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# Insecticide resistance status in *Aedes Albopictus* (Skuse, 1894), (Diptera: Culicidae) populations after the first invasion of Türkiye during two years

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**Abstract**
An invasive *Aedes Albopictus* (Skuse, 1894) (Diptera: Culicidae) is considered one of the most crucial disease vectors. In this study, we aimed to monitor gradual changes in insecticide resistance profiles of *Ae. Albopictus* populations have recently invaded the Turkish territory. We collected *Ae. Albopictus* specimen from ten localities in the Black Sea, Marmara and Aegean regions in subsequent years. Adult *Ae. Albopictus* insecticide susceptibility to deltamethrin (0.05%), cyfluthrin (0.15%), etofenprox (0.5%), bendiocarb (0.1%), fenitrothion (1%) and dichloro diphenyl trikloroetan (DDT) (4%) was determined by the susceptibility test of World Health Organisation. Results showed common pyrethroid susceptibility. Bendiocarb and fenitrothion resistance was more common in the study. Interestingly, DDT resistance was still high in some populations despite the prohibition of the insecticide in Türkiye. A comparison of resistance status in two years demonstrated that populations were not under pyrethroid selection pressure in the study area. The fact that the bendiocarb and fenitrothion resistance, which was present when the populations were first established, continues after two years, demonstrates that the populations are under selection pressure in the study area. We believe that the findings in the study would help resistance management which is a major component of *Ae. Albopictus* control efforts.

**Keywords:** Insecticide resistance, pyrethroids, carbamate, organophosphate, aedes Albopictus

## 1. Introduction

*Aedes Albopictus* (Skuse, 1894) (Diptera: Culicidae), is of paramount importance in public health all around the world. It can transmit different kind of viruses such as yellow fever virus (YFV), dengue virus (DENV), and chikungunya virus (CHIKV) as well as pathogen nematodes called *Dirofilaria immitis* [1]. The Global dengue burden is estimated at 3.9 billion people every year with 96 million having severe disease [2]. It is estimated that 109.000 people had severe infections and 51.000 deaths due to yellow fever infections in Africa and South America in 2018 [3]. Both CHIKV and Zika virus (ZIKV) caused 106.000 losses and 44.000 disability-adjusted life years between 2010-2019 globally [4]. West Nile encephalitis is a big problem around the balkanian country as well as Turkey and Aedes Albopictus is potential and competent vector of the West nile virus together with *Culex pipiens* complex species [5].

*Aedes Albopictus* is originating from Southeast Asia [6]. The species is thought to have entered the Indian Ocean with immigrants from Asia in the last few centuries [7]. Today, although this species is predominantly found in rural or semi-rural areas of Asia, it has been also reported in areas with dense vegetation such as Kuala Lumpur, Singapore, and Tokyo [8]. Ae. Albopictus was introduced to the Americas in 1985 [9] and is now found in many American countries from America to Argentina, in many Pacific islands such as Hawaii, Solomon, and Fiji, and in Australia [7]. The species was first detected in South Africa in 1989 and later in Nigeria, Cameroon, Equatorial Guinea, and Gabon [7]. In Europe, *Ae. Albopictus* was first recorded in Albania in 1979 and since then Bosnia-Herzegovina, Croatia, Belgium, Greece, Montenegro, France, Italy, Netherlands, Serbia, Slovenia, Spain and Switzerland, Bulgaria, Germany, San Marino, Malta, Monaco, and Georgia [10-12].

The eggs and larvae were found on the Türkiye-Greece border city for the first time in 2011 [13]. Then, sedentary populations have been reported from the North Eastern Black Sea region [14]. Finally, the Aegean region populations have been reported from Aydın and Muğla [15].

Currently, mosquito control management is a major strategy for the prevention and control of mosquito-borne diseases because of the lackesness of influential drugs and vaccines against most mosquito-borne diseases [16, 17]. Mosquito management activities have been maintained by the Turkish Ministry of Health, municipalities, and private companies and rely on both larvae and adult control in Türkiye [18]. Pyrethroids are used for indoor residual spraying for adult mosquito control while biological control agents such as *Bacillus thurungiensis* israilensis, *Bacillus sphaericus* and insect growth regulators such as pyriproxyfen and methoprene for larval control in Türkiye [19]. In addition to that, other pesticide and herbicides usage should not be ignored as a result of agricultural purposes since arable soils are widely distributed in different regions of the country. However, spraying large areas of mosquito habitats with chemical insecticides is not only costly but poses serious risks to human health and pollution of the environment. Comprehensive spraying has led to the insecticide resistance issue in mosquito populations in many regions of the world [20, 21].

World Health Organization's (WHO) insecticide susceptibility bioassay test is a practical tool that might be used in the field and laboratory to determine the resistance status of the target vector mosquitoes [22]. This method is highly recommended by WHO since it enables a standard evaluation method to compare different results from different parts of the World [23].

Monitoring of resistance levels in different vector mosquito species has often been performed in Türkiye as well as all over the World. There have been satisfying data sets about the resistance status of *Culex pipiens* (Linnaeus, 1758) (Diptera: Culicidae) [24-26], *Anopheles maculipennis* (Meigen, 1818) (Diptera: Culicidae) [27] *Anopheles sacharovi* (Favre, 1903) (Diptera: Culicidae) [28] populations from different regions of Türkiye. However, despite the quick invasion of *Ae. Albopictus* in Türkiye there have been data set of insecticide resistance of *Ae. Albopictus* Türkiye populations except for the one study reported from the Aegean populations [15].

