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Original article (Orijinal araştırma)

Determination of insecticide residues in soils from Troia agricultural fields by the QuEChERS method[1]

QuEChERS yöntemi ile Troia tarım alanları topraklarında insektisit kalıntılarının belirlenmesi

Burak POLAT[2*] **Osman TİRYAKİ[2]**

Abstract

Extensive and misuse of pesticides can cause to toxicity to humans and pollution in the environment. The primary objective of this study was to determine insecticide load of agricultural soils of Troia, located in Troia National Park of Çanakkale Province (Türkiye) by the QuEChERS method. For method verification, blank soil samples were spiked at two levels of pesticides. The overall recovery was 84.8% with a relative standard deviation of 13.0% (n = 230), with the values within acceptable recovery (60-140%) and repeatability (≤20%) ranges set by SANTE. Forty-nine soil samples were collected in the study area in 2020. Thirty-six samples had insecticide residues at varying concentrations. Overall, 23 insecticide residues were detected at different frequencies. The most frequent pesticides were: chlorantraniliprole> imidacloprid> pyridaben> clothianidin> indoxacarb (in decreasing order). Mean concentration of insecticide residues in soils varied between 0.99-77.7 µg/kg. Imidacloprid residues were detected in all fields, except cabbage fields. The highest imidacloprid concentration (23.3 µg/kg) was detected in pepper fields. Imidacloprid was detected in 21 samples with a mean concentration of 6.20 µg/kg. Persistent insecticides with the long half-lives, such as chlorantraniliprole, imidacloprid, and clothianidin, were detected in almost all samples.

Keywords: Insecticide residues, pesticide load, soil samples, Troia National Park

Öz

Pestisitlerin yoğun ve yanlış kullanımı, insanlar ve çevre için toksisiteye neden olabilir. Bu çalışmanın temel amacı, Troia Milli Parkı-Çanakkale İli (Türkiye) 'ndeki Troia tarım topraklarının insektisit yükünün QuEChERS metodu ile belirlenmesidir. Yöntem doğrulaması için, pestisit içermeyen toprak numuneleri pestisitler ile 2 seviyede spike edilmiştir. Yöntemin geri kazanımı, SANTE tarafından belirlenen kabul edilebilir geri kazanım (%60-140) ve tekrarlanabilirlik (≤%20) aralıkları içindeki değerler ve %13.0'lük bir RSD (n = 230) ile %84.8 bulunmuştur. 2020 yılında çalışma alanından 49 toprak örneği toplanmıştır. Bunlardan 36 adedinde farklı konsantrasyonlarda insektisit kalıntısı bulunmuştur. Topraklarda toplam 23 adet insektisit kalıntısı farklı sayıda örneklerde tespit edilmiştir. En fazla sayıda örnekte tespit edilme sırası şöyledir; chlorantraniliprole> imidacloprid> pyridaben> clothianidin> indoxacarb. Toprakta insektisit kalıntılarıları ortalama konsantrasyonları 0.99- 77.7 µg/kg arasında değişmiştir. Lahana ekili alan dışında tüm alanlarda imidacloprid kalıntısı bulunmuştur. En yüksek imidacloprid konsantrasyonu (23.30 µg/kg) biber ekili alanlarda bulunmuştur. İmidacloprid tespit edilen örnek sayısı 21 ve ortalama konsantrasyon 6.20 µg/kg olarak bulunmuştur. Chlorantraniliprole, imidacloprid ve clothianidin gibi uzun yarılanma ömrüne sahip kalıcı insektisitler neredeyse tüm örneklerde tespit edilmiştir.

Anahtar sözcükler: İnsektisit kalıntısı, pestisit yükü, toprak örnekleri, Troia Milli Parkı

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Introduction

Pesticides are essential components of modern farming. Pesticides reduce pests-induced losses in agricultural production and then increase yield levels. However, excessive and improper uses of pesticides and their prolonged persistence in environment may lead to soil pollution, toxicity to humans and animals, and undesirable residues in the environment and in living tissues (Tiryaki et al., 2010; Tiryaki & Temur, 2010; Hathout et al., 2022).

Soils are contaminated with pesticides through various means including direct applications, accidental spills, incorporation of pesticide-treated plant residues into the soils, runoff from pesticide-applied surfaces. Herbicides pose a greater risk of pollution on soils. Behavior of pesticides in soil affects complex chemical, physical and dynamic biological systems. These include absorption, desorption, evaporation, degradation, surface runoff and leaching. It has been reported that 14-80% of pesticides used for pests and disease control reached to soil depending on application technique, phenological period of the plant and plant density (Çılgı & Jepson, 1992; Temur et al., 2012). Pesticides can also bioaccumulate in soil, leading to even greater possible risks for environment. European Commission states that soil conservation was vital for long-term sustainable agricultural process. Therefore, soil pesticide levels should systematically be monitored and relevant measures should be taken over time (Karasali et al., 2016; Bhandari et al., 2019).

Pesticides (especially organochlorines) can persist in environment for long durations and may pose serious health risks on human health and environment, thus, several pesticides have been banned for use in agricultural fields. Such prohibitions increased the significance of tests for pesticide residues on foodstuff and in the environment (Liu et al., 2016). Excessive use of pesticides may destroy rich biodiversity, ecological cycles and soil health (Bhandari et al., 2019).

Contamination of soil with pesticides affects agroecosystems. Such contaminations influence soil microbial community, bacterial diversity, microorganisms, nitrogen transformation and soil enzymes (Andersch & Anderson, 1991). Excessive use of pesticides is the primary source of pollution in agricultural lands (Balderacchi et al., 2014). It has been reported that 70% of pesticides used in agriculture end up in the soil and seriously contaminate farmlands (Sun, 2000). Therefore, agricultural soil quality is closely related to crop quality and food safety, which are thereby associated with human health.

Half-lives determine the fate of pesticides in the soil. Half-life (DT_{50}) indicates the time or duration in which a pesticide degrades by half and is usually expressed in days, months or years. With the use of half-life, it is possible to see if a pesticide tends to accumulate in soils. Based on half-lives, pesticides can be divided into three persistence groups as of: low (<16 days), moderate (between 16-59 days) and high (>60 days). Short half-lived pesticides accumulate less in soils than the long half-lived pesticides, with the latter a greater risk to soil and water resources (Anonymous, 2022). Despite their highly toxic nature, organophosphorus insecticides have half-lives of <30 days, thus they do not pose long-term risks to soil and water resources; however, neonicotinoids with quite a long half-life may pose serious risk of pollution especially in soils (Seagraves & Lundgren, 2012; DiBartolomeis et al., 2019).

In Türkiye, annual total pesticide use was 39 kt in 2015, but increased to 54 kt in 2020 (TUIK, 2021), with 8 and 12 kt of that, respectively, being insecticides. The average application of pesticides was about 1.7 kg of active ingredients per ha in 2018. In Çanakkale Province, Türkiye, agricultural activities are largely practiced around and within Troia National Park. Tomato, maize, sunflower, wheat, pepper, rice, cabbage and beans are predominant crops grown in Troia. Considering these products, there is an intensive use of insecticides against many insects. A total of 1.6 kt of solid/liquid pesticides (223 t insecticide) were used throughout Çanakkale in 2021 and 23.4% of the pesticides were used in the Central District (Anonymous, 2021). In the previous study, 1.80 mg/kg of imidacloprid and 2.71 mg/kg of emamectin benzoate residues

were found in areas where conventional tomato growing is conducted in Troia. Both residue levels were trace amounts and less than MRL (Polat & Tiryaki, 2019).

In a study conducted by Yıldırım & Özcan (2007) in 2003, 14 soil samples were taken from the borders of Troia National Park, where agricultural production was conducted. Soil samples were analyzed by the standard method 6630 (Greenberg et al., 1998; USEPA, 2007a) and gas chromatography. Captan (100-230 ppb), cypermethrin (20-80 ppb), endosulfan (16.7-230 ppb), ethion (1-6 ppb), mancozeb (2 ppb), trifluralin (20 ppb) pesticides were detected. The residues of endosulfan and captan were higher than the others.

QuEChERS method is generally used to analyze pesticide residues on agricultural commodities (Anastassiades et al., 2003; Lehotay, 2007) and has proven to be efficient in detection of pesticide residues in fruit and vegetables (Çatak & Tiryaki, 2020; Polat, 2021). The method has also proven to be efficient in pesticide residue analyses of soil samples (Nagel, 2009; Temur et al., 2012; Zaidon et al., 2019; Vickneswaran et al., 2021). Analyses were conducted with LC-MS/MS system.

The primary objective of this study was to determine insecticide load of agricultural soils of Troia, located in Troia National Park of Çanakkale Province by the QuEChERS method. Method validation was done by using relevant validation criteria (Hu et al., 2018, SANTE, 2020; Zaidon et al., 2019).

Materials and Methods

Chemicals and reagents

Analytical standards for pesticide analysis were supplied from Chem Service (West Chester, PA, USA) and Dr. Ehrenstorfer GmbH (Wesel, Germany). QuEChERS extraction kits (1.5 g NaOAC and 6 g $MgSO_4$) and QuEChERS clean-up kits [400 mg C_{18}, 400 mg primary secondary amines (PSA, 40 µm particle size), 1.2 g $MgSO_4$] and 0.22 µm nylon syringe filter (Membrane Solutions, Plano, TX, USA) were used. The other solvents and reagents including acetonitrile (MeCN) and acetic acid (HAc) were chromatographic grade.

Apparatus and chromatographic conditions

Insecticide detection was conducted in an LC-MS/MS device. Separation was made with the use of an Acquity UPLC BEH C_{18} column (1.7 mm, 100 x 2.1 mm) under flow rate of 0.35 mL min^{-1}, injection volume of 1 µL, and total run time of 15 min. The gradient program included 10 mM $NH_4CH_3CO_2$ in methanol (B) and 10 mM $NH_4CH_3CO_2$ in water pH 5 (A). Transition groups (precursor and fragment ion) and retention times of insecticide were provided in Table 1.

Study area and sample collection

The study area, Troia, is located in the Central District of Çanakkale Province, where agricultural activities are practiced intensively (Figure 1). The sampling area included six villages: Kumkale, Halileli, Tevfikiye Çıplak, Kalafat and Pınarbaşı. Soil samples were taken from tomato, maize, sunflower, wheat, pepper, rice, cabbage and bean fields. Forty-nine soil samples were collected from 5-25 cm deep after at the growing period (November 2020). Soils in the sampling area have organic matter between 0.49-2.75%, clay 8-54%, pH 7.7-8.2 (Yıldırım & Özcan, 2007). Samples were placed in labeled clean plastic polythene bags (Adeyinka et al., 2019, Zaidon et al., 2019), transported to laboratory in an icebox and kept frozen (-20ºC) until the analyses. Air-dried soils were passed through 2 mm sieve (USEPA, 2007b). Blank soil samples were collected from the study area, which is known to be pesticide free, for recovery experiment and matrix-matched calibration.

Table 1. Retention times (tR) calibration line equations (5 point), concentration ranges and correlation coefficients (R^2)

Insecticide	tR (min)	Precursor ion / Fragment ion (m/z)	Calibration curve equation*	Concentration range (ppb)	R^2
Acetamiprid	5.1	223.1 >125.9 / 223.1 > 55.9	$y = -71.68 x^2 + 108657 x + 1294.5$	1-200	0.99975
Chlorantraniliprole	8.2	482.0 > 283.9 / 482.0 > 450.9	$y = -9.73 x^2 + 14384 x + -708.4$	1-200	0.99987
Clofentezine	10.0	302.9 > 137.9 / 302.9 > 101.9	$y = -37.75 x^2 + 33418.1 x + -61.8$	1-200	0.99987
Clothianidin	4.6	250.0 > 131.9 / 250.0 > 169.0	$y = -11.01 x^2 + 15197.3 x + 2078.3$	1-200	0.99967
Cyhalothrin-lambda	11.3	467.2 > 225.0 / 467.2 > 141.0	$y = -0.03 x^2 + 1418.16 x + 1139.4$	10-2000	0.99999
Cypermethrin	11.4	433.1 > 190.9 / 435.1 > 192.9	$y = -0.004 x^2 + 2962.79 x + 1248.4$	10-200	0.99991
Deltamethrin	11.4	523.0 > 280.9 / 523.0 > 506.0	$y = -0.27 x^2 + 3382.47 x + -515.6$	1-200	0.99983
Etoxazole	11.1	360.1 > 140.9 / 360.1 > 112.9	$y = -175.9 x^2 + 237612 x + 16003.1$	1-200	0.99975
Flubendiamide	9.6	680.9 > 253.9 / 680.9 > 274.0	$y = -11.12 x^2 + 11290.5 x + 1704.8$	1-200	0.99884
Hexythiazox	10.9	353.0 > 227.9 / 353.0 > 168.0	$y = -19.65 x^2 + 37913 x + 926.1$	1-200	0.99972
Imidacloprid	4.6	256.0 > 175.0 / 256.0 > 209.0	$y = -5.76 x^2 + 12261.9 x + -36.0$	1-200	0.99982
Indoxacarb	10.3	528.0 > 202.9 / 528.0 > 249.0	$y = -7.27 x^2 + 7726.08 x + -571.2$	1-200	0.99999
Lufenuron	10.8	508.9 > 325.9 / 508.9 > 174.9	$y = -8.63x^2 + 2235.18 x + 422.2$	1-200	0.99988
Metaflumizone	10.7	507.1 > 178.0 / 507.1 > 115.9	$y = 0.10 x^2 + 3653.8 x + 511.9$	10-2000	0.99999
Methoxyfenozide	8.9	369.1 > 149.0 / 369.1 > 313.1	$y = -297.83 x^2 + 82874.9 x + -3970.2$	1-200	0.99999
Novaluron	10.4	493.0 > 158.0 / 493.0 > 141.0	$y = -0.70 x^2 + 6529.42 x + 323.1$	1-200	0.99968
Pirimicarb	7.6	239.1 > 71.9 / 239.1 > 182.1	$y = -86.99 x^2 + 180575 x + -2331.0$	1-200	0.99999
Pymetrozine	3.8	218.0 > 104.9 / 218.0 > 77.9	$y = -53.47 x^2 + 123182 x + -6110.0$	1-200	0.99997
Pyridaben	11.5	365.1 > 147.0 / 365.1 > 309.0	$y = -127.96 x^2 + 116395 x + 8582.8$	1-200	0.99975
Tebufenpyrad	10.7	334.1 > 116.9 / 334.1 > 145.0	$y = -27.83 x^2 + 37081.3 x + 1796.9$	1-200	0.99982
Teflubenzuron	10.8	378.9 > 338.9 / 378.9 > 358.9	$y = -10.64 x^2 + 5571.07 x + 3575.8$	1-200	0.99764
Thiamethoxam	3.9	292.0 > 211.0 / 292.0 > 181.0	$y = -12.48 x^2 + 32248.7 x + -634.9$	1-200	0.99998
Triflumuron	10.0	359.0 > 155.9 / 359.0 > 138.9	$y = -24.38 x^2 + 29410.2 x + 3210.6$	1-200	0.99963

* Ordinary calibration curve was used.

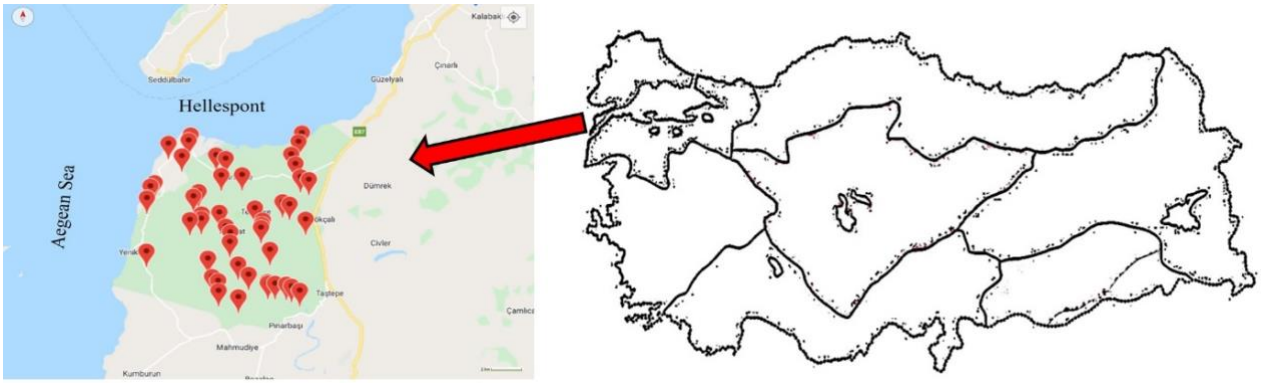

Figure 1. Study area (Troia, Türkiye).

Analyses

Analyses were all completed within three months after sample collection. The modified QuEChERS method was used for the analysis of spiked and sampled soils (Adeyinka et al., 2019; Zaidon et al., 2019; Vickneswaran et al., 2021).

About 10 g sieved sample was placed into 50 mL tubes, supplemented then with 100 µL of HAc and shaken vigorously. Samples were spiked with 100 µL of pesticide spike solutions corresponding 1x LOQ (limits of quantification) and 10x LOQ spike level. Sample tubes were supplemented with 15 mL MeCN and shaken for 15 s. QuEChERS extraction pouch kits (6 g $MgSO_4$ and 1.5 g NaOAC) were then supplemented into the samples and vortexed for 1 min. Resultant extracts were centrifuged at 3.000 rpm for 10 min. Supernatant aliquots (8 mL) were supplemented with QuEChERS clean-up kit (1.2 g $MgSO_4$, 400 mg C_{18} and 400 mg PSA) and shaken for 15 s. Sample tubes were centrifuged again at 3,000 rpm for 10 min, resultant supernatant was filtered through 0.22 µm nylon syringe into 2 mL vials and analyzed in an LC-MS/MS device.

Verification of analysis method

Method verification was performed with the use of linearity, recovery, precision and LOQ parameters. Recovery tests were conducted at two spiking levels (1x LOQ and 10x LOQ) of each pesticide for method accuracy and precision. Tests were conducted in five replicates. Percent recovery (%) was calculated as:

$$Recovery\ (\%) = \frac{Determined\ concentration\ (\frac{\mu g}{kg})}{Spiked\ concentration\ (\frac{\mu g}{kg})} X100 \qquad (1)$$

For accurate results, matrix-matched calibration curve was used to quantify insecticides.

Results and Discussion

Method verification

Calibration curves of 23 pesticide standards were linear over the various concentration ranges (soil matrix-matched calibration), with various correlation coefficient (R^2) (Table 1). Retention times, quantification and confirmation ion and matrix-matched calibration line equations (5-point level), used in MRM mode for pesticide detection, are also shown in Table 1.

Percent recovery together with relative standard deviations (RSDs) and limit of quantification values are provided in Table 2. Percent recoveries varied between 60.6 and 107% with RSDs of between 1.73 and 29.2% (Table 2). Number of recovery data (n) was 10 for each insecticide. Method overall recovery was identified as 84.8% with an RSD of 13.0% (n = 230). These recovery values validated the accuracy of the method as listed in Table 2 and the values were within the acceptable ranges indicated as between 60-140% in SANTE (2020). The LOQ values (Table 2) also revealed that the method could detect pesticide residues lower than the MRL set by the EU (2020). The findings revealed that the QuEChERS method can serve as an accurate and rapid tool for detection of insecticide residues in soil samples.

Table 2. Percent recovery together with RSDs and LOQ values

Insecticide	Spiking level				Mean		LOQ µg/kg
	1xLOQ		10xLOQ				
	Recovery %*	RSD (%)	Recovery %*	RSD (%)	Recovery % (As a tool for trueness)	RSD % (As a tool for precision)	
Acetamiprid	86.8	2.6	91.3	3.4	89.1	3.9	1
Chlorantraniliprole	96.0	4.2	87.9	4.5	92.0	6.2	1
Clofentezine	75.8	3.9	75.3	7.3	75.6	5.5	1
Clothianidin	101	7.1	88.2	4.5	94.4	9.0	1
Cyhalothrin-lambda	88.9	1.7	63.7	29.2	76.3	15.5	10
Cypermethrin	69.5	7.4	82.8	9.1	76.2	8.3	10
Deltamethrin	82.2	2.8	85.0	4.7	83.6	4.1	1
Etoxazole	87.8	2.6	80.4	5.0	84.1	5.9	1
Flubendiamide	78.0	6.9	94.7	5.5	86.3	11.7	1
Hexythiazox	98.2	3.1	88.2	3.2	93.2	6.4	1
Imidacloprid	105	3.5	90.8	3.6	98.1	8.5	1
Indoxacarb	70.6	17.9	80.9	2.6	75.8	13.4	1
Lufenuron	87.0	9.3	91.0	4.5	89.0	7.2	1
Metaflumizone	60.6	2.5	85.0	6.9	72.8	18.5	10
Methoxyfenozide	96.0	2.4	89.4	4.5	92.7	5.0	1
Novaluron	69.6	12.7	90.6	6.7	80.1	16.5	1
Pirimicarb	76.8	5.6	90.7	9.4	83.7	7.5	1
Pymetrozine	60.6	8.2	67.1	11.8	63.9	10.0	1
Pyridaben	107	3.9	99.3	25.0	102.8	17.7	1
Tebufenpyrad	87.4	5.7	88.4	4.7	87.9	5.0	1
Teflubenzuron	88.0	12.9	89.8	5.4	88.9	9.3	1
Thiamethoxam	78.8	3.9	74.5	12.2	76.6	23.1	1
Triflumuron	78.4	9.0	93.9	6.7	86.1	11.9	1
Method overall recovery (accuracy): 84.8 % (n=230; SD=11.00; RSD%=13.0)							

* Mean of 5 replicates (analytical portions).

Analytical results of soil samples

Concentrations of insecticide residues detected in soil samples are provided in Table 3. Of the 49 samples, 36 (~75%) contained insecticide residues at varying concentrations. Overall, 23 insecticide residues were detected in different frequencies. The detection frequencies of 23 insecticides are given in Table 3. The most frequent pesticides (first 10) were: chlorantraniliprole > imidacloprid > pyridaben > clothianidin > indoxacarb > methoxyfenozide > clofentezine > cypermethrin > novaluron > thiamethoxam (in decreasing order). Mean concentration of insecticide residues in soils varied between 0.99 and 77.7 µg/kg with the lowest value (0.90 µg/kg) for acetamiprid and the highest value (204 µg/kg) for pyridaben in soil from tomato fields. Chlorantraniliprole and pyridaben were detected in soil samples sunflower, wheat and rice fields (crops for which they are not licensed).

Acetamiprid, clothianidin, imidacloprid and thiamethoxam residues, included in the neonicotinoid group of IRAC classification (IRAC, 2022), were detected in present samples. Neonicotinoid insecticides have negative effects on non-target organisms and wildlife, thus they have recently been banned in the EU. Use of imidacloprid in greenhouses will be terminated on 1 June 2022 in Türkiye (PPPDA, 2022). Imidacloprid residues were found in all agricultural lands, except for cabbage fields. The greatest imidacloprid concentration (23.3 µg/kg) was seen in pepper fields. Detection frequency of imidacloprid was 21. Acetamiprid residues were detected only in four samples, all from tomato fields, with mean and maximum concentrations of 2.69 µg/kg and 4.41 µg/kg, respectively. Bonmatin et al. (2021) detected at least one neonicotinoid in 80% of the soil samples and three insecticides in 64% of the samples. While

imidacloprid was detected in all samples, clothianidin and thiamethoxam were the other common insecticides detected in 69 and 73% of the soil samples, respectively. Amin et al. (2021) detected nine pesticides such as cypermethrin, chlorpyrifos, propachlor, carbofuran, metachlore, endosulfan, cyhalothrin, difenoconazole and acetamiprid in soil samples and reported pesticide concentrations of between 6.77 and 32.0 µg/kg. Clothianidin residues were detected in 14 samples with the mean and maximum concentrations of 4.12 µg/kg and 8.93 µg/kg, respectively. Clothianidin has been banned in the 31 July 2019 in Türkiye (PPPDA, 2022). The presence of clothianidin may due to illegal use or application of previous season. The European Food Safety Authority following clothianidin for all field uses, a high risk was identified in the next crop scenario, or a high risk was not ruled out (EFSA, 2016). Thiamethoxam residues were detected in 5 samples with mean and maximum concentrations of 8.29 µg/kg and 27.6 µg/kg, respectively. Prado-Lu (2015) took soil samples from 26 different farms and detected insecticide residues in 11 samples. Chlorpyrifos, cypermethrin, malathion, profenophos and triazophos residues were detected on four farms.

