Turkish Journal of Zoology

http://journals.tubitak.gov.tr/zoology/

Research Article

Turk J Zool
(2021) 45: 223-234

doi:10.3906/zoo-2102-20

First report on mild insecticide resistance in newly established Aegean *Aedes albopictus* populations of Turkey

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Received: 15.02.2021 • **Accepted/Published Online:** 07.05.2021 • **Final Version:** 21.05.2021

Abstract: The invasive nature of *Aedes albopictus,* a competent vector of dengue, zika, yellow fever, and chikungunya viruses, has spread to many parts of the world. To date, there have been no report regarding the insecticide resistance status of *Aedes albopictus* populations along the Aegean coasts of Turkey. This study aims to confirm the occurrence of *Aedes albopictus* populations in the Aegean region of Turkey and test their susceptibility against different insecticide classes. Bioassay for susceptibility to Dikloro difenil trikloroetan (DDT) (4%), propoxur (0.1%), fenitrothion (1%), bendiocarb (0.1%), permethrin (0.75%), and deltamethrin (0.05%) of each population was assessed through the World Health Organization (WHO) standard bioassay test. A total of 675 adult *Aedes albopictus* samples were analysed from four populations in addition to control group and laboratory population. Results showed that all of the populations were resistant or at least possible resistant to DDT. In addition to that, all of the populations showed susceptibility against bendiocarb, propoxur, and fenitrothion except the Didim population, which is a possible resistant to propoxur and fenitrothion and the Bodrum population which is a possible resistant to fenitrothion. Results also indicated reduced mortality against permethrin in the Kuşadası and Güzelçamlı populations, while all of the populations were susceptible to deltamethrin. This study might help public health authorities to choose proper insecticide in control activities before insecticide resistance spread other populations and fix in these populations.

Key words: *Aedes albopictus*, insecticide resistance, Aegean region

1. Introduction

Mosquitoes affect more than half of the world's population because they transmit pathogens such as viruses, protozoa and bacteria, which infect both humans and animals (Caraballo, 2014). *Aedes* mosquitoes are among the most important global vectors of these human and animal diseases. *Aedes (Stegomyia) albopictus* (Diptera: Culicidae) is a vector of a wide variety of human diseases such as zika, yellow fever, dengue fever, and chikungunya fever (Leta et al., 2018). Dengue fever is one of the fastest spreading mosquito-borne diseases, prevalent in more than 100 countries in tropical and subtropical regions of the world. A sevenfold increase has been reported in global incidence of dengue between 1990 to 2003 (Stanaway et al., 2016). Chikungunya virus affects one third of the human population in areas where the virus continues its cycle. At the end of 2017, more than 2.6 million suspected cases of chikungunya were reported in the US alone (CDC, 2017). The virus still continues its cycle today and causes individual diseases as well as periodic epidemics worldwide (CDC, 2020). Zika virus (ZIKV) disease is estimated to cause about 500.000 cases per year in the United States (Franklinos, 2019). Between 2015 and 2018, a total of 2.474 ZIKV cases were reported from European Union/European Economic Area (EU/EEA) countries (ECDC, 2019a).

Ae. albopictus is native to southeast Asia (Gratz, 2004). However, its invasive nature has allowed it to spread from East Asia and Indian Ocean islands (subtropical / tropical areas) to all continents except Antarctica (Caminade et al., 2012; Benedict et al., 2007). The invasion potential of this species and its easy spread to the world is due to its ecological plasticity (Scholte & Schaffner, 2007). The range and distribution of *Ae. albopictus* has also increased due to a combination of factors, notably climate change, increased tourism, and growth of global trade (Whitehorn and Yacoub et al., 2019). *Ae. albopictus* has been reported in 26 countries in Europe and has established wide populations in 20 of these countries (ECDC, 2019 b, c). The existence of *Ae. albopictus* was first recorded in Turkey (Edirne) in 2011 (Öter et al., 2013). Akiner et al. (2016) reported widely distributed *Ae. albopictus* populations in Artvin, Rize, and Trabzon provinces in 2015 in the Black Sea region (Akiner et al., 2016). Then, distribution range

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has been reported to widen throughout the European and Anatolian side of İstanbul and the Marmara region including Kocaeli province (Şakacı, 2021). Finally, its distribution was also determined around the Aliağa port in İzmir (Akıner, 2019). To date, there has been no reported data regarding its distribution in other parts of Turkey.