The development of insecticide resistance in vector mosquito populations may result in a decline in effective control strategies [20]. Therefore, it is highly necessary to monitor insecticide resistance regularly before these populations widen their spread into larger areas. In this study, we aimed to detect the insecticide resistance status of *Ae. Albopictus* populations from the Black Sea, Marmara and Aegean regions of Türkiye. Additionally, we aimed to detect gradual changes in resistance status in two years (2018-2019). We believe that this comparison might shed light on the knowledge of which insecticides were used after the populations settled in Türkiye and which insecticides were used before in the source populations from which they came.

## 2. Materials and Methods

### 2.1. Study sites

This study was carried out with *Ae. Albopictus* samples were collected from seven cities at the end of the mosquito season in the Black Sea region, Marmara region, and Aegean region of Türkiye in two consequtive years (2018-2019) (1) Artvin (Artvin-Merkez and Artvin-Hopa), 2) Rize (Rize-Pazar and Rize-Merkez), 3) Trabzon (Trabzon-Merkez and Trabzon-Vakfıkebir) are located in the Black Sea region, 4) Kocaeli (Kartepe), 5) İstanbul (Rumeli), 6) Kırklareli (İğneada) are located in the Marmara region and 7) İzmir (Aliağa) is located in the Aegean region (Figure 1).

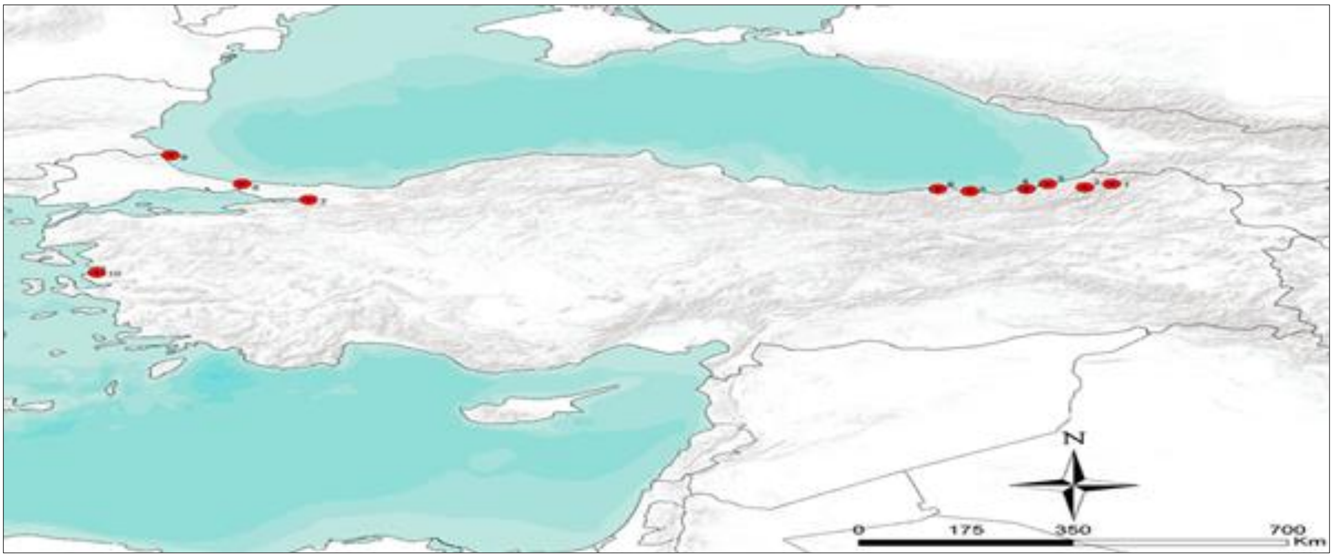


**Fig 1:** Sampling localities of *Aedes Albopictus* populations (1:Artvin-Merkez, 2:Artvin-Hopa, 3:Rize-Pazar, 4:Rize-Merkez, 5:Trabzon-Merkez, 6:Trabzon-Vakfıkebir, 7:Kocaeli-Kartepe, 8:İstanbul-Rumeli, 9:Kırklareli-İğneada, 10:İzmir-Aliağa)

### 2.2. Mosquito strains and collection

Fieldwork has been performed in ten localities from seven cities in Türkiye. Sampling was performed by checking used tire storage areas, tire shops, and plastic containers filled with water, especially at points close to residential areas. Larval sampling was performed through larval dippers and ovitraps. Mouth aspirator and BG sentinel Trap was used for adult sampling. Larvae were taken into plastic bottles while adult individuals were taken into cardboard cups to transfer to the vector ecology and control laboratory of Recep Tayyip Erdoğan University. Both larval and adult identification have been performed using the key of Schaffner *et al.* (2001).

Larvae were reared to adults in plastic containers by feeding with fish food (Tetramin®) in an insectarium maintained at 26±2 °C, 14:12 h photoperiod with 72%±5 humidity. Adult mosquitoes were fed in net cages by feeding 10% sugar solution. Non-blood fed, 3-5 day old F2 and F3 generation female mosquitoes were used for further analysis.

