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Original article (Orijinal araştırma)

Neonicotinoid resistance in *Bemisia tabaci* (Genn., 1889) (Hemiptera: Aleyrodidae) populations from Antalya, Turkey[1]

Bemisia tabaci (Genn., 1889) (Hemiptera: Aleyrodidae)'nin Antalya, Türkiye popülasyonlarında neonikotinoid direnci

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Summary

Bemisia tabaci (Genn., 1889) (Hemiptera: Aleyrodidae) is one of most important pests of many crops worldwide. Furthermore, it has the ability to rapidly develop resistance to diverse group of insecticides, hence controlling this pest is problematic. The aim of the current study was to investigate neonicotinoid resistance status of different whitefly populations collected from Antalya, Turkey during summer 2011. *Bemisia tabaci* populations from five different locations in Antalya were all biotype B. Resistance to acetamiprid was between 4.4 and 30.4 times the LC_{50} of a susceptible population. Similarly, resistance to thiamethoxam was between 8.6 and 31.8 times compared to susceptible population. With the exception of one population, there was high correlation ($r^2 = 0.92$) between LC_{50} for acetamiprid and thiamethoxam. The LC_{50} confidence limits for all field populations overlapped for both neonicotinoids. Furthermore, LC_{90} values of all field populations for both neonicotinoids were higher than the recommended doses. These findings indicate development of uniform neonicotinoid resistant for whitefly in the region. The resistance homogeneity and cross resistance in the region sampled is discussed.

Keywords: *Bemisia tabaci*, neonicotinoid, biotype, whitefly, Antalya

Özet

Bemisia tabaci (Genn., 1889) (Hemiptera: Aleyrodidae) tüm dünyada birçok kültür bitkisinde görülen çok önemli zararlılardan biridir. Ayrıca, zararlı birçok gruptan insektisite karşı hızla direnç geliştirme kabiliyetine sahip olması nedeniyle mücadelesi problemlidir. Bu çalışmanın amacı, 2011 yılı yaz döneminde Antalya (Türkiye)'dan toplanan farklı beyazsinek popülasyonlarının neonikotinoid grubu insektisitlere karşı direnç durumunu araştırmaktır. Antalya'nın beş farklı bölgesinden toplanan beyazsinek popülasyonlarının hepsinin B biyotipi olduğu belirlenmiştir. Acetamiprid için hassas bir popülasyona göre LC_{50} dayanıklılık katsayılarının 4,4 ve 30,4 kat arasında olduğu görülmüştür. Benzer şekilde, hassas popülasyonla karşılaştırıldığında thiamethoxam için dayanıklılığın 8,6 ve 31,8 kat arasında değiştiği belirlenmiştir. Bir popülasyon dışında, acetamiprid ve thiamethoxam LC_{50} değerleri arasında yüksek bir korelasyon ($r^2 = 0.92$) olduğu tespit edilmiştir. Her iki neonikotinoid içinde, araziden toplanan tüm popülasyonlara ait LC_{50} değerlerinin güven aralıklarının iç içe geçtiği belirlenmiştir. Ayrıca, her iki neonikotinoid içinde, LC_{90} değerlerinin arazi popülasyonlarında tavsiye dozunun üzerinde olduğu görülmektedir. Bu bulgular bölgede neonikotinoidler için homojen bir dayanıklılığın gelişmekte olabileceğine işaret etmektedir. Beyazsinek popülasyonlarında görülen dayanıklılığın homojenliği ve çapraz direnç konuları test edilen neonikotinoidler için tartışılmıştır.