Although chemical insecticides pose serious risks to human health and environment, current control of *Ae. albopictus* is still heavily dependent upon the use of chemical insecticides. The use of chemical insecticides is costly and not sustainable and, besides this, extensive spraying has also resulted in the development of insecticide resistance in *Ae. albopictus* populations as well as other insect species around the world (Moyes et al., 2017). Insecticide resistance is defined as an ability of mosquitoes to survive exposure to a standard dose of insecticide. This might be achieved by behavioural (such as exophily instead of endophily) or physiological adaptation (WHO, 2016). Physiological resistance mechanisms are divided into two groups including metabolic resistance via increased detoxification enzyme levels (esterase, glutathione-s-transferase, mixed function oxidase) and target site mechanism, which includes mutations in voltage-gated sodium channel (VGSC), acetylcholinesterase (AChE), and γ-aminobutyric acid (GABA) receptor genes (Hemingway et al., 2004). Genetic testing (polymerase chain reaction) and biochemical testing (enzyme activity assays) are used to detect underlying mechanisms of insecticide resistance (WHO, 2016). Prior to detection of underlying resistance mechanisms, the World Health Organization WHO insecticide susceptibility bioassay test is highly recommended by WHO to detect the resistance status of the tested populations to insecticides (WHO, 2016). According to this protocol, mosquitoes are exposed to known concentrations of an insecticide for a fixed period of time using insecticide impregnated papers. Then, they are transferred to holding tubes in which the mosquitoes never contact to any insecticide residue, and the mortality is reported after 24 h. This is a simple direct response to exposure test and used as a field and laboratory surveillance tool by researchers all around the world (Aizoun et al., 2013).

The emergence of insecticide resistance in mosquito populations might lead to ineffective control strategies and resurgence of mosquito-borne diseases (Liu, 2015). Therefore, monitoring the insecticide resistance levels regularly has huge importance against possible resistance that may occur in populations. The Aegean region is located in the western part of Turkey and has been localized along the coasts of Aegean Sea. The Aegean region is well developed in terms of agriculture and tourism sector and has many ports, which are very active in import and export mobility. In addition, many cruise ships carry passengers to many European countries, especially Greece and Italy during the tourism season. This study reports the widespread presence of *Ae. albopictus* populations in the Aegean region of Turkey and assesses the insecticide resistance status of these *Ae. albopictus* populations.

2. Material and methods

2.1. Mosquito collection and rearing

Considering the *Ae. albopictus* distribution risk in the Aegean region based on climatic suitability, intense human mobility, close geographical localisation to Aegean islands, and recent distribution in the Marmara and Black Sea region of Turkey, a field work has been performed in Aydın and Muğla during September-October 2020. Plastic cups, flower vases, puddles on the marble tombstone in graveyards were carefully searched for the presence of *Ae. albopictus* larvae. Larval samples were collected using larval dippers and brought to the laboratory in plastic containers. A total of four *Ae. albopictus* populations were collected from different localization sites (Figure 1). Collected larval samples were brought to vector insect laboratory of Aydın Adnan Menderes University and reared to adults under the standard conditions at 26-28 °C, 12:12 h photoperiod and 70%-80% relative humidity in an insectarium. Morphological identification was carried out using the available keys of Schaffner et al. (2001) and Becker et al. (2003). Populations were reared in Bugdorms (30*30*30 cm) until F_1 generations were obtained.

2.2. Insecticide susceptibility bioassay

Bioassays were performed using WHO diagnostic susceptibility bioassay tubes according to WHO protocols (WHO, 2016). Insecticide impregnated papers were supplied by the WHOPES collaborating Centre at Universiti Sains Malaysia. Susceptibility was assessed against the following insecticides: DDT (4%), propoxur (0.1%), fenitrothion (1%), bendiocarb (0.1%), permethrin (0.75%), and deltamethrin (0.05%). Insecticide concentrations were determined based on the dosages most frequently used in the literature for *Ae. albopictus;* this also allows us to compare the results reliably with previous studies (Arslan et al., 2016; Kushwah et al., 2015; Pichler et al., 2019). Based on the larval abundance four *Ae. albopictus* (Aydın-Kuşadası, Aydın-Güzelçamlı, Aydın-Didim, Muğla-Bodrum), populations were put to insecticide susceptibility tests (Figure 1). Bioassays were performed in insectarium conditions under which mosquito colonies were reared. Each test tube included 25 unfed, 3-5 days old, F_1 generation *Ae. albopictus* females. Assays were carried out in triplicate. The mosquitoes were exposed to insecticides for 1 h. After 1 h, they were transferred into the holding tubes and fed on 10% sugar solution for 24 h. Control groups were exposed to insecticide free papers impregnated only with the excipient, and the experiment

Figure 1. Sampling localities of *Ae. albopictus* along the Aegean Sea coasts of Turkey. (1: Aydın-Kuşadası, 2: Aydın-Güzelçamlı, 3: Aydın-Didim, 4: Muğla-Bodrum).

for control group was performed in the same way as the test tubes lined with insecticide impregnated filter papers. A laboratory strain (LAB population) (F_{60} generation), which had not been exposed to any insecticides for approximately 5 years, was also used as a laboratory reference strain for comparing the results of each tested population. Dead mosquitoes were recorded, and the mortality rates were calculated based on the mean values of three replicates after 24 h holding period. Populations were evaluated as 'susceptible' if the mortality rates were ≥ 98%; 'possible resistant' if mortality rates between 90%-97%; 'resistant' when the mortality rates were ≤ 90% as described by WHO guideline (WHO, 2016).

