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Relationship Between Resistance Against Neonicotinoids and Esterase Enzyme for *Myzus persicae* (Sulzer) (Hemiptera:Aphididae) Populations in South of Turkey

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ABSTRACT
In this study, the development of imidacloprid and acetamiprid resistance in *Myzus persicae* populations and the relationship between neonicotinoid resistance and esterase enzyme were investigated in seven *Myzus persicae* populations which is collected from greenhouse pepper production areas in 2018. In order to determine the resistance ratios of aphid populations against the insecticides, 1 control and 6 doses were used. Each insecticide dose was used in 3 replicates and 25 adult female individuals were used in each replication. For imidacloprid, the highest and the lowest resistance ratios were found to be 6.88 and 3.19-fold, in K-4 and D populations, respectively. For acetamiprid, the highest and the lowest resistance ratios were found to be 7.35 and 2.72-fold, in K-1 and E-2 populations, respectively. Also, highest and lowest esterase activities were found to be 2.60 and 1.75 mOD min^{-1} mg^{-1} in K-4 and E-2 populations, respectively. According to the results of this study, imidacloprid and acetamiprid resistance determined in some *Myzus persicae* populations may be related to esterase enzyme. However, detailed studies are required to establish a clear relationship between resistance and enzyme.

Keywords: *Acetamiprid, Esterase, Imidacloprid, Myzus persicae, Resistance*

1. Introduction

Myzus persicae (Sulzer) (Hemiptera: Aphididae), also known as green peach aphid, is a pest that causes significant crop loss in vegetables, tobacco, fruit and ornamental plants worldwide. This pest, harms the plant by absorbing its sap and releases a sweet-sticky substance during feeding. They cause fumagine as a result of saprophyte fungi adhere to this secretion. (Van Emden & Harrington 2007). *M. persicae* easily resists environmental pressures as a result of the high genetic diversity achieved through sexual reproduction. In addition, it is active throughout the year especially in areas with homogeneous conditions such as greenhouse production areas and can cause great damage by increasing the population density with parthenogenetic reproduction. (Blackman & Eastop 2007). *M. persicae* is one of the most important aphid pests in the world because of its host diversity, mechanism of damage, life cycle, as well as its ability to spread rapidly, transmit viral diseases and easily develop resistance to insecticides (Pavela 2018).

Insecticides are used commonly in Turkey to control against *M. persicae* due to their rapid efficacy. However the pest, develops resistance to insecticides in a short time due to its favorable biology. Thus, *M. persicae* has been reported to develop resistance to more than 75 chemicals (Sparks & Nauen 2015). *M. persicae* develops metabolic resistance to organophosphate and carbamate by increasing E4 or FE4 carboxylesterase levels (Devonshire & Moores 1982). In addition, it is possible to develop resistance to pyrethroid (kdr and super-kdr) and dimethyl carbamate (MACE) by target region mutations. (Eleftherianos et al. 2008). Neonicotinoid insecticides have been also used in the world for the last twenty years in the control of many pests including *M. persicae*. Neonicotinoid group insecticides are known as nicotinergic acetylcholine receptors and they inhibit the basic chemical transmission in the central nervous system of insects. (Afzal et al. 2015). Neonicotinoids belong to the group 4A in the IRAC MoA classification and are known as NACHR agonist. Especially in recent years, there are many records about *M. persicae's* neonicotinoid resistance in the world (Charaabi et al. 2016; 2018; Voudouris et al. 2017; de Little et al. 2017). Therefore, it is important to examine the resistance developed by *M. persicae* against neonicotinoids in detail.

In this study, resistance status of *M. persicae* field against imidacloprid and acetamiprid, two neonicotinoid insecticides commonly used in Turkey, have been examined. The levels of esterase enzyme that is known as an important detoxification enzyme has been also investigated.

2. Material and Methods

2.1. Myzus persicae populations

M. persicae populations were collected from greenhouse pepper production areas in Southern Turkey in April and May, 2018 (Table 1). Seven *M. persicae* populations were collected during the survey studies. The comparison population (susceptible population) was used to determine the resistance levels of the field populations. The susceptible population has collected from pepper production areas in 2006 and has been grown in climate controlled rooms without any pesticide application since then. *M. persicae* populations were cultured on hazelnut radish (*Raphanus sativus*) in climatic chambers with 26±1 °C, 60-65% humidity and 16:8 photoperiodic conditions.

