Pakistan J. Zool., vol. 51(2), pp 703-707, 2019. DOI: http://dx.doi.org/10.17582/journal.pjz/2019.51.2.703.707

Evaluation of some Synthetic Pyrethroids and Piperonyl Butoxide Combinations against Turkish House Fly (*Musca domestica* L.) Populations

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Article Information
Received 15 March 2018
Revised 13 May 2018
Accepted 12 July 2018
Available online 01 March 2019

Authors' Contribution
HC and OK designed the experiment, HC analyzed the data and wrote the article. EO, SK, YP, KA, HC and OK conducted the experiments.

Key words
House fly, *Musca domestica*, Piperonyl butoxide, Synthetic pyrethroid, Toxicity.

ABSTRACT

The house fly, *Musca domestica* L. (Diptera: Muscidae) is known as a mechanical vector of many pathogenic bacteria, fungi and parasites. The use of synthetic pyrethroid (SP) insecticides is recommended by the World Health Organization for the control of adult houseflies. Despite the fact that SPs have been used effectively, this insect has developed resistance in many regions/countries of the world. The use of synergistic substances to overcome insecticide resistance is widespread. Piperonyl butoxide (PBO) is used as a synergist in combination with SPs for the control of houseflies. The aim of this research was to determine the effect of SPs (cypermethrin, permethrin and deltamethrin) and their combination with different rates of PBO (1: 0.125, 1:0.25, 1:0.5 and 1:1) against four Turkish house fly populations (Ankara, Antalya, Gaziantep and Şanlıurfa) in laboratory conditions. According to results the use of PBO in combination with insecticide active substances causes an increase in the mortality rates after 24 h. Also the toxicities of tested chemicals, based on their KDT_{50} (Knock-down 50 times) values, increased when mixed with PBO.

INTRODUCTION

Synthetic pyrethroid insecticides have been used for more than fifty years to control of agricultural, veterinary and forestry insect pests in the world (Pasay *et al*., 2009; Scoot, 2017). These insecticides are also recommended by World Health Organization (2006) against the various public health pests for indoor and outdoor conditions. The synthetic pyrethroid family includes many compounds; some of them are cypermethrin, deltamethrin and permethrin that available commercially worldwide.

Many insecticide/acaricide formulations used against agricultural/public health pests including mosquitoes, ticks, mites and fleas contain the active ingredient Piperonyl butoxide (PBO). There are many products with a large variety of different mixtures of PBO and synthetic pyrethroids (Glynne-Jones, 1998). PBO is not a pesticide itself, but enhances the properties of insecticides or acaricides. PBO is mainly used as synergistic agent for pesticides and it is often combined with synthetic pyrethroids (Khan *et al*., 2013). PBO inhibits cytochrome P450 monooxygenases and the use of PBO as synergized with pyrethroids recommended by WHO (2006) in cold and thermal fog formulations.

House fly, *Musca domestica* L. (Diptera: Muscidae) is known as mechanical vectors of many pathogens including bacteria, fungi, viruses, helmints and protozoans. This insect picks up disease-causing organisms via its body surface, mouthparts, feces and vomits (Calibeo-Hayes *et al*., 2002; Macovei *et al*., 2008). House flies have developed resistance against a large number of insecticide groups and there are many reports on insecticide resistance of *M. domestica* from different regions of the world (Acevedo *et al*., 2009; Cetin *et al*., 2009; Khan *et al*., 2017; Ma *et al*., 2017).

The detection of knock down resistance in the field populations may have severe consequences for sustained use of pyrethroids in house fly control. Therefore the aim of the present study was to determine the effect of some synthetic pyrethroids (cypermethrin, permethrin and deltamethrin) and their combination with different rates of piperonyl butoxide (PBO) against four Turkish house fly (*M. domestica*) populations (Ankara, Antalya, Gaziantep and Şanlıurfa) in laboratory conditions.