### 2.3. Adult resistance bioassays

WHO standard tube test was used for susceptibility studies against diagnostic doses in adult trials. Test kits and insecticide-impregnated papers provided by WHO were obtained from WHO's reference laboratory at Universiti Sains Malaysia. 6 different insecticides (deltamethrin (0.05%), cyfluthrin (0.15%), etofenprox (0.5%) from the pyrethroid (PY) group, bendiocarb (0.1%) from the carbamate (CB) group, fenitrothion (1%) from the organophosphate (OP) group, and DDT (4%) from the organochlorine (OC) group were applied to adults with the help of standard kits. Descriptive doses for each insecticide were dereferenced by WHO (WHO, 2012). Deltamethrin, cyfluthrin, etofenprox, and bendiocarb are selected to use since they have been widely used in recent years. The reason for the selection of fenitrothion and DDT to test is the fact that their wide use in Türkiye in the past. The main reason for this is to investigate the existence of resistance that may arise from these groups or that has become permanent. Experiments were carried out with F2 and F3 generations in 4 repetitions with 20 individuals for each test using 3-5 days old non-blood-fed females. The tests were carried out in by standard specified as 1 hour for each insecticide. Individuals were taken to holding tubes at the end of the 1 hr contact period. Food support was provided by placing cotton impregnated with 10% sugar solution in the resting cups. Control groups were subjected to free insecticide-impregnated papers instead of the active insecticide. The experiments of the control group were carried out in the same way as the experiments of the test groups. In addition to insecticide-free group, a laboratory strain which will be stated as LAB population from now on, which had not been subjected to any insecticides for about 47 generations, was also added to experiments as a control-reference strain. Dead mosquitoes were counted after 24 hr holding period and percentage mortality rates were measured depending on the mean values of four replicates. The evaluation of the susceptibility was stated as 'susceptible' if the mortality rates are ≥ 98%; 'possible resistant' if the mortality range between 90-97% and 'resistant' if the range ≤ 90% as it was suggested by WHO [22, 29].

### 2.4. Statistical analysis

Mean mortality rates were compared between populations and years by the one-way analysis of variance (ANOVA). Then, Tukey's honestly significant post hoc test (HSD) was used to compare the means to find out changes in mortality rates between 2018-2019. Statistica ver. 12.0 (StatSoft, Inc. USA) was used for statistical analysis. The results were stated as statistically significant if $p<0.05$.

## 3. Results

### 3.1. Deltamethrin

*Ae. Albopictus* adult populations in the five districts (Artvin-Hopa, Rize-Pazar, Kocaeli-Kartepe, İstanbul-Rumeli, İzmir-Aliağa) were possible resistant to deltamethrin while they were susceptible in the other five districts (Artvin-Merkez, Rize-Merkez, Trabzon-Vakfıkebir, Trabzon-Merkez and Kırklareli-İğneada) in 2018. All populations were susceptible to deltamethrin in 2019 except for the İğneada which was possibly resistant Mortality rates for the reference LAB strain were 100% (Figure 2). A Tukey's HSD test post-hoc test indicated no significant mortality rate changes between two years in the Artvin-Merkez, Rize-Pazar, Rize-Merkez, Trabzon-Vakfıkebir, Trabzon-Merkez and Kocaeli-Kartepe populations (p>0.05). The mortality rates of deltamethrin, which were statistically significant between two years based on Tukey's HSD posthoc test, are given in Table 1.

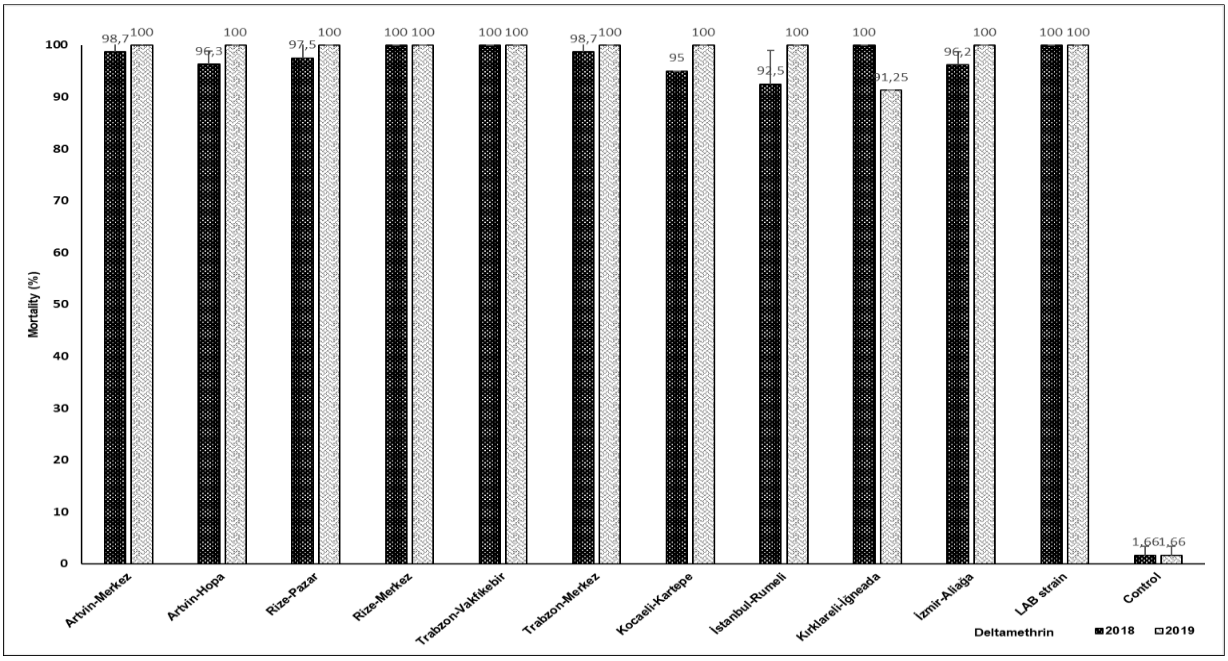


**Fig 2:** The mortality rates of *Ae. Albopictus* populations against deltamethrin between 2018-2019. Mortality rates were calculated based on mean mortality rates. Error bars indicate Standard deviations (SD)