Table 1- Collection dates, coordinates and hostplants of *Myzus persicae* populations

Populations	*Collection dates*	*Host plants*	*Coordinates*
K-1	01.05.2018	Pepper	36°32'39"N 30°27'41"E
K-2	01.05.2018	Pepper	36°28'33"N 30°35'25"E
K-3	01.05.2018	Pepper	36°31'09"N 30°35'23"E
K-4	01.05.2018	Pepper	36°30'83"N 30°34'72"E
E-1	01.05.2018	Pepper	36°67'63"N 29°91'43"E
E-2	01.05.2018	Pepper	36°63'82"N 29°88'22"E
D	01.05.2018	Pepper	36°25'59"N 30°02'74"E

2.2. Leaf-dip bioassays

In order to determine LC_{50} values of two insecticides that contains imidacloprid and acetamiprid against *M. persicae*, we used method no 19 that is recommended by IRAC (Insecticide Resistance Action Committee). In bioassay experiments, Confidor SC 350 (Bayer Crop Science) with imidacloprid active substance and Mospilan 20 SP (Sumi Agro) with acetamiprid active substance were used. First, 1% agar powder was boiled with distilled water and allowed to cool. Agar medium was poured into a 9 cm petri dish at a height of approximately 4 mm and waited to medium be hardened. To determine the LC_{50} and LC_{90} values in all aphid populations, a preliminary study yielded a dose of approximately 90-99%. In order to determine the resistance ratios of aphid populations, 1 control and 6 doses were used. Pure water was applied to the control group. Each dose of the insecticides was applied 3 replications and 25 *M. persicae* adult female invidivuals were used in each replication. Hazelnut radish leaves with a diameter of 3 cm were immersed to the previously prepared insecticide concentrations for 10 seconds using the leaf-dip method. The leaf discs were then placed into the petri dish with agar medium by means of forceps. For each population, 25 individuals from the aphid adults were placed on the leaf discs under binocular. The petri dishes were left to climate cabinets having 26±1 °C, 60-65% humidity and 16:8 photoperiodic conditions. Dead-live counting procedure was performed after 72 hours.

2.3. Statistical analysis

LC_{50} and LC_{90} values were determined in the POLO computer package program (LeOra Software 1994) by using the measurement data determined after 72 hours of *M. persicae* populations. For all populations used in the experiment, the LC_{50} and LC_{90} values were compared to the LC_{50} and LC_{90} values of the susceptible population and the resistance ratios of the populations were obtained for each insecticide. Resistance values were determined by the ratio of LC_{50}values determined in field populations to LC_{50}value of the susceptible population.

2.4. Esteraese activity

For determination of esterase enzyme activity in *M. persicae* populations, Devonshire et al. (1992) method was used. Each well of the microplates were loaded in with 20 mM phosphate buffer (pH:7) that containing 50 LL of 0.1% Triton X-100 (Boehringer Mannheim, especially purified) by using a multichannel micropipette. Adult aphids belonging to the populations to be tested were transferred to each well with a brush. The aphids were homogenized using a multiple homogenizer and the tissue was allowed to dissolve for 15 minutes.30 mg of Fast Blue RR Salt was weighed and then phosphate buffer (pH:6) added to 50 mL. After filtration through a No:1 Whatman filter, 500 L of 100 mM 1-naphthyl acetate solution was added. 200 µL of the prepared dye-substrate solution was taken into all wells with a multi-channel micropipette. Optical density (O. D.) values were obtained by reading in the kinetic microplate reader for a total of 5 minutes at 450 nm wavelength at 10 second

intervals. To calculate the total protein in the samples, Bradford's protein assay method was used, and bovine serum albumin (BSA) was used as a standard (Bradford 1976).

2.5. Detection carbocylesterase activity by electreophoresis

In the *M. persicae* populations, Ornstein and Davis (1964) method was used for the determination of carboxyl esterase by electrophoresis. For this purpose, one wingless adult aphids were homogenized in 25 µL homogenization solution (0.1 g sucrose, 1 mL 1.6% Triton X-100, 0.001% bromocresol purple) and 15 µL homogenate was loaded into each gel well. The gel, run at 250 volts for 1.5 hours, was taken up in 50 mL of Fast Blue RR salt solution [0.1 g of Fast Blue RR salt, 50 mL of 0.2 M phosphate buffer (pH:6)] containing 1 mL of 100 mM 1-naphthyl acetate. After staining for about 15 minutes, the gel was placed in 7% acetic acid for fixation. After 24 hours, it was photographed by using the imaging device.