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0030-9923/2019/0002-0703 $ 9.00/0

MATERIALS AND METHODS

Insecticides and PBO

In efficacy tests, cypermethrin (92% Tagros Chem. India Ltd., CAS Number: 52315-07-8), permethrin (94% Tagros Chem. India Ltd., CAS Number: 52645-53-1), deltamethrin (98% Tagros Chem. India Ltd., CAS Number: 52918-63-5) and Piperonyl butoxide (94% Endura Spa CAS Number: 51-03-6) technical substances were used. Tested chemicals were dissolved in acetone (99.8% Merck CAS Number: 67-64-1).

Flies

Five *M. domestica* populations were used in this research: the susceptible population, obtained from Hacettepe University in 2002, has been reared in our laboratory without exposure to any insecticides and four field populations were collected by using sweep nets from livestock farms and garbage dumps in Ankara, Antalya, Gaziantep and Şanlıurfa cities from Turkey in the summer of 2015 (Fig. 1).

Fig. 1. House fly sampled cities from Turkey.

In field sampling areas, the synthetic pyrethroids have been widely used for at least ten years against mosquitoes and houseflies. The collected houseflies were transported in fine muslin cages (22 × 22 × 22 cm) including cube sugar and wetted cotton pads to the Vector Control an Ecology Laboratory, Akdeniz University, Antalya within 24 h. Houseflies were kept under standard laboratory conditions (24±2°C, 60±10% RH and a 12h:12h light:dark photoperiod) and supplied with water, sugar and milk.

Toxicity tests

Toxicity tests were made by using glass jar residual surface method used by Cetin *et al.* (2010) and recommended by WHO. In the tests, glass jars with 160 cm² treatment areas were used as application surface and at volume of 250 ml. Doses of pyrethroids were determined according to WHO recommendations. Deltamethrin 0.0075 g ai/m², Permethrin 0.0625 g ai/m², Cypermethrin 0.025 g ai/m²), and four combinations with PBO (1: 0.125, 1:0.25, 1:0.5 and 1:1) were used in the assays (Table I).

Pyrethroid or pyrethroid+PBO combination solutions at the application doses (1 ml test solution) were put at the bottom of each jar, and the jars rotated until the acetone was vaporized and the test solution was deployed on the inner surface as a film. For control only jars containing acetone are prepared. Application of test chemicals to the jars was done 24 h before the tests.

After rearing for two generations in laboratory, the house flies were used for toxicity tests. Twenty five 3–5-day-old house flies were taken into treated jars that were covered by clean net and rubber in order to provide air transfer. After 15 min exposure flies were transferred to clean jars. Knockdown rates were determined at 5 min intervals for 1 h. The top of the jars were closed by net, wet cotton was put on the net in order to provide humidity. Final mortality was assessed at 24 h post-exposure to insecticides and all tests were replicated three times. Toxicity tests were conducted under standard laboratory conditions; 24±2°C, 60±10% RH and a 12h: 12h light: dark photoperiod.

Data analysis

Knock down fifty times (KDT_{50}) values were determined using probit analysis program. The KDT resistance ratios (Resistant/Susceptible) were determined by dividing the KDT_{50}, for the populations tested by the KDT_{50} for the standard reference population (WHO).

RESULTS AND DISCUSSION

The KDT_{50} values, KD resistance rates and 24 h mortality rates of test chemicals (cypermethrin, deltamethrin and permethrin) alone and different combinations with PBO against house fly (*M. domestica*) are shown in Tables I and II.

When alone permethrin compared with other two synthetic pyrethroids generally lower KDT_{50} values and high-mortality rates were obtained because of the its high KD affect. The KDT_{50} were more decreased by insecticide+PBO combinations than insecticides that are used alone (Table I). The effect of the PBO synergist on the toxicity of pyrethroids was variable. The highest KDT_{50} values for Permethrin, Deltamethrin and Cypermethrin without PBO were determined for Şanlıurfa, Ankara and Şanlıurfa strains, respectively. The highest KDT_{50} values of active substances with PBO combinations were Permethrin and Deltamethrin for Şanlıurfa strain and Cypermethrin for Ankara strain except the combination 1:0.25 rate (Table I).

Table I.- Effect of different synthetic pyrethroids administrated in combination PBO with knock-down time (min) and KD resistance rates different strains of *Musca domestica*.

