EFFECT OF PIPERONYL BUTOXIDE ON THE BIOLOGICAL ACTIVITY OF SOME SYNTHETIC PYRETHROID INSECTICIDES USED IN PUBLIC HEALTH IN TURKEY

TÜRKİYE'DE HALK SAĞLIĞI'NDA KULLANILAN BAZI SENTETIK PİRETROİT İNSEKTİSİTLERİN BİYOLOJİK AKTİVİTESİ ÜZERİNE PİPERONİL BUTOKSİT'İN ETKİSİ

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ÖZET

Bu çalışmada sipermetrin, deltametrin ve permetrinin biyolojik aktiviteleri üzerine piperonil butoksitin (PBO) etkisini göstermek ve laboratuvar (WHO) ve saha (Ankara) karasinek popülasyonlarında kullanılan insektisitler arasında etkileri ile ilgili farklılıkların var olup olmadığını belirlemek açmaçlandı. Sipermetrin, deltametrin ve permetrin etken maddeleri tek başına ve PBO sinerjist bileşiği ile 1:1 ve 1:3 oranında kombinasyonları olarak 3 grup oluşturuldu. 4-6 günlük yaşta karasinekler üzerine bu etken maddelerin biyolojik etkileri tespit edildi. Deneysel işlem sırasında knockdown (bayıltıcı) ve mortalite yüzdeleri, knockdown zamanı ve knockdown direnç katsayıları belirlendi. Mortalite ve knockdown yüzdelerinin tek başına insektisit kullanılan grupta WHO karasinek popülasyonlarına göre Ankara karasinek popülasyonlarında oldukça düşük olduğu bulundu. PBO, Ankara karasinek popülasyonlarında insektisitlerin knockdown ve mortalite yüzdelerini önemli düzeyde artırdı ve ayrıca knockdown zamanlarını azalttı. Ayrıca PBO'nun insektisitlerle karıştırılması Ankara karasinek popülasyonlarında knockdown direnç katsayılarını azalttı. Dolayısıyla PBO'nun karasinek popülasyonlarında insektisitlerin biyolojik etkilerini artırmada ve knockdown direncinin ortaya çıkışını önlemede önemli fonksiyona sahip olduğu bulundu.

Anahtar Kelimeler: Karasinek, Sentetik piretroit insektisit, Piperonil butoksit, Biyolojik aktivite, Direnç.

ABSTRACT

In this study, it was aimed to show the effect of piperonyl butoxide (PBO) on the biological activities of cypermethrin, deltamethrin and permethrin and to determine whether there are differences in respect to the effects among insecticides used on laboratory (WHO) and field (Ankara) populations of houseflies. Three groups were included as cypermethrin, deltamethrin, and permethrin alone and combinations with PBO synergist compound at a 1:1 and 1:3 ratio. The biological effects of these active ingredients in 4-6 days old houseflies were determined. During the experimental process, knockdown and mortality percentages, knockdown time and knockdown resistance coefficients were determined. Mortality and knockdown percentages were found to be very lower in Ankara housefly populations than WHO housefly populations using insecticides alone. PBO highly increased the knockdown and mortality percentages of insecticides on Ankara housefly populations and as well as decreased knockdown times. In addition, the mixing of PBO with insecticides decreased knockdown resistance coefficients on Ankara housefly populations.

Thus, it was found that PBO had important function in preventing the occurrence of knockdown resistance and in increasing the biological effects of insecticides on housefly populations.

Keywords: Housefly, Synthetic pyrethroid insecticide, Piperonyl butoxide, Biological activity, Resistance.