3. Results and Discussion

3.1. Resistance results

The LC_{50} values and resistance ratios determined for imidacloprid in *M. persicae* populations are given in Table 2. The highest resistance rate to imidaclopride was determined in K-4 population with 6.88 fold and the lowest resistance rate was 2.26 fold in E-2 population. A low level of resistance to imidacloprid was detected in the populations D, E1 and E2. The LC_{50} values and resistance ratios determined for acetamiprid in *M. persicae* populations are given in Table 3. At the end of the study, 7.35, 6.80, 7.25, 6.51, 2.78, 2.72, 7.25 fold resistance development was determined for acetampirid in K1, K2, K3, K4, E1, E2 and D populations respectively. The highest resistance rate for acetamiprid was determined in the K1 population with 7.35 fold and the lowest resistance rate was found in the E2 population with 2.72 fold. Among the *M. persicae* populations, a lower level of resistance to acetamiprid was detected in E1 and E2 populations.

Table 2- LC_{50} values and resistance ratios determined against imidacloprid in *Myzus persicae* populations

Population	*n**	*Slope±SE*	*LC_{50} (mg a.i l^{-1}) (95% CL)*	*R***
K1	411	1.85±0.13	2.85 1.55-4.35	6.78
K2	419	1.79±0.13	2.32 1.11-3.68	5.52
K3	369	1.96±0.16	1.78 0.73-3.04	4.23
K4	411	1.96±0.13	2.89 1.70-4.24	6.88
D	409	1.80±0.13	1.34 0.52-2.32	3.19
E1	410	1.69±0.24	1.06 0.24-1.87	2.52
E2	405	1.56±0.23	0.95 0.22-1.70	2.26
Susceptible	402	0.95±0.34	0.42 0.03-0.96	-

*; Number of individuals used in the experiment, **; resistance ratio

Development of resistance by *M. persicae* against insecticides has been known since the early 1970s. This caused to problems in the control of aphids which becomes resistant to insecticides in the late 1970s and early 1980s (Moores 1995). Therefore, there are studies in which *M. persicae* has developed resistance to many insecticide groups including carbamates, organophosphates and neonicotinoids (Moores et al. 1996; Denholm & Jespersen 1998; Barber et al. 1999; Cassanell et al. 2005; Criniti et al. 2008). However, the number of studies that determine the development of resistance and resistance mechanisms against *M. persicae* insecticide Group in Turkey is quite limited.

In recent years, neonicotinoid insecticides have been used extensively in *M. persicae* control worldwide. In our study, resistance against imidacloprid and acetamiprid active ingredients have ranged 2.26-6.88 fold and 2.72-7.35 fold, respectively. Foster et al. (2008) reported that resistance development by *M. persicae* against imidacloprid, thiamethoxam, thiacloprid, clothianidin and dinotefuran was 11-, 18-, 13-, 100- and 6- folds, respectively. In another study, such values were found is 27.5- 30.14-and 41.31 folds for imidacloprid, thiamethoxam and thiacloprid, respectively (Puinean et al. 2010). Panini et al. (2014) identified 11.7-fold imidacloprid resistance in the 92H6 population of *M. persicae*. Therefore, neonicotinoid resistance by *M. persicae* populations collected from Turkey was found in accordance with the literature. There is no high level neonicotinoid resistance in *M. persicae* populations collected from pepper fields in Turkey. It is thought that the reason is the

rotation of neonicotinoid insecticides and other group insecticides using throughout the season. Therefore, it is thought that the resistance levels to insecticides with different mode of action should be investigated in future studies

Table 3- Determined LC_{50} values and resistance ratios to acetamiprid in *Myzus persicae* populations

Population	*n**	*Slope ±SE*	*LC_{50} (mg a.i l^{-1}) (95% CL)*	*R***
K1	418	0.79±0.13	3.13 1.65-4.86	7.35
K2	415	0.82±0.13	2.90 1.54-4.47	6.80
K3	416	0.74±0.12	3.09 1.54-4.91	7.25
K4	413	0.82±0.13	2.77 1.46-4.29	6.51
D	414	0.82±0.13	2.85 1.55-4.35	7.25
E1	413	0.87±0.22	1.18 0.21-2.41	2.78
E2	413	0.92±0.21	1.15 0.26-2.25	2.72
Susceptible	406	1.07±0.29	0.42 0.03-1.06	-

*; Number of individuals used in the experiment, **; Resistance ratio

3.2. Esterase activity results

The results of esterase enzyme activity determined by microplate assay method in *M. persicae* populations are given in Table 4. The highest esterase enzyme activity was detected in the K2 population with a value of 2.38 mOD min^{-1} mg^{-1} protein, while the lowest esterase enzyme activity was found in the E2 population with a value of 1.75 mOD min^{-1} mg^{-1}protein. The esterase enzyme levels of K1, K2, K3 and K4 populations were found to be statistically different when compared to the susceptible population ($P<0.05$). The esterase enzyme activities of the populations D, E1 and E2 were statistically within the same group as well as the susceptible population ($P<0.05$). In addition, esterase enzyme activities were higher in K1, K2, K3 and K4 populations for both imidacloprid and acetamiprid.