Strain	Permethrin (1:0)		Permethrin + 0.125 PBO (1:0.125)		Permethrin + 0.25 PBO (1:0.25)		Permethrin + 0.5 PBO (1:0.5)		Permethrin + PBO (1:1)	
	KDT_{50} (min)	Resistance rates	KDT_{50} (min)	Resistance rates	KDT_{50} (min)	Resistance rates	KDT_{50} (min)	Resistance rates	KDT_{50} (min)	Resistance rates
Susceptible	<1.0	1.0	<1.0	1.0	<1.0	1.0	<1.0	1.0	<1.0	1.0
Ankara	1.33	1.3	5.21	5.5	2.33	2.3	2.06	2.0	<1.0	0.6
Antalya	2.79	2.8	2.97	3.1	<1.0	1.0	1.0	1.0	<1.0	1.0
Gaziantep	1.88	1.9	3.67	3.9	2.49	2.5	1.98	1.9	3.69	3.7
Şanlıurfa	9.62	9.6*	8.46	8.9*	6.97	7.0*	6.58	6.6*	7.30	7.3*
Test doses (gr ai/m^2)	0.0625 + 0.0		0.0625+0.0078125		0.0625 + 0.015625		0.0625 + 0.03125		0.0625 + 0.0625	
	Deltamethrin (1:0)		**Deltamethrin + 0.125 PBO (1:0.125)**		**Deltamethrin + 0.25 PBO (1:0.25)**		**Deltamethrin + 0.5 PBO (1:0.5)**		**Deltamethrin + PBO (1:1)**	
Susceptible	2.39	1.0	7.02	1.0	5.69	1.0	3.76	1.0	4.45	1.0
Ankara	20.51	8.6*	10.72	1.5	6.37	1.1	7.39	1.9	6.32	1.4
Antalya	6.74	2.8	6.53	0.9	6.68	1.2	6.71	1.8	6.15	1.4
Gaziantep	10.88	4.5	9.17	1.3	6.97	1.2	6.06	1.6	5.77	1.3
Şanlıurfa	16.31	6.8	11.51	1.6*	11.27	1.9*	8.96	2.4*	8.18	1.8*
Test doses (gr ai/m^2)	0.0075 + 0.0		0.0075 + 0.0009375		0.0075 + 0.001875		0.0075 + 0.00375		0.0075 + 0.0075	
	Cypermethrin (1:0)		**Cypermethrin + 0.125 PBO (1:0.125)**		**Cypermethrin + 0.25 PBO (1:0.25)**		**Cypermethrin + 0.5 PBO (1:0.5)**		**Cypermethrin + PBO (1:1)**	
Susceptible	<1.0	1.0	<1.0	1.0	<1.0	1.0	<1.0	1.0	<1.0	1.0
Ankara	4.84	4.8	9.80	9.8*	7.75	7.7	6.22	6.2*	6.60	6.6*
Antalya	9.31	9.3	5.06	5.0	3.28	3.3	3.42	3.4	1.45	1.4
Gaziantep	7.81	7.8	8.03	8.0	6.53	6.5	5.68	5.7	5.33	5.3
Şanlıurfa	12.84	12.8*	8.14	8.1	8.28	8.3*	6.13	6.1	5.18	5.2
Test doses (gr ai/m^2)	0.025 + 0.0		0.025 + 0.003125		0.025 + 0.00625		0.025 + 0.0125		0.025 + 0.025	

*Highest knock-down (KD) resistance rates.

Table II.- Response of field populations of *Musca domestica* to different combinations of insecticides after 24 h.

Combinations (Pyrethroid+PBO)	Mean mortalities (%)			
	Ankara	Antalya	Gaziantep	Şanlıurfa
1 Permethrin+0.0 PBO	100	100	97.0	65.3
1 Permethrin+0.125 PBO	100	100	100	100
1 Permethrin+0.25 PBO	100	100	100	100
1 Permethrin+0.5 PBO	100	100	100	100
1 Permethrin+1.0 PBO	100	100	100	100
1 Deltamethrin+0.0 PBO	10.9	71.5	70.7	8.0
1 Deltamethrin+0.125 PBO	50.0	90.7	88.7	25.3
1 Deltamethrin+0.25 PBO	68.7	86.7	88.3	46.7
1 Deltamethrin+0.5 PBO	75.0	100	100.0	66.7
1 Deltamethrin+1.0 PBO	95.0	100	98.0	76.3
1 Cypermethrin+0.0 PBO	93.4	68.3	27.7	17.7
1 Cypermethrin+0.125 PBO	58.2	100	27.7	94.3
1 Cypermethrin+0.25 PBO	97.1	100	100	91.7
1 Cypermethrin+0.5 PBO	100	100	100	100
1 Cypermethrin+1.0 PBO	98.6	100	100	100