2.3. Statistical analysis

One-way analysis of variance (ANOVA) was used to compare means of mortality rates based on WHO's susceptibility test results. Then, Tukey's honestly significant post hoc test (HSD) was used to compare the means to find out which specific groups were different comparing to each other, according to the homogeneity of variances. Statistica software version 12.0 (StatSoft, Inc. USA) was used for data analysis. The results were considered statistically significant when $p < 0.05$.

3. Results

3.1. DDT resistance

WHO susceptibility bioassay results showed that mortality rates against DDT ranged between 80%-96.6% in *Ae. albopictus* populations of the Aegean region. The Kuşadası, Güzelçamlık, and Bodrum *Ae. albopictus* populations were DDT resistant and the Didim population were possible resistant. The One-way ANOVA results indicated that the mortality rates were statistically significant between the populations [F (5, 12) =142.38; p =0.000] (Figure 2). A Tukey's HSD post-hoc test showed that the Bodrum population was statistically different from the control group (p = 0.000159), the Didim population was statistically different from the Güzelçamlı (p = 0.0038), and Kuşadası (p = 0.019) populations and also control group (p = 0.000159), the Güzelçamlı population was statistically different from the Didim population (p= 0.038) and control group (p = 0.000159), the Kuşadası population was significantly different from the Didim (p = 0.019), and LAB population (p = 0.043) and control group (p = 0.000159) (Table 1).

3.2. Propoxur resistance

All of the populations were susceptible against propoxur except the Didim population which was possible resistant against the given insecticide and mortality rates changed between 96.6%-98.3%. Mortality rates were statistically significant between the populations [F (5, 12) = 420.80; p = 0.000] (Figure 3). A Tukey's HSD post-hoc test showed that all of the populations were statistically different from the control group (p = 0.000159) (Table 2).

3.3. Bendiocarb resistance

All of the populations in study areas were almost susceptible against bendiocarb and mortality rates were between 98.3%-100%. Mortality rates were statistically significant between the populations [F (5, 12) =1729.2; p = 0.000] (Figure 4). A Tukey's HSD post-hoc test showed that all of the populations were statistically different from the control group (p = 0.000159) (Table 3).

3.4. Fenitrothion resistance

The Kuşadası and Güzelçamlık populations were susceptible to fenitrothion, while the Didim and Bodrum populations were possible resistant. Mortality rates ranged between 91.6%-100%. The One-way ANOVA results showed that the mortality rates were statistically

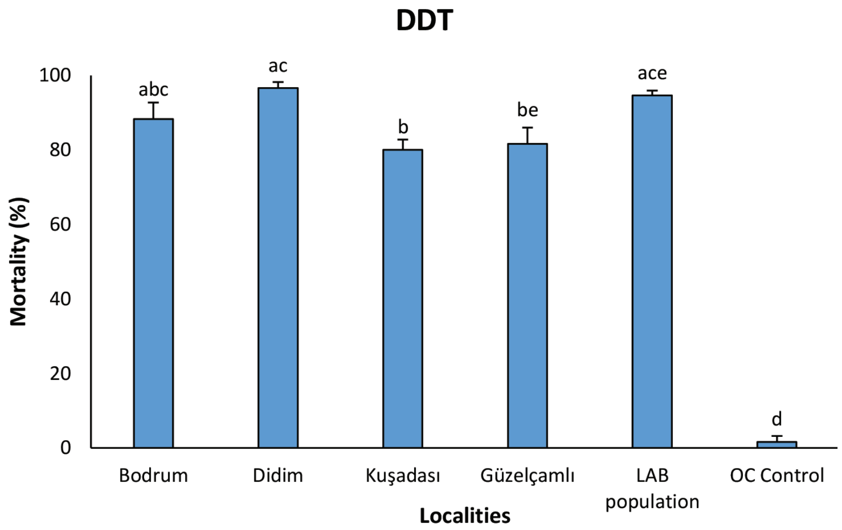

Figure 2. Mortality rates against DDT in *Aedes albopictus* populations of the Aegean region (Mean ± S.E. Same lower case letters above error bars indicates nonsignificant difference at $p > 0.05$ based on the ANOVA results followed by Tukey's HSD test).

Table 1. Tukey's HSD post-hoc test results for DDT.