### 3.2. Cyfluthrin
The Artvin-Hopa, Rize-Pazar, Rize-Merkez, Trabzon-Vakfıkebir and Kocaeli-Kartepe populations were possible resistant to cyfluthrin in 2018 while the İstanbul population were resistant. The remaining populations were susceptible to cyfluthrin in 2018. Most of the populations were susceptible to cyfluthrin except for the Kocaeli-Kartepe, İstanbul-Rumeli, and İzmir-Aliağa which were possibly resistant in 2019. The mortality rate was 100% for the reference LAB strain (Figure 3). A Tukey's post-hoc HSD test did not show significant differences in mortality rates between two years in the Artvin-Merkez, Rize-Pazar, Trabzon-Vakfıkebir, Trabzon-Merkez, Kocaeli-Kartepe, Kırklareli-İğneada and İzmir-Aliağa populations ($p>0.05$). A statistically significant change in cyfluthrin resistance between three seasons is given in Table 1.

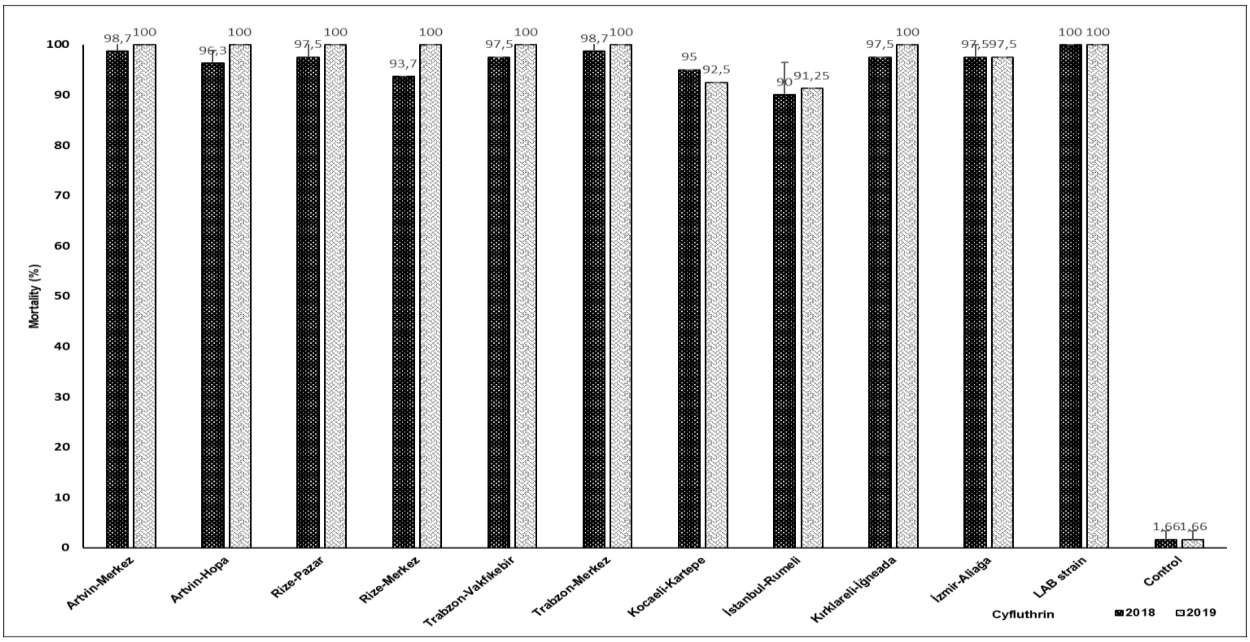


**Fig 3:** The mortality rates of *Ae. Albopictus* populations against cyfluthrin between 2018 and 2019. Mortality rates were calculated based on mean mortality rates. Error bars iindicate Standard deviations (SD)

### 3.3. Etofenprox
The Artvin-Hopa, Trabzon-Vakfıkebir, Trabzon-Merkez and Kırklareli-İğneada populations were susceptible to etofenprox while the remaining were possible resistant in 2018. The Kocaeli-Kartepe was resistant and the İzmir-Aliağa were possibly resistant to etofenprox in 2019. The remaining populations were all susceptible to etofenprox in 2019. The mortality rate for etofenprox was 100% for the reference LAB strain (Figure 4). There was no statistically significant difference in mortality rates in the Artvin-Merkez, Artvin-Hopa, Rize-Sanayi, Trabzon-Vakfıkebir, Trabzon-Sanayi, İstanbul-Rumeli, Kırklareli-İğneada, İzmir-Aliağa populations between two years based on the Tukey's HSD posthoc test analysis ($p>0.05$). Statistically significant changes in etofenprox mortality rates between three consecutive seasons are given in Table 1.