There was no significant difference between Gaziantep and Antalya strains in terms of KDT_{50} values in the combinations of active substances with PBO at 1:0.125 and 1:0.25 ratios. Of four field strains of *M. domestica* tested against permethrin and deltamethrin with all combinations of PBO, the strain from Şanlıurfa showed the highest level of KD resistance (1.6-8.9 folds). Şanlıurfa is an important production area for agricultural crops such as barley, pistachio, wheat, and sesame. In this region there is a very high use of agricultural pesticides against many pests. Livestock is also one of the fastest growing sectors in the area. Houseflies used in this research were collected from livestock farm in region where the synthetic pyretroids were applied frequently and at high rates. Both farmers and municipal vector control teams have been tried to control of house flies in these farms. Improper and high use of pesticides in area affects KD resistance development (Dhang *et al*., 2015). The detection of high knock down resistance rates especially in the Şanlıurfa strain may have severe consequences for sustained use of pyrethroids in house fly control. In general, the least sensitive population was determined as the Antalya population according to the percent mortality values obtained after 24 h (Table II). In control groups; no mortality and knock down were observed in adults.

Generally the use of PBO in combination with insecticide active substances causes an increase in the mortality rates obtained after 24 h, while the percent mortality rates achieved despite the rising PBO rates for some active substances are below the WHO success criteria (<80% mortality). Also the toxicities of tested chemicals, based on their KDT_{50s}, increased when mixed with PBO. For this reason, considering that the contribution of the PBO ratios preferred by the institutions to the biological activity of the active substance and the population may be different, the biological efficacy tests should be performed and the product should be supplied.

The toxicity of cypermethrin, deltamethrin, and permethrin active substances combined with PBO at the rate of 1:1 on some other Turkish house fly populations (Ankara, Istanbul, Izmir, and Adana) was studied by Cakir *et al*. (2008). They reported that PBO extensively promoted the ratio of knockdown and killing effect values of the insecticides.

The toxic effects of PBO+pyrethroid combinations were investigated on mosquitoes and other pests also. The toxicity of deltamethrin+PBO combination at the rate of 1:6 was studied by Fakoorziba *et al*. (2008) on *Aedes*, *Anopheles* and *Culex* mosquitoes. They found that PBO was found highly active synergist. Dadzie *et al*. (2017) reported that PBO significantly enhanced the susceptibility of resistant *Anopheles gambiae* to deltamethrin and permethrin in Ghana. Findings from this study showed that the use of PBO significantly enhanced the susceptibility of *An*. *gambiae* mosquitoes in most of the sentinel sites. It is recommended that vector control strategies incorporating PBO as a synergist can be effective in killing mosquitoes in the presence of resistance. Cetin *et al*. (2010) reported that with the addition of PBO at rate of 25% of the active substance to the formulation knock down and mortality rates reached to 100% on *Drosophila melanogaster* (Diptera: Drosophilidae) adults.

CONCLUSION

Our results showed that the addition of PBO to the synthetic pyrethroid insecticide formulations is very useful in order to achieve the biological efficiency level required and the optimum rate of PBO in pesticide formulations should be determined by efficacy tests according to the region, pest species and resistance ratios. Despite high combination rates of PBO with pyrethroids we determined lower mortalities for some populations and other mechanisms than metabolic resistance may be responsible for the development of resistance (Khan *et al*., 2016).

ACKNOWLEDGEMENTS

The research is supported by the Scientific Research Projects Coordination Unit of Akdeniz University. No conflict of interest was declared by the authors.

Statement of conflict of interest

Authors have declared no conflict of interest.

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