistance level to esfenvalerate (62.6-fold). Furthermore, while it showed moderate resistance level to bifenthrin (20-fold) and beta-cyfluthrin (16-fold), it showed low resistance level to lambda-cyhalothrin (7.5-fold). Moreover, it showed low resistance level to azinphos-methyl (organophosphate, 2.96-fold), as well. However, there was no resistance for indoxacarb (oxadiazine, 0.45-fold) and methomyl (carbamate, 0.52-fold) insecticides.

It was reported similar results from Australia that *H. armigera* resistant to synthetic pyrethroid insecticides with resistance to esfenvalerate, zeta-cypermethrin, bifenthrin and lambda-cyhalothrin ranging between 12- and 32-fold (Gunning *et al.,* 1999 and 2007). Moreover, in Australia, resistance monitoring studies in 2001-2002 season for *Helicoverpa armigera* was shown that there was no resistance to amitraz, indoxacarb and the mectins (Gunning, 2002), as well. Furthermore, results from the 2011-12 seasons showed that *Helicoverpa armigera* showed resistance to indoxacarb (oxadiazine) remained consistently low and resistance to carbamate (methomyl) remains at moderate and stable levels. However, there has been a significant increase in resistance to synthetic pyrethroids (Anonymous, 2012). In addition, Torres-Vila *et al.* (2002a) reported that *H. armigera* resistant to bifentrin and lambda-cyhalothrin insecticides in Spain ranging between 1- and 117-fold. Torres-Vila (2002a) also reported that *H. armigera* showed resistance ratios for azinphos-methyl, indoxacarb and methomyl insecticides ranging between 1- and 15-fold. It was also reported that *H. armigera* field strain in Spain showed low resistance level for methomyl, chlorpyrifos and lambda-cyhalothrin ranging between 1.9- and 6-fold (Avilla *et al.,* 2010).

There were similar results for resistance to synthetic pyrethroid insecticides, tralomethrin and lambda-cyhalothrin in our previous findings (Ugurlu *et al.,* 2007; Ugurlu and Gurkan, 2007). In Turkey, *H. armigera* strains from Adana, Antalya and Hatay (collected in 1999) were somewhat resistant to synthetic pyrethroids, but there was no significant resistance to carbamates, organophosphate and cyclodiene insecticides. Resistance ratios of Adana, Hatay and Antalya strains of *H. armigera* for tralomethrin were 24.7-, 19.7-,and 15.7-fold, respectively, and for lambda cyhalothrin 41-,20-, and 40-fold, respectively (Ugurlu and Gurkan, 2007). Furthermore, Adana and Antalya strain (collected in 2002) showed resistance ratios for 3- and 98-fold for lambda-cyhalothrin, respectively; and for esfenvalerate, 3.3-fold and 92.3-fold, respectively, with respect to the susceptible strain (Ugurlu *et al.,* 2007).

Compared this study results with previous results, it is clearly seen that Adana strain resistance level for esfenvalerate changed from low resistance level (3.3-fold, Ugurlu *et al.,* 2007) to high resistance level (36.6-fold, in this study). Adana strain also showed high resistance ratio for other tested synthetic pyrethroids such as beta-cyfluthrin (67-fold) and bifenthrin (59-fold). Moreover, Mardin strain showed high resistance level (62.6-fold) for esfenvalerate, too. Both Adana and Mardin strains displayed similar resistance level (low resistance, 6- and 7.5-fold) for lambda-cyhalothrin as reported previously (Ugurlu *et al.,* 2007; Avilla *et al*., 2010).

In addition, it was found similar results, low resistant or susceptible, for methomyl insecticide as reported by Gunning *et al.,* 1999, Torres-Vila (2002a), Ugurlu and Gurkan, (2007) and Avilla *et al.,* (2010).

One of the main reasons of resistance development is the constant use of the same insecticides over long periods of time. Unfortunately, in our country until recent years, synthetic pyrethroid insecticides have been used commonly for the controlling of *H. armigera* in cotton fields.

CONCLUSIONS

In summary, the results obtained in this study indicated that strains of *H. armigera* from Adana and Mardin field strains showed moderate to high resistance levels for synthetic pyrethroid insecticides, especially for beta-cyfluthrin, bifenthrin and esfenvalerate. However, they showed low resistance levels for lambda-cyhalothrin (pyrethroid) and azinphos-methyl (organophosphate) insecticides. In addition, they did not show significant resistance for indoxacarb (oxadiazine) and methomyl (carbamate) insecticides.

ACKNOWLEDGEMENTS

This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) and Republic of Turkey Ministry of Food, Agriculture and Livestock. I would like to thank to Ralf NAUEN (Bayer CropScience, Germany) for sending the susceptible strain of *Helicoverpa armigera.*

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Received: March 26, 2012 Accepted: January 14, 2013