Anahtar sözcükler: *Bemisia tabaci*, neonikotinoid, biyotip, beyazsinek, Antalya

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Introduction

Bemisia tabaci (Genn., 1889) (Hemiptera: Aleyrodidae) is a cosmopolitan pest causing substantial economic loss in many crops (Menn, 1996) The main damage is caused by direct feeding on plant tissues, excretion of large amounts of honeydew and transmission of several important plant viruses (Bedford et al.,1994; Brown et al., 1995; Denholm et al., 1998; Horowitz et al., 2003; Jones, 2003). Insecticide application has been one the main strategies to minimize the damage potential of pest in many cropping system. However, heavy insecticide use creates substantial selection pressure on the pest, which is known to have 34 biotypes (Tay et al., 2012). As a result, the pest has become resistant to several groups of insecticides shortly after their common use in the field. Consequently, *B. tabaci* resistance was reported from more than 20 countries for 35 active ingredients (Roditakis et al., 2005). Neonicotinoids, a relatively a new class of insecticides, are no exception for this pest and have lost their initial efficacies for certain *B. tabaci* populations around the world (Elbert & Nauen, 2000; Rauch & Nauen, 2003; Wang et al., 2010; Vassiliou et al., 2011). Furthermore, neonicotinoid resistance was reported to be associated with *B. tabaci* biotypes replacing each other completely in areas with heavy neonicotinoid usage (Simon et al., 1999; Pan et al., 2010; Sun et al., 2013). The present study was initiated to determine the neonicotinoid resistance status of whitefly populations from Antalya, Turkey where extensive vegetable growing and pesticide applications take place.

Material and Methods

Insects

Bemisia tabaci populations were initially collected from five different locations in Antalya Province, Turkey during summer 2011 (Table 1). The adults were reared on eggplant in cages maintained at 25-28°C with a 16L:8D h photoperiod for two generations until they reached adequate numbers and homogeneity. A population from southwestern Turkey (Koçarlı, Aydın Province), where pesticide applications have been less than average, was collected in 2009 and maintained at the Entomology Laboratory of Akdeniz University. This population was used as the susceptible reference in bioassays. Also, a lab population originally collected in 2009 from Konaklı, Antalya, where pesticide applications were frequent, was included in the study.

Table 1. Sources of *Bemisia tabaci* populations used in neonicotinoid bioassays

Collection Location	Collection Date	Host	Code
Serik	31 May 2011	Squash	Ser
Aksu	28 July 2011	Sesame	Aks
Tosmur	31 May 2011	Tomato	Tos
Konaklı	31 May 2011	Tomato	Kon
Kumluca	03 June 2011	Tomato	Kum
Koçarlı (susceptible reference population)	2009 and lab maintained since collection	Cotton	Lab1
Konaklı (additional reference population)	2009 and lab maintained since collection	Tomato	Lab2

Insecticides and leaf-dip bioassay

The study used two formulated neonicotinoid insecticides, acetamiprid 200 g L^{-1} (Mospilan SL, Nippon Soda Co, Tokyo, Japan) and thiamethoxam 240 g L^{-1} (Actara SC, Syngenta, Basel, Switzerland) for adult whitefly bioassays. Dose-response bioassays were based on Insecticide Resistance Action Committee suggested procedure. Briefly, cotton leaf discs (39 mm in diameter) were immersed in aqueous solution of insecticide containing 0.02% Triton X-100 for 5 s. The leaf discs were allowed to dry and placed upside down on Petri dish containing a thin layer of sterile agar (2%) layer. Between 18 to 22 *B. tabaci* females were placed on each leaf disc after a brief anesthetization using carbon dioxide. The dishes were inverted for the insects to orientate on abaxial side of the leaf disc and placed in a growth chamber (26±1°C, 16L:8D h). Four concentrations of each insecticide were tested to ensure mortality between 0 and 100%, along with a control concentration containing only 0.02% Triton X-100. The mortality data was corrected for each insecticide using Abbott's formula (Abbott, 1925). Each pesticide concentration was replicated three times and final mortality was assessed 48 h after insecticide exposure.

Biotype determination

Mitochondrial cytochrome oxidase I (*mtCOI*) gene was used to determine the biotypes of the pest. Adult whitefly DNA was extracted individually based on the method of Doyle & Doyle (1987). The primers C1-J-2195 and L2-N-3014 were used for *mtCOI* amplification in a thermal cycler according to Frohlich et al. (1999). Amplicons were directly sequenced in both directions using DTCS kit according to manufacturer's instructions (Beckman Coulter 8000 Genetic Analysis System, Brea, CA, USA). Five different individuals from each population were sequenced for *mtCOI* region and the sequences were compared to known biotypes from representative GenBank accessions and previously reported biotypes from the region sampled (Ikten et al., 2007). A divergence criteria <3.5 was used for inclusion in a biotype (Dinsdale et al., 2010).

Statistical analysis

PoloPlus program was used for probit analyses of the concentration-dependent mortality data (LeOra Software, 1987). Resistance ratios were calculated by dividing LC_{50} and LC_{90} values of field collected whitefly populations by the values obtained for the susceptible reference population (Lab1).