Populations	Tukey HSD test for DDT; MS = 27.278, df = 12.000					
	Bodrum	Didim	Güzelçamlık	Kuşadası	LAB-Population	Control
Bodrum		0.418	0.634	0.418	0.679	0.000159*
Didim	0.418		0.038*	0.019*	0.996	0.000159*
Güzelçamlık	0.634	0.038*		0.0998	0.083	0.000159*
Kuşadası	0.418	0.019*	0.998		0.043*	0.000159*
LABpopulation	0.679	0.996	0.083	0.0437*		0.000159*
Control	0.000159*	0.000159*	0.000159*	0.000159*	0.000159*	

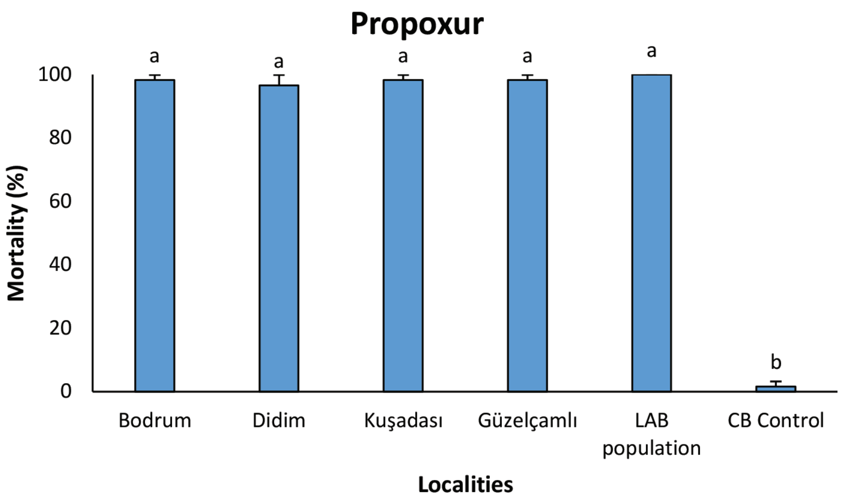

Figure 3. Mortality rates against propoxur in *Aedes albopictus* populations of the Aegean region (Mean ± S.E. Same lower case letters above error bars indicates nonsignificant difference at $p > 0.05$ based on the ANOVA results followed by Tukey's HSD test).

Table 2. Tukey's HSD post-hoc test results for propoxur.

Populations	Tukey HSD test for propoxur; MS = 11.111 , df = 12.000					
	Bodrum	Didim	Güzelçamlık	Kuşadası	LAB-Population	Control
Bodrum		0.988	1.000	1.000	0.988	0.000159*
Didim	0.988		0.988	0.988	0.817	0.000159*
Güzelçamlık	1.000	0.988		1.000	0.988	0.000159*
Kuşadası	1.000	0.988	1.000			0.000159*
LABpopulation	0.988	0.817	0.988	0.988		0.000159*
Control	0.000159*	0.000159*	0.000159*	0.000159*	0.000159*	

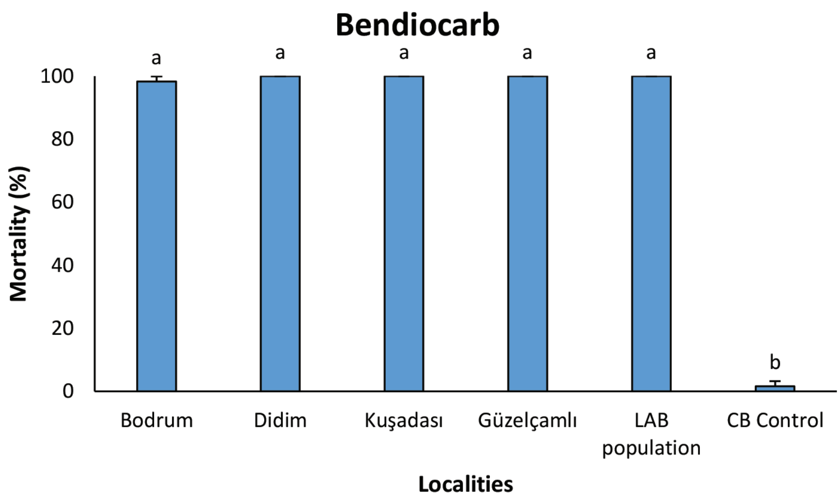

Figure 4. Mortality rates against bendiocarb in *Aedes albopictus* populations of the Aegean region (Mean ± S.E. Same lower case letters above error bars indicates nonsignificant difference at $p > 0.05$ based on the ANOVA results followed by Tukey's HSD test).

Table 3. Tukey's HSD post-hoc test results for bendiocarb.