Chlorantraniliprole, clofentezin, fenbutatin-oxide, flubendiamide, imidacloprid, pyridaben and thiamethoxam insecticides were detected above LOQ levels in soil samples taken from wheat fields. In a previous study, p,p'-DDE, diazinon, chlorfenapyr, difenoconazole pesticides were detected above LOQ in soil samples taken from wheat fields (Salem et al., 2021).

Persistence of pesticides in soil is an important factor in such studies. The DT_{50} of the studied insecticides are provided in Table 3 (PPDB, 2022). DT_{50} varied between 3 days (acetamiprid) and 204 days (chlorantraniliprole). Chlorantraniliprole, imidacloprid and clothianidin are the insecticides with the longest half-lives in soil and were detected in almost all fields (Figure 2). In addition to soil, imidacloprid has been identified as one of the more persistent pesticides in water systems (Braschi et al., 2022). Pyridaben (moderate half-life of 29 days) residues were also detected in almost all agricultural fields. These residues may be resulted from insecticides applied in previous seasons.

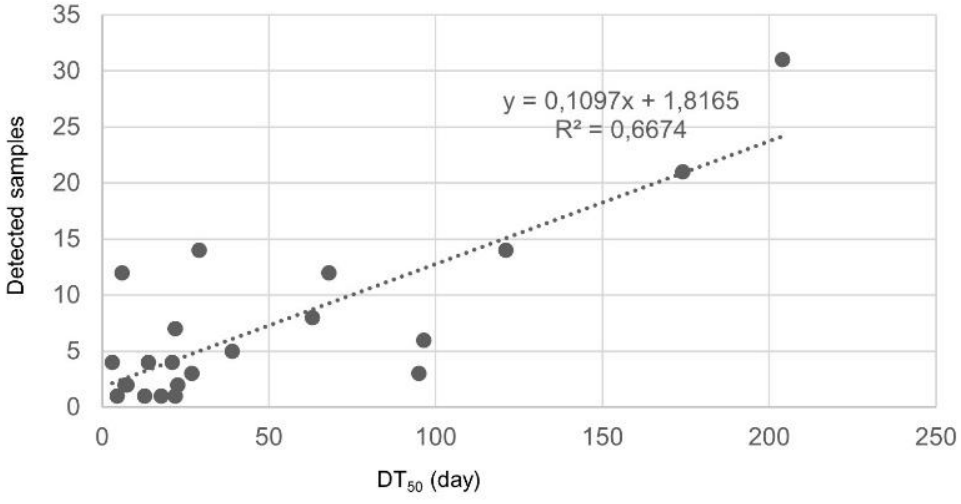

Figure 2. Relationship between number of detected samples and DT_{50}.

Table 3. Insecticide residues (µg/kg) in soils sampled from various cultivation areas

Insecticide	Type of agriculture products grown on agricultural lands								Mean conc.
	Tomato (8)*	Corn (10)	Sunflower (9)	Wheat (7)	Pepper (5)	Rice (4)	Cabbage (3)	Beans (3)	
Acetamiprid	2.69;0.9-4.4;4**								2.69
Chlorantraniliprole	23.8; 2.27-78.7;7	5.34; 1.72-15.3; 7	1.42;1.3-1.5;1	8.73;0.8-93; 6	3.33;0.8-11.8;3	1.31;1. 5-1.3; 2	5.65;1.7-13.0; 3	1.62;1. 2-2.2; 2	6.40
Clofentezine	25.1;9.2-41;2	5.43;0.9-20; 4		1.06;0.9-1.1; 1	5.92;5.4-6.2;1				9.38
Clothianidin	3.96;3.3-4.41	2.87;0.9-7.0; 7	2.95;1.8-4.2; 2		8.55;8.3-8.9;1		2.27;1.4-3.5; 3		4.12
Cyhalothrin-lambda	19.9;14.2-24.4;2						10.7; 8.5-12.5; 1		6.12
Cypermethrin	113;25-243;6						23.1;18.2-32; 1		68.1
Deltamethrin	4.56;1.1-10.3;4								4.56
Etoxazole	4.19;3-5.8;1							0.93;0. 8-1.1; 1	2.56
Fenbutatin-oxide		9.67; 1.4-20.6; 2		1.07; 0.9-1.1; 1					5.37
Fenpyroximate	1.2;0.9-1.3;1				0.97; 0.7-1.1;1				1.08
Flubendiamide	79.6;1.5-177;7	7.43; 2.45-13.2; 3		5.82; 5.4-6.0; 1		39.2;38.8-39.4; 1			33.0
Hexythiazox						0.99; 0.9-1.04;1			0.99
Imidacloprid	5.2;1.6-11.5;4	3.12; 1.3-6.1; 6	9.85; 9.3-10.2; 1	1.9; 1.4-2.6; 3	11.9; 2.4-23.3;4	3.92; 3.4-4.2; 2		7.52;7. 2-7.6; 1	6.20
Indoxacarb	26.5;1.3-83.5;7				3.06; 2.1-4.1;2	9.22; 8.7-10; 1	1.18; 0.7-1.4; 2		9.99
Metaflumizone	77.7; 12-203; 4								77.7
Methoxyfenozide	74.8;10.4-146; 5	11; 2.49-18.95; 5				22; 20.8-23.4; 1	33.5;33-34; 1		35.32
Novaluron	16.9;3.09-39.3;6								16.90
Pymetrozine					18.2; 1.1-39.6;2				18.20
Pyridaben	59.3;1.9-204;6	1.91; 0.7-4.7; 2	2.13; 1.6-2.4; 1	66; 2.61-192; 1	28.5; 0.2-160;3	22; 16.8-26.1; 1			30.0
Tebufenpyrad					2.69; 2.5-2.8;1				2.69
Teflubenzuron						3.25; 3.1-3.4 ;1			3.25
Thiamethoxam			0.98; 0.9-1.0; 1	2.38; 2.3-2.4; 1	2.49; 0.6-10.2;2		27.3; 27-27.6; 1		8.29
Triflumuron	2.77;2.4-3.1;1								2.77

*Number of soil samples taken from tomato fields;
** Mean residue; min. residue-max. residue; number of soil samples with pesticide residue.

Conclusion

In this study, QuEChERS analytical method was verified for pesticide residue detection in soil samples using an LC-MS/MS system. Present linearity, limit of quantification, accuracy, precision and matrix effect parameters revealed that QuEChERS analytical method may offer an accurate and rapid tool for pesticide residue detection in soils.

Neonicotinoids with negative impacts on non-target organisms and wildlife were also detected in varying concentrations in the soil samples. Insecticides with the longest half-lives were detected in soil samples taken from almost all fields. Based on these findings, it is concluded that the environmental risk of pesticides with high persistence should be given greater consideration. The higher the DT_{50}, the more environmental risk occurs.

Insecticide load of soil either comes from the application of the current year or from accumulation from previous years. Therefore, farmers need to be more aware of the effects of pesticides on environment and human health. They should also be encouraged to practice more judicious and conscious pesticide application.

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References

Adeyinka, G. C., B. Moodley, G. Birungi & P. Ndungu, 2019. Evaluation of organochlorinated pesticide (OCP) residues in soil, sediment and water from the Msunduzi River in South Africa. Environmental Earth Sciences, 78 (6): 1-13.

Amin, M., A. R. Gurmani, M. Rafique, S. U. Khan, A. Mehmood, D. Muhammad & J. H. Syed, 2021. Investigating the degradation behavior of cypermethrin (CYP) and chlorpyrifos (CPP) in peach orchard soils using organic/inorganic amendments. Saudi Journal of Biological Sciences, 28 (10): 5890-5896.

Anastassiades, M., S. J. Lehotay, D. Stajnbaher & F. J. Schenck, 2003. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and “dispersive solid-phase extraction” for the determination of pesticide residues in produce. Journal of AOAC international, 86 (2): 412-431.

Andersch, I. & J. P. E. Anderson, 1991. Influence of pesticides on nitrogen transformations in soil. Toxicological & Environmental Chemistry, 30 (3-4): 153-158.

Anonymous, 2021. Çanakkale Directorate of Provincial Agriculture and Forestry Data. Çanakkale Directorate of Provincial Agriculture and Forestry, 1 pp (in Turkish).

Anonymous, 2022. National pesticide information center; Pesticide half-life. (Web page http://npic.orst.edu/factsheets/half-life.html) (Date accessed: March 2022).

Balderacchi, M., M. Filippini, A. Gemitzi, B. Klöve, M. Petitta, M. Trevisan, P. Wachniew, S. Witczak & A. Gargini, 2014. Does groundwater protection in Europe require new EU-wide environmental quality standards? Frontiers in Chemistry, 2 (32):1-6.

Bhandari, G., P. Zomer, K. Atreya, H.G. Mol, X. Yang & V. Geissen, 2019. Pesticide residues in Nepalese vegetable and potential health risks. Environmental Research, 172: 511-521.

Bonmatin, J. M., E. A. Mitchell, G. Glauser, E. Lumawig-Heitzman, F. Claveria, M. B. Lexmond & F. Sánchez-Bayo, 2021. Residues of neonicotinoids in soil, water and people's hair: A case study from three agricultural regions of the Philippines. Science of the Total Environment, 757 (143822): 1-10.

Braschi, I., S. Blasioli, S. Lavrnić, E. Buscaroli, K. Di Prodi, D. Solimando & A. Toscano, 2022. Removal and fate of pesticides in a farm constructed wetland for agricultural drainage water treatment under Mediterranean conditions (Italy). Environmental Science and Pollution Research, 29 (5): 7283-7299.

Çatak, H. & O. Tiryaki, 2020. Insecticide residue analyses in cucumbers sampled from Çanakkale open markets. Turkish Journal of Entomology, 44 (4): 449-460.

Çılgı, T. & P. C. Jepson, 1992. The use of tracers to estimate the exposure of beneficial insects to direct pesticide spraying in cereals. Annals of Applied Biology, 121 (2): 239-247.

DiBartolomeis, M., S. Kegley, P. Mineau, R. Radford & K. Klein, 2019. An assessment of acute insecticide toxicity loading (AITL) of chemical pesticides used on agricultural land in the United States. PLOS One, 14 (8): e0220029.

EFSA, 2016. Peer review of the pesticide risk assessment for the active substance clothianidin in light of confirmatory data submitted. EFSA Journal, 14 (11): 1-34.

EU, 2020. EU-Pesticides database (Web page: https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/mrls/?event=search.pr) (Date accessed: March 2022).

Greenberg, A. E., L. S. Clesscerі & A. D. Eaton, 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edition. APHA/AWWA/WPCF, Washington, 1325 pp.

Hathout, A. S., E. Saleh, O. Hussain, M. Amer, A.-T. Mossa, A. A. Yassen & A. S. M. Fouzy, 2022. Determination of pesticide residues in agricultural soil samples collected from Sinai and Ismailia Governorates, Egypt. Egyptian Journal of Chemistry, 65 (3): 415-425.

Hu, M., J. Qiu, H. Zhang, X. Fan, K. Liu, D. Zeng & H. Tan, 2018. Method development and validation of indaziflam and its five metabolites in soil, water, and fruits by modified QuEChERS and UHPLC-MS/MS. Journal of Agricultural and Food Chemistry, 66 (39):10300-10308.

IRAC, 2022. Mode of action classification scheme. Insecticide Resistance Action Committee (IRAC). Version 10.2 (Web page: https://irac-online.org/documents/moa-classification) (Date accessed: April 2022).

Karasali, H., A. Marousopoulou & K. Machera, 2016. Pesticide residue concentration in soil following conventional and low-input crop management in a Mediterranean agro-ecosystem in central Greece. Science of the Total Environment, 541: 130-142.

Lehotay, S. J., 2007. Determination of pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulphate: collaborative study. Journal of AOAC International, 90 (2): 485-520.

Liu, Y., S. Li, Z. Ni, M. Qu, D. Zhong, C. Ye & T. Tang, 2016. Pesticides in persimmons. jujubes and soil from China: Residue levels. risk assessment and relationship between fruits and soils. Science of the Total Environment, 542: 620-628.

Nagel, T. G., 2009. The QuEChERS method-a new approach in pesticide analysis of soils. Journal of Horticulture, Forestry and Biotechnology, 13: 391.

Polat, B., 2021. Reduction of some insecticide residues from grapes with washing treatments. Turkish Journal of Entomology, 45 (1): 125-137.

Polat, B. & O. Tiryaki, 2019. Determination of some pesticide residues in conventional-grown and IPM-grown tomato by using QuEChERS method. Journal of Environmental Science and Health, Part B, 54 (2): 112-117.

PPDB, 2022. Pesticides Properties Data Base. (Web page: https://sitem.herts.ac.uk/aeru/ppdb/en/atoz.htm) (Date accessed: March 2022).

PPPDA, 2022. Plant Protection Product Database Application. (Web page: https://bku.tarim.gov.tr) (Date accessed: March 2022) (in Turkish).

Prado-Lu, D. & J. Leilanie, 2015. Insecticide residues in soil, water, and eggplant fruits and farmers' health effects due to exposure to pesticides. Environmental Health and Preventive Medicine, 20 (1): 53-62.

Salem, S. H. E., A. E. Fatah, G. N. E. Abdel-Rahman, U. Fouzy & D. Marrez, 2021. Screening for pesticide residues in soil and crop samples in Egypt. Egyptian Journal of Chemistry, 64 (5): 2525-2532.

SANTE, 2020. SANTE Guidelines. Guidance document on pesticide analytical methods for risk assessment and post-approval control and monitoring Purposes SANTE/2020/12830, Rev.1. (Web page https://ec.europa.eu/food/system/files/2021-02/pesticides_mrl_guidelines_2020-12830.pdf) (Date accessed: January 2022).

Seagraves, M. P. & J. G. Lundgren, 2012. Effects of neonicitinoid seed treatments on soybean aphid and its natural enemies. Journal of Pest Science, 85 (1): 125-132.

Sun, W. Z. M., 2000. The pesticide pollution problem of food in China. Pesticides, 7 (1): 1-4.

Temur, C., O. Tiryaki, O. Uzun & M. Basaran, 2012. Adaptation and validation of QuEChERS method for the analysis of trifluralin in wind-eroded soil. Journal of Environmental Science and Health Part B, 47 (9): 842-850.

Tiryaki, O., R. Canhilal & S. Horuz, 2010. Tarım ilaçlari kullanımı ve riskleri. Erciyes Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 26 (2):154-169 (in Turkish).

Tiryaki, O. & C. Temur, 2010. The fate of pesticide in the environment. Journal of Biological and Environmental Sciences, 4 (10): 29-38.

TUIK, 2021. Turkish Statistical Institute. (Web page: https://biruni.tuik.gov.tr/medas/?kn=92&locale=tr) (Date accessed: January 2022) (in Turkish).

USEPA, 2007a. Method 8081B. Organochlorine pesticides by gas chromatography. Environmental Protection Agency, Washington, USA. (Web page https://www.epa.gov/sites/default/files/2015-12/documents/8081b.pdf) (Date accessed: June 2022).

USEPA, 2007b. Method 1699: Pesticides in water, soil, sediment, biosolids, and tissue by HRGC/HRMS: Environmental Protection Agency, Washington, USA EPA-821-R-08-001, 96 pp. (Web page https://www.nemi.gov/methods/method_summary/9690) (Date accessed: March 2022).

Vickneswaran, M., J. C. Carolan & B. White, 2021. Simultaneous determination of pesticides from soils: a comparison between QuEChERS extraction and Dutch mini-Luke extraction methods. Analytical Methods, 13 (46): 5638-5650.

Yıldırım, İ. & H. Özcan, 2007. Determination of pesticide residues in water and soil resources of Troia (Troy). Fresenius Environmental Bulletin, 16 (1): 63-70.

Zaidon, S. Z., Y. Ho, H. Hamsan, Z. Hashim, N. Saari & S. M. Praveena, 2019. Improved QuEChERS and solid phase extraction for multi-residue analysis of pesticides in paddy soil and water using ultra-high performance liquid chromatography tandem mass spectrometry. Microchemical Journal, 145: 614-621.

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Original article (Orijinal araştırma)

Insecticide resistance status of *Bemisia tabaci* (Genn., 1889) (Hemiptera: Aleyrodidae) populations to cyantraniliprole, pyriproxyfen and spirotetramat in Antalya (Türkiye)[1]

Antalya (Türkiye)'dan *Bemisia tabaci* (Genn., 1889) (Hemiptera: Aleyrodidae) popülasyonlarının cyantraniliprole, pyriproxyfen ve spirotetramata direnç düzeyleri

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Abstract

In the study, the susceptibility of twelve *Bemisia tabaci* (Genn., 1889) (Hemiptera: Aleyrodidae) MEAM1 and MED populations collected from tomato and pepper greenhouses in Antalya Province (Türkiye) in 2019 and 2020 to spirotetramat, pyriproxyfen and cyantraniliprole were determined. To determine the lethal concentrations (LC_{50}) for the populations, spirotetramat and pyriproxyfen were applied using leaf dipping method to second instar and eggs, respectively, while a systemic uptake method was used for testing the susceptibility of whitefly instars to cyantraniliprole. The resistance ratios were calculated by dividing the LC_{50} of the populations by the LC_{50} of a susceptible population. The LC_{50} of the populations ranged from 0.28 to $1.70x10^3$ mg a.i./l for pyriproxyfen, from 1.76 to 228 mg a.i./l for spirotetramat, and from 0.103 to 0.382 mg a.i./l for cyantraniliprole. Resistance ratios for pyriproxyfen were particularly high. For spirotetramat and cyantraniliprole resistance varied between 2.38 and 309, and 4.68 to 17.4 times, respectively. All populations were susceptible to cyantraniliprole, but some populations highly resistance to pyriproxyfen and spirotetramat. The results will be a valuable reference for future monitoring and management of insecticide resistance.

Keywords: Biotype, insecticide, resistance, susceptibility, whitefly

Öz

Çalışmada, Antalya İli (Türkiye)'nden 2019 ve 2020 yıllarında toplanan *Bemisia tabaci* (Genn., 1889) (Hemiptera: Aleyrodidae)'nin oniki farklı MEAM1 ve MED popülasyonlarının spirotetramat, pyriproxyfen ve cyantraniliprole karşı duyarlılık düzeyleri belirlenmiştir. Popülasyonların lethal konsantrasyon (LC_{50}) değerleri spirotetramat ve pyriproxyfen için yaprak daldırma yöntemi ile sırasıyla 2. larva ve yumurta dönemine uygulanarak ve cyantraniliprole için sistemik alım yöntemi ile larva dönemine uygulanarak belirlenmiştir. Çalışmada popülasyonların LC_{50} değerlerinin duyarlı popülasyonunun LC_{50} değerine bölünmesiyle popülasyonların direnç katları belirlenmiştir. Popülasyonların LC_{50} değerleri, pyriproxyfen için 0.28 ila $1.70x10^3$ mg a.i./l, spirotetramat için 1.76 ila 228 mg a.i./l ve cyantraniliprole için 0.103 ila 0.382 mg a.i./l aralıklarında belirlenmiştir. Popülasyonların pyriproxyfen için direnç katları çok yüksek seviyede tespit edilmiştir. Direnç oranlarının spirotetramat için 2.38 ile 309 kat arasında ve cyantraniliprole için 4.68 ile 17.4 kat arasında değiştiği tespit edilmiştir. Sonuçlara göre, tüm popülasyonların cyantraniliprole karşı duyarlı olduğu tespit edilirken, pyriproxyfen ve spiroteramata karşı bazı popülasyonlarda yüksek düzeyde direnç tespit edilmiştir. Sonuçlar, insektisit direncinin gelecekte izlenmesi ve yönetimi için referans verileri içermektedir.

Anahtar sözcükler: Biyotip, insektisit, direnç, hassasiyet, beyazsinek

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Introduction

The cotton whitefly, *Bemisia tabaci* (Genn., 1889) (Hemiptera: Aleyrodidae), is a polyphagous pest that causes significant damage to many vegetables and ornamental plants in tropical and subtropical regions (Frohlich et al., 1999; De Barro et al., 2000). *Bemisia tabaci* is a species complex that has been reported in all regions of the world, except Antarctica, because of its ready adaptability to new hosts, geographic regions and has been found in association with 600 plant species. (Martin et al., 2000). By feeding on the plant, it can cause about 50% loss of yield and promote sooty mold by secreting honeydew (Horowitz et al., 2003). It is also a vector of plant viruses such as tomato yellow leaf curl virus (TYLCV) (Horowitz et al., 2003).

Several biotypes of *B. tabaci* have been identified in different parts of the world (Perring, 2001) and this indicates that *B. tabaci* is a complex of species, genetic groups or biotypes. Differences in biological properties of the biotypes cause differences in sensitivity to insecticides (Perring, 2001; Abdullahi et al., 2003). Middle East-Asia Minor1 (MEAM1) (formerly B biotype) and Mediterranean (MED) (formerly Q biotype) are the two most widespread and devastating genetic groups in the Mediterranean Basin (Horowitz et al., 2005). The MEAM1 and MED were reported from Türkiye (Erdoğan et al., 2008; Yükselbaba et al., 2012; Karut et al., 2017; Dağlı et al., 2020a).

Chemicals are widely used to control this pest because they are easy to apply and have a quick effect. *Bemisia tabaci* populations have acquired resistance to insecticides and insect growth regulators such as organophosphates, pyrethroids, neonicotinoids, carbamates and juvenile hormone analogs as a result of widespread chemical use (Denholm et al., 1996; Horowitz et al., 1998, 2005; Elbert & Nauen, 2000; Gravalos et al., 2015). *Bemisia tabaci* ranks fifth of the 12 insect species with the highest insecticide resistance reported worldwide (APRD, 2018). Due to resistance problems, there is a need for a new chemical compound that are especially effective against the target pest and have low toxic effects on the environment. Spirotetramat is a novel insecticide belonging to the new chemical class of tetramic acid derivatives (Bielza et al., 2019). Tetramic acid derivatives affect the second and third instars of whiteflies, and its mode of action appears by inhibiting the lipid metabolism enzyme, acetyl-CoA-carboxylase, causing a decrease in total lipids (Bretschneider et al., 2003; Nauen et al., 2005). Diamides are the most exciting new class of insecticides developed recently. Diamide insecticides have a novel mode of action that acts on the ryanodine receptor in insects, no other synthetic insecticide has ever been used at this site of the insect, and have very low mammalian toxicity due to their specificity for insect ryanodine receptors (Gravalos et al., 2015). Pyriproxyfen is a juvenile hormone analog that inhibits hatching of eggs and suppresses adult emergence in whiteflies and other insects (Horowitz et al., 1999; Li et al., 2012).

Overall, resistance monitoring studies are important tools for early detection of a decrease in susceptibility to insecticides in pests known to be prone to development of resistance. There were studies conducted on determination of the resistance of *B. tabaci* to cyantraniliprole, spirotetramat and pyriproxyfen in a number of countries. Hopkinson & Pumpa (2019) studied the toxicity of spirotetramat, cyantraniliprole and dinotefuran to Australian *B. tabaci* MEAM1 populations. Gravalos et al. (2015) investigated the resistance and cross-resistance of *B. tabaci* Mediterranean strains collected from Greece, Italy and Spain to cyantraniliprole. Bielza et al., (2019) determined the resistance and cross-resistance status of Spanish *B. tabaci* populations against spiromesifen and spirotetramat compounds. Resistance of *B. tabaci* populations against pyriproxyfen have been documented from several countries including China, Egypt, Israel, Spain and the USA (Horowitz et al., 1999, 2002, 2005; El Kady & Devine, 2003; Fernandez et al., 2009).