### 3.4. Bendiocarb
The Artvin-Merkez, Rize-Pazar, and İzmir-Aliağa populations were resistant to bendiocarb while the remaining populations were all possibly resistant to bendiocarb in 2018. All populations were resistant against bendiocarb in 2019. However, reference LAB-strain was also resistant to bendiocarb which had a 90% mortality rate (Figure 5). There was no statistically significant change between any of the years in the İzmir-Aliağa population ($p>0.05$). Statistically significant changes in mortality rates against bendiocarb between two years are given in Table 2.

**Table 1:** Insecticide bioassay results against pyrethroids for *Aedes Albopictus* in 2018, 2019

| Site | Insecticide | Yearly mortality rates and p value of the yearly changes | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2018 | 2019 | P | | 2018 | 2019 | P | | 2018 | 2019 | P |
| Artvin-Merkez | Deltamethrin | 98.75 | 100 | 0.67 | Cyfluthrin | 98.75 | 100 | 0.46 | Etofenprox | 97.5 | 100 | 0.14 |
| Artvin-Hopa | | 96.25 | 100 | 0.01* | | 96.25 | 100 | 0.01* | | 98.75 | 100 | 0.46 |
| Rize-Pazar | | 97.5 | 100 | 0.14 | | 97.5 | 100 | 0.62 | | 95 | 100 | 0.03* |
| Rize-Merkez | | 98.75 | 100 | 0.46 | | 93.75 | 100 | 0.02* | | 96.25 | 95 | 0.80* |
| Trabzon-Vakfıkebir | | 98.75 | 100 | 0.46 | | 97.5 | 100 | 0.14 | | 98.75 | 100 | 0.46 |
| Trabzon-Merkez | | 98.75 | 100 | 0.46 | | 98.75 | 100 | 0.80 | | 98.75 | 100 | 0.46 |
| Kocaeli-Kartepe | | 95 | 100 | 0.07 | | 95 | 92.5 | 0.62 | | 96.25 | 65 | 0.00* |
| İstanbul-Rumeli | | 92.5 | 100 | 0.04* | | 90 | 91.25 | 0.80 | | 97.5 | 100 | 0.14 |
| Kırklareli-İğneada | | 100 | 91.25 | 0.00* | | 97.5 | 100 | 0.14 | | 98.75 | 100 | 0.46 |
| İzmir-Aliağa | | 96.25 | 100 | 0.01* | | 97.5 | 97.5 | 1.00 | | 97.5 | 96.25 | 0.80 |

Asterisk (*) indicates significant differences between two years based on Tukey's HSD post-hoc test, p indicates significance value of the changes between years

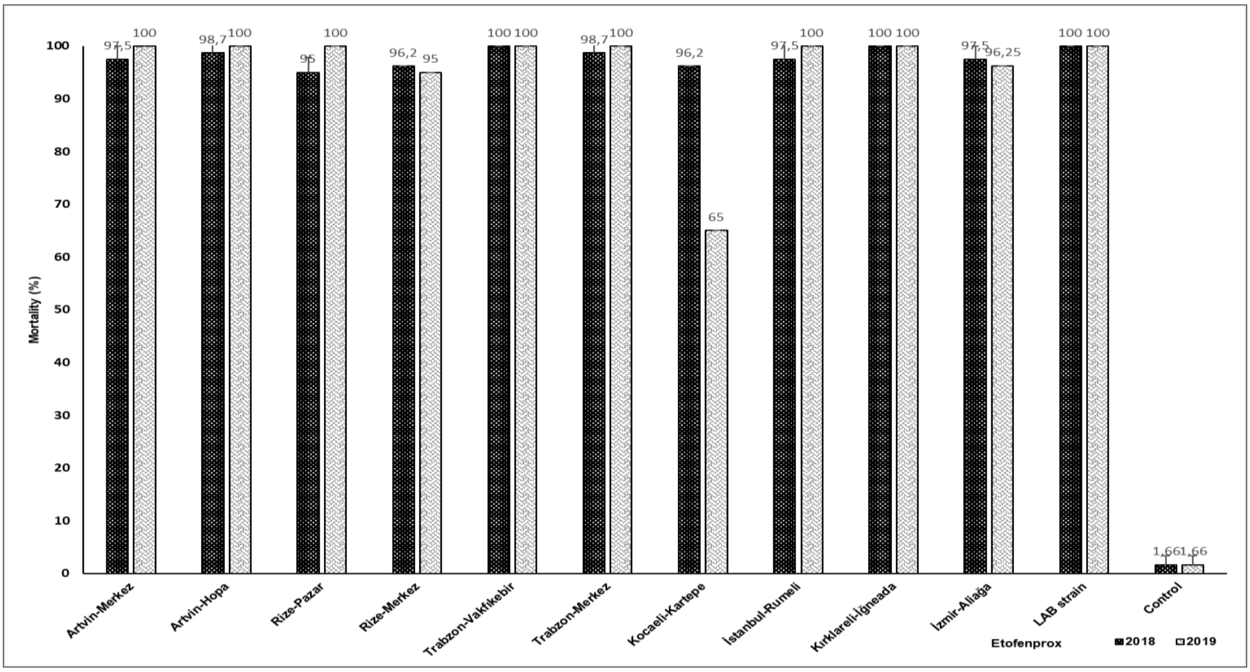


**Fig 4:** The mortality rates of *Ae. Albopictus* populations against etofenprox between 2018-2019. Mortality rates were calculated based on mean mortality rates. Error bars indicate Standard deviations (SD)

### 3.5. Fenitrothion

The Artvin-Merkez and İzmir-Aliağa were resistant to fenitrothion whilst the remaining populations were all possibly resistant in 2018. All populations were resistant against fenitrothion in 2019 except for the İzmir-Aliağa which was possible resistant. The reference LAB-Strain was also possible resistant to fenitrothion which had a 93, 3% mortality rate (Figure 6). A Tukey's HSD posthoc test showed no significant difference in mortality rates against fenitrothion in the İzmir-Aliağa population between two years (p>0.05). A statistically significant alteration in mortality rates against fenitothion is given in Table 2.

