J. Entomol. Res. Soc., 13(2): 45-51, 2011 *ISSN:1302-0250*

Susceptibility of Different Strains of the Sunn Pest, *Eurygaster integriceps* Put. and *Eurygaster maura* L. (Heteroptera: Scutelleridae) to Fenitrothion and Alpha-cypermethrin in Turkey

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ABSTRACT

The Sunn pest are a group of insects representing several genera of the shield bug (Scutelleridae) families, including the species *Eurygaster integriceps* Put. and *Eurygaster maura* L. (Hemiptera: Scutellaridae) being the most economically important pests in Middle Anatolia Souteast Anatolia and Thrace in Turkey. Chemical control has been the main method used against these pests. Chlorinated hydrocarbon, organophosphorus and pyrethroid insecticides have been used since 1955. The aim of this study was to determine the resistance levels of sunn pest, *Eurygaster spp*. collected from wheat fields in different regions of Turkey to alpha-cypermethrin and fenitrothion. Insecticide toxicity was measured using a topical bioassay method using new generation adults. Insects were treated on the pronotum with one microlitre aliquots of insecticide in acetone for treatments and acetone alone for the control. After treatment, insects were maintained at laboratory conditions and mortality was recorded after 48 hours. Results were analysed and LD_{50} values were determined by using probit analysis. The results of bioassays showed that there was no resistance in *E. integriceps* Put strains to alpha-cypermethrin and fenitrothion. The resistance ratio for alpha-cypermethrin were determined 1.7 and 1.9 fold in the Diyarbakır and Tekirdağ strains of *E. integriceps* Put, respectively. For fenitrothion, the resitance ratios were determined 2.3 and 1.4 fold in the Diyarbakır and Şanlıurfa strains, respectively. In *Eurygaster maura* (L) strains, resistance ratios varied from 3.3 to 7.6 for alpha cypermethrin and 1.05 to 1.9 for fenitrothion. The results obtained showed that there was no resistance in *E. integriceps* Put. strains to alpha-cypermethrin and fenitrothion. However, Yozgat, Kırıkkale and Konya strains of *E.maura* from Middle Anatolia had some resistance development to alpha cypermethrin but there was no evidence of resistance development to fenitrothion.

Keywords: Sunn pest, *Eurygaster spp*., insecticide resistance, alpha-cypermethrin, fenitrothion.

INTRODUCTION

Wheat production places an important role in agricultural production and is a major source of income for most people in Turkey. In 2009 total wheat production in Turkey was 16.860.000 tonnes (Anonymous, 2010). Sunn Pest, *Eurygaster integriceps* Put. and *Eurygaster maura* (L.) (Heteroptera:Scutellaridae) are the most harmful pests of wheat in Turkey as well as neighbouring countries in the Middle and Near East (Kinaci and Kinaci 2004). This pest threatens food security and reduces the stability

of traditionally wheat-based agricultural systems. Pre-harvest bug damage to wheat caused by *Eurygaster spp*. and *Aelia spp*. occurs in many countries of the Middle East, Eastern Europea, and North Africa (Paulian and Popov 1980) and yields grain with reduced bread making quality, due to pre-harvest insect attack, have been reported from Germany, Russia, Spain, Hungary, Czechoslovakia, former Yugoslavia, Italy, Turkey, Iran, Iraq, Syria and New Zeland (Jordon and Pascoe, 1996; Critchley, 1998, Hairi *et al*., 2000; Parker *et al*.,2003; Bandani *et al*.,2005; Kazzazi *et al*.,2005; Abdullahi *et al*.,2010) . Althought annual losses in cereal crops are approximately 20-30% in barley this can be up to 100% in wheat (Bandani *et al*. 2005).

The sunn pest, *Eurygaster integriceps*, underwent outbreaks in 1927-1929 in South Anatolia, and in 1939-1941 in Southeast Anatolia. Populations of *E. integriceps* have increased since 1982 in Thrace, where they peaked in 1987, causing significant damage to the crop. The Sunn pest, *E. maura* has became a problem in Central Anatolia, especially in the provinces of Konya, Aksaray, Kırşehir and Ankara since 1995 (Şimşek *et al*.,1996).