Table 4- Esterase enzyme activities in *Myzus persicae* populations

Population	*n**	*specific activity mOD min^{-1} mg^{-1} protein*	*R/S***
Susceptible	4	1.80 b***	
K1	4	2.35 a	1.30
K2	4	2.38 a	1.32
K3	4	2.01 a	1.11
K4	4	2.60 a	1.44
D	4	1.85 b	1.02
E1	4	1.95 b	1.08
E2	4	1.75 b	<1

*; Number of repetitions, **; Enzyme activity of tested population/enzyme activity of susceptible population, ***; The same letters indicate the same group statistically ($P<0.05$)

3.3. Elektroforesis

The gel results of carboxylesterase enzyme examined by Polyacrylamide Gel Electrophoresis (PAGE) are given in Figure 1. The esterase gel concentrations determined in the field populations of *M. persicae* were higher than the susceptible populations. Especially in the populations of K1, K2, K3 and K4, which have high resistance to imidacloprid and acetamiprid, revealedesterase gel bands with higher density than those of other populations. In the E2 population, esterase gel band with

enzyme density was determined. In addition, esterase enzyme activity in the E2 population was lower than that of other populations. Therefore, esterase enzyme activity and electrophoresis results have supported each other.

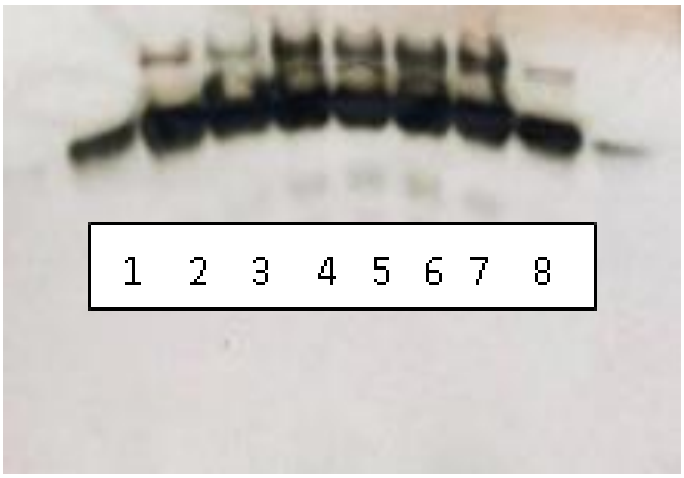

Figure 1- Carboxylesterase gel bands in *Myzus persicae* populations (1: susceptible; 2: K1; 3: K2; 4: K3; 5: K4; 6: D; 7: E1; 8: E2)

The esterase enzyme activities of *M. persicae* populations were found to be <1-1.44 fold compared to the susceptible population. Indeed, many studies, revealed a role for acetylcholinesterase, carboxylesterase or other esterase enzymes in insecticide resistance in aphids (Gao et al. 1992; Song et al. 1995).Wang et al. (2002) reported that the 8.1-fold imidacloprid resistance of *Aphis gossypii* (Hemiptera:Aphididae) may be linked with esterase enzyme. However, a 108.9-fold multiple resistance to fenveralate was also determined in this imidacloprid-resistant population. Therefore, both neonicotinoid and pyrethroid resistance increase the esterase enzyme activities. As a matter of fact, recent studies have shown that neonicotinoid resistance in *M. persicae* may be related to the increase of cytochrome P450 enzyme and R81T mutation in nAChR (Bass et al. 2011; 2014). It is noteworthy thatcarbamate, organophosphate and pyrethroid group insecticides have been also used to control pests in areas where *M. persicae* populations are collected in our study. Therefore, one could say that the increase of esterase enzyme could be related to also carbamate, organaphosphate and pyrethroid group insecticides as a result of cross-resistance development. Thus, *M. persicae* have been reported to develop metabolic resistance to organophosphate and carbamate by increasing carboxylesterase levels of E4 or FE4 (Devonshire 1989). Therefore, further studies would be helpful in considering this matter.

4. Conclusions

Taking these results into consideration, even if the development of neonicotinoid resistance in *M. persicae* populations is not very high, it is possible that the resistance will increase as selection pressure continues. This shows that *M. persicae*, who has rapid reproductive ability, may have problems in the control in the future. Therefore, at intervals of neonicotinoid development of resistance in *M. persicae* populations collected from production areas in Turkey it is thought that should be monitored regularly. In addition, further studies are needed to determine the mechanisms of resistance in *M. persicae* neonicotinoids in Turkey. In addition, rotation of insecticides which have different mode of action that will be use in production areas is one of the insecticide resistance management method.

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