There are limited studies on the resistance of *B. tabaci* to these insecticides in Türkiye. Erdoğan et al. (2008) determined the resistance level of *B. tabaci* populations to organophosphates, synthetic pyrethroids, and insect growth regulator in 2000 and 2001. Dağli et al. (2020b) determined the susceptibility of *B. tabaci* Mediterranean and Aegean populations collected between 2005 and 2006 to endosulfan, lambda-cyhalothrin

and imidacloprid. Bahşi et al. (2012) determined the resistance level of *B. tabaci* populations collected from Antalya and its districts to the chlorpyrifos-ethyl, cypermethrin and acetamiprid in between 2007 and 2009. Satar et al. (2018) studied the resistance of five *B. tabaci* populations collected from vegetable and cotton fields in provinces of Mediterranean Region of Türkiye to neonicotinoids in 2009. Mohammed et al. (2020) determined the resistance of *B. tabaci* populations collected from greenhouses in Mersin Province to the acetamiprid, imidacloprid, thiamethoxam, spinetoram, spinosad and sulfoxaflor in 2018.

Studies on the susceptibility of *B. tabaci* populations have shown that the risk of the development of resistance is high in regions where repeated insecticide applications are common during the growing season to control the pest (Wang et al., 2020). In these cases, the necessity of appropriate and regular resistance screening studies becomes clear. To the best of our knowledge, is that no research has been conducted in Türkiye on the susceptibility of *B. tabaci* populations to spirotetramat, and cyantraniliprole. This study is aimed to determine the susceptibility and current resistance of whitefly populations to spirotetramat, pyriproxyfen, and cyantraniliprole in the Western Mediterranean Region of Türkiye to design strategies for the control of *B. tabaci* and discuss the sustainability of these strategies. Based on the data obtained as a result of the research, the study is aimed to contribute to the design of resistance management methods to delay and prevent the development of resistance. The data obtained in this study will be informative in terms of comparison of the resistance level to the specified active substances. Additionally, it can be a valuable resource for researchers on this subject and will provide data containing important information from Türkiye

Materials and Methods

Insecticides

For all insecticide bioassays, commercial formulations of the diamide group- cyantraniliprole (Circaden 200SC 200 g/l, USA), tetramic acid spirotetramat (Movento SC100 100 g/l, Germany) and insect growth regulator pyriproxyfen (Admiral 10 EC 100 g/l, France) were used in the study.

Insects

Bemisia tabaci populations were collected from tomato and pepper greenhouses in Alanya, Demre, Gazipaşa, Gaziler, Kumluca, and Serik districts in Antalya Province, with at least 200 whiteflies in 2019 and 2020 (Table 1). Antalya Province is located in the Western Mediterranean Region of Türkiye. Antalya is greenhouse vegetable cultivation center of Türkiye with has 37% of the country's total greenhouse area with 27.8 kha of greenhouse cultivation and 48% of the greenhouse vegetable production (TUIK, 2019). In Türkiye, the amount of pesticide uses in 2018 was 60 kt and regionally, pesticides were mostly used in the Mediterranean Region with 29%. Antalya Province ranked first place with 8.6% of total pesticide use in Türkiye (Anonymous, 2021). The collected *B. tabaci* populations were maintained on cotton plants in climate chambers with 26 ± 1°C temperature, 60 ± 10% relative humidity (RH) and 16:8 h L:D photoperiod without any insecticide application. An insecticide susceptible reference population (SUD-S) was also used in the study. SUD-S, initially collected on cotton in Sudan in 1978, was obtained from Raulf Nauen (Crop Science Division R&D Bayer AG, Germany) where it has been maintained in the absence of insecticides for the past 40 years. SUD-S was maintained on cotton as above conditions.

Determination of the genetic groups of *Bemisia tabaci* populations

Genetic groups of *B. tabaci* populations used in the study were identified using sequence information of the mitochondrial cytochrome oxidase I (mtCOI) region. The mtCOI were amplified with specific primers "C1-J-2195 5'-TTGATTTTTTGGTCATCCAGAAGT-3', "TL2-N-3014 5'-TCCAATGCACTAATCTGCCATATTA-3' (Frohlich et al., 1999), as stated by Yükselbaba & Göçmen (2016). According to the protocol specified in Omega EZNA SQ Tissue DNA isolation kit, DNA isolation was made from 10 individual female *B. tabaci*

adults subjected from each population. Following DNA isolation, amplification of the mtCOI region was determined according to Yükselbaba & Göçmen (2016). The PCR products obtained were sequenced in both directions by BM Labosis (Ankara, Türkiye). Using the BLAST tool, the nucleotide sequences were compared to those in the GenBank database (www.ncbi.nlm.nih.gov). Sequences were added to the GenBank database under accession numbers ON738324- ON738335 (Table 1).

Insecticide bioassay

The LC_{50} and LC_{90} of the populations were determined separately for each active substance. Lethal concentrations were determined by using at 5 or more concentrations of each insecticide, giving mortality between 10 and 90%. For populations collected in 2019 and 2020 each dose was applied to 5 or more replicates.

Determination of susceptibility of *Bemisia tabaci* populations to pyriproxyfen

The baseline susceptibility of *B. tabaci* populations to pyriproxyfen were determined by applying leaf dipping method to *B. tabaci* eggs as described by Horowitz et al. (1999), Ma et al. (2010) and IRAC (2019) method 16. At least twenty whitefly adults from the population were aspirated with a mouth aspirator and placed in small cages. The cages were attached to a young cotton plant leaves with a clip and kept in climate chambers at 26 ± 1°C, 60 ± 10% RH and 16:8 h L:D photoperiod for 24 h to allow whiteflies to deposit eggs on the cotton leaves. Then the adults were removed and the total number of eggs on the leaves were determined and noted. There were at least 30 eggs per leaf. The leaves with whitefly eggs were dipped for 10 s in insecticide solutions containing Triton-X in a volume of 100 ml and dipped a pure Triton-X solution as a negative control. The treated leaves were checked about 8 days after the insecticide application. Mortality of the eggs was calculated by subtracting the live instars from the total number of eggs.

Determination of the susceptibility of *Bemisia tabaci* populations to spirotetramat

For spirotetramat, the leaf dipping method described by Bielza et al. (2019) was used with slight modification. At least 20 whitefly adults taken from the populations were placed in cages and young cotton plant leaves were attached to the cages to allow whiteflies to deposit eggs on the cotton leaves for 24 h. After 24 h the whiteflies were removed, and the young cotton plants were kept at 26°C, 60% RH and a16:8 h L:D photoperiod for 10 days to allow second instars to develop. Then, using a microscope, different stages of the whitefly on the leaves were removed and the second instar numbers were noted. There were at least 25 second instars per leaf. Then the leaves with second instars were dipped in 100 ml serial dilutions of insecticide solutions containing Triton-X and a negative control for 10 s. The treated leaves were checked 6 days after the application. Instars that developed to pupal stage were considered to be living, dried nymphs as well as those that did not develop and remained as 2nd instars were considered as affected and dead ones.

Determination of the susceptibility of *Bemisia tabaci* populations to cyantraniliprole

The lethal concentrations of *B. tabaci* populations against cyantraniliprole were determined by using the systemic uptake method described by Li et al. (2012) with some modifications. At least twenty adult whiteflies were taken from the populations and placed in cages. Young cotton plant leaves were attached to the cages to allow them to deposit eggs on the leaves for 24 h. After that whiteflies were removed and the number of eggs on the leaves were counted. There were at least 30 eggs per leaf. The shoots of the cotton plants were cut into ~13 cm lengths with the help of clean scissors and placed in tubes containing 20 ml of serial doses of cyantraniliprole containing Triton-X and a negative control. These tubes with cotton plant shoots were kept in a climate chamber with 26°C, 60% RH and a16:8 L:D photoperiod for 12 days after the application. The nymphs that developed to second instars were considered as alive. Mortality was determined by subtracting the number of living second instars from the total number of eggs (Li et al., 2012).

Data analysis

Lethal concentrations, 95% confidence interval and related parameters of the populations were determined by probit analysis POLO-PC (Leora Software 2008, Petulama, CA, USA). The resistance ratio (RR_{50}) of the populations was calculated by dividing their LC_{50} by the LC_{50} of SUD-S. *Bemisia tabaci* populations were considered significantly different when the 95% confidence interval of the LC_{50} of the *B. tabaci* populations did not overlap (Nauen et al., 2005; Gravalos et al., 2015). The insecticide resistance level was classified according to the criteria: low resistance (RR_{50} = 2-10); moderate resistance (RR_{50} = 11-30); high resistance (RR_{50} = 31-100); very high resistance (RR_{50} > 100) (Torres-Vila et al., 2002; Peng et al., 2017).

Results

In the study, the genetic groups of *B. tabaci* populations used in insecticide bioassays were determined as MED and MEAM1 groups (Table 1).

Table 1. Details of *Bemisia tabaci* populations used in the study

Population code	Location	Sampling date	Coordinates	Host	Group	Accession
ALN19	Alanya	27.06.2019	36°35'37" N, 31°51'33" E	*Solanum lycopersicum*	MEAM1	ON738334
ALN20	Alanya	20.06.2020	36°35'38" N, 31°51'45" E	*S. lycopersicum*	MEAM1	ON738335
DMR19	Demre	28.06.2019	36°24'24" N, 30°00'20" E	*S. lycopersicum*	MED	ON738326
DMR20	Demre	21.06.2020	36°25'51" N, 30°02'15" E	*Capsicum annuum*	MED	ON738327
GZP19	Gazipaşa	27.06.2019	36°16'46" N, 32°20'28" E	*S. lycopersicum*	MEAM1	ON738332
GZP20	Gazipaşa	20.06.2020	36°15'46" N, 32°19'28" E	*S. lycopersicum*	MEAM1	ON738333
GLR19	Gaziler	24.06.2019	36°99'58" N, 30°77'80" E	*S. lycopersicum*	MEAM1	ON738330
GLR20	Gaziler	19.06.2020	36°98'32" N, 30°76'05" E	*S. lycopersicum*	MEAM1	ON738331
KML19	Kumluca	28.06.2019	36°22'23" N, 30°17'50" E	*S. lycopersicum*	MED	ON738324
KML20	Kumluca	21.06.2020	36°30'23" N, 30°35'50" E	*C. annuum*	MED	ON738325
SRK19	Serik	27.06.2019	36°56'36" N, 31°2'28" E	*S. lycopersicum*	MED	ON738328
SRK20	Serik	19.06.2020	37°00'44" N, 31°03'53" E	*S. lycopersicum*	MED	ON738329
SUD-S	Sudan	1978	-	*Gossypium hirsutum*	-	

In the study, pyriproxyfen was applied to *B. tabaci* eggs. The LC_{50} of *B. tabaci* populations ranged between 0.28 and $1.70x10^3$ mg a.i./l (Table 2). The highest LC_{50} was found in GLR19 population, while GZP20 population had the lowest LC_{50}. Table 2 shows that the resistance ratios of the populations ranged from 350 to $2.12x10^6$. There were four orders of magnitude differences between the populations. The confidence interval of all populations did not overlap with the confidence interval for SUD-S.

The LC_{50} of the populations to spirotetramat were between 1.76 and 228 mg a.i./l (Table 3). The highest LC_{50} was determined in the GZP19 population, while the lowest LC_{50} was determined in the KML19 population. The resistance ratios of the populations ranged from 2.38 to 309. The confidence interval of SRK20 overlapped with the confidence interval of SUD-S. All other populations were significantly different from SUD-S.

Table 2. Susceptibility status of *Bemisia tabaci* populations to pyriproxyfen

Population	n	Slope ± SE	LC_{50} (mg a.i./l) (95% CL)	LC_{90} (mg a.i./l) (95% CL)	H	X^2	df	RR_{50}
ALN19	1856	1.03 ± 0.08	0.33 (0.18-0.51)	5.85 (3.86-10.4)	2.29	68.8	30	412
ALN20	1497	1.05 ± 0.05	0.34 (0.19-0.55)	5.70 (3.31-11.7)	3.89	97.0	25	425
DMR19	2182	1.34 ± 0.07	0.71 (0.34-1.15)	6.36 (3.75-5.23)	9.42	273	29	887
DMR20	1347	0.63 ± 0.04	3.65 (1.96-6.86)	967 (362-3.48x10^3)	2.14	44.9	21	4.56x10^3
GZP19	3080	0.90 ± 0.05	0.85 (0.16-2.24)	22.6 (8.73-117)	15.0	511	34	1.06x10^3
GZP20	1710	1.03 ± 0.05	0.28 (0.15-0.45)	4.96 (2.98-9.38)	3.97	103	26	350
GLR19	3805	0.38 ± 0.03	1.70x10^6 (711-5.06x10^3)	3.94x10^6 (499x10^3-12.2x10^6)	3.61	148	41	2.12x10^6
GLR20	1160	0.46 ± 0.04	291 (94.1-984)	186x10^3 (24.6x10^3-10.3x10^6)	3.20	70.5	22	363x10^3
KML19	3766	0.42 ± 0.03	243 (105-528)	121x10^3 (34.5x10^3-773x10^3)	5.73	223	39	304x10^3
KML20	2518	2.81 ± 0.32	564 (467-674)	1.61x10^3 (1.20x10^3-2.81x10^3)	1.99	37.8	19	705x10^3
SRK19	3415	0.48 ± 0.03	1.01x10^3 (308-4.19x10^3)	1.19x10^6 (104x10^3-300x10^6)	7.09	269	38	1.26x10^6
SRK20	2451	0.49 ± 0.03	134 (62.5-261)	57.9x10^3 (19.2x10^3-291x10^3)	3.43	85.7	25	167x10^3
SUD-S	2400	1.92 ± 0.18	0.0008 (0.0001-0.011)	0.0036 (0.0021-0.37)	11.7	351	30	1.00

n, number of individuals used in bioassay; $RR_{50,}$ ratio of LC_{50} of the test population and the susceptible population; H, heterogenity; X^2, Chi-square; and df: degrees of freedom

Table 3. Susceptibility status of *Bemisia tabaci* populations to spirotetramat

Population	n	Slope ± SE	LC_{50} (mg a.i./l) (95%CL)	LC_{90} (mg e.m./l) (95% CL)	H	X^2	df	RR_{50}
ALN19	1479	0.45 ± 0.03	10.8 (4.34-22.9)	7.56x10^3 (2.56x10^3-35.5x10^3)	2.04	69.4	34	14.6
ALN20	1357	0.59 ± 0.04	2.83 (1.12-5.84)	413 (201-1.03x10^3)	1.96	58.7	30	3.82
DMR19	2079	1.05 ± 0.09	156 (63.3-260)	2.59x10^3 (1.53x10^3-6.73x10^3)	3.36	141	42	211
DMR20	1107	0.52 ± 0.04	43.9 (24.2-79.8)	12.9x10^3 (4.65x10^3.-52.2x10^3)	1.68	40.4	24	59.3
GZP19	2108	1.21 ± 0.12	228 (124-346)	2.61x10^3 (1.36x10^3-10.3x10^3)	3.99	172	43	309
GZP20	1165	0.81 ± 0.11	18.2 (2.16-53.1)	699 (295-2.50x10^3)	2.23	62.6	28	24.6
GLR19	1782	0.99 ± 0.05	4.15 (2.51-6.27)	81.1 (52.4-138)	2.64	89.8	34	5.60
GLR20	986	0.51 ± 0.05	6.24 (1.04- 22.4)	2.05x10^3(517-16.7x10^3)	2.95	79.7	27	8.43
KML19	3102	1.18 ± 0.05	1.76 (1.25-2.38)	21.7 (15.5-32.7)	2.96	145	49	2.38
KML20	836	0.46 ± 0.06	92.7 (41.7-184)	57.7x10^3 (17.1x10^3-372 x10^3)	0.89	17.8	20	125
SRK19	2197	0.68 ± 0.03	2.32 (1.06-4.50)	182 (83.9-505)	5.80	203	35	3.14
SRK20	1462	0.59 ± 0.04	2.01 (0.93-3.89)	308 (132-970)	2.63	78.8	30	2.72
SUD-S	1792	1.29 ± 0.09	0.74 (0.46-1.02)	7.26 (4.93-13.2)	3.04	94.1	31	1.0

n, number of individuals used in bioassay; $RR_{50,}$ ratio of LC_{50} of the test population and the susceptible population; H, heterogenity; X^2, Chi-square; and df: degrees of freedom.

The LC_{50} of cyantraniliprole for the populations ranged from 0.103 to 0.382 mg a.i./l, and the LC_{50} of SUD-S was determined as 0.022 mg a.i./l (Table 4). KML20 population had the highest LC_{50}, while ALN19 population had the lowest LC_{50}. Based on the LC_{50} of the SUD-S population Table 4 shows that the resistance ratios of the populations ranged from 4.68-17.4 times. The confidence interval of the populations did not overlap with the confidence interval of SUD-S.

Table 4. Susceptibility status of *Bemisia tabaci* populations to cyantraniliprole

Population	n	Slope ± SE	LC_{50} (mg a.i./l) (95%CL)	LC_{90} (mg e.m./l) (95%CL)	H	X^2	df	RR_{50}
ALN19	1845	2.04 ± 0.19	0.103 (0.08-0.127)	0.44 (0.37-0.57)	1.44	36.1	25	4.68
ALN20	582	2.34 ± 0.31	0.155 (0.109-0.202)	0.55 (0.37-1.46)	2.81	64.6	23	7.04
DMR19	1288	2.12 ± 0.16	0.289 (0.241-0.343)	1.16 (0.89-1.70)	1.94	68	35	13.1
DMR20	929	1.64 ± 0.14	0.304 (0.257-0.360)	1.83 (1.35-2.76)	0.71	14.2	20	13.8
GZP19	2130	2.70 ±0.17	0.262 (0.167-0.364)	0.78 (0.54-1.47)	9.73	350	36	11.9
GZP20	1218	2.26 ± 0.15	0.301 (0.227-0.410)	1.11 (0.73-2.32)	5.26	121	23	13.7
GLR19	2364	2.65 ± 0.12	0.298 (0.264-0.333)	0.91 (0.78-1.10)	2.06	78.3	38	13.5
GLR20	1015	2.55 ± 0.19	0.272 (0.241-0.309)	0.87 (0.71-1.12	0.55	11.0	20	12.4
KML19	3300	2.84 ± 0.13	0.283 (0.193-0.423)	0.80 (0.52-1.76)	15.39	570	37	12.9
KML20	801	1.54 ± 0.13	0.382 (0.218-0.651)	2.59 (1.28-12.67)	6.34	146	23	17.4
SRK19	2749	2.04 ± 0.09	0.353 (0.313-0.398)	1.50 (1.21-2.00)	2.09	73	35	16.0
SRK20	1637	2.14 ± 0.13	0.346 (0.285-0.429)	1.37 (1.00-2.16)	2.41	57.9	24	15.8
SUD-S	1888	2.21 ± 0.11	0.022 (0.013-0.041)	0.083 (0.043-0.505)	26.97	566	21	1.0

n, number of individuals used in bioassay; $RR_{50,}$ ratio of LC_{50} of the test population and the susceptible population; H, heterogenity; X^2, Chi-square; and df: degrees of freedom.

Discussion

The idea of biotypes in *B. tabaci* was introduced in 1950s after *B. tabaci* populations could not be separated morphologically due to different biological characteristics (Perring, 2001). It has been suggested that host associations, virus-carrying capacity, as well as different susceptibility and resistance to insecticides, resulted in biological differences between biotypes (Horowitz et al., 2005; De la Rua et al., 2006). Khasdan et al. (2005) suggested that different resistance to insecticides in *B. tabaci* B and Q biotypes have an impact the spread and dynamics of the biotypes. Kontsedalov et al. (2012) associated the biotype changes with different susceptibility of biotypes to insecticides. In this study the populations were determined as MED and MEAM1 genetic groups. Confidence interval of MEAM1 and MED populations with the highest LC_{50} for pyriproxyfen were overlapped, similarly, no difference was observed in the confidence intervals of MEAM and MED populations with the lowest LC_{50} for pyriproxyfen. There was no difference in the confidence interval of MEAM and MED populations with highest LC_{50} and the lowest LC_{50} for spirotetramat and cyantraniliprole. No differences were observed in the susceptibility to these insecticides in the MEAM1 and MED populations.

Pyriproxyfen resistance was observed in this study for the first time in Türkiye, with a very high level of resistance in all populations compared to the LC_{50} of the SUD-S population. Despite the high resistance ratios, the LC_{90} of the populations collected from Alanya, Demre and Gazipaşa remained below the recommended dose. Thus, pyriproxyfen has a high chance of controlling these populations. Additionally, the LC_{90} of Gaziler, Kumluca and Serik populations were above the recommended dose. Therefore, it has been conducted that pyriproxyfen might not be effective for controlling *B. tabaci* in these three regions.

Similarly, Horowitz et al. (2002) observed high resistance (>500 times) to pyriproxyfen after three consecutive applications in rose greenhouses one year after pyriproxyfen was introduced in Israel. Fernandez et al. (2009) determined the susceptibility of six *B. tabaci* populations to pyriproxyfen in Spain in 2006. it was determined that the LC_{50} of the populations ranged from 15.4 to 402 mg a.i./l with resistance ratios between 0.7 and 19.3 times. Despite high LC_{50}, low resistance ratios were reported due to the reference population used in their studies. Hopkinson et al. (2020) determined the pyriproxyfen susceptibility of *B. tabaci* populations from cotton fields in Australia in 2017 with LC_{50} between 0.001 and 2.1 mg a.i./l and resistance ratios ranging from 0.10 to 96.9 times compared to the susceptible population. Wang et al. (2020) reported the LC_{50} of six *B. tabaci* populations collected from Shangdong Province, China for pyriproxyfen ranged from 15.3 to 59.0 mg a.i./l with resistance ratios between 1.44 and 5.55 times. In comparison to other studies, the LC_{50} they determined were higher, although they described the resistance of populations as low. Ma et al. (2007) determined the resistance level of six *B. tabaci* populations collected from the Xinjiang Province, China in 2004 and 2005 for pyriproxyfen. They found, the LC_{50} of the populations ranged from 0.021-0.037 a.i./l with resistance ratios between 22 and 37. Toscano et al. (2001) determined the LC_{50} of *B. tabaci* populations collected in Arizona and California, USA between 1997 and 1999 were in between 0.003 and 9.7 mg a.i./ml for pyriproxyfen. In their study, they found over three orders of magnitude variant in susceptibility to pyriproxyfen. Although LC_{50} and resistance ratios reported in some of these studies partially overlap to the LC_{50} of some populations in our study, our study differed in that it includes populations with higher LC_{50} and resistance ratios. In our study, we obtained high LC_{50} such as $1.70x10^3$ and $1.01x10^3$ mg a.i./l to pyriproxyfen. The primary reason for this high value is the licensed and extensive use of pyriproxyfen in the management of *B. tabaci* since 1995 in Türkiye. Very high resistance ratios were observed in our study. SUD-S is highly susceptible because it has been maintained for many years without being exposed to insecticide under laboratory conditions which resulted in high resistance ratios we recorded. Similarly, Bielza et al. (2007) reported about $3x10^6$ times resistance to spinosad in *F. occidentalis* populations. They noted that the highly sensitive laboratory strain results in very high rates of resistance in field populations.

Tetronic and tetramic acid derivatives, often known as ketoenols, have been approved for use against *B. tabaci* in Türkiye since 2009. In Türkiye, no research on the susceptibility of *B. tabaci* to spirotetramat has been conducted. We conduct the first study on spirotetramat susceptibility in *B. tabaci* populations in Türkiye. In the study, low to very high resistance in *B. tabaci* to spirotetramat were observed. Based on the LC_{90} of the populations, it was found that all populations except KML19 had LC_{90} above the recommended dose. Based on our findings, it was determined that spirotetramat could have a low success rate in controlling *B. tabaci* in the sampling regions. In parallel with our study, high resistance was reported in China and Spain. Peng et al. (2017) studied the resistance changes in *B. tabaci* Q biotype to spirotetramat from 2012 to 2016 in China. They determined that all populations showed an increase in resistance from a low level in 2012 to a moderate or high level in 2016. They found that the resistance of two populations had increased to 184 (1.40 mg a.i./l) and 544 (4.13 mg a.i./l) times in 2016. Bielza et al. (2019) determined the susceptibility of 19 *B. tabaci* field populations for spirotetramat in Spain. They reported that the LC_{50} of the most susceptible and resistant field populations were 14.2 and 306 mg a.i./l with resistance ratio of 6-130, respectively. Other research (mentioned below) partially overlap with our study's low LC_{50} and resistance ratios. Hopkinson & Pumpa (2019) reported the susceptibility status of *B. tabaci* populations to spirotetramat ranged from 2.80 to 5.98 mg a.i./l with a 2.1 times difference. Prabhaker et al. (2014) determined the susceptibility status of *B. tabaci* in Arizona and California to spirotetramat. They reported that Arizona and California *B. tabaci* populations had LC_{50} ranged from 1.02 to 7.02 µg a.i/ml, and from 0.91 to 13.5 µg a.i./ml, with a 7-14 times difference in resistance between populations, respectively.