The first chemical application to control this pest were carried out on 222,000 da by airplane and on 25,000 da with ground applicators in Southeastern Anatolia in 1955. Insecticide applications started in Thrace in 1987 then insecticide aplication were continued in Thrace and Central Anatolia and the Aegean regions in 1988. Approximately 180 million da have been sprayed by airplane from the first applications up to today. After the introduction of Ultra Low Volume (ULV) application systems, treated areas increased to a maximum in 2003 of 18.8 million da. The Ministry of Agriculture and Rural Affairs has started to take some steps in order to reduce sprayed area. Subsequently, aerial applications in cereal fields were completely banned in 2006.

The insecticides, used widely against the Sunn pest, are DDT, diazinon, fenitrothion, fenthion, isochlorthion, parathion methyl, trichlorphon, alpha-cypermethrin, cypermethrin, deltamethrin, zeta cypermethrin and gamma cyhalothrin in Turkey. Some of them are still using nowadays. Fenitrothion and alpha-cypermethrin were used extensively in order to conrol these pests between 1981-2002 and 1993-2006, respectively, in Turkey (Koçak and Yılmaz, 2006). As a consequence of intensive use of these pesticides, there were some complains about insecticide performance and sprayed areas increased year by year.

In spite of the use of a high quantity of pesticides to manage these pests, there have been no detailed insecticides resistance studies related with Sunn pest since the work by Temizer (1976) on Sunn pest, and other studies on cereal bug, *Aelia rostrota* Boh. (Öden *et al*. (1972; Ünal *et al*. 1994) in Turkey. Resistance development studies are important because resistance development can have great economical, ecological and social importance in plant protection.

The aim of this study was to determine the current resistance levels of Sunn pest strains collected from different cereal farms to fenitrothion and alpha-cypermethrin. This work should permit the identification of situations where it may be necessary to use alternative insecticides for the control of this pest in Turkey.

MATERIALS AND METHODS

Insects

Adults of *E. integriceps* and *E. maura* were collected from different locations in Turkey. Strains were named according to the nearest city where they were collected. Adult new generation of *E. integriceps* populations were collected from wheat fields in Diyarbakır and Urfa provinces in June 2005 and from Tekirdağ province in Thrace in 2007. *E. maura* populations were collected from the Middle Anatolian provinces of Konya, Yozgat, Çankırı, Aksaray, Kırıkkale in 2006-2008. The insects collected were transferred to the Ankara Plant Protection Central Reseach Institute laboratory and maintained on wheat plants at 25± 2°C under a 14 h light:10 h dark (LD14:10) photoperiod.

Chemicals

The insecticides used in this study were fenitrothion 95.6% obtained from Bayer Crop Science, Germany and alpha-cypermethrin 97.3% obtained from Basf Chemical Company, Germany. Acetone obtained from Merck, Turkey was used as solvent.

Bioassays

Insecticide toxicity was measured by using a topical bioassay method (Öden, 1972). Active substances of insecticide were dissolved in acetone and for the bioassays following six concentrations of alpha-cypermethrin and fenitrothion were tested: 0.268, 0.134, 0.066, 0,033, 0.016, 0.008µg/µl and 2, 1, 0.5, 0.25, 0.125 and 0.063µg/µl, respectively. Insects were treated on the pronotum with one microlitre aliquots of insecticide in acetone for treatments or acetone alone for control using a micro applicator. In total, 30 adults were used without taking into account the gender for each concentration. After teatment, ten insects were placed in a plastic box (60x90 mm) containing green grass and they were maintained under laboratory conditions of 25±2°C and LD 14:10. Mortality was recorded after 48 hours. Insects were considered dead if they were unable to move or could not get to the upright position when they were placed on one side. Data were analyzed and median Lethal Dose (LD_{50}) values for fenitrothion and alpha-cypermethrin were determined by probit analysis using POLO PC Software (LeOra Software,1994). The statistic *g* was used to estimate the confidence intervals at probability levels of 90, 95, and 99. If *g* is > 0.5 at any of these probability levels, the value of lethal dose may lie outside the limits (Robertson and Preisler, 1992).

Resistance ratios (RRs) were estimated at the LD_{50} level by dividing the LD_{50} value of each field strain by the LD_{50} value of the susceptible strain. The strain had the lowest LD_{50} values was accepted as an susceptible strain, due to the unavailability of a suitable reference susceptible strain normally used to calculate resistance ratios. Experiments where mortality rates for the control were higher than 10%, were not taken into account.