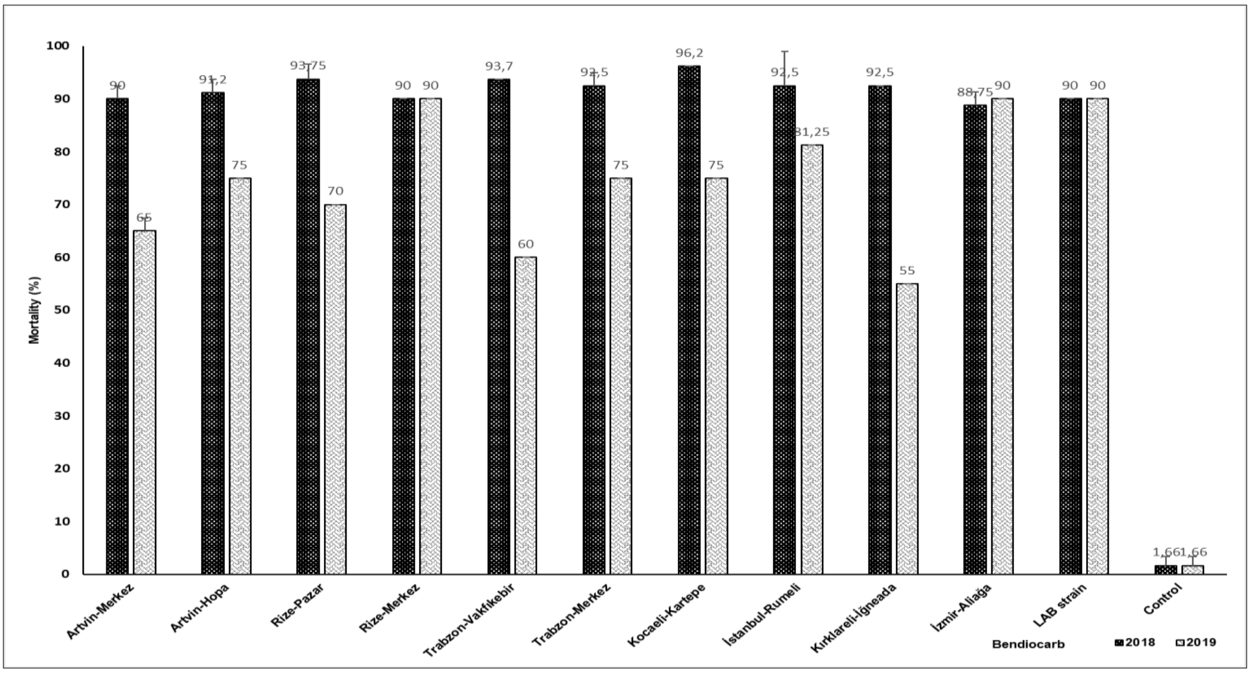


**Fig 5:** The mortality rates of *Ae. Albopictus* populations against bendiocarb between 2018-2019. Mortality rates were calculated based on mean mortality rates. Error bars indicate Standard deviations (SD)