***Note:** This study abstract was accepted as oral presentation to the 5th International Biocidal Congress, on 5-7 March 2021 after reviewer evaluation, and this manuscript was summarized from graduate thesis with the same title.*

INTRODUCTION

Pesticides are widely used against mosquito, housefly, other flies, cockroach, flea, bedbug, louse, mites, tick, house dust mite, bumblebee, mollusk, ant, and rodents for preventing public health (Britch et al., 2019; Sankoh et al., 2016; WHO, 1988). Many compounds synthesized from natural pyrethrins have recently become insecticide group that is mostly used in plant protection, veterinary medicine, and public health. Synthetic pyrethroids are chosen because they are safe and have biological effects to many pests (Castilla-Castano et al., 2019; Deletre et al., 2019; Papanikolaou et al., 2018).

Pyrethroids cause the stabilization of the open state of sodium channels and prolonged channel opening for neurotoxic effects (Field et al., 2017; Silver et al. 2014). They have knockdown effects in pests. Restlessness, convulsion, paralysis and death occur in pests exposed to poisonous amounts at single dose. Marked knockdown effects are associated with paralysis in muscles of flies (Kaya and Bilgili, 2000; Sun et al., 2016; WHO, 1990a, 1990b).

Pest resistance in the result of use of pesticides in a manner of intense, unlimited and careless is an important concern for pest management programs (Scott, 2017). According to the data of World Health Organization (WHO), resistance was taken place against various pesticides in almost all the pests with risk for publich health in 1992 (WHO, 1992). There are various resistance modes concerning synthetic pyrethroids on pests (Dong et al., 2014). The resistance of houseflies has been explained by mechanisms with regard to voltage-sensitive sodium channel mutations and insecticide detoxification via cytochrome P450 monooxygenases (Scott, 2017). Voltage-sensitive sodium channel alleles such as knockdown resistance (kdr), kdr-his and super kdr are associated with housefly resistance to pyrethroids (Scott et al., 2013).

To reduce insecticide resistance in pests, besides implementing of various methods, the use of synergist components that increase the pesticide potent is an important practice (Wu et al., 2017). One of the important synergist components is piperonyl butoxide (PBO) (Gleave et al. 2018; Scott et al., 2013; Farnham, 1999; Stewart, 1998; Laszlo et al., 2001). This component is the most common used synergist in the formulations to protect public health. PBO is used in the formulations of pyrethrins and pyrethroids (Dadzie et al., 2017; Showyin, 1998). PBO can be solved in water and mineral oils and all the organic solvents. The mixture ratio of PBO to pesticide formulations is an important point. The recommended ratio of pyretroid to PBO varies from 1:5 to 1:20. Although PBO is present at 1:5 to 1:20 ratio in formulations, in this study, as standard active compounds with analytical purity that is not become a formulation are used and the investigation of the effect of PBO on insecticide biological activities and the role of PBO amount in the occurrence of this effect is aimed, insecticide and PBO mixture is used at 1:1 and 1:3 ratio.

In this study, it was aimed to show the effect of PBO on the biological activities of insecticide active ingredients and to determine whether there are differences in terms of effect between insecticides on laboratory and field populations of houseflies.

2. METHODS

In this study, cypermethrin (with an analytical purity of 94%), deltamethrin (with an analytical purity of 98%), and permethrin (with an analytical purity of 94%) by Changzhou Company, China; PBO (with an analytical purity of 93%) by Endura, Italy; acetone (C_3H_6O; m: 58.08 g/mol) by Merck, Germany and carbondioxide (CO_2) by HABAŞ, Turkey were supplied.

Test insecticides consisted of WHO susceptible population and Ankara population houseflies (*Musca domestica* L.). Housefly rearing was performed in 25x40x25 cm gauze cages (at 26 °C, 55% relative humidity, 12 h dark and light cycle). Field and laboratory larvae were fed milk and wheat bran, and pupae were transferred in cage and fed water and sugar. The experiments were made on adult houseflies at 4-6 days of age.

This study consisted of three groups for evaluating the biological effects of cypermethrin, deltamethrin, permethrin active compounds, and PBO synergist component in houseflies as follows:

Group I: Cypermethrin, deltamethrin, permethrin.

Group 2: Cypermethrin+PBO, deltamethrin+PBO, permethrin +PBO with the ratio of 1:1.