Results

Amplification of COI region from all whitefly samples produced 850bp DNA fragments. Consensus sequences of each whitefly samples were compared to known biotypes with a divergence threshold of 3.5%. All COI sequences from the whiteflies was aligned using ClustalW and produced an identical haplotype. Comparison of single haplotype to known biotype sequences resulted in 100% identity to biotype B, hence all whitefly samples from different populations were assigned to biotype B.

Bioassay results showed thiamethoxam had varying degrees of toxicities for whitefly populations. The highest LC_{50} of 115.8 mg/L was obtained from Tos population, whereas Lab1 had an LC_{50} of 3.7 mg/L for thiamethoxam. Furthermore, Lab2 had an even lower LC_{50} of 1.2 mg/L (Table 2). The resistance ratios ranged from 8.6 to 31.8 for LC_{50} and 9.9 to 40.8 for LC_{90} based on Lab1 (Table 2). Using Lab2, the resistance ratios were 3-7 times higher. Moreover, all field collected whitefly populations had LC_{90} values of higher than the recommended field dose of thiamethoxam. Whereas, LC_{90} values of susceptible Lab1 and Lab2 populations were much lower (10% or lower) than recommended field dose (240 µg ai ml^{-1}).

The highest LC_{50} for acetamiprid (97.5 mg/L) was found in the Aks population. Lab1 population had an LC_{50} of 3.2 mg/L, giving a 30 times resistance ratio for Aks (Table 3). As in the thiamethoxam assays, Lab2 had an even lower LC_{50} (0.3 mg/L) than that of Lab1. The range for resistance ratios among the field collected populations was 4.4 to 30.4 for LC_{50} based on Lab1 (Table 3). These values increased by up to 10 times when based on Lab2. Also, LC_{90} values of all field collected whitefly populations were more than two times higher than the recommended field dose (60 µg ai ml^{-1}). The LC_{90} values of Lab1 and Lab2 populations were only 5 and 0.5% of the recommended field dose for acetamiprid.

Table 2. Toxicity values of *Bemisia tabaci* populations from Antalya for thiamethoxam

Population	n	Slope±SE	LC_{50} (µg ai ml^{-1}) (95% CL)	LC_{90} (µg ai ml^{-1}) (95% CL)	RF_{50}	RF_{90}
Ser	261	1.0±0.2	31.4 (10.7-115.0)	812.5 (185.2-66149.2)	8.6	24.6
Aks	182	1.0±0.2	73.8 (27.2-443.9)	1349.6 (275.4-144033.2)	20.3	40.8
Kum	191	1.4±0.3	45.7 (22.0-90.6)	370.1 (163.9-2068.4)	12.5	11.2
Tos	237	2.9±1.0	115.8 (24.7-166.5)	325.9 (238.0-927.1)	31.8	9.9
Kon	293	1.2±0.2	38.3 (12.8-96.9)	457.4 (155.5-12570.7)	10.5	13.8
Lab1	237	1.3±0.1	3.7 (1.5-10.8)	33.1 (11.1-319.2)	1.0	1.0
Lab2	180	2.2±0.3	1.2 (0.8-2.2)	4.7 (2.6-15.4)	0.3	0.1

n: Number of whiteflies tested; SE: Standard Error; LC: Lethal Concentration; CL: Confidence Limits;
RF: Resistance Factor calculated as (LC_{50} of field population) / (LC_{50} of Lab1 population)

Table 3. Toxicity values of *Bemisia tabaci* populations from Antalya for acetamiprid

Population	n	Slope±SE	LC_{50} (µg ai ml^{-1}) (95%CL)	LC_{90} (µg ai ml^{-1}) (95% CL)	RF_{50}	RF_{90}
Ser	276	1.4±0.2	14.2 (3.8-117.1)	118.5 (28.3-32091.5)	4.4	11.6
Aks	224	1.3±0.28	97.5 (41.9-1261.0)	920.5 (211.2-986546.3)	30.4	90.4
Kum	128	0.9±0.2	57.0 (16.1-970.8)	1704.2 (234.3-41302279.2)	17.8	167.3
Tos	122	1.1±0.2	25.2 (9.0-138.4)	400.9 (86.7-18284.0)	7.9	39.4
Kon	149	1.0±0.2	21.2 (7.1-111.1)	362.1 (78.8-24972.2)	6.6	35.6
Lab1	157	2.6±0.4	3.2 (2.1-4.7)	10.2 (6.6-20.5)	1.0	1.0
Lab2	150	3.0±0.4	0.3 (0.2-0.4)	0.8 (0.5-1.4)	0.1	0.1

n: Number of whiteflies tested; SE: Standard Error; LC: Lethal Concentration; CL: Confidence Limits;
RF: Resistance Factor calculated as (LC_{50} of field population) / (LC_{50} of Lab1 population)