Populations	Tukey HSD test for bendiocarb; MS = 2.7778 , df = 12.000					
	Bodrum	Didim	Güzelçamlık	Kuşadası	LAB-Population	Control
Bodrum		0.817	0.817	0.817	0.817	0.000159*
Didim	0.817		1.000	1.000	1.000	0.000159*
Güzelçamlık	0.817	1.000		1.000	1.000	0.000159*
Kuşadası	0.817	1.000	1.000		1.000	0.000159*
LABpopulation	0.817	1.000	1.000	1.000		0.000159*
Control	0.000159*	0.000159*	0.000159*	0.000159*	0.000159*	

significant between the populations [F (5, 12) = 832.05; p = 0.000] (Figure 5). A Tukey's HSD post-hoc test showed that the Bodrum population was statistically different from the Güzelçamlık (p = 0.0091), Kuşadası populations (p = 0.0091) and the control group (p = 0.000159), the Didim population was statistically different from the control group (p = 0.000159), the Güzelçamlık population was statistically different from the Bodrum population (p

= 0.0091) and control group (p = 0.000159), the Kuşadası population was significantly different from the Bodrum population (p = 0.0091) and control group (p = 0.000159) and the LAB population was significantly different from the control group (p = 0.000159) (Table 4).

3.5. Permethrin resistance

The Bodrum and Didim populations were susceptible against permethrin while the Kuşadası and Güzelçamlık populations were possible resistant. Mortality rates ranged between 96.6%-98.3%. Mortality rates were statistically significant between the populations [F (5, 12) = 865.2; p = 0.000] (Figure 6). A Tukey's HSD post-hoc test showed that all of the populations were statistically different from the control group (p = 0.000159) (Table 5).

3.6. Deltamethrin resistance

Finally, mortality rates for deltamethrin were calculated between 98.3%-100%. All of the populations were susceptible against deltamethrin. Mortality rates were statistically significant between the populations [F (5, 12) =1776.8; p = 0.000] (Figure 7). A Tukey's HSD post-hoc test showed that all of the populations were statistically different from the control group (p = 0.000159) (Table 6).

4. Discussion

This study reports the first evidence of distribution of *Ae. albopictus* in cities of Aydın and Muğla in Turkey. Besides reporting the distribution data, it also includes the insecticide resistance status of these Aegean *Ae. albopictus* populations against DDT, propoxur, temephos, bendiocarb, fenitrothion, permethrin, and deltamethrin. To date, no detailed data on either larvicide or adulticide usage against *Ae. albopictus* populations of the Aegean region has been reported. However, pyriproxyfen and Bti/Bs mixture or Bti alone has been used in larval breeding sites while different kinds of pyrethroids (cypermethrin, lambda cyhalothrin and tetramethrin) and secondarily

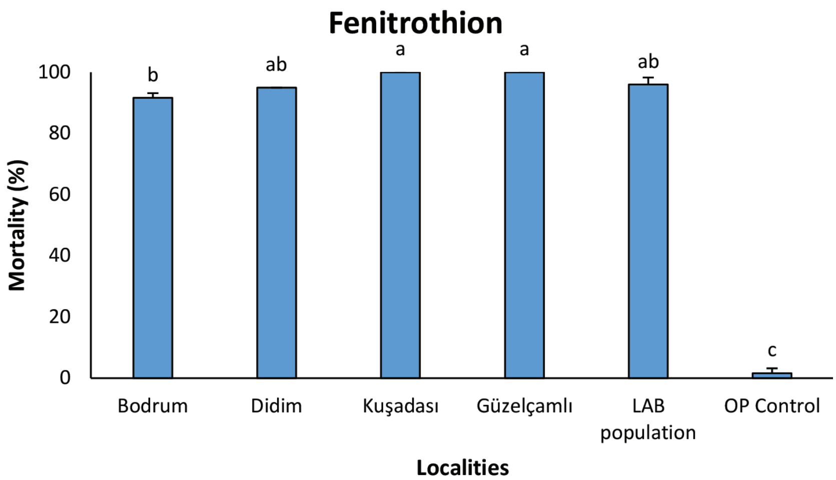

Figure 5. Mortality rates against fenitrothion in *Aedes albopictus* populations of the Aegean region (Mean ± S.E. Same lower case letters above error bars indicates nonsignificant difference at $p > 0.05$ based on the ANOVA results followed by Tukey's HSD test).

Table 4. Tukey's HSD post-hoc test results for fenitrothion.