Group 3: Cypermethrin+PBO, deltamethrin+PBO, permethrin +PBO with the ratio of 1:3.

In Group I, cypermethrin, permethrin and deltamethrin stock solutions were prepared separetely. 0.1 ml was obtained from each stock solutions, and added to 9.9 mL acetone. 1 ml was taken from each of these solutions and introduced into the jars of 144 cm^2. Cypermetrin at a dose of 24,936 mg/m^2, permethrin at a dose of 60,381 mg/m^2, and deltamethrin at a dose of 74,8611 mg/ m^2 were used in this study.

The recommendation of WHO regarding permanent surface treatment dose for houseflies is 0,025-0,1 g/m^2 for cypermethrin; 0,0625 g/m^2 for permethrin, and 0,075-0,015 g/m^2 for deltamethrin (WHO, 1997).

In Group II and Group III, PBO doses were in a ratio of 1:1 or 1:3 according to cypermethrin, permethrin and deltamethrin doses. 1 ml solution was prepared for each insecticide and introduced into the jar of 144 cm^2.

The version of standard permanent surface method recommended by WHO was used for the biological efficacy experiment (Busvine, 1971; WHO, 1981; WHO, 1985). According this method, 1 ml of each prepared solutions was introduced into the jars of 144 cm^2.

After houseflies at 4-6 days of age were made unconscious using carbondioxide, 25-35 houseflies were introduced into the each jar with insectice solution and the jars were closed using gauze in a manner of allowing air circulation. The numbers of houseflies knocked down were recorded at 0, 5, 10 and 15 min.

After insecticide contact of 15 min, houseflies were transferred to another labelled clean jars. These jars were closed using gauze. Soaked cottons were introduced on gauze for supplying humidity. Houseflies were kept for 24 h. After this process, died flies were recorded.

The biological efficacy experiments were carried out using two different populations. The experiments were maintained during 3 generations of houseflies. Using the changes in knockdown ratio in 0, 5, 10, and 15 min, the time when houseflies were knocked down at 50% (KT_{50}) values was determined with probit analysis reported by Finney (1977).

Using KT_{50}, knock down resistance coefficient (R/S) values were determined as follows:

R/S= Ankara (field) population KT_{50} value/ WHO (susceptible) population KT_{50} value. R/S values were only determined in Ankara housefly populations. WHO population is considered 1,00 because there is no resistance.

2.1. Statistical Analysis

Statistical analyses were carried out for comparisons between insecticides by Kruskal-Wallis test, and comparisons between populations by Mann-Whitney U test.

3. RESULTS

The values and statistical comparisons of mortality percentage, knockdown percentage, 100% knockdown time, 50% knockdown time, and knockdown resistance coefficients on WHO housefly populations and Ankara housefly populations treated with cypermethrin, deltamethrin and permethrin alone and PBO combinations at a 1:1 and 1:3 ratio were given in Tables 1, 2, and 3.

PBO (at a 1:1 and 1:3 ratio) addition to the cypermethrin, deltamethrin and permethrin significantly increased the mortality and knockdown percentages on Ankara housefly populations compared to cypermethrin, deltamethrin and permethrin alone.

PBO (at a 1:3 ratio) addition to the cypermethrin significantly decreased the 100% knockdown time on WHO and Ankara housefly populations compared to cypermethrin alone.

PBO (at a 1:1 and 1:3 ratio) addition to the deltamethrin and permethrin significantly decreased the 50% knock-down time on WHO and Ankara housefly populations compared to deltamethrin and permethrin alone.

Knockdown resistance coefficient was significantly increased on Ankara housefly populations than WHO housefly populations treated with cypermethrin, deltamethrin and permethrin alone. In addition, there were no differences between WHO and Ankara housefly populations in terms of knockdown resistance coefficients by PBO (at a 1:1 and 1:3 ratio) addition to the cypermethrin, and permethrin (Tables 1, 2 and 3).