Based on the LC_{50} of the susceptible population used in this study, all *B. tabaci* populations showed low to moderate resistance to cyantraniliprole. The LC_{90} of the populations were much lower than the 100 mg/l of cyantraniliprole recommended dose (Table 4). The lethal concentrations obtained indicate that there is still a high susceptibility in the populations to cyantraniliprole. One possible reason for the moderate resistance could be that populations were exposed to insecticides from different groups during the growing season and the high resistance found in the other insecticides used in the study support this theory. Cyantraniliprole has been approved in Türkiye since 2015 and it is still too early to observe any resistance to this insecticide in *B. tabaci*. When the reasons described above are considered, the resistance ratios found in the study against cyantraniliprole can be explained as natural variation. Similar to our study, Gravalos et al. (2015) reported that the LC_{50} of cyantraniliprole in 14 *B. tabaci* populations collected from resistance-prone regions of Greece, Italy, and Spain ranged from 0.011 to 0.116 mg a.i./l, with a difference of 10.5 times between the most and least susceptible populations. They determined that the 10.5 times difference was a natural variation and this could be related to the previous use of chlorantraniliprole and flubendiamide in these regions. Li et al. (2012) found the LC_{50} of *B. tabaci* populations collected from Arizona in 2008 and 2009 for cyantraniliprole were in between 0.015 and 0.191 µg a.i./ml, with resistance ratios ranging from 0.94 to 2.63 times. They reported that the difference in susceptibility against cyantraniliprole between populations was low, and this was due to natural variation. Susceptibility in *B. tabaci* to cyantraniliprole has also been reported in studies from Australia, China and the USA (Caballero et al., 2013; Xie et al., 2014; Hopkinson & Pumpa, 2019). According to the studies afore mentioned above, *B. tabaci* populations were found to be susceptible to cyantraniliprole, however, cyantraniliprole resistance was reported in *B. tabaci* populations in China. Wang et al. (2018) determined the resistance of adult *B. tabaci* populations to cyantraniliprole by leaf dipping method between 2015 and 2016 in China. They found the LC_{50} of the populations were between 5.53 and 27.4 mg a.i./l and between 14.1 and 40.4 mg a.i./l in 2015 and 2016, respectively. They determined that the resistance ratios were between 7.01 and 25.8 in the 2016 populations. They noted a significant increase in resistance in *B. tabaci* against cyantraniliprole within 2 years. In their study, cyantraniliprole was applied to *B. tabaci* adults by a different method than ours. Cyantraniliprole is more toxic to *B. tabaci* nymphal stage than to the adult stage (Caballero et al., 2013; Gravalos et al., 2015). The difference between the findings could be due to these factors.

In the present study, all populations were found to be highly resistant to pyriproxyfen, but susceptible to cyantraniliprole. Low, moderate and high resistance were observed in *B. tabaci* populations to spirotetramat. Based on these findings, it is strongly recommended to be careful when using formulations containing spirotetramat and pyriproxyfen. Also, rotation of insecticides from different classes should be considered when it comes to managing *B. tabaci* resistance. Cyantraniliprole can be used in rotation with pyriproxyfen and spirotetramat in *B. tabaci* management. To avoid the development of cyantraniliprole resistance, it is also recommended to avoid repeated use of insecticides containing cyantraniliprole in the control of *B. tabaci*. Insecticide usage is the primary strategy in the control of *B. tabaci*, which has resulted in development of resistance to many classes of insecticides. Insecticide resistance evaluation should be conducted regularly in intensive insecticide-using areas to detect early signs of the development of resistance. Considering the findings of the study, it is recommended to give priority to biological control and biotechnical control methods in effective control of *B. tabaci*.

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References

Abdullahi, I., S. Winter, G. I. Atiri & G. Thottappilly, 2003. Molecular characterization of whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) populations infesting cassava. Bulletin of Entomological Research, 93 (2): 97-106.

Anonymous, 2021. Republic of Turkey Ministry of Agriculture and Forestry General Directorate of Food and Control. (https://www.tarimorman.gov.tr/GKGM/Belgeler/DB_Bitki_Koruma_Urunleri/Istatistik/Il_Duzeyinde_BKU_Kullanim_Miktar_2018.pdf) (Date accessed November 2021) (in Turkish).

APRD, 2018. Arthropod Pesticide Resistance Management *Bemisia tabaci*. (Web page: https://www.pesticideresistance.org/) (Date accessed: November 2018).

Bahşi, Ş. Ü., F. Dağlı, C. İkten & H. Göçmen, 2012. Susceptibility level of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) populations collected from Antalya to Acetamiprid, Chlorpyrifos-ethyl and Cypermethrin. Akdeniz Üniversitesi Ziraat Fakültesi Dergisi 25 (1): 17-22 (in Turkish with abstract in English).

Bielza, P., I. Moreno, A. Belando, C. Grávalos, J. Izquierdo & R. Nauen, 2019. Spiromesifen and spirotetramat resistance in field populations of *Bemisia tabaci* Gennadius in Spain. Pest Management Science 75 (1): 45-49.

Bielza, P., V. Quinto, J. Contreras, M. Torne, A. Martin & P. J. Espinosa, 2007. Resistance to spinosad in the western flower thrips, *Frankliniella occidentalis* (Pergande), in greenhouses of south-eastern Spain. Pest Management Science, 63: 682-687.

Bretschneider, T., J. Benetbuchhol, R. Fischer & R. Nauen, 2003. Spirodiclofen and spiromesifen novel acaricidal and insecticidal tetronic acid derivatives with a new mode of action. Chimia, 57 (11): 697-701.

Caballero, R., S. Cyman, D. J. Schuster, H. E. Portillo & R. Slater, 2013. Baseline susceptibility of *Bemisia tabaci* (Genn.) biotype B in southern Florida to cyantraniliprole. Crop Protection, 44: 104-108.

Dağlı, F., U. Yükselbaba, C. Ikten, N. Topakçı & H. Göçmen, 2020a. Molecular identification of cotton whitefly *Bemisia tabaci* Genn. (Hemiptera: Aleyrodidae) populations of Turkey based on mitochondrial cytochrome oxidase subunit I. Applied Ecology and Environmental Research 18 (2): 2351-2361.

Dağlı, F., U. Yükselbaba, C. Ikten, N. Topakçı & H. Göçmen, 2020b. Insecticide resistance in *Bemisia tabaci* (Genn.) Populations collected from the Mediterranean and Aegean regions of Turkey. Applied Ecology & Environmental Research, 18 (6): 7757-7768.

De Barro, P. J., F. Driver, J. W. H. Trueman & J. Curran, 2000. Phylogenetic relationships of world populations of *Bemisia tabaci* (Gennadius) using ribosomal ITS1. Molecular Phylogenetics and Evolution, 16 (1): 29-36.

De la Rua, P., B. Simon, D. Cifuentes, C. Martinez-Mora & J. L. Cenis, 2006. New insights into the mitochondrial phylogeny of the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) in the Mediterranean Basin. Journal of Zoological Systematics and Evolutionary Research, 44 (1): 25-33.

Denholm, I., M. Cahill, F. J. Byrne & A. L. Devonshire, 1996. "Progress with Documenting and Combating Insecticide Resistance in *Bemisia*, 507-603". In: *Bemisia*: 1995, Taxonomy, Biology, Damage, Control and Management (Eds. D. Gerling & R. T. Mayer). Intercept Ltd Andover, Hants, 702 pp.

El Kady, H. & J. G. Devine, 2003. Insecticide resistance in Egyptian populations of the cotton whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae). Pest Management Science, 59 (8): 865-871.

Elbert, A. & R. Nauen, 2000. Resistance of *Bemisia tabaci* (Homoptera: Aleyrodidae) to insecticides in southern Spain with special reference to neonicotinoids. Pest Management Science, 56 (1): 60-64.

Erdoğan, C., G. D. Moores, M. O. Gurkan, K. J. Gorman & I. Denholm, 2008. Insecticide resistance and biotype status of populations of the tobacco whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) from Turkey. Crop Protection, 27 (3-5): 600-605.

Fernandez, E., C. Grávalos, P. J. Haro, D. Cifuentes & P. Bielza, 2009. Insecticide resistance status of *Bemisia tabaci* Q-biotype in south-eastern Spain. Pest Management Science; 65 (8): 885-891.

Frohlich, D. R., I. Torrez-jerez, I. D. Bedford, P. G. Markham & J. K. Brown, 1999. A phylogeographical analysis of the *Bemisia tabaci* species complex based on Mitochondrial DNA markers. Molecular Ecology, 8 (10): 1683-1691.

Gravalos, C., E. Fernández, A. Belando, I. Moreno, C. Rosb & P. Bielza, 2015. Cross-resistance and baseline susceptibility of Mediterranean strains of *Bemisia tabaci* to cyantraniliprole. Pest Management Science, 71 (7): 1030-1036.

Hopkinson, J. E. & S. E. Pumpa, 2019. Baseline susceptibility of *Bemisia tabaci* MEAM 1 (Hemiptera: Aleyrodidae) in Australia to spiroteramat, cyantraniliprole and dinotefuran, with reference to pyriproxyfen cross-resistance. Austral Entomology, 58 (4): 762-771.

Hopkinson, J., S. Pumpa, S. Van Brunschot, C. Fang, M. Frese, W. T. Tay & T. Walsh, 2020. Insecticide resistance status of *Bemisia tabaci* MEAM1 (Hemiptera: Aleyrodidae) in Australian cotton production valleys. Australian Entomology, 59 (1): 202-214.

Horowitz, A. R., I. Denholm, K. Gorman, J. L. Cenis, S. Kontsedalov & I. Ishaaya, 2003. Biotype Q of *Bemisia tabaci* identified in Israel. Phytoparasitica, 31 (1): 94-98.

Horowitz, A. R. & I. Ishaaya, 2014. Dynamics of biotypes B and Q of the whitefly *Bemisia tabaci* and its impact on insecticide resistance. Pest Management Science, 70 (10): 1568-1572.

Horowitz, A. R., S. Kontsedalov, I. Denholm & I. Ishaaya, 2002. Dynamics of insecticide resistance in *Bemisia tabaci*: a case study with the insect growth regulator pyriproxyfen Pest Management Science, 58 (11): 1096-1100.

Horowitz, A. R., S. Kontsedalov, V. Khasdan & I. Ishaaya, 2005. Biotypes B and Q of *Bemisia tabaci* and their relevance to neonicotinoid and pyriproxyfen resistance. Archives of Insect Biochemistry and Physiology, 58 (4): 216-225.

Horowitz, A. R., Z. Mendelson, M. Cahill, I. Denholm & I. Ishaaya, 1999. Managing resistance to the insect growth regulator, pyriproxyfen, in *Bemisia tabaci*. Pest Management Science, 55 (3): 272-276.

Horowitz, A. R., Z. Mendelson, P. G. Weintraub & I. Ishaaya, 1998. Comparative toxicity of foliar and systemic applications of acetamiprid and imidacloprid against the cotton whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae). Bulletin of Entomological Research, 88 (4): 437-442.

IRAC (Insecticide Resistance Action Committee), 2019. Susceptibility test methods no: 016. (Web page: https://www.irac-online.org/methods) (Date accessed: July 2019).

Karut, K., M. M. Karaca, İ. Döker & C. Kazak, 2017. Analysis of species, subgroups, and endosymbionts of *Bemisia tabaci* (Hemiptera: Aleyrodidae) from southwestern cotton fields in Turkey. Environmental Entomology, 46 (4) 1035-1040.

Khasdan, V., I. Levin, A. Rosner, S. Morin, S. Kontsedalov, L. Maslenin & A. R. Horowitz, 2005. DNA markers for identifying biotypes B and Q of *Bemisia tabaci* (Hemiptera: Aleyrodidae) and studying population Dynamics. Bulletin of Entomological Research, 95 (6): 605-613.

Kontsedalov, S., F. Abu-Moch, G. Lebedev, H. Czosnek, A. R. Horowitz & M. Ghanim, 2012. *Bemisia tabaci* biotype dynamics and resistance to insecticides in Israel during the years 2008-2010. Journal of Integrative Agriculture, 11 (2): 312-320.

Li, X. C., B. A. Degain, V. S. Harpold, P. G. Marcon, R. L. Nichols, A. J. Fournier, S. E. Naranjo, J. C. Palumbo & P. C. Ellsworth, 2012. Baseline susceptibilities of B and Q biotype *Bemisia tabaci* to anthranilic diamides in Arizona. Pest Management Science, 68 (1): 83-91.

Ma, D., K. Gorman, G. Devine, W. Luo & I. Denholm, 2007. The biotype and insecticide-resistance status of whiteflies, *Bemisia tabaci* (Hemiptera: Aleyrodidae), invading cropping systems in Xinjiang Uygur Autonomous Region, northwestern China. Crop Protection, 26 (4): 612-617.

Ma, W., X. Li, T.J. Dennehy, C. Lei, M. Wang, B. A. Degain & R. L. Nichols, 2010. Pyriproxyfen resistance of *Bemisia tabaci* (Homoptera: Aleyrodidae) biotype B: metabolic mechanism. Journal of Economic Entomology, 103 (1): 158-165.

Martin, J. H., D. Mifsud & C. Rapisarda, 2000. The whiteflies (Hemiptera: Aleyrodidae) of Europe and the Mediterranean basin. Bulletin of Entomological Research, 90 (5): 407-448.

Mohammed, M. A., M. M. Karaca, İ. Döker & K. Karut, 2020. Monitoring insecticide resistance and endosymbiont composition, in greenhouse populations of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) from Mersin, Turkey. Phytoparasitica, 48 (4): 659-672.

Nauen, R., H. J. Schnorbach & A. Elbert, 2005. The biological profile of spiromesifen (Oberon) a new tetronic acid insecticide/acaricide. Pflanzenschutz-Nachrichten Bayer, 58: 417-440.

Peng, Z., H. Zheng, W. Xie, S. Wang, Q. Wu & Y. Zhang, 2017. Field resistance monitoring of the immature stages of the whitefly *Bemisia tabaci* to Field resistance monitoring of the immature stages of the whitefly *Bemisia tabaci* to spirotetramat in China. Crop Protection, 98 (2017): 243-247.

Perring, T. M., 2001. The *Bemisia tabaci* species complex. Crop Protection, 20 (9): 725-737.

Prabhaker, N., S. Castle & T. M. Perring, 2014. Baseline susceptibility of *Bemisia tabaci* B biotype (Hemiptera: Aleyrodidae) populations from California and Arizona to spirotetramat. Journal of Economic Entomology, 107 (2): 773-780.

Satar, G., M. R. Ulusoy, R. Nauen & K. Dong, 2018. Neonicotinoid insecticide resistance among populations of *Bemisia tabaci* in the Mediterranean region of Turkey. Bulletin of Insectology, 71 (2): 171-177.

Torres-Vila, L. M., M. C. Rodriguez-Molina, A. Lacasa-Plasencia & P. Bielza-Lino, 2002. Insecticide resistance of *Helicoverpa armigera* to endosulfan, carbamates and organophosphates: the Spanish case. Crop Protection, 21 (19): 1003-1013.

Toscano, N. C., N. Prabhaker, S. J. Castle & T. J. Henneberry, 2001. Inter-regional differences in baseline toxicity of *Bemisia argentifolii* (Homoptera: Aleyrodidae) to the two insect growth regulators, buprofezin and pyriproxyfen. Journal of Economic Entomology, 94 (6): 1538-1546.

TUIK, 2019. Turkish Statistical Institute: Crop production statistics. (Web page: http: //www.tuik.gov.tr) (Date accessed: January 2019) (in Turkish).

Wang, F., J. Liu, P. Chen, H. Li, J. Ma, Y. Liu & K. Wang, 2020. *Bemisia tabaci* (Hemiptera: Aleyrodidae) insecticide resistance in Shandong Province, China. Journal of Economic Entomology, 113 (2): 911-917.

Wang, R., J. D. Wang, W. N. Che & C. Luo, 2018. First report of field resistance to cyantraniliprole, a new anthranilic diamide insecticide, on *Bemisia tabaci* MED in China. Journal of Integrative Agriculture, 17 (1): 158-163.

Xie, W., Y. Liu, S. Wang, Q. Wu, H. Pan, X. Yang, L. Guo & Y. Zhang, 2014. Sensitivity of *Bemisia tabaci* (Hemiptera: Aleyrodidae) to several new insecticides in China: effects of insecticide type and whitefly species, strain and stage. Journal of Insect Science, 14 (1): 261-268.

Yükselbaba, U. & H. Göçmen, 2016. Determination of the resistance development potential to spiromesifen and the enzyme activities of the B and Q biotypes of cotton whitefly *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae). Fresenius Environmental Bulletin, 25 (7): 2461-2465.

Yükselbaba, U., C. İkten & H. Göçmen, 2012. “Determination of the biotypes of *Bemisia tabaci* (Genadius) (Hemiptera: Aleyrodidae) populations from Antalya Province of Turkey by sequence analysis of Mitochondrial Cytochrome Oxidase I (mtCOI) gene region, 29”. QBOL-EPPO Conference on DNA Barcoding and diagnostic methods for plant pests (21-25 June 2012, Haarlem, Netherlands), 71 pp.

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Original article (Orijinal araştırma)

Screening of the nematicidal potential of some spice extracts against root-knot nematode, *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Tylenchida: Meloidogynidae)[1]

Bazı baharat ekstraktlarının *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Tylenchida: Meloidogynidae)'ya karşı nematisidal potansiyellerinin araştırılması

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Abstract

Experiments were conducted in the laboratories and greenhouses of Plant Protection Department, Agricultural Faculty, Ondokuz Mayıs University in 2018 and 2019 to investigate the nematicidal effects of aqueous extracts of 13 spices on *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Tylenchida: Meloidogynidae). Spice extract concentrations of 0.5, 1 and 2% were tested in laboratory experiments for inhibition of egg hatching, mortality and immobility of second-stage juveniles (J2s). When used at a concentration of 2%, clove, *Syzygium aromaticum* L. (Myrtales: Myrtaceae) caused the greatest immobility and mortality of J2s. The extracts had a lesser effect on J2s than the egg hatching. For the pot experiment, five effective spices extracts were selected based on the laboratory experiments. These extracts were applied at 2% to 200 g of soil inoculated with 3,000 nematode eggs then susceptible tomato seedlings were transplanted into the soil. Forty-five days after inoculation, the gall index and the quantity of nematode eggs on roots were determined and reproduction factor of nematode calculated. All extracts, except cumin, *Cuminum cyminum* L. (Apiales: Apiaceae), reduced root gall index and the reproduction factor when compared to control. Basil, *Ocimum basilicum* L. (Lamiales: Lamiaceae) extract reduced nematode reproduction the greatest degree, followed by turmeric, *Curcuma longa* L. (Zingiberales: Zingiberaceae) and clove extracts.

Keywords: Egg hatching inhibition, J2 mobility, J2 mortality, nematicidal effect, spice extract

Öz

On üç baharattan elde edilen sulu ekstraktların *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Tylenchida: Meloidogynidae) üzerine nematicidal etkilerini belirlemek amacıyla Ondokuz Mayıs Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü Nematoloji Laboratuvarı ve seralarında, 2018 ve 2019 yıllarında denemeler yürütülmüştür. Laboratuvar çalışmalarında ekstraktların 3 farklı konsantrasyonunun (%0.5, 1, 2) yumurta açılımı, ikinci dönem larvaların (J2) hareketi ve canlılığına etkileri araştırılmıştır. J2'lerin hareketi ve canlılığına en fazla etkiyi %2'lik konsantrasyonda karanfil, *Syzygium aromaticum* L. (Myrtales: Myrtaceae) sağlamıştır. Genel olarak ekstraktların yumurta açılıma etkisi, larvalara olandan fazladır. Laboratuvar denemeleri sonucunda etkili bulunan 5 baharat ekstraktı saksı denemeleri için seçilmiştir. Ekstraktların %2 konsantrasyonları 3000 nematod yumurtası bulaştırılmış 200 g toprağa uygulanmış, sonrasında hassas domates fideleri şaşırtılmıştır. Nematod bulaştırılmasından 45 gün sonra, kök başına yumurta sayısı ve ur skalası bulunmuş, üreme faktörü hesaplanmıştır. Kimyon, *Cuminum cyminum* L. (Apiales: Apiaceae) hariç ekstraktların tamamı, ur skalası ve üreme faktöründe kontrole kıyasla azalmaya neden olmuştur. Nematodun üremesini en fazla azaltan baharat fesleğen, *Ocimium basilium* L. (Lamiales: Lamiaceae) olmuş onu zerdeçal, *Curcuma longa* L. (Zingiberales: Zingiberaceae) ve karanfil izlemiştir.

Anahtar sözcükler: Yumurta açılımı engelleme, J2 hareket, J2 ölüm, nematisidal etki, baharat ekstraktı

[1] This study was the summary of master thesis of first author and presented as an oral in The Seventh International Congress of Nematology (1-6 May 2022, Antibes Juan-Les-Pins, France).

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Introduction

Root-knot nematodes (*Meloidogyne* spp.) (RKNs) are sedentary endoparasites of over 3,000 plant species, cause significant economic losses and can be found in almost all agricultural soils and climates. RKNs are one of the most important nematode taxa that reduce the yield and quality of agricultural products in tropical and subtropical regions (Trudgill & Blok, 2001; Abad et al., 2003; Kiewnick & Sikora, 2006). The unusual root gall formation that alters water and nutrient uptake is the most obvious morphological response of susceptible plants to infection with RKNs and the name of the genus comes from this symptom (Jones et al., 2013). Like other plant-parasitic nematodes, RKNs reduce plant productivity while predisposing plants to fungal and bacterial infections (Zhou et al., 2016). RKNs infect a wide range of horticultural and field crops, especially vegetables, causing an estimated $157x10^9$ USD in annual damage worldwide (Abad et al., 2008). RKNs cause a 10% decline in annual vegetable yields (Koenning et al., 1999). However, yield loss in susceptible plants to this pest, such as tomatoes can reach 68% (Padilla-Hurtado et al., 2022).

Given their economic importance, there is a growing need for long-term management strategies to control RKNs. Cultural methods are widely used but have major limitations due to their broad host range and the presence of mixed populations of different RKN species in the field (Trudgill & Blok, 2001; Xiang et al., 2018). RKN-resistant cultivars have proven to be a useful management tool, but there are few commercially available resistant cultivars and the existence of resistance-breaking virulent populations has also been documented in many countries (Roberts, 1995; Devran & Söğüt, 2010; Xiang et al., 2018; Hajihassani et al., 2020). Given there are few effective chemicals that can be used on a large scale against plant-parasitic nematodes, and because resistant plant cultivars are not available for many species, they are among the most difficult pests to control (Jones et al., 2013). High molecular weight soil fumigants, carbamates and organic phosphorus compounds are commonly used for control, but several of these chemicals have been banned or restricted because of their broad spectrum of activity. Most of the nematicides are highly toxic, carcinogenic and leave residues in harvested products. They also have significant adverse effects on the environment, natural life, humans and animals (Dutta et al., 2019; Ebone et al., 2019). Given the negative effects of nematicides and the lack of supply of resistant plant cultivars, studies on alternative management methods have attracted considerable attention in recent years. The use of plant extracts as an alternative to synthetic pesticides for the management of RKNs has gained importance. Numerous plant species from 57 families, including Asteraceae, Lamiaceae, Lauraceae, Myrtaceae and Rutaceae, may contain nematicidal compounds (Andrés et al., 2012). The use of plant extracts against RKN has shown their efficacy in previous studies (Javed et al., 2007; Hassan et al., 2013; Curto et al., 2015; Xia et al., 2019). Some of the plant extracts are already used commercially for RKN management, especially in organic farming (Zaidat et al., 2020). Spice plants are also known to contain components that have a negative impact on nematodes (Oka, 2001; Abbas et al., 2009; Ntalli & Caboni, 2012; Nile et al., 2017; Zaidat et al., 2020).