Populations	Tukey HSD test for fenitrothion; MS = 5.4444 , df = 12.000					
	Bodrum	Didim	Güzelçamlık	Kuşadası	LAB-Population	Control
Bodrum		0.528	0.009*	0.009*	0.275	0.000159*
Didim	0.528		0.164	0.164	0.994	0.000159*
Güzelçamlık	0.091*	0.164		1.000	0.348	0.000159*
Kuşadası	0.091*	0.164	1.000		0.348	0.000159*
LABpopulation	0.275	0.994	0.348	0.348		0.000159*
Control	0.000159*	0.000159*	0.000159*	0.000159*	0.000159*	

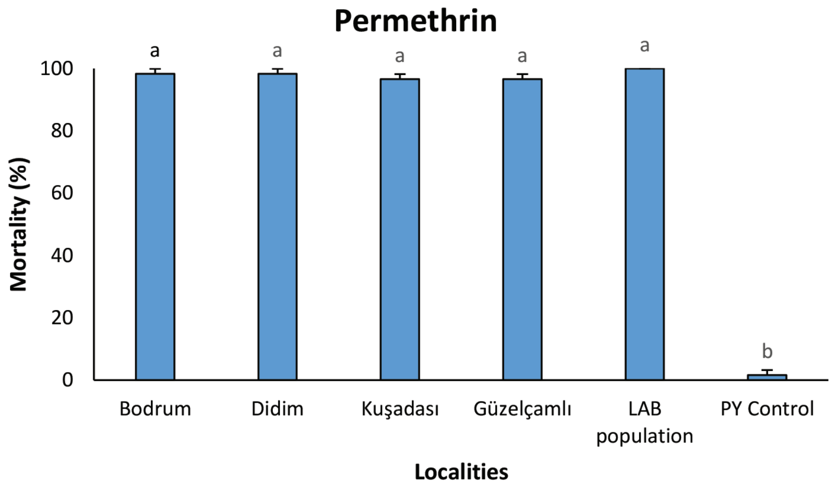

Figure 6. Mortality rates against permethrin in *Aedes albopictus* populations of the Aegean region (Mean ± S.E. Same lower case letters above error bars indicates non-significant difference at $p > 0.05$ based on the ANOVA results followed by Tukey's HSD test).

Table 5. Tukey's HSD post-hoc test results for permethrin.

Populations	Tukey HSD test for permethrin; MS = 5.5556, df = 12.000					
	Bodrum	Didim	Güzelçamlık	Kuşadası	LAB-Population	Control
Bodrum		1.000	0.947	0.947	0.947	0.000159*
Didim	1.000		0.947	0.947	0.947	0.000159*
Güzelçamlık	0.947	0.947		1.000	0.538	0.000159*
Kuşadası	0.947	0.947	1.000		0.538	0.000159*
LABpopulation	0.947	0.947	0.538	0.538		0.000159*
Control	0.000159*	0.000159*	0.000159*	0.000159*	0.000159*	

nicotine mimic insecticide combination with pyrethroids have been used to achieve adult control studies in the Black Sea region of Turkey (Akıner et al., 2018). Taken into consider that quick expansion and distribution range and high population density of invasive species in Turkish lands, regular monitoring of insecticide resistance has remarkable importance. It is also important to note that the Aegean region is highly touristic and insecticide spraying has been extensively conducted during summer season to avoid nuisance caused by other mosquito species.

Results showed that the Bodrum, Kuşadası, and Güzelçamlık populations were resistant to DDT, while the Didim population was possible resistant. Due to devastating damage to the ecosystems, the application of DDT was officially banned in Turkey in 1980s (Kolankaya, 2006). DDT resistance has been reported from other mosquito species including *Culex pipiens* (Linnaeus, 1758), *Aedes (Ochlerotatus) caspius* (Pallas, 1771), and *Anopheles sacharovi* (Favre, 1903) populations collected from the Aegean region of Turkey (Yavaşoğlu, 2019). Despite the forbidden of DDT, the ongoing DDT resistance in aforementioned species firstly was attributed to the historic and wide use of this insecticide leading to soil contamination and fixation of resistance alleles in target mosquito species. Secondly, it was attributed to the applications of dicofol, which contains 10% of DDT as an impurity and dicofol leaks from underground storage tanks to the environment (Turgut et al., 2013). The ecological niche of *Ae. albopictus* species is both related to natural (e.g., rock pools, tree holes) and artificial (discarded tyres, man-made plastic containers) freshwater collections in the urban and semi-urban environment (Ramasamy et al., 2011). This might explain the possible exposure of the individuals to DDT residue in the environment. Another explanation could be related to the state of the source population. Population genetic data