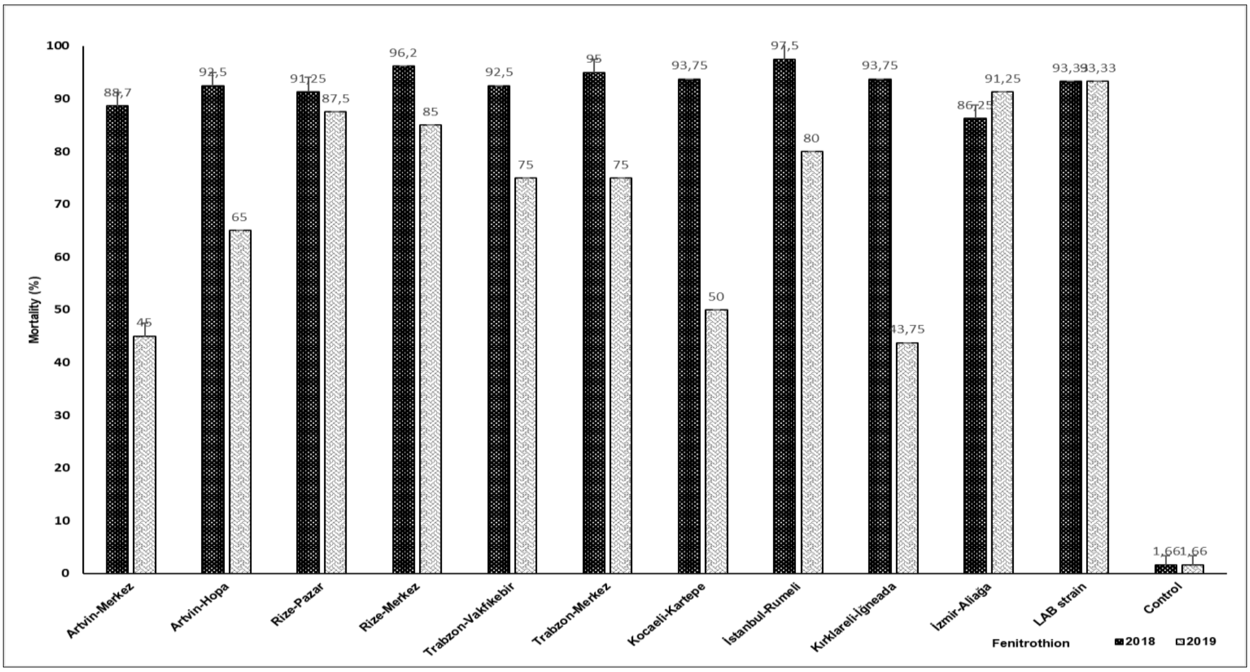


**Fig 6:** The mortality rates of *Ae. Albopictus* populations against fenitrothion between 2018-2019. Mortality rates were calculated based on mean mortality rates. Error bars indicate Standard deviations (SD)

### 3.6. DDT

All populations in the study area were possibly resistant against DDT except for the Artvin-Merkez which was resistant in 2018. However, Artvin-merkez and Kırklareli-İğneada populations were resistant to DDT in 2019, Trabzon-Vakfıkebir, Kocaeli-Kartepe, İzmir-Aliağa populations which were possible resistant to DDT. The remaining populations were susceptible to DDT in 2019 (Figure 7). The alteration of mortality rates between the seasons was not statistically significant in the Artvin-Hopa, Trabzon-Vakfıkebir, Kocaeli-Kartepe, and İzmir-Aliağa populations (p>0.05). In addition to that, statistically significant changes in mortality rates against DDT based on Tukey's HSD posthoc test are given in Table 2.

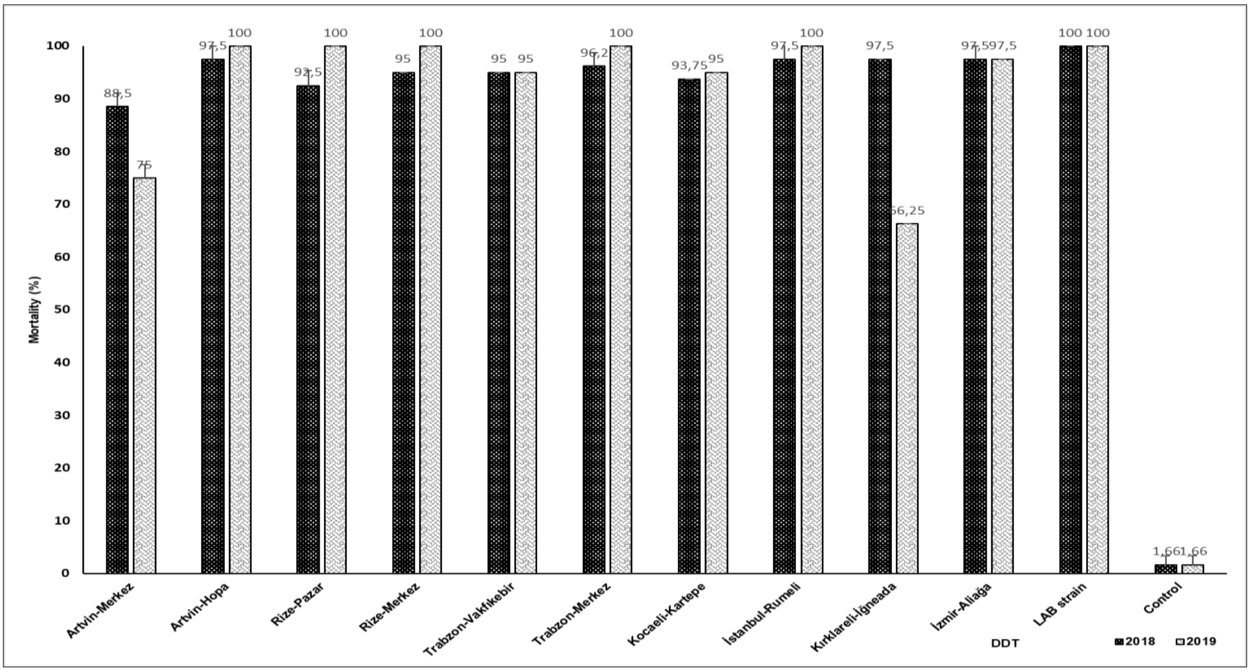


**Fig 7:** The mortality rates of *Ae. Albopictus* populations against DDT between 2018-2019. Mortality rates were calculated based on mean mortality rates. Error bars indicate Standard deviations (SD).