Spices have been used for many years as medicinal materials, in religious rituals, in cosmetics and perfumery, or as food. They have also been tested for their potential use as pesticides. Spices obtained by drying various plant parts such as roots, leaves and seeds, may be toxic to nematodes. Many studies show that extracts and oils derived from spice plants have negative effects on nematodes by inhibiting egg hatching, causing second-stage juvenile (J2) immobility, or being lethal (Oka et al., 2000; Ibrahim et al., 2006; Abbas et al., 2009; Aydınlı & Mennan, 2014; Youssef et al., 2015; El-Nagdi Wafaa et al., 2017). Therefore, in this study, the nematicidal potential of aqueous extracts from 13 spices plants for management of the root-knot nematode *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Tylenchida: Meloidogynidae) was investigated in the laboratory and in pot experiments. The effects of spice extracts on egg hatching, J2 mobility and mortality of *M. arenaria* were studied in the laboratory. Then, five most effective extracts were selected and used to study the effects of *M. arenaria* damage on tomato plants in pot experiments in a greenhouse.

Materials and Methods

Nematode inoculum

Meloidogyne arenaria was used in the study because it is the most abundant nematode species in greenhouses in the Black Sea Region of Türkiye (Aydınlı & Mennan, 2016). The population of the nematode species required for the study was obtained from nematode-susceptible tomato cultivars of Rio Grande (May Seed Company, Bursa, Türkiye) grown continuously as a mass culture in the greenhouses of the Nematology Laboratory of the Department of Plant Protection of Ondokuz Mayıs University (Aydınlı & Mennan, 2016).

The species of the root-knot nematode population was confirmed using morphological and biochemical methods. Female perineal patterns were used for morphological diagnosis (Taylor & Netscher, 1974), and the esterase enzyme phenotype was used for biochemical diagnosis (Esbenshade & Triantaphyllou, 1985). Females for both methods were collected from infested tomato plant roots using a stereomicroscope (Nikon SMZ1500). After evaluation of the perineal patterns of the females and the esterase enzyme phenotypes, it was confirmed that the root-knot nematode population used in the study was *M. arenaria*. The eggs and J2s of *M. arenaria* were obtained from this mass culture. For this purpose, tomato plants in mass culture pots were removed; the roots were washed with water, cut into 1-2 cm long pieces, and shaken for 3-5 min in a glass flask containing 0.5% NaOCl. This solution with the roots was sieved through a 200 mesh (75 µm) and 500 mesh (25 µm) sieve and the eggs in the lower sieve (500 mesh) were collected in a glass beaker (Hussey & Barker, 1973) and then counted under the stereo microscope. The J2s were collected daily from the eggs and stored at 15°C. The juveniles used for the experiments were less than 5 days old.

Preparation of the aqueous spice extract

For the laboratory experiments, 13 spice species were used (Table 1). The spices were purchased (Kaan Baharat A.Ş., Rize, Türkiye) and a 10% (w/v) stock solution of each spice was prepared. In a shaker, 10 g of spice were mixed with 90 ml of distilled water and shaken at 100 rpm in the refrigerator (4°C) (Heidolph, Unimax 2010). After 24 h in the shaker, the spice-water mixture was passed through a muslin cloth, then a 38-µm sieve, and lastly into a beaker. The supernatants were collected using Whatman No. 1 filter paper, transferred to dark plastic bottles, and kept refrigerated until as a stock solution (Oka et al., 2006). Stock solutions were used to make three concentrations (0.5, 1 and 2%) for each spice.

Laboratory experiments

Laboratory experiments were conducted to investigate the nematicidal effects of 0.5, 1 and 2% aqueous extracts of spice on egg hatching, J2 mobility, and mortality of *M. arenaria*.

Effect of spice extracts on egg hatching

The spice extract stock solution was immediately passed through a sterile 0.2 m syringe filter before use. All in vitro experiments were performed in 48-well cell culture plates (Sigma SIAL0548). Using a micropipette, 100 eggs, extracts and water were added to each well. As a result, the final volume of the prepared concentration was adjusted 100 µl. Each treatment was repeated four times. For 7 days, the plates were kept in a dark environment in an incubator at 24°C. To determine the effect of the treatments on egg hatching, the J2 and eggs in the wells of the plates were counted under a stereomicroscope at each day. The experiment was repeated once more under the same conditions (experiments 1 and 2). The inhibition rate of egg hatching was calculated at the end of the experiment by evaluating the unhatched eggs (Oka et al., 2000; Nile et al., 2017).

Table 1. Species, family, common name and plant part(s) from which the spice was made

Species	Family	Common name	Plant parts
*Anethum graveolens**	Apiacae	Dill	Fruit and leaves
Capsicum annium	Solanaceae	Chili pepper	Fruit
Cuminum cyminum	Apiaceae	Cumin	Fruit
Coriandrum sativum	Apiaceae	Coriander	Fruit and leaves
Curcuma longa	Zingiberaceae	Turmeric	Rhizomes
Helichrysium italicum	Asteraceae	Italian helichrysum, immortelle	Young shoots and leaves
Ocimium basilicum	Lamiaceae	Basil	Leaves
Piper nigrum	Piperaceae	Black pepper	Fruit
Prunus mahaleb	Rosaceae	Mahaleb cherry	Fruit
Rhus coriaria	Anacardiaceae	Sicilian sumac, tanner's sumac	Fruit
Syzygium aromaticum	Myrtaceae	Clove	Flower buds
Thymus vulgaris	Lamiaceae	Thyme	Young shoots and leaves
Zingiber officinale	Zingiberaceae	Ginger	Rhizomes

* Spice extracts were purchased from Kaan Baharat A.Ş., Rize, Türkiye.

Effect of spice extracts on J2 immobility and mortality

Tomato plants infested with *M. arenaria* were removed from mass culture and their roots were washed. Under a stereomicroscope, egg masses were collected from the roots with forceps to sterile water. The eggs were placed in an incubator at 24°C and checked every 2 days. Hatched J2s were collected and stored at 5°C until needed. J2s as young as 2 days old were used for extract applications (Ferris & Zheng, 1999; Oka et al., 2000). In 48-well plates, the effects of a 0.5, 1 and 2% aqueous spice extract on J2 immobility were studied using 100 J2 instead of eggs, as mentioned above. As controls, only water and J2s were used, with no extract application. After 48 h, the plates were examined under a stereomicroscope, and mobile and immobile J2s were counted and recorded (Zaidat et al., 2020). Treatments were applied to four replicates in an incubator at 24°C, and all experiments were repeated under the same conditions (experiments 1 and 2). To assess the effect of the aqueous spice extracts on J2 mortality, the extracts were removed with a micropipette and replaced with sterile water in the wells where J2s were counted. A second assessment was performed after 24 h, and they were classified as immobile and/or dead if the J2 was straight or slightly curved. The number of J2s in the sample was confirmed using small touches with a needle under a stereomicroscope, and the inactive J2s were considered dead. The treatment percentage mortality rate was calculated and compared to distilled water (Ferris & Zheng, 1999; Oka et al., 2000; Coltro-Roncato et al., 2018).

Pot experiment

The soil used in the pot experiments was heated for 150 min at 165°C for sterilization. For the experiments, the tomato cultivar Falcon (May Seed Company, Türkiye), which is known to be susceptible to root-knot nematodes, was used. Tomato seeds were sown and grown to the seedling stage with two to four leaves at a controlled temperature (25 ± 3°C). *Meloidogyne arenaria* eggs were obtained in the way described in the laboratory studies. After the laboratory studies, the five effective spices basil, clove, cumin, coriander, and turmeric were chosen for the pot experiments. The pot experiments consisted of seven applications with 5 spice extracts and negative and positive controls. Negative and positive controls were distilled water and nematicide (200 g/l ethoprophos), respectively. The stock solution of spice extracts (10% in 4 ml), 3000 nematode eggs (1,500 eggs/ml), and water (14 ml) were applied to 200 g of sterile sandy soil, resulting in a final concentration of spice extracts in the soil of 2%. For 1 week, soils were kept at room temperature (22-26°C). The soil was transferred to pots at the end of this period, and the susceptible tomato

seedlings were transplanted (Oka et al., 2006). The plants were grown in greenhouses at 25 ± 3°C applying daily routine requirements. The experiment was conducted using a randomized block design with eight replicates. Tomato plants were taken from pots 45 days after nematode inoculation and their roots were carefully cleansed. The gall index was determined using a 0-5 gall scale: 0, no galls; 1, traces of infestation with a few minor galls; 2, 25%; 3, 26-50%; 4 51-75%; and 5, >75% of the roots galled (Hussey & Janssen, 2002). The number of eggs in each root was counted under a stereomicroscope as reported before (Hussey & Barker, 1973). Subsequently, the reproduction factor (R_f) was calculated by the division of the final population of egg (P_f) by the initial population (3000 egg, P_i) (Oostenbrink, 1966).

Data analysis

The rates of egg hatching inhibition, the immobility and the mortality of J2s were expressed as a percent of total treatments. The percent inhibition in egg hatching was calculated by using the formula:

$$\% \text{ inhibition egg hatching} = ((C_0 - T_1) / C_0 \times 100)$$

where, C_0 is the number of juveniles hatched in control and T_1 is the number of juveniles hatched in each concentration of spice extract. In case of mortality, C_0 is the number of live nematodes in control and T_1 is the number of live nematodes after 24 and 72 h exposure (Khan et al., 2019). The raw data were $\log_{10}(x+1)$ transformed first to improve homogeneity for statistical analysis. The data obtained from the trials were evaluated in the SAS statistical program and Tukey's comparison test was applied to determine the means of different groups when variances were homogeneous ($P \leq 0.05$).

Results

Effect of spice extracts on egg hatching, J2 immobility and J2 mortality

Given there was no statistical difference between the values from the experiments 1 and 2, the results were combined and reported over eight replicates. The aqueous extracts of the 13 spices tested showed highly significant effects on egg hatching. It was found that all spice extracts inhibited egg hatching by 19.1-93.1% (Table 2). With increasing concentration, the rate of inhibition of egg hatching increased significantly. Two percent was used as the highest concentration; the inhibition rate of egg hatching was ranged from 33.3-93.1%. The lowest egg hatching inhibition rate was observed with immortelle extract (19.1%), followed by dill (19.4%) at concentrations of 0.5%. Pepper extract caused the highest inhibition of egg hatching at all concentrations, followed by basil and clove.

The effect of aqueous spice extract applications on larval immobility was evaluated with counts conducted 48 h after extract application; at the lowest concentration (0.5%) of spice extracts, the highest rate of immobile J2s was recorded for cloves at 16.8%, and the lowest rate was recorded for dill extract at 2.6% (Table 3). Coriander had the greatest effect after cloves (16.0%). Cloves were followed by sumac (12.5%), cumin (12.5%), black pepper (12.0%), thyme (11.6%), basil (9.8%), and hot pepper (8.0%), with no statistically difference ($P \leq 0.05$). At the 1% concentration of spice extract applications, the highest rate of J2 immobility was found in cloves at 27.9%, while the lowest rate of immobility was found in dill extract at 2.5%. Coriander (21.1%), thyme (20.1%), sumac (18.3%), cumin (18.3%), and basil (17.6%) had the highest immobility rates after cloves. Spice extracts, for which the highest and lowest J2 immobility rates were determined at concentrations of 0.5 and 1%, showed the same effect at a concentration of 2%. In general, as the concentration increased with each application of spice extracts, the J2 immobility rate also increased. Except for the applications of pepper, coriander, immortelle, basil and clove, the change in these increases was not statistically significant at all concentrations ($P \leq 0.05$).

Table 2. Inhibition rate of spice aqueous extracts at three concentrations on egg hatching of *Meloidogyne arenaria*

Spices		Concentrations					
		0.5%		1%		2%	
Anethum graveolens	Dill	19.4	C de*	28.4	B c-e	35.8	A c-e
Capsium annium	Pepper	82.3	C a	86.1	B a	93.1	A a
Cuminum cyminum	Cumin	39.5	B b-d	51.9	AB a-d	59.3	A a-d
Coriandrum sativum	Coriander	64.0	A a-c	68.3	A ab	73.6	A a-c
Curcuma longa	Turmeric	49.5	A a-d	55.8	A a-d	59.6	A a-d
Helichrysium italicum	Immortelle	19.1	C de	29.1	B c-e	33.3	A c-e
Ocimum basilium	Basil	70.4	B ab	78.4	AB ab	83.4	A ab
Piper nigrum	Black pepper	61.9	A a-c	67.1	A ab	70.6	A a-d
Prunus mahlep	Mahaleb	51.3	A a-d	55.9	A a-d	60.9	A a-d
Rhus coriaria	Sumach	37.0	A b-e	46.4	A bc	52.1	A bc
Syzygium aromaticum	Clove	69.1	B ab	78.4	A ab	82.0	A ab
Thumus vulgaris	Thyme	51.1	A a-d	58.5	A a-c	65.5	A a-d
Zingiber officinale	Ginger	50.9	A a-d	53.1	A a-d	54.6	A a-d
Control	D. W.	0.00	A f	0.00	A f	0.00	A f

* Data are given as the mean of 8 replicates. Data followed by the same letter are not significantly different at P ≤ 0.05. Shown with uppercase letters are comparable only within the rows, and the lowercase letters are only comparable for the values in the same column.

Table 3. Immobilization rate of spice aqueous extracts at three concentrations on J2s of *Meloidogyne arenaria*

Spices		Concentrations					
		0.5%		1%		2%	
Anethum graveolens	Dill	2.6	B cd*	2.5	B de	3.8	A cd
Capsium annium	Pepper	8.9	C a-d	16.9	B a-d	22.9	A a-c
Cuminum cyminum	Cumin	12.5	A ab	18.3	A a-d	22.9	A a-c
Coriandrum sativum	Coriander	16.0	C a	21.1	B ab	32.9	A ab
Curcuma longa	Turmeric	6.1	B b-d	8.9	A b-e	10.5	A cd
Helichrysium italicum	Immortelle	2.8	C cd	4.3	B c-e	9.1	A cd
Ocimum basilium	Basil	9.8	C a-c	17.6	B a-d	22.8	A a-c
Piper nigrum	Black pepper	12.0	B ab	15.5	B a-e	23.6	A a-c
Prunus mahlep	Mahaleb	4.9	B b-d	14.1	A a-e	19.6	A b-d
Rhus coriaria	Sumach	12.5	A ab	18.3	A ad	20.5	A a-c
Syzygium aromaticum	Clove	16.8	C a	27.9	B a	39.5	A a
Thumus vulgaris	Thyme	11.6	C a-c	20.1	AB a-c	23.6	A bc
Zingiber officinale	Ginger	3.5	BC b-d	10.6	AB b-e	16.4	A b-d
Control	D. W.	0.0	A e	0.0	A f	0.0	A e

* Data are given as the mean of 8 replicates. Data followed by the same letter are not significantly different at P ≤ 0.05. Shown with uppercase letters are comparable only within the rows, and the lowercase letters are only comparable for the values in the same column.

J2 mortality was more affected by all spices and concentrations than J2 immobility. Also, the extracts that were found to be effective in J2 immobility were effective in J2 mortality. The extracts with the greatest effect in each of the different concentrations of spice extracts were clove, coriander, thyme, cumin, and sumac (Table 4). Aside from these extracts, basil and hot pepper extracts showed statistically the same level of J2 mortality at 1 and 2% (P ≤ 0.05). J2 mortality increased with increasing concentration of spice extracts but was not statistically significant when the effects of each application at different concentrations

were considered. When the concentrations of the extracts of pepper (5.0-14.5%), coriander (10.5-19.4%), immortelle (0.37-4.25%), basil (5.3-14.6%), and clove (13.6-27.8%) were increased, the mortality rate increased significantly. Also, higher concentrations of dill, turmeric, black pepper, and mahaleb extracts resulted in a statistically significant mortality rate when compared to lower concentrations.

Table 4. Mortality rate of spice aqueous extracts at three concentrations on J2s of *Meloidogyne arenaria*

Spices		Concentrations					
		0.5%		1%		2%	
Anethum graveolens	Dill	0.8*	BC c	1.1	B de	2.1	A cd
Capsium annium	Pepper	5.0	C bc	11.0	B a-e	14.5	A a-d
Cuminum cyminum	Cumin	8.5	B ab	12.4	AB a-c	16.4	A a-c
Coriandrum sativum	Coriander	10.5	C ab	13.8	B a-c	19.4	A ab
Curcuma longa	Turmeric	2.8	B b	3.5	B b-e	5.9	A b-d
Helichrysium italicum	Immortelle	0.4	C c	2.4	B cd	4.3	A b-d
Ocimum basilium	Basil	5.3	C bc	10.5	B a-e	14.6	A a-d
Piper nigrum	Black pepper	6.1	B bc	8.9	B a-e	13.0	A a-d
Prunus mahlep	Mahaleb	3.0	BC bc	5.3	B b-e	10.3	A b-d
Rhus coriaria	Sumach	8.6	A ab	12.4	A a-c	15.0	A a-d
Syzygium aromaticum	Clove	13.6	C a	20.1	B a	27.8	A a
Thumus vulgaris	Thyme	8.5	BC ab	14.8	AB ab	19.0	A ab
Zingiber officinale	Ginger	2.9	BC bc	5.4	AB b-e	8.6	A bc
Control	D. W.	0.0	A c	0.0	A ef	0.0	A e

* Data are given as the mean of 8 replicates. Data followed by the same letter are not significantly different at $P \leq 0.05$. Shown with uppercase letters are comparable only within the rows, and the lowercase letters are only comparable for the values in the same column.

Pot experiments

Although the spice extract applications were not as effective as nematicides, they caused a significant decrease in galling index and egg count in tomato roots compared to the negative control ($P \leq 0.05$) (Table 5). No signs of phytotoxicity were observed on tomato plants during the growing season. Among the spice extracts, the lowest value of gall index was in the plants growing in the soils where basil and turmeric extracts were applied (1.12). In addition, the application of coriander and clove resulted in a significant decrease (2.0) in the gall index compared to the negative control (3.25). Plants treated with basil and turmeric extracts had the lowest number eggs in their roots, followed by clove, cumin and coriander. The R_f of the nematode was ranked similarly, and the plants with the least reproduction were those treated with basil, followed by turmeric and clove extracts. With the same statistical group, basil extract reduced *M. arenaria* reproduction by 84.0%, turmeric by 79.0%, and clove by 76.0%. Even coriander had the lowest R_f reduction, but it was still nearly 50% (49.6%). As a result, the R_f in all treated spice extracts is about half the R_f in the negative control (Figure 1). When compared to the controls, the application of the extracts resulted in a reduction in the R_f of 49.6 to 84.0%.

Table 5. Effect of the spice extracts on the gall index, eggs per root, and reproduction factor (R_f) of *Meloidogyne arenaria* on the roots of susceptible tomato plants in the greenhouse (25 ± 3°C)*

Spices	Gall index (0-5)[3]	Eggs x 10^3/root	R_f
Coriander (*Coriandrum sativum)*	2.0 bc	37.5 b	12.5 b
Cumin (*Cuminum cyminum*)	3.0 ab	23.1 c	7.7 c
Turmeric (*Curcuma longa*)	1.1 c	15.7 e	5.2 e
Basil (*Ocimium basilicum*)	1.1 c	11.9 f	4.0 f
Clove (*Syzygium aromaticum*)	2.0 bc	18.1 d	6.0 d
+ Control[1]	0.0 d	0.0 g	0.0 g
- Control[2]	3.3 a	74.4 a	24.8 a

***** The data are the averages of 8 replicates, and the values with the same letters in the column according to the Tukey test are not statistically different according to P ≤ 0.05.

[1] The positive control consisted of commercial nematicide with the active ingredient ethoprophos (200 g/l). [2]The negative control consisted of water without extracts. [3]0-5 gall scale: where 0 =no galling; 1 = trace infection with a few small galls; 2 =25% roots galled; 3 = 26 to 50%; 4 = 51 to 75%; and 5 = >75% roots galled (Hussey & Jenssen, 2002).

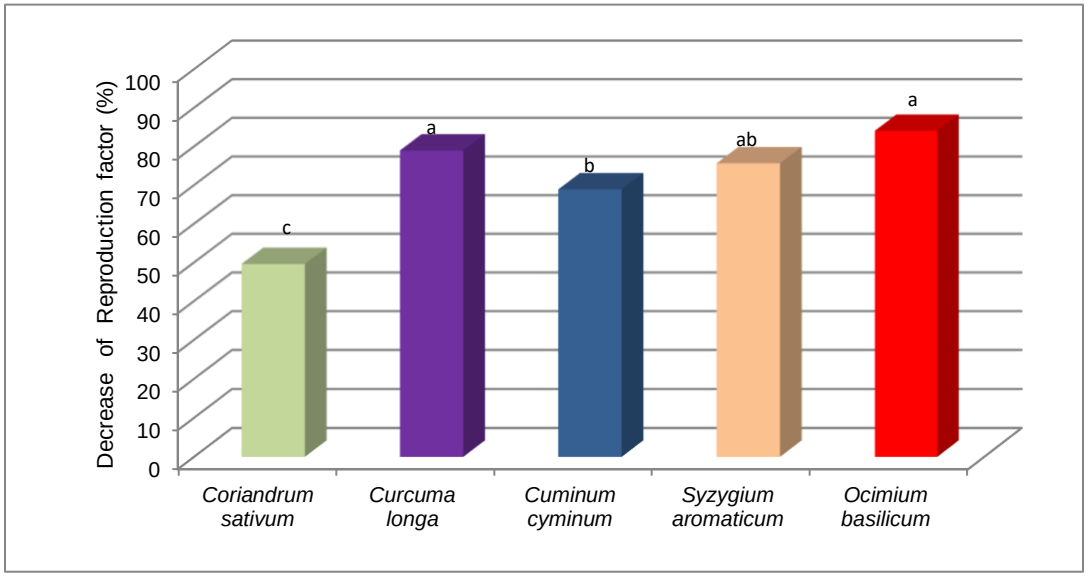

Figure 1. The effect of spice extracts on the reproduction factor of *Meloidogyne arenaria* in tomato plant roots.

Discussion

Spices are used as food additives, colorants, flavorings, and preservatives, as well as anthelmintic, antiseptic, antidiabetic and antipathogenic agents. The antimicrobial activity of spices was first described in 1880 and also nematicidal effects have been known (Rahman et al., 2011). In this study, the nematicidal potentials of 13 spice extracts were investigated in laboratory and pot experiments. Egg hatching tests are useful for screening nematicidal activity of extracts, because counting hatched juveniles is more accurate than counting juveniles in a particular J2 population (Oka et al., 2000). The highest inhibition rate of egg hatching was found to be 93.1% at a 2% concentration of pepper extract. When Abbas et al. (2009) investigated the effects of 50% and 100% aqueous concentrations of pepper spice extract on the hatching of *Meloidogyne javanica* (Treub, 1885) eggs, they found similar results. In the same study, the effects of cumin, coriander, turmeric, black pepper and ginger on egg hatching and larval mortality were investigated, and it was discovered that, contrary to the current study, the other spice extracts inhibited egg hatching more than black pepper. In our study, J2 mortality was also higher when these extracts applied. At all concentrations, black pepper extracts reduced egg hatching significantly (61, 67 and 70%, respectively).