The values and statistical comparisons of mortality percentage, knock-down percentage, 100% knock-down time, 50% knock-down time, and knock-down resistance coefficients on WHO populations and Ankara populations treated with cypermethrin, deltamethrin and permethrin alone in Group I were given in Table 4.

Mortality and knock-down percentages of cypermethrin, deltamethrin and permethrin significantly decreased on Ankara housefly populations than WHO housefly populations. The longest KT_{50} was determined in Ankara housefly populations treated with permethrin than cypermethrin and deltamethrin. The highest knockdown resistance coefficient was determined with permethrin treatment on Ankara housefly populations, and the lowest knockdown resistance coefficient was determined in Ankara housefly populations with deltamethrin treatment than cypermethrin and permethrin treatment (Table 4).

The values and statistical comparisons of mortality percentage, knockdown percentage, 100% knockdown time, 50% knockdown time, and knockdown resistance coefficients on WHO populations and Ankara populations treated with cypermethrin+PBO, deltamethrin+PBO and permethrin+PBO at a 1:1 ratio were given in Table 5.

There were no statistical differences in terms of mortality percentage and knockdown percentages, 100% knockdown time, 50% knockdown time, and knock down resistance coefficient between Ankara housefly populations with the addition of PBO at a ratio of 1:1 to cypermethrin, deltamethrin and permethrin. Mortality percentage of cypermethrin, and permethrin on Ankara housefly populations was increased by the addition of PBO as not observed difference between WHO and Ankara housefly populations. Knockdown resistance coefficiency was not statistically different between Ankara housefly populations treated with deltamethrin+PBO, cypermethrin+PBO and permethrin+PBO (Table 5).

Mortality and knockdown percentages were not statistically different between WHO and Ankara houseflies populations. 100% knockdown time and 50% knock-down time were not statistically different between Ankara housefly populations treated with cypermethrin+PBO, deltametrin+PBO, and permethrin+PBO at a 1:3 concentration. Knockdown resistance coefficient was lower in the treatment with deltamethrin+PBO at a 1:3 concentration compared to the treatment with cypermethrin+PBO and permethrin+PBO at a 1:3 concentration on Ankara housefly populations (Table 6).

4. DISCUSSION

The biological activity of cypermethrin plus PBO at a 1:1 and 1:3 concentrations was significantly increased on WHO and Ankara housefly populations as complying with that the insecticide and PBO combinations were reported to have positive effects in combat against flies (Khan et al., 2013; Khan et al., 2015). There were no mortality percentage differences between WHO and Ankara housefly populations with the use of cypermethrin and PBO at a 1:1 and 1:3 ratio. This suggests that mortality percentage of field (Ankara) population approaches the mortality percentage of susceptible (WHO) population. The use of cypermethrin plus PBO at a 1:1 and 1:3 ratio compared to cypermethrin alone significantly increased the knockdown percentage on Ankara housefly populations. This result was in line with other reports explaining that insecticide and PBO combinations were reported to have positive effects in combating flies (Gleave et al., 2018; Showyin, 1998). Cypermethrin plus PBO than cypermethrin alone significantly decreased 100% knock down time on WHO and Ankara housefly populations. Reduced knock-down time means increase in mortality on houseflies and to be successful in combat against flies (Lazslo, 2001; Tozzi, 1998).

Deltamethrin plus PBO at a 1:1 and 1:3 ratio significantly increased the mortality percentage on WHO and Ankara housefly populations. There was no mortality percentage difference between WHO and Ankara housefly populations with the use of deltamethrin and PBO at a 1:3 ratio. This suggests that mortality percentage of field (Ankara) population approaches the mortality percentage of susceptible (WHO) population. The use of deltamethrin plus PBO at a 1:1 and 1:3 ratio compared to deltamethrin alone significantly increased knockdown percentage on Ankara housefly population. The use of deltamethrin plus PBO than deltamethrin alone significantly decreased 100% knock down time on WHO housefly population. Deltamethrin plus PBO than deltamethrin alone decreased 50% knock down time on WHO and Ankara housefly populations. This suggest that reduced knockdown time means increase in mortality on houseflies and being successful in struggle against flies (Lazslo, 2001; Tozzi, 1998).