RESULTS

In this study, LD_{50} values for alpha-cypermethrin were found to be 0.011, 0,019 and 0.021µg /insect for Şanlıurfa, Diyarbakır and Tekirdağ strains, respectively. The results obtained from bioassays of insecticides with sunn pests, *E. integriceps* are shown in Table 1 and 2. The Şanlıurfa strain of *E. integriceps* had the lowest LD_{50} value for alpha-cypermethrin. Thus, we concluded that the LD_{50} of alpha-cypermethrin from the Şanlıurfa strain could be used as a reference value to calculate the resistance ratios for the other *E. integriceps* strains. Based on the LD_{50} value for the Şanlıurfa strain, the resistance ratio for alpha-cypermethrin were determined 1.7 fold in the Diyarbakır strain and 1.9 fold in the Tekirdağ strain. There for resistance ratios were low for this chemical.

With fenitrothion the LD_{50} values were calculated to be 0.359, 0.222, and 0.157 µg /insect in, Diyarbakır, Şanlıurfa and Tekirdağ, respectively. The Tekirdağ strain had the lowest LD_{50} value. That's why we concluded that the LD_{50} of fenitrothion from the Şanlıurfa strain could be used as a reference value to calculate the resistance ratios for the other *E. integriceps* strains. Based on the LD_{50} value for the Tekirdağ strain, the resitance ratios for fenitrothion were determined 2.3 fold in the Diyarbakır strain and 1.4 fold in Şanlıurfa strain.

According to these results, there was also a relatively low level of resistance development to fenitrothion in these strains although it was slightly higher overall.

Mean weight values of Diyarbakır, Şanlıurfa and Tekirdağ strains of *E. integriceps* were 0.141g, 0.137g and 0.143g, respectively.

The results obtained from bioassays of insecticides with sunn pest *E. maura* strains are shown in Table 2. Table 2 indicates that LD_{50} values and resistance ratios for alpha-cypermethrin and fenitrothion for field strains of *E. maura* collected from Middle Anatolia. As the Çankırı strain of *E. maura* had the lowest LD_{50} value for alpha-cypermethrin and it was used as a reference value to calculate resistance ratios. Resistance ratios for alpha cypermethrin varied from 3.3 to 7.6 for the field strains. This indicates a relatively moderate level of resistance development.

With fenitrothion the Kırıkkale strain had the lowest LD_{50} value. This value was used to calculate resistance ratios for field strains. Resistance ratios varied from 1.05 to 1.9 for fenitrothion in field strains. All strains therefore showed low resistance ratios for fenitrothion.

Mean weight values of *E. maura* strains were 0.124, 0.124, 0,121, 0.118 and 0.122 g in Konya, Kırıkkale, Yozgat, Çankırı and Aksaray, respectively.

DISCUSSION

The results of the research described in this paper demonstrate that resistance ratios were very low for alpha-cypermethrin in the Diyarbakır and Tekirdağ strains of *E. integriceps*. According to these data, there was no resistance in the *E. integriceps* strains tested to alpha-cypermethrin. We conclude that this insecticide can be still used to control of *E. integriceps* strains in the regions assayed in Turkey.

Table 1. Toxicity of topically applied insecticides to *Eurygaster integriceps* adults.

Strains	Insecticides	Insect Num. [a]	LD_{50} [b] (95%CL)[c]	g values	Slope±SE	Heterogenity	Resistance Ratios[d]
Diyarbakır	Alpha-cypermethrin	150	0.019 (0.015-0.025)	0.114	2.32±0.4	0.95	1.7
	Fenitrothion	150	0.359 (0.278- 0.450)	0.113	3.23±0.55	0.82	2.28
Şanlıurfa	Alpha-cypermethrin	150	0.011 (0.007- 0.014)	0.145	2.06±0.4	0.11	-
	Fenitrothion	180	0.222 (0.140- 0.462)	0.132	2.94±0.47	1.88	1.4
Tekirdağ	Alpha-cypermethrin	180	0.021 (0.016-0.026)	0.069	2.55 ±0.34	0.43	1.9
	Fenitrothion	180	0.157 (0.126-0.200)	0.072	2.52±0.35	0.49	-

[a] Insect number which was used in the experiments. [b] µg/insect.

[c] 95% confidence Limits of LD_{50} values.

[d] LD_{50} values of field strains / LD_{50} values of succeptible strains.