Nile et al. (2017) found that black pepper extracts significantly suppressed galls in tomatoes and reduced RKN population in roots. Black pepper is a very important spice due to its valuable medicinal and aromatic properties. Piperamides, the primary component of *P. nigrum*, have a wide range of biological activities, including antimicrobial, antioxidant, and insecticidal properties (Scott et al., 2005). Özdemir (2014), in a similar study, investigated the effect of basil, black pepper, and ginger essential oils on J2 mortality of *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1919 at three different concentrations (1, 3 and 5%) under laboratory conditions and found that black pepper treatments had the highest toxic effect (82%, 86-91%) with the highest mortality rate as a result of laboratory experiments.

All aqueous spice extracts had a greater effect on egg hatching than J2 immobilization and J2 mortality. At a 2% concentration of clove extract, the highest J2 mortality was found to be 27.75%, making clove the most successful extract in terms of J2 mortality. Salgado & Campos (2003) investigated the effects of aqueous clove extracts on J2 mortality of *Meloidogyne exigua* Goeldi, 1887, and it was discovered that clove extract killed more than 50% of the J2s compared to the control. Meyer et al. (2007) demonstrated in microwell tests that clove oil reduced *M. incognita* egg hatch and J2 viability. Clove oil has also been shown to have nematicidal effects on plant-parasitic nematodes (Sangwan et al., 1990; Pandey & Dwivedi, 2000). Previous research on the effects of clove oil on nematodes, mostly on taxa other than RKN has been conducted. Clove oil was nematotoxic to J2s of *Anguina tritici* (Steinbuch, 1799) Chitwood, 1935 (Tylenchida: Anguinidae), *Tylenchulus semipenetrans* Cobb, 1913 (Tylenchida: Tylenchulidae), *M. javanica*, and *Heterodera cajani* Koshy, 1967 (Tylenchida: Heteroderidae) (Sangwan et al., 1990). A commercial standard of eugenol was also toxic to *M. incognita* J2s (Chatterjee et al., 1982). Meyer et al. (2007) also reported that the volatiles in 5% clove oil reduced nematode egg hatching by 30% and the viability of hatched J2s of *M. incognita* by up to 100%. El Badri et al. (2008) used clove extracts to kill the larvae of *Bursaphelenchus xylophilus* (Steiner & Bührer, 1934) Nickle, 1970 (Tylenchida: Parasitaphelenchidae). Clove oil extract has been shown to inhibit egg embryogenesis and to have complete nematicidal activity against J2s both free and in egg masses. In a separate study, extracts from clove were found more effective in killing *M. incognita*, with an effective concentration EC50 which was 5-10 times lower than the EC50 of the synthetic pesticides, chlorpyrifos, carbosulfan, and deltamethrin according to Taniwiryono et al. (2009). Among plant essential oils, eugenol, the main component of clove oil extracted from clove buds and basil leaves, was found to be active against pathogenic organisms including plant-parasitic nematodes (Pandey & Dwivedi, 2000; Park et al., 2005; Meyer et al., 2007; Huang & Lakshman, 2010). So, the application of clove buds as a plant pesticide for future use against nematodes is promising. Clove has a high nematicidal activity for future use against RKN (Taniwiryono et al., 2009). These characteristics make this product an intriguing tool for a novel nematode management strategy (Carlotti et al., 2011).

In our study, cumin extracts inhibited hatching in 39.5-59.3% of eggs, immobilized 12.5-22.9% of J2s, and killed 8.5-16.4% of J2s. The effects of essential oil and hydrosol isolated from cumin seeds on the mobility, hatching, and survival of J2s of *M. incognita* and *M. javanica* were studied by Pardavella et al. (2020). Lower hatching of RKN eggs was observed with an increasing concentration of extracts, which is consistent with the current study. In general, the nematicidal effect increased with increasing extract concentration in laboratory experiments.

In our pot experiments, the most effective extracts were basil and turmeric. The gall index of basil and turmeric applied to tomato was 1.1, and these extracts reduced the reproductive factor by 84.0 and 78.9%, respectively. Oka et al. (2000) found that when they studied the influence of essential oils from 27 spice and aromatic plants, basil extracts reduced egg hatching (68%) and the immobile J2 rate was 18%, which is similar to our findings. Basil extracts also reduced *M. arenaria* egg hatching by 70-83% and reduced immobilization by 9-23%, which agrees with Oka et al. (2000). In trials conducted by Douda et al. (2010), commercially available basil plant essences reduced the gall index of *M. hapla* in carrots (*Daucus carota* L.) (Apiales: Apiaceae). These results also confirmed the findings of the present study.

Turmeric also resulted in a significant reduction in the gall index (65.5%) and eggs per root of tomato plants in the pot experiments compared to the nematicide-treated control. The nematicidal activity of turmeric against RKN has been known for a long time (Pillai & Desai, 1978). Pandey et al. (2001), also stated that the extract of turmeric, a very well-known medicinal plant, had strong nematicidal and nematode hatching inhibitory activity against *M. incognita*. These findings supported the conclusions of the current study. Under in vitro conditions, Neeraj et al. (2020) used methanolic and hexane extracts of turmeric and discovered different levels of mortality of *M. incognita* at different concentrations. The percent mortality of J2s and the suppression of egg hatching, as well as our experimental results, were shown to be directly proportional to the concentration of the extracts and the time of exposure. Turmeric ethanolic extracts have been found to be more effective than all other plant extracts in increasing mortality and inhibiting egg hatching (Mioranza et al., 2016; Neeraj et al., 2017). Aqueous extract, fresh juice, and essential oil of turmeric have also been shown to have biopesticidal properties (Saju et al., 1998). Constituents of turmeric have been shown to be effective, in both in vitro and in vivo studies, against also various plant pathogens. According to Nair et al. (2015), turmeric suppressed the number of *M. hapla* in the roots of tomato cv. Rutgers while increasing the number of beneficial nematodes in the soil with minimal negative effects on plant health and growth, and the components of turmeric leaf macerates and extracts suppressed the ability of *M. hapla* to infect plant hosts without affecting plant growth. According to Babu et al. (2012), curcumin, the main component of turmeric, has a high nematicidal potential, with 92.5% inhibition of the activity of the enzyme glutathione S-transferase of *M. incognita*, an enzyme responsible for nematode survival in host plants. Mioranza et al. (2016) found that an aqueous extract of turmeric at four concentrations (1, 5, 10 and 15%) reduced *M. incognita* J2 mobility in an in vitro assay. Borges et al. (2013) investigated the toxicity of a 10% aqueous extract of turmeric against J2s of *M. incognita* and found that it was completely lethal. According to Ulfa et al. (2021), turmeric extract in various solvents significantly inhibited RKN egg hatching and root penetration but had no effect on RKN development or reproduction. Rashid et al. (2021) used turmeric against *M. incognita* and found that while maximum mortality was achieved up to 20%, root gall severity and final nematode population were significantly suppressed, which is consistent with our findings. It was discovered that the use of turmeric is crucial for RKN management.

Spice extracts have a nematicidal effect because of their ability to penetrate cell walls, which are characterized by high levels of certain oxygenated compounds (Knobloch et al., 1989). The mechanisms of action of spice extracts are also explained by the fact that they cause ADP phosphorylation, protein denaturation and degradation, enzyme inhibition, and interference with electron flow in the respiratory chain (Konstantopoulou et al., 1994). The ability of spice extracts to penetrate cell walls, which are characterized by a high content of certain oxygenated compounds, accounts for their nematicidal action (Knobloch et al., 1989). Clove contains eugenol and eugenol acetate compounds, cumin contains aldehyde, thymol, carvacrol, menthol, and menthone compounds, coriander contains carbohydrate and geranyl acetate compounds, black pepper contains capsaicin, phellandrene, dipentene, and sesquiterpene compounds, pepper contains capsaicin compounds, ginger contains sesquiterpenoid hydrocarbons, turmeric contains curcumin, thiamine, riboflavin, niacin and ascorbic acid. These compounds have been found to be effective against pests and diseases (Peter, 2001). There is a clear need for extract component fractionation to test each compound individually. However, it is possible to generalize that the nematicidal activity of each extract against nematodes follows a multisite mode of action. This is since each extract contains a large number of compounds, each with a distinct functional group and mode of action (Kesba et al., 2021). As a result, future research will focus on these natural active compounds isolated from plants as new compounds with nematicidal properties (Ferraz & De Freitas, 2004). Plant extracts may have a stronger nematicidal effect than synthetic nematicides (Kesba et al., 2021). In the future, all active and effective components of spices particularly basil, clove, and turmeric, could be isolated and analyzed for use as environmentally-friendly biopesticides against RKN.

References

Abad, P., B. Favery, M. N. Rosso & P. Castagnone-Sereno, 2003. Root-knot nematode parasitism and host response: molecular basis of a sophisticated interaction. Molecular Plant Pathology, 4 (4): 217-224.

Abad, P., J. Gouzy, J-M. Aury, P. Castagnone-Sereno, E. G. J. Danchin, E. Deleury, L. Perfus-Barbeoch, V. Anthouard, F. Artiguenave, V. C. Blok, M-C. Caillaud, P. M. Coutinho, C. Dasilva, F. De Luca, M. F. Deau, T. Esquibet, J. V. Flutre, N. Goldstone, T. HamamouchHewezi, O. Jaillon, C. Jubin, P. Leonetti, M. Magliano, T. R. Maier, G. V. Markov, P. McVeigh, G. Pesole, P., Poulain M. Robinson-Rechavi, E. Sallet, B. Se´gurens, D. Steinbach, T. Tytgat, E. Ugarte, C. V. Ghelder, P. Veronico, T. J. Baum, M. Blaxter, T. Bleve-Zacheo, E. L. Davis, J. J. J. Ewbank, B. Favery, E. Grenier, B. Henrissat, J. T. Jones, V. Laudet, A. G. Manule., H. Quesneville,, M-N Rosso, T. Schiex, G. Smant, J. Weissenbach & P. Wincker, 2008. Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. Nature Biotechnology, 26 (8): 909-915.

Abbas, S., S. Dawar, M. Tariq & M. J. Zaki, 2009. Nematicidal activity of spices against *Meloidogyne javanica* (Treub) Chitwood. Pakistan Journal of Botany, 41 (5): 2625-2632.

Andrés, M. F., A. González-Coloma, J. Sanz, J. Burillo & P. Sainz, 2012. Nematicidal activity of essential oils: a review. Phytochemistry Reviews, 11 (4): 371-390.

Aydınlı, G. & S. Mennan, 2014. Effect of some plant extracts on *Meloidogyne arenaria* Neal, 1889 (Tylenchida: Meloidogynidae) and tom**a**to. Turkish Journal of Entomology, 38 (3): 414-420.

Aydınlı, G. & S. Mennan, 2016. Identification of root-knot nematodes (*Meloidogyne* spp.) from greenhouses in the Middle Black Sea Region of Turkey. Turkish Journal of Zoology, 40 (5): 675-685.

Babu, R. O., D. Moorkoth, S. Azeez & S. J. Eapen, 2012.Virtual screening and in vitro assay of potential drug like inhibitors from spices against glutathione-S-transferase of *Meloidogyne incognita*. Bioinformation, 8 (7): 319-325.

Borges, F. G., O. J. Kuhn, A. G. Battistus, R. L. Estevez & S. Coltro, 2013.Toxidade de tratamentos alternativos e químicos in vitro sobre *Tubixaba tuxaua* e *Meloidogyne incognita*. Scientia Agraria Paranaensis, Marechal Cândido Rondon, 12 (S): 440-449 (in Spanish with abstract in English).

Carlotti, B., F. Paci, L. Ambrogioni, C. Benvenuti & P. F. Roversi, 2011. Effects of clove oil extract on egg and second stage juveniles of *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949. Redia, XCIV (1): 13-19.

Chatterjee, A., N. C. Sukul, S. Laskar & S. Ghoshmajumdar, 1982. Nematicidal principles from two species of Lamiaceae. Journal of Nematology, 14 (1): 118-120.

Coltro-Roncato, S., J. R. Stangarlin, A. C. Gonçalves Jr., O. J. Kuhn, E. D. V. Gonçalves, O. D. F. Dildey & É. L. de Moraes, 2018. Flores effects of cucurbitacin A on mobility of *Meloidogyne incognita* second-stage juveniles. Research on Crops, 19 (3): 504-508.

Curto, G., E. Dallavalle, R. Santi, N. Casadei, L. D'Avino & L. Lazzeri, 2015. The potential of *Crotalaria juncea* L. as a summer green manure crop in comparison to Brassicaceae catch crops for management of *Meloidogyne incognita* in the Mediterranean area. European Journal Plant Pathology, 142 (2): 829-841.

Devran, Z. & M. A. Söğüt, 2010. Occurrence of virulent root-knot nematode populations on tomatoes bearing the Mi gene in protected vegetable growing areas of Turkey. Phytoparasitica, 38 (3): 245-251.

Douda, O., M. Zouhar, J. Mazáková, E. Nováková & R. Pavela, 2010. Using plant essences as alternative mean for northern root-knot nematode (*Meloidogyne hapla*) management. Journal of Pest Science, 83 (1): 217-221.

Dutta, T. K., M. R. Khan & V. Phani, 2019. Plant-parasitic nematode management via biofumigation using brassica and non-brassica plants: current status and future prospects. Current Plant Biology, 17 (4): 17-32.

Ebone, L. A., M. Kovaleski & C. C. Deuner, 2019. Nematicides: history, mode, and mechanism action. Plant Science Today, 6 (2): 91-97.

El Badri, G. A. A., L. Dong-Woon, J. C. Park, H. B. Yu, H. Y. Choo & S. M. Lee, 2008. Nematocidal screening of essential oils and herbal extracts against *Bursaphelenchus xylophilus*. The Plant Pathology Journal, 24 (2): 178-182.

El-Nagdi Wafaa, M. A., M. M. A. Youssef & D. G. Mona, 2017. Nematicidal activity of certain medicinal plant residues in relation to controlling root knot nematode, *Meloidogyne incognita* on cowpea. Applied Science Reports, 20 (2): 35-38.

Esbenshade, P. R. & A. C. Triantaphyllou, 1985. Use of enzyme phenotypes for identification of *Meloidogyne* species. Journal of Nematology, 17 (1): 1- 6.

Ferraz, S. & L. G. De Freitas, 2004. "Use of Antagonistic Plants and Natural Products, 931-977". In: Nematology: Advances and Perspectives. Vol. 2. Nematode Management and Utilization (Eds. Z. X. Chen, S. Y. Chen & D. W. Dickson) CABI Publishing, Wallingford, UK, 1234 pp.

Ferris, H. & L. Zheng, 1999. Plant sources of Chinese herbal remedies: Effects on *Pratylenchus vulnus* and *Meloidogyne javanica*. Journal of Nematology, 31 (3): 241-263.

Hajihassani, A., W. B. Rutter, T. Schwarz, M. Woldemeskel, M. E. Ali & N. Hamidi, 2020. Characterization of resistance to major tropical root-knot nematodes (*Meloidogyne* spp.) in *Solanum sisymbriifolium*. Phytopathology, 110 (3): 666-673.

Hassan, M. E., F. Zahra & I. H. L. Mina, 2013. Anti-nematode effect assessment of *Peganumharmala* based-products against *Meloidogyne javanica* on melon. Journal of Biology, 3 (5): 5-10.

Huang, Q. & D. K. Lakshman, 2010. Effect of clove oil on plant pathogenic bacteria and bacterial wilt of tomato and geranium. Journal of Plant Pathology, 92 (3): 701-707.

Hussey, R. S. & R. K. Barker, 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Diseases Reports, 57 (12): 1025-1028.

Hussey, R. S. & G. J. W. Janssen, 2002. "Root-Knot Nematodes: *Meloidogyne* species 43-71" In: Plant Resistance to Parasitic Nematodes (Eds. J. L. Starr, R. Cook & J. Bridge) CAB International, Wallingford, UK, 252 pp.

Ibrahim, S. K., A. F. Traboulsi & S. El-Haj, 2006. Effect of essential oils and plant extracts on hatching, migration and mortality of *Meloidogyne incognita*. Phytopathologia Mediterranea, 45 (3): 238-246.

Javed, N., S. R. Gowen, M. Inam-ul-Haq & S. A. Anwar, 2007. Protective and curative effect of neem (*Azadirachta indica*) formulations on the development of root-knot nematode *Meloidogyne javanica* in roots of tomato plants. Crop Protection, 26 (4): 530-534.

Jones, J. T., A. Haegeman, E. G. Danchin, H. S. Gaur, J. Helder, M. G Jones & R. N Perry, 2013. Top 10 plant-parasitic nematodes in molecular plant pathology. Molecular Plant Pathology, 14 (9): 946-961.

Kesba, H., A. Abdel-Rahman & S. Sayed, 2021. Screening the nematicidal potential of indigenous medicinal plant extracts against *Meloidogyne incognita* under lab. and greenhouse conditions. Egyptian Journal of Biological Pest Control, 31 (81): 1-12.

Khan, F., A. Mohd, K. Amir, T. Moh, A. Taruba, S. Mohammad & A. M. Siddiqui, 2019. Evaluation of the nematicidal potential of some botanicals against root-knot nematode, *Meloidogyne incognita* infected carrot: In vitro and greenhouse study. Current Plant Biology, 20: 100115.

Kiewnick, S. & R. A. Sikora, 2006. Biological control of the root-knot nematode *Meloidogyne incognita* by *Paecilomyces lilacinus* strain 251. Biological Control, 38 (2): 179-187.

Knobloch, K., A. Pauli, B. Iberl, H. Weigand & N. Weis, 1989. Antibacterial and antifungal properties of essential oil components. Journal of Essential Oil Research, 1 (3): 119-128.

Koenning, S. R., C. Overstreet, J. W, Noling, P. A., Donald, J. O. Becker & B. A. Fortnum, 1999. Survey of crop losses in response to phytoparasitic nematodes in the United States for 1994. Nematology, 31 (4S): 587-618.

Konstantopoulou, I., L. Vassilopoulou, P. P. Mawogantisi & G. Scouras, 1994. Insecticidal effect of essential oils: A study of essential oils extracted from eleven Greek aromatic plants on *Drosophila auroria*. Experientia, 48: 616-619.

Meyer, S. L., F. Dilip, K. Lakshman, I. A. Zasada, B. T. Vinyard & D. J. Chitwood, 2007. Dose-response effects of clove oil from *Syzygium aromaticum* on the root-knot nematode *Meloidogyne incognita*. Pest Management Science, 64: 223-229.

Mioranza, T. M., M. A. Müller, A. M. Inagaki, F. Fuchs, S. Coltro-Roncato, J. R. Stangarlin & O. J. Kuhn, 2016. Potencial nematicida e nematostático do extrato de *Curcuma longa* sobre *Meloidogyne incognita*. Revista de Ciências Agroambientais, Alta Floresta, 14 (1): 104-109 (in Portuguese with abstract in English).

Nair, M. G., N. Seenivasan, Y. Liu, R. Feick & H. Melakeberhan, 2015. Leaf constituents of *Curcuma* spp. suppress *Meloidogyne hapla* and increase bacterial-feeding nematodes. Nematology, 17: 353-361.

Neeraj, N. K., A. Kumar, V. Kumar, M. Sindhu, M. S. Goel, S. R. & G. Singh, 2020. Bio-nematicidal effect of botanicals against root-knot nematode (*Meloidogyne incognita*) in tomato (*Solanum lycopersicum*). Indian Journal of Agricultural Sciences, 89 (12): 2096-2101.

Neeraj, S. G, A. Kumar, S. Ram & V. Kumar, 2017. Ethanolic extracts of medicinal plants to *Meloidogyne incognita* evaluation of nematicidal activity (Kofoid and White) Chitwood under lab conditions. International Journal of Pure Applied Bioscience, 5 (1): 827-831.

Nile, S. H., A. S. Nile, Y. S. Keum, V. Baskar & S. Ramalingam, 2017. In vitro and in planta nematicidal activity of black pepper (*Piper nigrum* L.) leaf extracts. Crop Protection, 100: 1-7.

Ntalli, N. G. & P. Caboni, 2012. Botanical nematicides: A review. Journal of Agricultural and Food Chemistry, 60 (40): 9929-9940.

Oka, Y., 2001. Nematicidal activity of essential oil components against the root-knot nematode *Meloidogyne javanica*. Nematology, 3 (2): 159-164.

Oka, Y., B. H. Ben-Daniel & Y. Cohen, 2006. Control of *Meloidogyne javanica* by formulations of *Inula viscosa* leaf extracts. Journal of Nematology, 38 (1): 46-51.

Oka, Y., S. Nacar, E., Putievsky, U. Ravid, Z. Yaniv & Y. Spiegel, 2000. Nematicidal activity of essential oils and their components against the root-knot nematode. Phytopathology, 90 (7): 710-715.

Oostenbrink, M., 1966. Major characteristics of the relation between nematodes and plants. Mededelingen Van Delandbouwhogeschool Te Wageningen, 66 (4): 1-46.

Özdemir, E., 2014. Bazı Bitkisel Uçucu Yağların Kök-ur Nematodu *Meloidogyne incognita* (Kofoid & White, 1919) (Nemata: Meloidogynidae) Üzerinde Etkinliğinin Belirlenmesi. Çanakkale Onsekiz Mart Üniversitesi Fen Bilimleri Enstitüsü Bitki Koruma Anabilim Dalı, (Basılmamış) Yüksek Lisans Tezi, Çanakkale, 61 s (in Turkish with abstract in English).

Padilla-Hurtado, B., Y. Morillo-Coronado, S. Tarapues, S. Burbano, M. Soto-Suárez, R. Urrea & N. Ceballos-Aguirre, 2022. Evaluation of root-knot nematodes (*Meloidogyne* spp.) population density for disease resistance screening of tomato germplasm carrying the gene Mi-1. Chilean Journal of Agricultural Research, 82 (1): 157-166.

Pandey, R. C. & B. K. Dwivedi, 2000. Comparative study of different plant extracts for their nematicidal potential. Current Nematology, 11 (1/2): 39-43.

Pandey, R., N. Pant, D. C. Jain & A. Kalra, 2001. Medicinal plant extracts as potent source of nematicidal activities. Nematology Mediterranea, 29 (1): 19-21.

Pardavella, I., D. Daferera, T. Tselios, P. Skiada & I. Giannakou, 2020. The use of essential oil and hydrosol extracted from *Cuminum cyminum* seeds for the control of *Meloidogyne incognita* and *Meloidogyne javanica*. Plants-Basel, 10 (1): 46 (1-14).

Park, I. K., J. Y. Park, K. H. Kim, K. S. Choi, I. H. Choi & C. S. Kim, 2005. Nematicidal activity of plant essential oils and components from garlic (*Allium sativum*) and cinnamon (*Cinnamomum verum*) oils against the pine wood nematode (*Bursaphelenchus xylophilus*). Nematology, 7 (5): 767-774.

Peter, K.V., 2001. Handbook of Herbs and Spices, Woodhead Publishing Limited, Cambridge, England, 322 pp.

Pillai, S. N. & M. V. Desai, 1978. Effect of turmeric isolated from *Curcuma longa* on root-knot nematode. Pesticides, 12: 49-50.

Rahman, S., A. K. Parvez, R. Islam & M. H. Khan, 2011. Antibacterial activity of natural spices on multiple drug resistant *Escherichia coli* isolated from drinking water. Bangladesh Annals of Clinical Microbiology and Antimicrobials, 10 (1): 1-10.

Rashid, U., A. Panhwar, A. Farhan, M. Akhtar, N. Jalbani & D. R. Hashmi, 2021. Nematicidal effects *Curcuma longa* of various fractions against *Meloidogyne incognita* (root knot nematodes). Turkish Journal of Agricultural Engineering Research, 2 (1): 175-182.

Roberts, P. A., 1995. Conceptual and practical aspects of variability in root-knot nematodes related to host plant resistance. Annual Review of Phytopathology, 33 (1): 199-221.

Saju, K. A., M. N. Venugopal & M. J. Mathew, 1998. Antifungal and insect repellant activities of essential oil of turmeric (*Curcuma longa* L.). Current Science India, 75 (7): 660-662.

Salgado, S. M. & V. P. Campos, 2003. Hatching and mortality of *Meloidogyne exigua* in extracts and in natural products. Fitopatologia Brasileira, 28 (2): 166-170.

Sangwan, N. K., B. S. Verma, K. K. Verma & K. S. Dhindsa, 1990. Nematicidal activity of some essential plant oils. Pesticide Science, 28 (3): 331-335.