Permethrin plus PBO significantly increased the mortality percentage on WHO and Ankara housefly populations. There were no mortality percentage differences between WHO and Ankara housefly populations with the use of permethrin and PBO at a 1:1 and 1:3 ratio. This suggests that the mortality percentage of field population approaches the mortality percentage of susceptible population. The use of permethrin plus PBO compared to permethrin alone increased knockdown percentage, decreased 100% knock down time and 50% knockdown time. This study suggests that reduced knockdown time means increase in mortality on flies and being successful in struggle against flies (Lazslo, 2001; Tozzi, 1998).

The use of cypermethrin, deltamethrin and permethrin significantly increased the knockdown resistance coefficients on Ankara housefly populations. Similarly, other studies reported rapid resistance development in *M. domestica* caused by deltamethrin (Khan et al., 2013; Khan, 2019a), and permethrin (Khan, 2019b). This study suggests that knockdown resistance occurs in Ankara housefly populations with the use of cypermetrin, deltamethrin and permethrin.

This study revealed that PBO addition to insecticide mixtures used in this study provided increase in mortality and knockdown percentages, decrease in knockdown times and knockdown resistance

coefficients on housefly populations. Increase in PBO amounts in the insecticide mixtures markedly brought out these effects.

Mortality and knockdown percentages on Ankara housefly populations were not statistically different among insecticides in group I; among insecticides plus PBO at a 1:1 ratio in group II; and among insecticides plus PBO at a 1:3 ratio in group III. However, there were statistical differences in knockdown resistance coefficients among insecticides in group I; and among insecticides plus PBO at a 1:3 ratio in group III. This reveals variable resistance concerns on Ankara houseflies against insecticides as determined with using cypermethrin, deltamethrin and permethrin. Thus, as resistance concerns on houseflies against insecticides are determined in other studies in Turkey and the world (Akıner and Çağlar, 2012; Kocisova et al., 2002; Tezok et al., 1973), resistance concerns should be noted in combat programmes against houseflies.

In this study, mortality and knockdown percentages were found to be very lower in Ankara housefly populations than WHO house fly populations. This might be attributed to preexposure of Ankara housefly populations to insecticide (Kristensen et al., 2000; WHO, 1997). In addition, PBO was determined to highly increase the effects of mortality and knockdown percentages of the insecticides on susceptible and field housefly populations. Thus, it was observed that PBO was beneficial for combating pest. PBO decreased knockdown time, and this is a factor for success in combating housefly. Moreover, PBO was observed to decrease knockdown resistance coefficient via its addition to the insecticide formulations. Thus, PBO was determined to have an important function for preventing knockdown resistance of houseflies.

5. CONCLUSION

It was concluded that PBO addition to insecticide formulations would be vey beneficial, especially in protection of human and domestic animal health, and environment, being economics through increase in success rate, decrease in knockdown resistance, reduction in insecticide use in combating pests via synthetic pyrethroid insecticides with these results.

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Table 1. The results of the biological activities of cypermethrin alone and its PBO combinations on WHO and Ankara housefly populations with three generations.