**Table 2:** Insecticide bioassay results against bendiocarb, fenitrothion and DDT for *Aedes Albopictus* in 2018, 2019

| Site | Insecticide | Yearly mortality rates and p value of the yearly changes | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2018 | 2019 | P | | 2018 | 2019 | P | | 2018 | 2019 | P |
| Artvin-Merkez | Bendiocarb | 90 | 65 | 0.02* | Fenitrothion | 88.75 | 45 | 0.00* | DDT | 87.5 | 75 | 0.01* |
| Artvin-Hopa | | 91.25 | 75 | 0.01* | | 92.5 | 65 | 0.00* | | 97.5 | 100 | 0.14 |
| Rize-Pazar | | 93.75 | 70 | 0.00* | | 91.25 | 87.5 | 0.65 | | 92.5 | 100 | 0.04* |
| Rize-Merkez | | 90 | 90 | 1.00 | | 96.25 | 85 | 0.01* | | 95 | 100 | 0.03* |
| Trabzon-Vakfikebir | | 93.75 | 60 | 0.00* | | 92.5 | 75 | 0.00* | | 95 | 95 | 1.00 |
| Trabzon-Merkez | | 92.5 | 75 | 0.00* | | 95 | 75 | 0.00* | | 96.25 | 100 | 0.01* |
| Kocaeli-Kartepe | | 96.25 | 75 | 0.00* | | 93.75 | 50 | 0.00* | | 93.75 | 95 | 0.87 |
| İstanbul-Rumeli | | 92.5 | 81.25 | 0.02* | | 97.5 | 80 | 0.00* | | 97.5 | 100 | 0.54 |
| Kırklareli-İğneada | | 92.5 | 55 | 0.00* | | 93.75 | 43.75 | 0.00* | | 97.5 | 66.25 | 0.00* |
| İzmir-Aliağa | | 88.75 | 90 | 0.91 | | 86.25 | 91.25 | 0.25 | | 97.5 | 97.5 | 1.00 |

Asterisk (*) indicates significant differences between two years based on Tukey's HSD post-hoc test, p indicates significance value of the changes between years

## 4. Discussion

Results indicated that the field samples are generally highly sensitive to the PY group or possibly resistant in some populations. Populations had varying degrees of resistance against insecticides from the other group and especially high resistance against the insecticides from the CB and OP group. Although some populations were sensitive to DDT, which is prohibited in Türkiye, the existence of possible resistance and resistance has been determined. It might not be the result of insecticide pressure in Türkiye but might be because of the persistence of the resistance throughout the generations since the times when DDT was allowed to use. In a summary, *Ae. Albopictus* populations might still maintain the resistance they gained as a result of the insecticide pressure they encountered in their original areas.

Results did not indicate significant fluctuations in mortality rates against PYs between the years except for deltamethrin resistance in the Kırklareli-İğneada and etofenprox resistance in the Kocaeli-Kartepe populations. This might be proof of etofenprox use in the Kocaeli-Kartepe population after the first invasion. One of the reasons for the decrease in the mortality rate against deltamethrin in the Kırklareli-İgneada population may be the use of deltamethrin in the control after the population has settled in İğneada.

The results show that the populations in the studied area, except for the İzmir-Aliağa, were resistant or at least possibly resistant to bendiocarb and fenitrothion in 2018, the year when the newly established. It shows that after one year, mortality rates against bendiocarb and fenitrothion, which are present in almost all populations, have decreased significantly and all of them have become completely resistant to these two insecticides. In this case, it shows that the existing resistance, or at least possible resistance, in the source populations is also maintained in Türkiye as bendiocarb and fenitrothion are subject to selection pressure. In the İzmir-Aliağa population, which is an exception, there was no significant fluctuation in bendiocarb and fenitrothion resistance between the years.

Vector control studies for the invasive Aedes species are not at the desired level worldwide and the recent Dengue and Chikungunya epidemics observed in Europe support this situation [30]. *Aedes Albopictus* species has spread almost all over the world and is accepted as the species responsible for the mentioned epidemics in Europe [31, 32]. Resistance records for this species are also based on data from Asia [33], America [34], and partially Africa [35]. Especially in Asia, resistance development has been reported against 4 insecticide classes, and there are reports of OP resistance from the USA [21]. In recent studies, there are resistance studies from Greece [36], Italy [37], and Spain [38] from the European continent. Data from Asia show that sensitivity to CB and OP groups is decreased [21]. CB and OP resistance were observed in varying proportions in the populations we tested. This suggests that the main origins of the populations in our country are of Asian origin and that they reached our country after the first European invasion [39]. Suter *et al.* [40] showed in their study reported that susceptibility against DDT was low. Considering that DDT is prohibited in Europe, it is concluded that there is a molecular resistance originating from the origin of the species and this may cause problems for PY [40]. Pichler *et al.* [37] stated in their study that after the 26th year of invasion in Italy, the sensitivity to permethrin and alpha-cypermethrin decreased and the development of resistance began. DDT and pyrethroids, which choose the same target region, may come to a position where difficulties may be experienced in combating due to cross-resistance.

## 5. Conclusions

It should be known that passive transport, which is the main factor in the spread of the species so quickly, poses a risk and it is not surprising that similar results are obtained in resistance situations. Grigoraki *et al.* [41] mentioned this in their study and showed that while the haplotypic polymorphism of the resistance-associated carboxylesterase gene region was high in *Ae. Albopictus* collected from Florida and Athens. Phylogenetic studies showed the presence of a correlation between the geographic origin and haplotypic similarity. Our results about the *Aedes Albopictus* and *Aedes aegypti* invasion situation in Turkey showed the correlation with geographic origin and supported this situation [42, 43]. Although the results we obtained are not molecular or biochemical, the classical bioassay test results obtained are similar to the results from the distribution areas of the species. However, it is necessary to conduct more detailed analyses of the resistance profile of the species and to monitor the resistance systematically.

## 6. Acknowledgement

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