Scott, I. M., N. Gagnon, L. Lesage, B. J. R. Philogène & J. T. Arnason, 2005. Efficacy of botanical insecticides from *Piper* spp. (Piperaceae) extracts for control of European chafer (Coleoptera: Scarabaeidae). Journal of Economic Entomology, 98 (3): 845-855.

Taniwiryono, D., H. Berg, J. A. G. Riksen, I. M. C. M. Rietjens, S. R. Djiwantia, J. E. Kammenga & A. J. Murk, 2009. Nematicidal activity of plant extracts against the root-knot nematode, *Meloidogyne incognita*. Open Natural Products Journal, 2 (1): 77-85.

Taylor, D. P. & C. Netscher, 1974. An improved technique for preparing perineal patterns of *Meloidogyne* spp. Nematologica, 20 (2): 268-269.

Trudgill, D. L. & V. C. Blok, 2001. Apomictic, polyphagous root-knot nematodes: exceptionally successful and damaging biotrophic root pathogens. Annual Review of Phytopathology, 39: 53-77.

Ulfa, M., T. Himawan & H. Tarno, 2021. Nematicidal activity of turmeric extract against nematodes *Meloidogyne* spp. Research Journal of Life Science, 8 (1): 48-56.

Xia, Y., Y. Qi., X. Yu, B. Wang, R. Cao & D. Jiang, 2019. Nematicidal effect against *Bursaphelenchus xylophilus* of harmine quaternary ammonium derivatives, inhibitory activity and molecular docking studies on acetylcholinesterase. European Journal of Plant Pathology, 153: 111-122.

Xiang, N., K. S. Lawrence & P. A. Donald, 2018. Biological control potential of plant growth-promoting rhizobacteria suppression of *Meloidogyne incognita* on cotton and *Heterodera glycines* on soybean. Annual Review Journal Journal of Phytopathology, 166 (7-8): 449-458.

Youssef, M. M. A., W. M. A. El-Nagdi & M. G. Dawood, 2015. Population density of root knot nematode, *Meloidogyne incognita* infecting eggplant as affected by medicinal plant aqueous extracts. Applied Science Reports, 10 (1): 8-11.

Zaidat, S. A. E., F. Mouhouche, D. Babaali, N. Abdessemed, M. de Cara & M. Hammache, 2020. Nematicidal activity of aqueous and organic extracts of local plants against *Meloidogyne incognita* (Kofoid and White) Chitwood in Algeria under laboratory and greenhouse conditions. Egyptian journal of Biological Pest Control, 30 (1): 46-52.

Zhou, L., G. Yuen, Y. Wang, L. Wei & G. Ji, 2016. Evaluation of bacterial biological control agents for control of root-knot nematode disease on tomato. Crop Protection, 84: 8-13.

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Original article (Orijinal araştırma)

Seasonal abundance and diversity of family Drosophilidae (Diptera) and records of some other dipterans in fruit orchards in Aydın Province (Türkiye)

Aydın İli (Türkiye) meyve bahçelerindeki Drosophilidae (Diptera) familyası türlerinin mevsimsel yoğunlukları ve tür çeşitliliği ve birlikte saptanan diğer Diptera türleri

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Abstract

The composition and seasonality of the populations of Drosophilidae (Diptera) species were evaluated, along with some other dipteran species, in three fruit orchards in Aydın Province. Bait traps with grape vinegar were used for collecting drosophilids from September 2018 to January 2020. The family Drosophilidae was represented by 11 species, and additionally, 10 other fly species from seven families were found in the same traps. The dominant drosophilid species was *Drosophila subobscura* Collin, 1936 among 1 964 individuals trapped in the three orchards, followed by *Drosophila immigrans* Sturtevant, 1921, *Drosophila melanogaster* Meigen, 1830, *Zaprionus tuberculatus* Malloch, 1932 and *Drosophila suzukii* Matsumura, 1931. The highest number of drosophilids were trapped in April 2019, 1 836 specimens in total. The population of drosophilids varied with season, with the first peak in April 2019 and the second in November-December in 2019. Drosophilids were trapped in low numbers during the summer months. As part of this study, *Aulacigaster falcata* Papp, 1997 (Diptera: Aulacigastridae) was recorded in Türkiye for the first time.

Keywords: *Aulacigaster falcata*, Drosophilidae, *Drosophila suzukii*, fruit orchards, seasonal abundance

Öz

Bu çalışmada Aydın İli'ndeki üç meyve bahçesinde Drosophilidae (Diptera) familyası türlerinin belirlenmesi ve bunların mevsimsel yoğunluklarının araştırılması amaçlanmıştır. Aynı zamanda çalışmada saptanan diğer diptera türleri de incelenmiştir. Çalışmalar Eylül 2018-Ocak 2020 tarihleri arasında içerisinde üzüm sirkesi bulunan besin cezbedici tuzaklar kullanılarak yürütülmüştür. Çalışma sonunda, tuzaklarda 11 Drosophilidae türü ve ayrıca yedi familyadan 10 farklı sinek türü belirlenmiştir. Drosophilidae türlerinden *Drosophila subobscura* Collin, 1936 toplam 1 964 birey olarak çalışma bahçelerinde belirlenmiş ve en çok yakalanan tür olmuştur. Bunu sayısal olarak *Drosophila immigrans* Sturtevant, 1921, *Drosophila melanogaster* Meigen, 1830, *Zaprionus tuberculatus* Malloch, 1932 ve *Drosophila suzukii* Matsumura, 1931 izlemiştir. Bahçelerde en çok drosophilid 1 836 birey ile Nisan (2019) ayında elde edilmiştir. Drosophilid türleri sayısal olarak birlikte dikkate alındığında, mevsimsel dalgalanmalar göstermiş olup, bunlardan ilk tepe noktası Nisan (2019) ayında ve ikincisi Kasım-Aralık (2019) aylarında ortaya çıkmıştır. Ancak, drosophilid türleri yaz ayları süresince oldukça düşük sayılarda tuzaklara yakalanmıştır. Çalışmada saptanan *Aulacigaster falcata* Papp, 1997 (Diptera: Aulacigastridae) Türkiye faunası için ilk kayıt niteliğindedir.

Anahtar sözcükler: *Aulacigaster falcata*, Drosophilidae, *Drosophila suzukii*, meyve bahçeleri, mevsimsel yoğunluk

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Introduction

Drosophilidae is a species-rich family of Diptera comprising more than 4 500 species (Bachli, 2020). These minute flies are distributed throughout the world in various climates and habitats in all biogeographic regions (Brake & Bachli, 2008). Drosophilids are also crucial organisms for their essential role in genomic studies (Schmitz et al., 2007).

The fauna of the Drosophilidae has been extensively studied in many countries (Watabe et al., 1993; Bachli, et al., 2005; Miller, 2015; Obona et al., 2019; Tidon et al., 2019; Yuzuki & Tidon, 2020). Many drosophilid species are strongly attracted to various volatile compounds produced from fermenting or decaying organic substrates (Atkinson, 1977). The majority of drosophilid species are saprophagous and known to be substantial consumers of decaying plant materials (Schmitz et al., 2007). Unlike other drosophilids, *Drosophila suzukii* Matsumura, 1931 females can deposit eggs into ripening fruit by inserting ovipositor through the fruit skin (Walsh et al., 2011). *Drosophila suzukii* is an invasive and destructive pest that originated from East-Asia (Rota-Stabelli et al., 2013). It has been reported as a crucial pest of berries and stone fruits in many countries of Asia, the Americas and Europe (Lee et al., 2011; Calabria et al. 2012; Depra et al., 2014; Kinjo et al., 2014). The biology, pest status, distribution and geographic expansion of the species and related biological control studies were reviewed by Asplen et al. (2015). *Drosophila suzukii* was found on strawberries in Erzurum Province as the first record in Türkiye in 2014 (Orhan et al., 2016). It has recently been reported in many agricultural areas of Türkiye. After the *D. suzukii* first appeared in Türkiye, numerous investigations were conducted on its pest status (Tozlu et al., 2018; Efil, 2018; Kasap & Özdamar, 2019; Zengin & Karaca, 2019; Agbaba et al., 2020; Kaçar, 2020; Özbek-Çatal et al., 2021).

Many drosophilid species have been reported in Türkiye (Şengün & Kocabay, 1967; Özar et al., 1985; Akşit et al., 2003; Gençer et al., 2005; Koçak & Kemal, 2013; Kocatepe, 2019; Zengin, 2020; Özbek-Çatal et al., 2021). However, the Drosophilidae fauna still needs to be investigated.

The study aimed to evaluate the occurrence and seasonal variation of the Drosophilidae species in fruit orchards and to determine the abundance of *D. suzukii,* the recently introduced invasive pest. Additionally, some other dipteran species captured in the traps were also determined.

Materials and Methods

The faunistic studies were undertaken from September 2018 to January 2020 to determine the Drosophilidae fauna in orchards in Aydın Province. Traps were placed in trees in three orchards to capture flies. Between September 2018 and April 2019, these traps replaced with new ones in irregular intervals and from April 2019 onwards they were replaced regularly once a week. Flies in the traps were counted and data obtained throughout the study were used to determine the fauna of the drosophilid species in the orchards and data obtained after April 2019 were used to evaluate seasonal abundance.

Three orchards were chosen for the study in Aydın Province: fig orchard (cv. Bursa Black) size of 2 ha, (37°75' N, 27°78' E), plum (Angelino) and quince of 1.5 ha (37°83' N, 27°77' E) and mixed fruit orchard of 2 ha comprising of apple, pear, quince, plum, grape and peach (37°76' N, 27°75' E) (Figure 1). Samplings for monitoring and faunistic studies were conducted with bait traps, wrapped with a red-sticky-plastic band as a color attractant material from bottom to mid of 500 ml transparent plastic bottle. They were perforated with eight holes (2 mm in diameter) placed in the upper quarter of the bottle as entry for drosophilids, and 100 ml of grape vinegar (Tariş^{TM}) was added into the traps as bait. In each orchard, plastic bottle traps were set up randomly in the orchards in the canopy of trees at 1.5-2.0 m above the ground on the southern side of the tree. One trap per tree was installed, and three traps were placed in each orchard and replaced with new traps weekly. Sampling materials were inspected under stereomicroscope, and Drosophilidae samples were separated and counted in the laboratory. They were deposited in Eppendorf tubes of 10 ml with 70% ethanol and stored in the fridge for the identification.

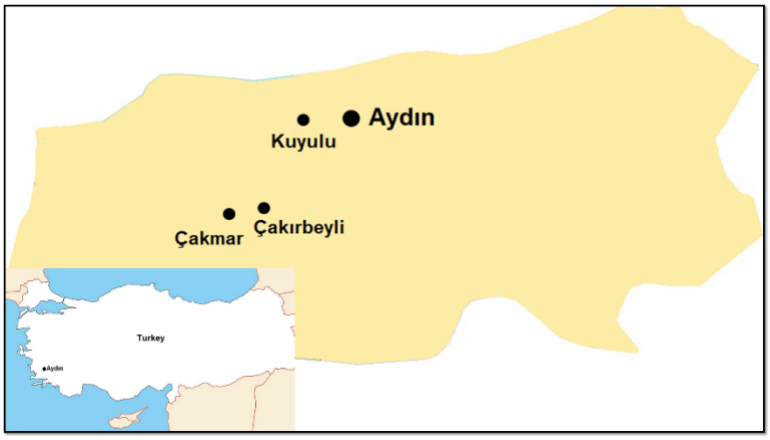

Figure 1. Position of the study area in Aydın Province.

Results and Discussion

Twenty-one species from the families Drosophilidae, Asteiidae, Aulacigastridae, Chloropidae, Ephydridae, Milichiidae, Odiniidae and Phoridae were determined. The family Drosophilidae represented by 11 species was also the most numerous (Table 1).

Table 1. Dipteran species recorded from three fruit orchards in Aydın Province

Family	Species
Drosophilidae	*Drosophila busckii* Coquillett, 1901
	Drosophila funebris (Fabricius, 1787)
	Drosophila hydei Sturtevant, 1921
	Drosophila immigrans Sturtevant, 1921
	Drosophila melanogaster Meigen, 1830
	Drosophila subobscura Collin, 1936
	Drosophila suzukii Matsumura, 1931
	Hirtodrosophila confusa (Staeger, 1844)
	Scaptodrosophila rufifrons (Loew, 1873)
	Scaptomyza sp. Hardy, 1849
	Zaprionus tuberculatus Malloch, 1932
Asteiidae	*Asteia amoena* Meigen, 1830
Aulacigastridae	*Aulacigaster falcata* Papp, 1997
Chloropidae	*Rhopalopterum femorale* (Collin, 1946)
	Chlorops sp. Meigen, 1803
Ephydridae	*Psilopa* sp. Fallen, 1823
Milichiidae	*Desmometopa microps* Lamb, 1914
	Desmometopa sp. Loew, 1866
	Milichiella lacteipennis (Loew, 1866)
Odiniidae	*Odinia meijerei* Collin, 1952
Phoridae	*Megaselia* sp. Rondani, 1856

Previously in Türkiye, Koçak & Kemal (2013) reported 36 drosophilid species from different geographical region of Türkiye and Zengin (2020) has recorded 21 688 drosophilid specimens from 13 species and seven genera in Uşak Province in Türkiye. Akşit et al. (2003) and Gençer et al. (2005) have revealed some drosophilid species in fig orchards, and Özbek-Çatal et al. (2021) identified 11 species of drosophilids in various fruit orchards in Eastern Mediterranean Region of Türkiye. The European fauna of Drosophilidae comprises more than 100 species (Bachli et al., 2013; Nartshuk, 2014; Maca et al., 2015). The Brazilian fauna of drosophilids

has been studied, and more than 300 species were recorded (Tidon et al., 2019). According the number of the species being considered, Drosophilidae fauna is still needed to be investigated in Türkiye.

In the present study, 4 217 drosophilid individuals were captured across the three orchards. The abundance of captured flies varied remarkably between months. The drosophilids were the most numerous in April (1,836 specimens representing 43.5% of the total), followed by May (616, 14.6%), November (470, 11.2%), December (466, 11.1%), January (213, 5.1%), and October (179, 4.2%). In August and September less number of drosophilids, only 38 and 81 specimens, respectively, were trapped (Table 2). In addition, the change in population of drosophilids varied seasonally, with the first peak in April 2019 (1,836 across the three orchards) followed by second peak in November and December (470 and 466, respectively) (Table 2).

Table 2. Abundance of the Drosophilidae species according to months in the examined fruit orchards

Species	Total numbers in all traps										
	Apr 19	May 19	Jun 19	Jul 19	Aug 19	Sep 19	Oct 19	Nov 19	Dec 19	Jan 20	Total
D. immigrans	394	204	7	0	2	0	0	58	128	20	813
D. subobscura	1 326	337	12	58	13	4	27	39	43	105	1 964
D. suzukii	13	13	75	29	14	3	32	39	49	9	276
D. melanogaster	72	10	34	51	8	31	20	98	119	49	492
D. busckii	29	2	6	0	0	0	0	12	23	11	83
H. confusa	1	4	1	0	0	0	0	0	15	5	26
Z. tuberculatus	0	0	0	0	1	43	100	222	88	6	460
Others	6	46	15	25	0	0	0	2	1	8	103
Total	1 836	616	150	163	38	81	179	470	466	213	4 217

Drosophila subobscura Collin, 1936 was the most common species with 1964 specimens (46.6%), followed by *Drosophila immigrans* Sturtevant, 1921 (808, 19.2%), *Drosophila melanogaster* Meigen, 1830 (492, 11.7%), *Zaprionus tuberculatus* Malloch, 1932 (460, 10.9%), and *D. suzukii* (276, 6.5%). Other drosophilid species were found in smaller number: *Drosophila busckii* Cocquillett, 1901 (83, 2.0%) and *Hirtodrosophila confusa* (Staeger, 1844) (26, 0.6%). The changes in numbers of drosophilids reflected the peaks of predominant species in the present study similarly to the changes described by Toda (1973).

The changes in monthly occurrence and abundance of the species varied between the three orchards. Some of the drosophilid species were not continuously present and disappeared after some months; *D. immigrans* in July, September and October; *D. busckii* in July, August, September and October; *H. confusa* in July, September, October and November; *Z. tuberculatus* in April, May, June and July were not trapped (Table 2). It seems that the period of their presence in orchards depended on food availability and climatic conditions. *Drosophila subobscura*, *D. melanogaster*, and *D. suzukii* were captured continuously in the traps over whole study period. *Drosophila subobscura* and *D. melanogaster* have been reported as fruit specialist species having the ability to colonize in rural are which domesticated fruit trees (Atkinson & Shorrocks, 1977) and *D. melanogaster* has been reported to be facultatively carnivorous (Yang, 2018), so generally does not face a shortage of food. Additionally, it has been reported that cold-hardening could enhance the ability of *D. melanogaster* to remain active at lower temperatures (Kelty & Lee, 2001). Of other species, *D. suzukii* is a pest of soft fruits. It can be expected that *D. suzukii* can maintain its population constantly because food was available in the orchards during the study period. However, *D. suzukii* had a lower population density than *D. subobscura* and *D. melanogaster*. The reason of this needs to be investigated in detail. *Drosophila suzukii* adults were captured throughout the year with spring and late autumn peaks in a coastal area in Greece, which is relatively close to our study region in Türkiye. However, only a single peak was observed in the mainland in autumn (Papanastasiou et al., 2020). In

Central Europe, large populations of *D. suzukii* were observed in September and October, but the species was almost absent before July, and it was suggested that the long-distance migration might be essential for it to re-establish following the high mortality in winter (Deutsch & Kiss, 2021).

During the present study, it was found that other common species, such as *D. immigrans* in July, September and October, and *Z. tuberculatus* in April, May, June and July, disappeared from orchards. Seasonal abundance observed among the drosophilid species was classified either unimodal or bimodal (Toda, 1973) according to sampling data of the species in this study. *Drosophila immigrans*, *D. subobscura* and *D. busckii* were bimodal with first peak in spring with second, lower peak in autumn. The other abundant species *Z. tuberculatus* was unimodal with a peak in late autumn (Table 2). These results could be a consequence of interspecific difference of microhabitat preference.

Drosophilids were captured in low numbers between June and September in the fruiting period (Figure 2). One of the possible reasons could be that the adverse effect of high temperatures in summer influenced on drosophilid populations. In this period, the daily mean temperatures were around 30°C, and the maximum temperatures during some days exceeded 40°C. At the same time, almost no precipitation was recorded, and the RH was only 40-50% (Figure 3). These conditions might have negative influence of food resources of certain drosophilids. Additionally, adverse effect of the summer temperature might stimulate the migration of drosophilids to cooler highlands to find more suitable conditions. Wakahama (1962) showed that the number of *Drosophila* species was more abundant in lower altitudes in spring and autumn, but it was higher at high altitudes in summer. Summer heat at low altitudes, and low winter temperatures at high altitudes may adversely affect the abundance of some drosophilids, so they migrate seasonally between lowland and highland areas (Kimura et al., 1977; Kimura & Beppu, 1993; Tait et al., 2018).

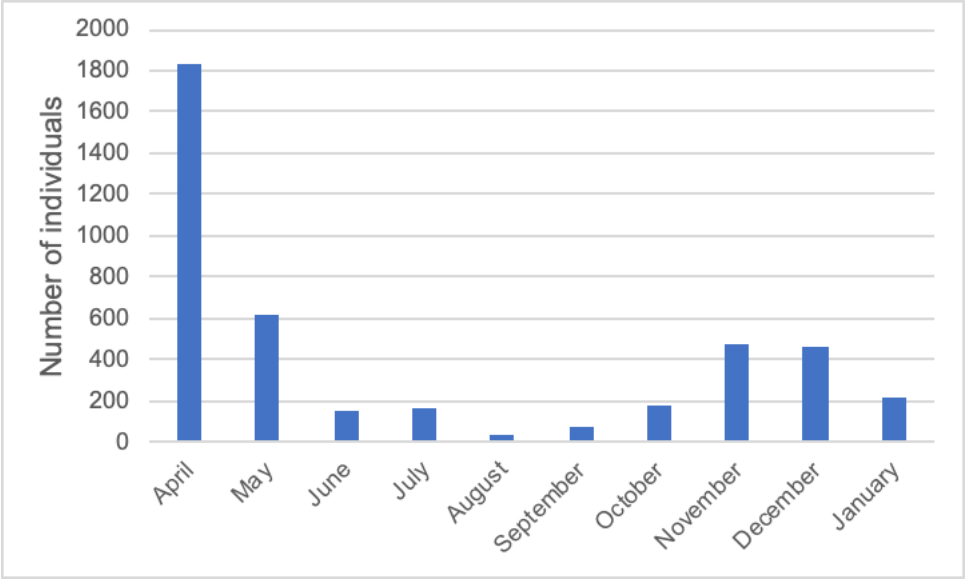

Figure 2. Seasonal changes of drosophilid numbers in three study orchards through April 2019 to January 2020.

The number of drosophilid species captured during the study period is presented in Table 2. The dominant species was *D. subobscura,* which was found in the traps in every month.

Previous studies have demonstrated interspecific co-existance of the larvae of drosophilid species (Heed, 1971; Atkinson, 1977; Atkinson & Shorrocks, 1977). However, different species of drosophilids can survive in the same habitats by sharing the same sources, which may be favorable for the one in the first stage and for another in a later time (Merrell, 1951).

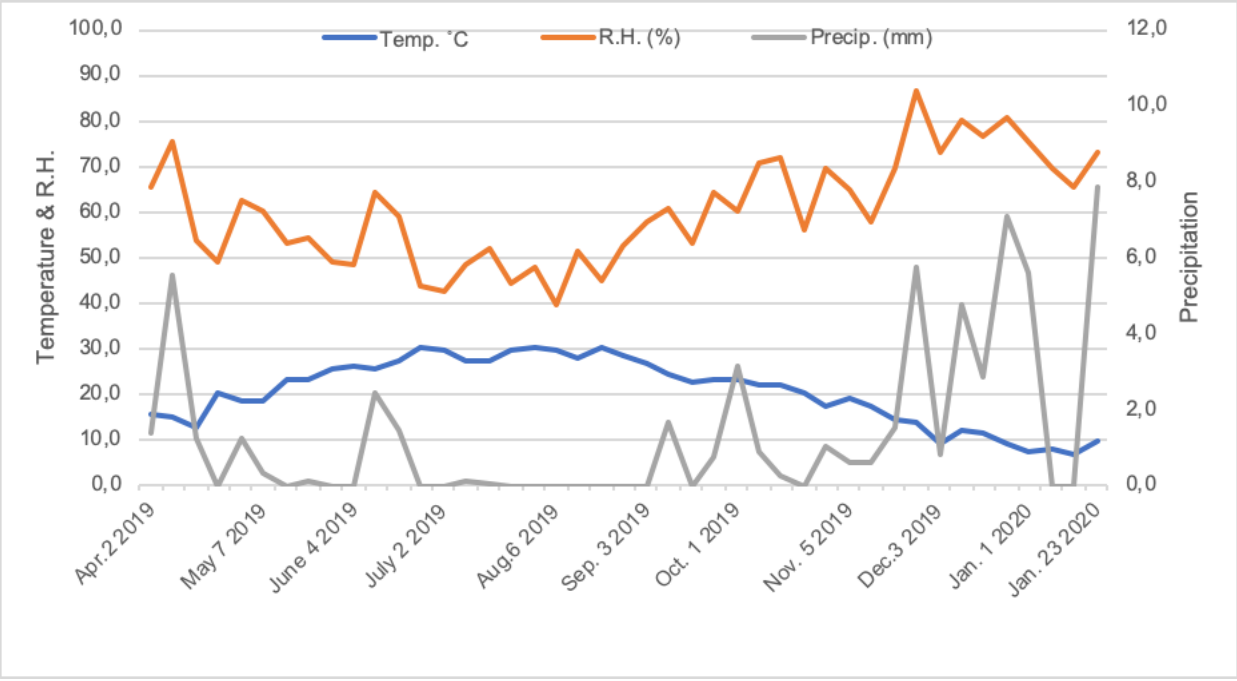

Figure 3. Weather conditions as daily mean values in Aydın Province during the study period.