		Cypermethrin Mean±SEM	Cypermethrin+PBO (1:1 ratio) Mean±SEM	Cypermethrin+PBO (1:3 ratio) Mean±SEM
dx%	WHO	71.1333 ± 4.2978^{ax}	95.3667 ± 3.9540^{b}	99.2667 ± 0.7333^{c}
	Ankara	26.5333 ± 8.9773^{ay}	73.9667 ± 12.7714^{b}	95.7667 ± 3.2405^{c}
KD%	WHO	95.8333 ± 2.3681^{x}	100.0000 ± 0.0000	100.0000 ± 0.0000
	Ankara	35.0000 ± 12.9620^{ay}	81.9667 ± 14.1775^{b}	100.0000 ± 0.0000^{c}
100% KDT	WHO	14.3333 ± 0.6667^{a}	9.3333 ± 0.3333^{bx}	8.3333 ± 0.6667^{cx}
	Ankara	$>15.0000\pm0.0000^{a}$	$>15.0000\pm0.0000^{ay}$	11.3333 ± 1.3333^{by}
KT_{50}	WHO	8.2700 ± 0.3672	5.3167 ± 5976^{x}	5.2000 ± 0.4102
	Ankara	16.0333 ± 1.0035	10.5000 ± 1.2235^{y}	7.5367 ± 0.9198
R/S	WHO	1.0000 ± 0.0000^{x}	1.0000 ± 0.0000	1.0000 ± 0.0000
	Ankara	2.9300 ± 5.2922^{y}	2.0433 ± 0.4083	1.4833 ± 0.2834

Note: a,b,c: Different letters in the same line reveal statistical significance between groups ($p<0.05$).

x,y: Different letters in the same column reveal statistical significance between groups ($p<0.05$).

Mean±SEM= Mean±Standard Error of the Mean

dx%: Mortality percentage

KD%: Knock down percentage

100% KDT: The time when knock-down effect occurred in all the houseflies (minute).

KT_{50}: The time when knock-down effect occurred in 50% of the houseflies (minute).

R/S: Knock down resistance coefficient.

Table 2. The results of the biological activities of deltamethrin alone and its PBO combinations on WHO and Ankara housefly populations with three generations.

		Deltamethrin Mean±SEM	Deltamethrin+PBO (1:1 ratio) Mean±SEM	Deltamethrin+PBO (1:3 ratio) Mean±SEM
dx%	WHO	77.9000 ± 6.0103^{ax}	92.3667 ± 2.1497^{bx}	99.5667 ± 0.4333^{c}
	Ankara	45.7000 ± 14.3598^{ya}	58.8333 ± 19.1339^{yb}	87.8000 ± 10.1264^{c}
KD%	WHO	94.5333 ± 2.7630^{x}	100.0000 ± 0.0000^{x}	100.0000 ± 0.0000
	Ankara	26.9333 ± 13.5740^{ay}	68.4333 ± 5.4871^{by}	94.0667 ± 5.9333^{c}
100% KDT	WHO	$>15.0000\pm0.0000^{a}$	11.0000 ± 0.5774^{bx}	10.0000 ± 1.1547^{cx}
	Ankara	$>15.0000\pm0.0000$	$>15.0000\pm0.0000^{y}$	14.0000 ± 0.5774^{y}
KT_{50}	WHO	9.0667 ± 0.6718^{ax}	8.7533 ± 0.9467^{b}	5.9300 ± 1.0584^{cx}
	Ankara	14.8667 ± 0.1333^{ay}	13.4033 ± 0.7204^{by}	7.9867 ± 1.3453^{cy}
R/S	WHO	1.0000 ± 0.0000^{x}	1.0000 ± 0.0000^{x}	1.0000 ± 0.0000^{x}
	Ankara	1.6500 ± 0.1250^{y}	1.5500 ± 0.1234^{y}	1.3667 ± 0.1338^{y}

Note: a,b,c: Different letters in the same line reveal statistical significance between groups ($p<0.05$).

x,y: Different letters in the same column reveal statistical significance between groups ($p<0.05$).

Mean±SEM= Mean±Standard Error of the Mean

dx%: Mortality percentage

KD%: Knock down percentage

100% KDT: The time when knock-down effect occurred in all the houseflies (minute).

KT_{50}: The time when knock-down effect occurred in 50% of the houseflies (minute).

R/S: Knock down resistance coefficient.

Table 3. The results of the biological efficacy of permethrin alone and its PBO combinations on WHO and Ankara housefly populations with three generations.