SE:Standard Error

Table 2. Toxicity of topically applied insecticides to *Eurygaster maura* adults.

Strains	Insecticides	Insect Num.[a]	LD_{50} [b] (95%CL)[c]	g Values	Slope±SE	Heterogenity	Resistance Ratio[d]
Konya	Alpha-cypermethrin	180	0.013 (0.08- 0.020)	0.141	1.16±0.22	0.41	4.3
	Fenitrothion	150	0.198 (0.166 -0.237)	0.082	3.98±0.58	0.03	1.9
Yozgat	Alpha-cypermethrin	150	0.023 (0.015-0.039)	0.24	1.42±0.36	0.17	7.6
	Fenitrothion	150	0.11 (0.089-0.138)	0.093	2.98±0.47	0.17	1.05
Çankırı	Alpha-cypermethrin	180	0.003 (0.000-0.006)	0.462	1.38±0.48	0.05	-
	Fenitrothion	150	0.132 (0.090-0.163)	0.30	4.95±1.38	0.29	1.26
Aksaray	Alpha-cypermethrin	240	0.01 (0.007-0.013)	0.097	1.79±0.83	0.48	3.3
	Fenitrothion	150	0.118 (0.084-0.168)	0.117	1.99±0.35	0.75	1.13
Kırıkkale	Alpha-cypermethrin	180	0.016 (0.012-0.021)	0.084	2.03±0.30	0.36	5.3
	Fenitrothion	180	0.104 (0.092-0.120)	0.092	5.43±0.84	0.35	-

[a] Insect number which was used in the experiments. [b] µg/insect

[c] 95% confidence Limits of LD_{50} values.

[d] LD_{50} values of field strains / LD_{50} values of succeptible strains.

SE:Standard Error

For fenitrothion in Diyarbakır and Tekirdağ strains of *E. integriceps* there was also no resistance development. The first study that was related to resistance in *E. integriceps* was carried out by Temizer (1976) in Turkey. He found that LD_{50} values of fenitrothion were 0.047 and 0.046 mg/insect in 1970 and 1972, respectively. These values are significantly lower than the values we recorded. He also found some variations in susceptibility between the stages to some insecticides. Fenitrothion was used to control Sunn pests in cereal areas from 1981 to 2002 in Turkey. As a consequence of intensive use of these pesticides, there were still some complains about insecticide performance and sprayed areas increased year by year.

The resistance ratios of *E. maura* strains collected from Middle Anatolia ranged from 3.3 to 7.6 for the alpha-cypermethrin. According to this result, we conclude that *E. maura* strains may have incipient resistant to alpha-cypermethrin. It is suggested that different mode of action insecticides should be used rotationally for the control of this pest to prevent the increasing of the resistance development in the future.

Croft(1991) emphasized that it is important to use insecticides rotationaly to increase the effectiveness of pesticides over time and to delay the resistance onset. Alternating the use of pesticides with different mode of action must be put into practice to reduce resistance development.

There are a limited number of studies related to resistance in Sunn pest in other countries. Investigations on resistance or sensitivity of three pests to insecticides in Romania indicated incipient resistance of *Eurygaster sp*. to trichlorfon and sensitivity to dimethoate (Alexandrescu *et al*. 1990). Bandani *et al*. (2005) showed that LD_{10}, LD_{50}, and LD_{90} were 34.7, 90.43, and 235.5 ppm, respectively, for the fenitrothion in *E. integriceps in* Iran. In Russia, a further increase in number of pest species resistant to OPs and pyrethroids was observed in the 1990s. One of these resistant pests was Sunn pest (*E. integriceps*) in the North Caucasus Region (Sukhouruchenko and Dolzhenko, 2008).

In conclusion, the results of this study showed that there was no evidence of resistance development in *E. integriceps* strains tested in this study for alpha-cypermethrin but that they may be some evidence of low level resistance development for fenitrothion. On the contrary, *E. maura* strains had no resistance to fenitrothion, but incipient resistance to alphacypermethrin.

ACKNOWLEDGEMENTS

This study were supported by Turkish Republic Prime Ministry State Planning Organization (SPO) and Ministry of Agriculture and Rural Affairs, Plant Protection Central Research Institute. We acknowledge the contribution of test substances from the following agrichemical companies: Bayer CropScience and Basf Chemical Company.

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Received: July 06, 2010 Accepted: March 21, 2011