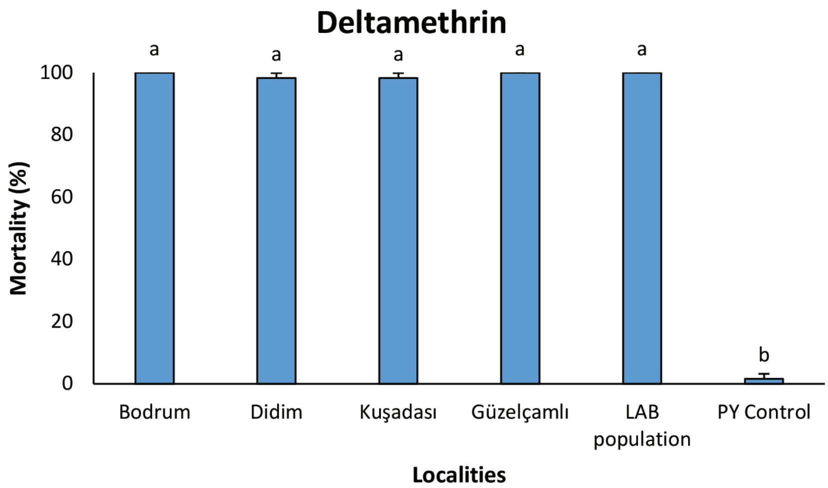

Figure 7. Mortality rates against deltametrin in *Aedes albopictus* populations of the Aegean region (Mean ± S.E. Same lower case letters above error bars indicates nonsignificant difference at $p > 0.05$ based on the ANOVA results followed by Tukey's HSD test).

Table 6. Tukey's HSD post-hoc test results for deltamethrin.

Populations	Tukey HSD test for deltamethrin; MS = 2.7778, df = 12.000					
	Bodrum	Didim	Güzelçamlık	Kuşadası	LAB-Population	Control
Bodrum		0.817	1.000	0.817	1.000	0.000159*
Didim	0.817		0.817	1.000	0.817	0.000159*
Güzelçamlık	1.000	0.817		0.817	1.000	0.000159*
Kuşadası	0.817	1.000	0.817		0.817	0.000159*
LABpopulation	1.000	0.817	1.000	0.817		0.000159*
Control	0.000159*	0.000159*	0.000159*	0.000159*	0.000159*	

set would give information about the invasion route of the populations distributed in the study area. Although, Cytochrome oxidase I (*COI*) and NADH dehydrogenase 5 (*ND5*) sequence analysis referred to close relationship between Russian and Italian strains of *Ae. albopictus* strains collected from the Black Sea region (Türkozan, 2019), we do not have enough data to track the migration route for the Aegean populations, yet. Population genetics study would present if insecticide resistance genes of *Ae. albopictus* have migrated from Europe or other parts of the World to Turkey. For instance, long distance of resistance genes has been reported for *Culex pipiens* using population genetics data (Chevillon et al., 1999). Additionally, it would be interesting to know DDT resistance status of *Ae. albopictus* populations distributed in neighbouring populations where the populations would possibly move from. However, there is no reported DDT resistance data set on DDT resistance from the Aegean islands, close to the populations included in this study, from where they might have distributed to the study area. However, reduced mortality rates against DDT have been also reported in *Ae. albopictus* populations in Swiss-Italian border (Suter et al., 2017), Thailand (Somboon et al., 2013), Japan 194 (Kawada et al., 2010), and Malaysia (Ishak et al., 2015).

Results showed that all of the populations were susceptible against bendiocarb, a carbamate insecticide. However, reduced mortality rates were observed against propoxur in the Didim population. Similarly, reduced mortality rates were observed against fenitrothion in the Bodrum and Didim populations. Although, OP and CB insecticides are not the main component of mosquito control studies, reduced mortality rates against these insecticides reveals selection pressure on *Ae. albopictus* populations as a result of possible extensive use of OP and CB in study area. In this study, the collection sites were graveyards where herbicides are usually used for graveyard care services. Although there has been no clear report regarding the use of specific herbicides types in these graveyards, it has been reported that the first active ingredient used in herbicides worldwide is glyphosate from the OP group. Glyphosate is the most widely licensed active ingredient in noncultivated areas in Turkey in 2016 with a rate of 7.2% (Torun, 2017). This situation might partially explain the selection pressure for OP insecticides in these populations. These findings are similar to those observed in different parts of European *Ae. albopictus* populations. For example, Spanish *Ae. albopictus* populations were susceptible against bendiocarb, while they were resistant against pirimiphos-methyl (organophosphate) (Paajmans et al., 2019). The Swiss-Italian *Ae. albopictus* populations were susceptible against bendiocarb and malathion (Suter et al., 2017). On the contrary, malathion resistance has been reported in Greek *Ae. albopictus* populations possibly due to the use of OPs in agriculture (Balaska et al., 2020). Additionally, OP and CB resistance has been reported from the Asian and African countries. For instance, propoxur resistance in *Ae. albopictus* populations has also been reported from India (Bharati et al., 2019) and China (Li et al., 2017). Fenitrothion resistance has also been reported in one populations of Central African Republic, while most of the populations has been susceptible to fenitrothion and propoxur (Ngoagouni et al., 2016).