		Permethrin Mean±SEM	Permethrin+PBO (1:1 ratio) Mean±SEM	Permethrin+PBO (1:3 ratio) Mean±SEM
dx%	WHO	92.1667 ± 4.1450^{x}	100.0000 ± 0.0000	100.0000 ± 0.0000
	Ankara	22.50000 ± 8.4161^{ay}	89.4667 ± 5.7981^{b}	99.5000 ± 0.5000^{c}
KD%	WHO	97.6333 ± 2.3667^{x}	100.0000 ± 0.0000^{x}	100.0000 ± 0.0000
	Ankara	11.4333 ± 5.3480^{ay}	71.4000 ± 5.8275^{by}	98.5000 ± 1.5000^{c}
100% KDT	WHO	13.3333 ± 0.8819^{a}	10.3333 ± 0.8819^{bx}	8.6667 ± 0.3333^{cx}
	Ankara	$>15.0000\pm0.0000$	$>15.0000\pm0.0000^{y}$	14.3333 ± 0.3333^{y}
KT_{50}	WHO	7.8700 ± 0.7853^{a}	5.5133 ± 0.5965^{b}	3.9467 ± 0.9623^{c}
	Ankara	38.2633 ± 14.0972^{a}	11.3400 ± 0.5650^{b}	7.9433 ± 9.2442^{c}
R/S	WHO	1.0000 ± 0.0000^{x}	1.0000 ± 0.0000	1.0000 ± 0.0000
	Ankara	5.2000 ± 2.3672^{y}	2.1000 ± 0.2409	2.2933 ± 0.6178

Note: a,b,c: Different letters in the same line reveal statistical significance between groups ($p<0.05$).

x,y: Different letters in the same column reveal statistical significance between groups ($p<0.05$).

Mean±SEM= Mean±Standard Error of the Mean

dx%: Mortality percentage

KD%: Knock down percentage

100% KDT: The time when knock-down effect occurred in all the houseflies (minute).

KT_{50}: The time when knock-down effect occurred in 50% of the houseflies (minute).

R/S: Knock down resistance coefficient.

Table 4. The results of the biological activities of Group I insecticides on WHO and Ankara housefly populations with three generations.

		Cypermethrin Mean±SEM	Deltamethrin Mean±SEM	Permethrin Mean±SEM
dx%	WHO	71.1333 ± 4.2978^{x}	77.9000 ± 6.0103^{x}	92.1667 ± 4.1450^{x}
	Ankara	26.5333 ± 8.9773^{y}	45.7000 ± 14.3598^{y}	22.5000 ± 8.4161^{y}
KD%	WHO	95.8333 ± 2.3681^{x}	94.5333 ± 2.7630^{x}	97.6333 ± 2.3667^{x}
	Ankara	35.0000 ± 12.9620^{y}	26.9333 ± 13.5740^{y}	11.4333 ± 5.3480^{y}
100% KDT	WHO	14.3333 ± 0.6667	$>15.0000\pm0.0000$	13.3333 ± 0.8819
	Ankara	$>15.0000\pm0.0000$	$>15.0000\pm0.0000$	$>15.0000\pm0.0000$
KT_{50}	WHO	8.2700 ± 0.3672	9.0667 ± 0.6718^{x}	7.8700 ± 0.7853
	Ankara	16.0333 ± 1.0035^{a}	14.8667 ± 0.1333^{by}	38.2633 ± 14.0972^{c}
R/S	WHO	1.0000 ± 0.0000^{x}	1.0000 ± 0.0000^{x}	1.0000 ± 0.0000^{x}
	Ankara	2.9300 ± 5.2922^{ay}	1.6500 ± 0.1250^{by}	5.2000 ± 2.3672^{cy}

Note: a,b,c: Different letters in the same line reveal statistical significance between groups ($p<0.05$).

x,y: Different letters in the same column reveal statistical significance between groups ($p<0.05$).

Mean±SEM= Mean±Standard Error of the Mean

dx%: Mortality percentage

KD%: Knock down percentage

100% KDT: The time when knock-down effect occurred in all the houseflies (minute).

KT_{50}: The time when knock-down effect occurred in 50% of the houseflies (minute).