Discussion

Neonicotinoid resistance has been documented in different populations of whitefly all around the world (Byrne et al., 2003; Nauen & Denholm, 2005; Wang et al., 2009; Schuster et al., 2010; Vassiliou et al., 2011; Ünal Bahşi et al., 2012). Furthermore, the resistance in biotype Q is reported in several studies to be higher than in biotype B (Horowitz et al., 2005; Luo et al., 2010; Rao et al., 2012). In the current study, the only biotype found was biotype B. However, previous studies have found sympatric presence of biotypes B and Q, and the sole presence of biotype Q in several areas of Antalya Province (Ikten et al., 2007; unpublished data). There are several studies reporting biotype B replacement by biotype Q under neonicotinoid pressure (Simon et al., 1999; Pan et al., 2010; Sun et al., 2013). However, the neonicotinoid resistant populations from the field and lack of biotype Q findings in current study contradict these reports as the region sampled was known to have biotype Q extensively (Ikten et al., 2007). The discrepancy may indicate that either a factor other than neonicotinoid pressure has decisive role in whitefly fitness or biotype B has a genetic pool as wide as that of biotype Q in the region sampled. However, due to the presence of both acetamiprid and thiamethoxam resistance in all whitefly populations collected in this study, it seems biotype B has as wide genetic plasticity than biotype Q to overcome neonicotinoid exposure.

For all populations, except Tos, a high correlation (r^2=0.92) was found between LC_{50} for acetamiprid and thiamethoxam indicating cross resistance develops in the field for neonicotinoids. Cross resistance among neonicotinoids in whitefly was previously shown in different regions of the world (Rauch & Nauen, 2003; Nauen et al., 2002; Wang et al., 2009; Schuster et al., 2010; Yuan et al., 2012). However, lack of neonicotinoid cross resistance was also indicated in some studies (Horowitz et al., 2004; Prabhaker et al., 2005; Vassiliou et al., 2011). Hence, it seems there may be more than one mechanism for neonicotinoid resistance and *B. tabaci* populations may gain neonicotinoid resistance through different genetic combinations.

The results of the neonicotinoid bioassays indicate moderate to high resistance levels in field collected *B. tabaci* populations. Compared to current study, lower LC_{50} values were reported for acetamiprid, whereas thiamethoxam values were higher in *B. tabaci* populations in Israel (Horowitz et al., 2004). Similarly, thiamethoxam toxicity was higher, and acetamiprid resistance was comparable for *B. tabaci* populations collected in Cyprus (Vassiliou et al., 2011). However, lower acetamiprid and thiamethoxam toxicity were reported for populations from West Africa (Houndete et al., 2010). With the exception of one population, lower acetamiprid toxicities were reported for populations collected in 2009-2010 from similar localities to those sampled in the current study (Ünal Bahşi et al., 2012). The difference may be an indication of subsequent resistance development in the region.

Resistance ratios were up to 30 times for both neonicotinoids based on Lab1, and 300 times higher based on Lab2. Furthermore, LC_{50} values of the populations collected from field showed overlapping confidence limits (95% CL) for both neonicotinoids. These two findings indicate homogenous resistant development over the region sampled. Similarly, uniform responses for neonicotinoid resistance were reported for different *B. tabaci* populations from Cyprus (Vassiliou et al., 2011). However, a heterogeneous response to acetamiprid was indicated by populations collected in Antalya between 2007 and 2009 (Ünal Bahşi et al., 2012). This indicates that neonicotinoid use on *B. tabaci* populations between 2007 and 2011 exerts contiguous pressure, hence resistance appears all over the region. Furthermore, LC_{90} values higher than recommended field doses for both neonicotinoids warrant careful consideration of other management options.

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