Reduced mortality rates against permethrin suggested possible permethrin resistance in the Kuşadası and Güzelçamlık populations, while the Bodrum and Didim populations were both susceptible against permethrin. This might be explained by the local use of permethrin in the Kuşadası and Güzelçamlık populations or the state of the source population. Additionally, all of the populations were still susceptible against deltamethrin. Evidence of lower susceptibility to different pyrethroids (α-cypermethrin and permethrin) were also indicated in Italian *Ae. albopictus* populations. Fortunately, all Italian populations were susceptible to deltamethrin (Pichler et al., 2017). A possible resistance to permethrin and deltamethrin were also reported in *Ae. albopictus* populations in Spain (Bengoa et al., 2017). Balaska et al. (2020) reported full susceptibility to deltamethrin in *Ae. albopictus* populations collected from 19 different locations of Greece. Furthermore, Fotakis et al. (2020) detected *kdr* mutation (1534C) at an allele frequency of 28% in Greece and the *kdr* mutation (V1016G) has been reported in Italy recently (Pichler et al., 2019), which might pose a potential threat against the effectiveness of pyrethroid insecticides in Europe.

Distribution data of *Ae. albopictus* populations in Aydın and Muğla has been reported for the first time in this article. Moreover, based on the information obtained from the local people, it is estimated that these species have recently settled in these areas. Although we do not ignore the insecticides that may have been applied locally and privately, to date, no control method has been applied specifically against these species by the municipalities in these areas. However, the occurrence of the reduced susceptibility in the Kuşadası and Güzelçamlık populations could be explained by an origin of these populations although we do not have population genetic data set to track the gene flow of resistance strain and support this idea. The presence of cross-resistance between DDT and permethrin in DDT resistant Kuşadası and Güzelçamlık populations could be another explanation for current permethrin resistance of these two populations as already reported for other mosquito species (Brengues et al., 2003). Similar to that result, Kawada et al. (2010) suggested possible cross-resistance between pyrethroids and DDT in *Ae. albopictus* populations collected from Nagasaki, Japan (Kawada et al., 2010). This situation was attributed to massive larvicidal treatment of graveyards containers with DDT formulations in the 1950s and resulted with development of resistance against pyrethroids. In addition to that, possible cross resistance between pyrethroids and DDT was explained by mutations in voltage gated sodium channel (VGSC) and increased cytochrome P450 monooxygenase activity in *Culex pipiens* populations in Japan (Kasai et al., 2007). However, further studies including both underlying biochemical and molecular mechanisms of the Aegean *Ae. albopictus* populations are needed to explain the relationship clearly.

This study has some limitations. First, we used specific diagnostic insecticide dosages based on data for Anopheline mosquitoes recommended by WHO (2016) since there has been no clearly reported discriminating insecticide concentrations for adult *Ae. albopictus* species. This might not represent 'true' resistance but rather indicate a 'reduced mortality rate'. The lack of precise diagnostic dosage also limited the interpretation of results across reported studies from different parts of the World. Secondly, WHO recommends to test at least 100 adults for each insecticide. However, there have been no enough adult F_1 female, and testing F_2 or F_3 generations would lead genetic inbreeding problem in laboratory colonies and would not reflect the reality. Thirdly, the LAB population, which was used as a laboratory reference strain, was possible resistant against DDT and fenitrothion even though it was susceptible against other insecticides. Although this population has been raised under insectarium condition without any exposure to any kind of insecticides since at least 50 generations, it appears that DDT and fenitrothion resistance has been fixed in this population. Finally, biochemical and molecular mechanisms underlying the DDT resistance and possible propoxur, fenitrothion and permethrin resistance would be more informative to evaluate the results reported in this study, clearly. Further studies regarding the target-site resistance mechanisms and altered detoxification enzyme levels will be performed in the future for more fundamental explanations.

5. Conclusion

This study is expected to fulfil a gap in knowledge about insecticide resistance of invasive *Ae. albopictus* populations in the Aegean region. There is no reported reliable data set regarding the insecticide usage against these populations in the Aegean region until the specimen collection has been carried out. However, the fact that the new establishment of *Ae. albopictus* populations in this area reduces the possibility that insecticides have been used in the region before. Other possibility refers that resistance to the most commonly used pyrethroids is arising where the species has been well established and moved from this source to the given populations in that study. Coupled with possible resistance reported recently in Greece (Fotakis et al., 2019) and Italy (Pichler et al., 2019), results serve as a warning for Turkey and Europe and urge the need for regular insecticide resistance studies. Mosquito-borne diseases are spreading to many countries that did not have these diseases previously and are increasing day by day both in Europe and in every continent of the world with the effect of various factors. These diseases and outbreaks clearly highlight the urgency of more extensive studies to better understand and monitor the spread of resistance phenotypes with larger spatial areas. For this reason, effective control measures could be performed before resistant individuals are widespread in larger areas.

Acknowledgment

I am grateful to Prof. Dr. Fatih Mehmet Şimşek, Prof. Dr. Mustafa Akıner, and Mustapha Touray for their help in developing the manuscript.

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