R/S: Knock down resistance coefficient.

Table 5. The results of the biological activities of Group II insecticides and their PBO combinations on WHO and Ankara housefly populations with three generations.

		Cypermethrin+PBO (1:1 ratio) Mean±SEM	Deltamethrin+PBO (1:1 ratio) Mean±SEM	Permethrin+PBO (1:1 ratio) Mean±SEM
dx%	WHO	95.3667±3.9540	92.3667±2.1497^{x}	100.0000±0.0000
	Ankara	73.9667±12.7714	58.8333±19.1339^{y}	89.4667±5.7981
KD%	WHO	100.0000±0.0000	100.0000±0.0000^{x}	100.0000±0.0000^{x}
	Ankara	81.9667±14.1775	68.4333±5.4871^{y}	71.4000±5.8275^{y}
100% KDT	WHO	9.3333±0.3333^{x}	11.0000±0.5774^{x}	10.3333±0.8819^{x}
	Ankara	>15.0000±0.0000^{y}	>15.0000±0.0000^{y}	>15.0000±0.0000^{y}
KT_{50}	WHO	5.3167±5976^{x}	8.7533±0.9467^{x}	5.5133±0.5965
	Ankara	10.5000±1.2235^{y}	13.4033±0.7204^{y}	11.3400±0.5650
R/S	WHO	1.0000±0.0000	1.0000±0.0000^{x}	1.0000±0.0000
	Ankara	2.0433±0.4083	1.5500±0.1234^{y}	2.1000±0.2409

Note: a,b,c: Different letters in the same line reveal statistical significance between groups ($p<0.05$).

x,y: Different letters in the same column reveal statistical significance between groups ($p<0.05$).

Mean±SEM= Mean±Standard Error of the Mean

dx%: Mortality percentage

KD%: Knock down percentage

100% KDT: The time when knock-down effect occurred in all the houseflies (minute).

KT_{50}: The time when knock-down effect occurred in 50% of the houseflies (minute).

R/S: Knock down resistance coefficient.

Table 6. The results of the biological activities of Group III insecticides and their PBO combinations on WHO and Ankara housefly populations with three generations.

		Cypermethrin+PBO (1:3 ratio) Mean±SEM	Deltamethrin+PBO (1:3 ratio) Mean±SEM	Permethrin+PBO (1:3 ratio) Mean±SEM
dx%	WHO	99.2667±0.7333	99.5667±0.4333	100.0000±0.0000
	Ankara	95.7667±3.2405	87.8000±10.1264	99.5000±0.5000
KD%	WHO	100.0000±0.0000	100.0000±0.0000	100.0000±0.0000
	Ankara	100.0000±0.0000	94.0667±5.9333	98.5000±1.5000
100% KDT	WHO	8.3333±0.6667^{x}	10.0000±1.1547^{x}	8.6667±0.3333^{x}
	Ankara	11.3333±1.3333^{y}	14.0000±0.5774^{y}	14.3333±0.3333^{y}
KT_{50}	WHO	5.2000±0.4102^{a}	5.9300±1.0584^{bx}	3.9467±0.9623^{c}
	Ankara	7.5367±0.9198	7.9867±1.3453^{y}	7.9433±9.2442
R/S	WHO	1.0000±0.0000	1.0000±0.0000^{x}	1.0000±0.0000
	Ankara	1.4833±0.2834^{a}	1.3667±0.1338^{by}	2.2933±0.6178^{c}

Note: a,b,c: Different letters in the same line reveal statistical significance between groups ($p<0.05$).

x,y: Different letters in the same column reveal statistical significance between groups ($p<0.05$).

Mean±SEM= Mean±Standard Error of the Mean

dx%: Mortality percentage

KD%: Knock down percentage

100% KDT: The time when knock-down effect occurred in all the houseflies (minute).

KT_{50}: The time when knock-down effect occurred in 50% of the houseflies (minute).

R/S: Knock down resistance coefficient.