Drosophilid assemblage abundance was the highest in the mixed orchard with 2,436 drosophilid individuals captured during study period (57.8%), followed by the plum+quince, and fig orchards with 1398 (33.1%) and 383 (9.1%), respectively (Tables 3 & 4). *Drosophila subobscura* was the most abundant in the three orchards, followed by *D. immigrans*, *D. melanogaster*, *D. tuberculatus* and *D. suzukii.* Other drosophilids, such as *D. busckii* and *H. confusa*, were captured in smaller numbers. However, the number of drosophilids were relatively low in the fig orchard compared to mixed and plum+quince orchards. The diversity of the fruit species in the orchards could be important for the abundance of drosophilids, as the availability of food and breeding sites increase with an increased range of fruit species. However, the impact of agricultural practice such as irrigation and fertilization might affect the circumstances of breeding sites, that is, the availability and duration of the favorable conditions for the drosophilids may differ in the orchards.

Asteia amoena Meigen, 1830 (Asteiidae), *Aulacigaster falcata* Papp, 1997 (Aulacigastridae), *Odinia meijerei* Collin, 1952 (Odiniidae), *Rhopalopterum femorale* (Collin, 1946) (Chloropidae), *Chlorops* sp. Meigen, 1803 (Chloropidae), *Psilopa* sp. Fallen, 1823 (Ephydridae), *Desmometopa microps* Lamb, 1914 (Milichiidae), *Megaselia* sp. Rondani, 1856 (Phoridae) were recorded (Table 1). *Aulacigaster falcata* was recorded for the first time in Türkiye.

Kahanpää (2014) has reported 18, 4 and 14 species from the families Asteiidae, Aulacigastridae and Odiniidae, respectively, in the checklist of the smaller families of Opomyzoidea. The family Chloropidae is distributed worldwide and may be found in different vegetation types (Karpa, 2001). The family Ephydridae was catalogued as having 1,747 species with their geographical distribution information (Mathis & Zatwarnicki, 1995). The family Milichiidae were reported as small and usually black flies (Sabrosky, 1973); many of them are commensal or kleptoparasitic relationships with predatory insects and mites (Sabrosky, 1973; Landau & Gaylor, 1987). Phoridae family is known to be inhabited in a wide range of habitats with described 4,000 species; many of them exploit decaying organic materials (Merritt et al., 2009). The species of these families recorded during the present study can be considered as common species with global distributions. Fruit orchards with decaying material and fruit can provide a favorable feeding source and habitat for many other dipterous insects.

Table 3. Occurrence of the Drosophilidae species trapped by month in the three fruit orchards

	Apr 19	May 19	Jun 19	Jul 19	Aug 19	Sep 19	Oct 19	Nov 19	Dec 19	Jan 20	Total
Mixed orchard (apple, pear, quince, plum, grape and peach)											
D. immigrans	227	50	4	0	2	0	0	56	108	19	466
D. subobscura	926	170	6	7	9	1	6	22	15	38	1 200
D. suzukii	12	10	57	16	12	2	3	28	41	2	183
D. melanogaster	14	3	17	12	2	13	7	65	94	19	246
D. busckii	1	0	0	0	0	0	0	12	22	8	43
H. confusa	0	2	0	0	0	0	0	0	2	2	6
Z. tuberculatus	0	0	0	0	0	0	0	158	72	5	235
Others	1	32	13	8	0	0	0	2	1	0	57
Total	1 181	267	97	43	25	16	16	343	355	93	2 436
Fig orchard											
D. immigrans	7	5	2	0	0	0	0	0	1	1	16
D. subobscura	55	9	1	41	1	0	0	17	10	42	176
D. suzukii	0	0	6	1	2	0	3	5	3	7	27
D. melanogaster	0	1	5	9	2	5	4	21	6	28	81
D. busckii	0	0	5	0	0	0	0	0	0	3	8
H. confusa	1	0	0	0	0	0	0	0	2	1	4
Z. tuberculatus	0	0	0	0	0	0	0	47	3	1	51
Others	0	2	2	8	0	0	0	0	0	8	20
Total	63	17	21	59	5	5	7	90	25	91	383
Plum + quince orchard											
D. immigrans	160	149	1	0	0	0	0	2	19	0	331
D. subobscura	345	158	5	10	3	3	21	0	18	25	588
D. suzukii	1	3	12	12	0	1	26	6	5	0	66
D. melanogaster	58	6	12	30	4	13	9	12	19	2	165
D. busckii	28	2	1	0	0	0	0	0	1	0	32
H. confusa	0	2	1	0	0	0	0	0	11	2	16
Z. tuberculatus	0	0	0	0	1	43	100	17	13	0	174
Others	5	12	0	9	0	0	0	0	0	0	26
Total	597	332	32	61	8	60	156	37	86	29	1 398

Table 4. Abundance of Drosophilidae species in the examined fruit orchards

Species	Total numbers captured in the all traps			
	Mixed plantation	Fig	Plum + Quince	Total
D. immigrans	466	16	331	813
D. subobscura	1 200	176	588	1964
D. suzukii	183	27	66	276
D. melanogaster	246	81	165	492
D. busckii	43	8	32	83
H. confusa	6	4	16	26
Z. tuberculatus	235	51	174	460
Others	57	20	26	103
Total	2 436	383	1 398	4 217

Conclusions

Fruit orchards provide favorable microhabitats for many Drosophilidae species. Thus, they can survive and establish high populations in the season. The changes in abundance and incidence of the species reflect interspecific differences in microhabitat preference.

The predominant species can reach high population numbers in human-modified habitats, like fruit orchards. The diversity of plants at the sampling sites is likely to provide make conditions more suitable for these species.

Drosophila subobscura, D. immigrans and *D. melanogaster* were the most abundant species in all sampled orchards; this supports the idea that these species are fruit specialist. Also, these species were determined as the most numerous in the mixed-orchards compared to the other two orchards. It is assumed that a mixture of fruit hosts contributes to the succession of the food availability for these drosophilids. In general, the numbers of the drosophilids trapped in early spring and late autumn could be indicate their abundance is dependent on the climatic conditions as well as the availability of food source.

The invasive pest species, *D. suzukii* was abundant in all orchards, and its population was maintained almost throughout the study period. There are many fruit orchards in the study area and they are located side by side, so breeding areas and food source are likely to be available year-round, providing of suitable habitat for *D. suzukii*. However, there were no complaints made by growers and no evidence of damage caused by *D.suzukii* in the study area, which is known actually as a serious pest on many economically important fruit species

However, there were no complaints made by growers and no evidence of damage caused by *D. suzukii*, which is known as a serious pest on many economically important fruit species in the study area. We conclude that *D. suzukii* can establish large populations at varying times depending on favorable conditions in different geographic areas. Our results showed that *D. suzukii* densities were low compared to other common drosophilids, such as *D. subobscura* and *D. melanogaster.* One possible reason for this might be that *D. suzukii* breed in other sites to reach higher population levels. However, this needs further investigation.

Earlier studies have already shown that the family Drosophilidae is particularly rich and comprises of thousands of species that are distributed worldwide in many different habitats. So, taking into consideration the richness of species, it is expected that there are other species still to be found.

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References

Agbaba, B., H. Tunaz & A. Erdoğan, 2020. The status of *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) in apricot and investigation of the possibilities of management strategies. Acta Horticulturae, 1290 (16): 89-94.

Akşit, T., F. Özsemerci & İ. Çakmak, 2003. Studies on determination of harmful fauna in fig orchards in Aydin province (Turkey). Turkish Journal of Entomology, 27 (3): 181-183 (in Turkish with abstract in English).

Asplen, M. K., G. Anfora, A. Biondi, D. S. Choi, D. Chu, K. M. Daane, P. Gibert, A. P. Gutierrez, K. A. Hoelmer, W. D. Hutchison, R. Isaacs, Z. L. Jiang, Z. Karpati, M. T. Kimura, M. Pascual, C. R. Philips, C. Plantamp, L. Ponti, G. Vetek, H. Vogt, V. M. Walton, Y. Yu, L. Zappala, & N. Desneux, 2015. Invasion biology of spotted wing *Drosophila* (*Drosophila suzukii*): a global perspective and future priorities. Journal of Pest Science, 88 (3): 469-494.

Atkinson, W., 1977. Ecological Studies of the Breeding Sites and Reproductive Strategies of Domestic Species of *Drosophila*. The University of Leeds Department of Pure and Applied Zoology, (Unpublished) PhD Thesis, Leeds, England, 130 pp.

Atkinson, W. & B. Shorrocks, 1977. Breeding site specificity in the domestic species of Drosophila. Oecologia, 29 (3): 223-232.

Bachli, G., 2020. TaxoDros: The database on taxonomy of Drosophilidae. (Web page: https://www.taxodros.uzh.ch) (Date accessed: December 2021).

Bachli G., C. Bystrowski & V. A. Richter, 2013. “Fauna Europaea: Drosophilidae”. In: Fauna Europaea: Diptera Brachycera. Fauna - Europaea version 2.6.2 (Eds. T. Pape & P. Beuk). (Web pages: https://www.fauna-eu.org) (Date accessed: March 2019).

Bachli, G., F. Viljoen, S. A. Escher & A. Saura, 2005. The Drosophilidae (Diptera) of Fennoscandia and Denmark. Series: Fauna Entomologica Scandinavica, 39. Brill NV, Leiden, The Netherlands, 362 pp.

Brake, I. & G. Bachli, 2008. Drosophilidae (Diptera). World Catalogue of Insects. Vol. 9. Apollo Books, Stenstrup, Denmark, 412 pp.

Calabria G., J. Máca, G. Bächli, L. Serra, & M. Pascual, 2012. First records of the potential pest species *Drosophila suzukii* (Diptera: Drosophilidae) in Europe. Journal of Applied Entomology, 136 (2012): 139-147.

Depra, M., J. L. Poppe, H. J. Schmidtz, D. D. Toni & V. L. S. Valente, 2014. The first records of the invasive pest *Drosophila suzukii* in the South American continent. Journal of Pest Science, 87 (3): 379-383.

Deutsch, F. & B. Kiss, 2021. "Seasonal abundance changes of spotted wing Drosophila in neighbouring habitats in Hungary. 1-6", The 1st International Electronic Conference on Entomology (1-15 July 2021, Web page: https://sciforum.net/event/IECE).

Efil, L., 2018. Çanakkale İli çilek alanlarında yeni bir zararlı *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae)'nin yayılış alanları ve bulaşıklığı. Türk Tarım ve Doğa Bilimleri Dergisi, 5 (3): 280-284 (in Turkish with abstract in English).

Gençer, N., K. Coşkuncu & N. Kumral, 2005. Determination of harmful and beneficial fauna in fig orchards in Bursa province. Journal of Agricultural Faculty of Ondokuz Mayıs University (Anadolu Journal of Agricultural Sciences), 20 (2): 24-30 (in Turkish with abstract in English).

Heed, W. B., 1971. Host plant specificity in Hawaiian *Drosophila*. Taxon, 20 (1): 115-121.

Kaçar, G., 2020. New records of the parasitoids of *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) in newly invaded areas in Turkey: molecular identification. Turkish Journal of Entomology, 44 (1): 71-79.

Kahanpää, J., 2014. Checklist of the smaller families of Opomyzoidea, Anthomyzidae, Asteiidae, Aulacigastridae, Clusiidae, Odiniidae, Opomyzidae and Periscelididae (Diptera) of Finland. ZooKeys, 441 (Special issue): 285-290.

Karpa, A., 2001. Revision of Chloropidae collection of B. A. Gimmerthal and a checklist of Latvian Chloropidae (Diptera). Latvijas Entomologs, 38: 44-49.

Kasap, İ. & E. Özdamar, 2019. Population development of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in vineyards of Çanakkale Province. Turkish Journal of Entomology, 43 (1): 57-62.

Kelty, J. D. & R. E. Lee, 2001. Rapid cold-hardening of *Drosophila melanogaster* (Diptera: Drosophiladae) during ecologically based thermoperiodic cycles. Journal of Experimental Biology, 204 (9): 1659-1666.

Kimura, M. T. & K. Beppu, 1993. Climatic adaptations in the *Drosophila immigrans* species group-seasonal migration and thermal tolerance. Ecological Entomology, 18 (2): 141-149.

Kimura, M. T., M. J. Toda, A. Beppu & H. Watabe, 1977. Breeding sites of drosophilid flies in and near Sapporo, northern Japan, with supplementary notes on adult feeding habits. Kontyu, 45 (4): 571-582.

Kinjo, H., Y. Kunumi & M. Nakai, 2014. Effects of temperature on the reproduction and development of *Drosophila suzukii* (Diptera: Drosophilidae). Applied Entomology and Zoology, 49 (2): 297-304.

Koçak, A. Ö. & M. Kemal, 2013. Diptera of Turkey, Priamus (Supplement 28). Centre for Entomological Studies Ankara, Ankara, 411 pp.

Kocatepe, O. 2019. Marmaris'te Çilekte Bulunan *Drosophila* Türleri, Popülasyon Yoğunlukları ve Zarar Oranlarının Belirlenmesi. Aydın Adnan Menderes Üniversitesi, Fen Bilimleri Enstitüsü, Bitki Koruma Anabilim Dalı, (Unpublished) Yüksek Lisans Tezi, Aydın, 63 pp (in Turkish with abstract in English).

Landau, G. D. & M. J. Gaylor, 1987. Observations on commensal Diptera (Milichiidae and Chloropidae) associated with spiders in Alabama. The Journal of Arachnology, 15 (2): 270-272.

Lee, J. C., D. J. Bruck, A. J. Dreves, C. Loriatti, H. Vogt & P. Baufeld, 2011. In Focus: Spotted wing drosophila, *Drosophila suzukii*, across perspectives. Pest Management Science, 67 (11): 1349-1351.

Maca, J., J. Rohacek, C. R. Vilela & M. Brezikova, 2015. New and interesting records of Drosophilidae (Diptera) from the Czech Republic and Slovakia. Acta Musei Silesiae Scientiae Naturales, 64 (2): 101-106.

Mathis, W. N. & T. Zatwarnicki, 1995. A world catalog of the shore flies (Diptera: Ephydridae). Memoirs on Entomology, International v. 4. Associated Publishers, Gainesville, Florida, 423 pp.

Merrell, D. J., 1951. Interspecific Competition between *Drosophila funebris* and *Drosophila melanogaster*. The American Naturalist, 85 (822): 159-169.

Merritt, R. W., G. W. Courtney & J. B. Keiper, 2009. Diptera: (Flies, Mosquitoes, Midges, Gnats). In: Encyclopedia of Insects (Eds: V. H. Resh & R. T. Carde). Academic Press, 1168 pp.

Miller, M., 2015. A review of the Drosophila (Diptera: Drosophilidae) Species of Northeastern North America. The University of Guelph, Guelph, Ontario, Canada, 443 pp.

Nartshuk, E. P., 2014. Fruit flies (Diptera: Drosophilidae) of the Russian Arctic. Zoosystematica Rossica, 23 (2): 256-263.

Obona, J., L. Dvorak, J. P. Haenni, L. Hrivniak, B. Japoshvili, J. Jezek & P. Manko, 2019. New and interesting records of Diptera from Azerbaijan and Georgia. Zoosystematica Rossica, 28 (2): 277-295.

Orhan, A., R. Aslantaş, B. Ş. Önder & G. Tozlu, 2016. First record of the invasive vinegar fly *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) from eastern Turkey. Turkish Journal of Zoology, 40 (2): 290-293.

Özar, A. İ., P. Önder, A. Sarıbay, T. Demir, S. Özkut, Y. Arınç, T. Azeri, M. Gündoğdu & H. Genç, 1985. Ege Bölgesi İncirlerinde Görülen Hastalık ve Zararlılarla Savaşım Olanaklarının Saptanması ve Geliştirilmesi Üzerinde Araştırmalar. Bornova Zirai Mücadele Araştırma Enstitüsü, İzmir. TÜBİTAK Proje No: TOAG-429 (Proje Nihai Raporu) 133 s (in Turkish).

Özbek-Çatal, B, A. F. Çalişkan Keçe & M. R. Ulusoy, 2021. Distribution and host plants of Drosophilidae (Diptera) species detected in fruit orchards of the Eastern Mediterranean Region of Turkey. Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi, 26 (2): 431-442 (in Turkish with abstract in English).

Papanastasiou, S. A., V. G. Rodovitis, E. P. Bataka, E. Verykouki & N. T. Papadopoulos, 2020. Population Dynamics of *Drosophila suzukii* in Coastal and Mainland Sweet Cherry Orchards of Greece. Insects, 11 (9): 621 (1-19).

Rota-Stabelli, O., M. Blaxter & G. Anfora, 2013. *Drosophila suzukii*. Current Biology, 23 (1): 8-9.

Sabrosky, C. W., 1973. Family Milichiidae. In: Papavero, N. (Ed.), A catalogue of the Diptera of the Americas South of the United States. Museu de Zoologia, Universidade de Sao Paulo, Sao Paulo, 12 pp.

Schmitz, H. J., V. L. S. Valente & P. R. P. Hofmann, 2007. Taxonomic survey of Drosophilidae from Mangrove Forest of Santa Catarina Island, Southern Brazil. Neotropical Entomology, 36 (1): 53-64.

Şengün, A. & M. Kocabay, 1967. İstanbul ve Civarı *Drosophila* Türleri. İstanbul Üniversitesi Yayınları, İstanbul, 71 pp (in Turkish).

Tait, G., A. Grassi, F. Pfab, C. M. Crava, D. T. Dalton, R. Margarey, L. Ometto, S. Vezzuli, M. V. Rossi-Stacconi, A. Gottardello, A. Pugliese, G. Firraro, V. M. Walton & G. Anfora, 2018. Large-scale spatial Dynamics of *Drosophila suzukii* in Trentino, Italy. Journal of Pest Science, 91 (4): 1214-1224.

Tidon, R., M. S. Gottschalk, H. J. Schmitz & M. B. Martins, 2019. Drosophilidae, Catálogo Taxonômico da Fauna do Brasil. (Web page: http://fauna.jbrj.gov.br/fauna/faunadobrasil/2) (Date accessed: February 2019).

Toda, M. J., 1973. Seasonal activity and microdistribution of drosophilid flies in Misumai in Sapporo. Journal of the Faculty of Science, Hokkaido University Series VI Zoology, 18 (4): 532-550.

Tozlu, E., N. Tekiner, G. Tozlu, R. Kotan & H. Öğütçü, 2018. Bacterial communities of *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) damaged in strawberry in Turkey. Universal Journal of Microbiology Research, 6 (2): 35-42.

Wakahama, K. I.,1962. Studies on the seasonal variation of population structures in *Drosophila,* II. The Effect of Altitude on Seasonal Activity of *Drosophila,* with a Note on the Monthly Numerical Variation of Species. Journal of the Faculty of Science, Hokkaido University Series VI Zoology, 15 (1): 65-73.

Walsh, D. B., M. P. Bolda, R. E. Goodhue, A. J. Dreves, J. Lee, D. J. Bruck, V. M. Walton, S. D. O'Neal & F. G. Zalom, 2011. *Drosophila suzukii* (Diptera: Drosophilidae): Invasive Pest of Ripening Soft Fruit Expanding its Geographic Range and Damage Potential. Journal of Integrated Pest Management, 2 (1): 1-7.

Watabe, H., M. J. Toda, G. C. Li, C. L. Duan, R. Imitty, B. Entomack & A. Muhtar, 1993. Drosophilid fauna (Diptera, Drosophilidae) of Chinese Central Asia. Japanese Journal of Entomology, 61 (3): 525-545.

Yang, D., 2018. Carnivory in the larvae of *Drosophila melanogaster* and other Drosophila species. Scientific Reports, 8 (1): 15484.

Yuzuki, K. & R. Tidon, 2020. Identification key for drosophilid species (Diptera, Drosophilidae) exotic to the Neotropical Region and occurring in Brazil. Revista Brasileira de Entomologia, 64 (1): 1-9.

Zengin, E., 2020. Occurrence of invasive species and seasonal dynamics of fruit flies (Diptera: Drosophilidae) species in Uşak province, Turkey. Revista de la Sociedad Entomológica Argentina, 79 (1): 21-30.

Zengin, E. & İ. Karaca, 2019. Dynamics of trapped adult populations of *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) and its parasitoids in Uşak Province, Turkey. Egyptian Journal of Biological Pest Control, 29 (43): 1-6.

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Original article (Orijinal araştırma)

Tachinid (Diptera: Tachinidae) fauna of Manisa Province of Türkiye with new records[1]

Manisa (Türkiye) İli'nin yeni kayıtlar ile Tachinid (Diptera: Tachinidae) faunası

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Abstract

This study was conducted between 2016-2020 to investigate the Tachinidae (Diptera) fauna of Manisa Province of Türkiye. For this purpose, Tachinidae samples were collected from the cultural and natural areas of five districts (Salihli, Sarıgöl, Selendi, Soma and Şehzadeler) selected to represent the province. Thirty-six species were determined and identified. These were four genera and five species in the subfamily Exoristinae, five genera and eight species in the subfamily Tachininae, three genera and four species in the subfamily Dexiinae, nine genera and 19 species in the subfamily Phasiinae. Among these, *Estheria cristata* (Meigen, 1826), *Cistogaster globosa* (Fabricius, 1775) and *Cylindromyia gemma* (Richter, 1972) (Diptera: Tachinidae) were recorded for the first time in Türkiye. The distributions and hosts in Türkiye of the identified species are given. In addition, the definitions of the species determined as new records for Türkiye are also included. This study is the first detailed study on the family Tachinidae in Manisa Province.

Keywords: Fauna, Manisa, new records, Tachinidae, Türkiye

Öz

Bu çalışma Manisa (Türkiye) İli'nin Tachinidae (Diptera) faunasını ortaya koymak için 2016-2020 yılları arasında gerçekleştirilmiştir. Bu amaçla ili temsil edecek şekilde seçilen 5 ilçenin (Salihli, Sarıgöl, Selendi, Soma ve Şehzadeler) kültür ve doğal alanlarından Tachinidae örnekleri toplanmıştır. Toplam 36 tür belirlenmiş ve tanımlanmıştır. Bunlar, Exoristinae alt familyasına ait 4 cins ve 5 tür, Tachininae alt familyasına ait 5 cins ve 8 tür, Dexiinae alt familyasına ait 3 cins ve 4 tür, Phasiinae alt familyasına ait 9 cins ve 19 türdür. Bunlar içerisinden *Estheria cristata* (Meigen, 1826), *Cistogaster globosa* (Fabricius, 1775) ve *Cylindromyia gemma* (Richter, 1972) (Diptera: Tachinidae) ülkemiz için yeni kayıt niteliğindedir. Tespit edilen türlerin konukçuları ve Türkiye'deki dağılışları hakkında bilgiler verilmiştir. Ayrıca ülkemiz için yeni kayıt olarak belirlenen türlerin tanımlarına da yer verilmiştir. Bu çalışma Tachinidae familyasına yönelik Manisa İli'nde yapılan ilk detaylı çalışmadır.

Anahtar sözcükler: Fauna, Manisa, yeni kayıtlar, Tachinidae, Türkiye

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Introduction

The Tachinidae are one of the largest families of the order Diptera, with around 8 600 known species globally and over 2 100 species in the Palearctic region (O'Hara et al., 2021). Türkiye has 341 species belonging to this family (Kara et al., 2020). All species identified in the family are parasitoids and most of their hosts are insect pests. Lepidopteran pests are common hosts. Others include members of the orders Coleoptera, Diptera, Hemiptera, Hymenoptera and Orthoptera. They provide important natural regulation of important insect pest populations (Grenier, 1988; Stireman et al., 2006; Tschorsnig, 2017). Kara & Tschorsnig (2003) and Tschorsnig (2017) provide detailed information on the Palearctic and Turkish hosts of tachinids, respectively.

Although there are some detailed studies conducted to reveal the species richness of the Tachinidae in Türkiye, the number of these studies is low given the size of the country (Doğanlar, 1975; Kara, 1998; Aksu, 2005; Korkmaz, 2007; Atay & Kara, 2014; Balkan et al., 2015; Lekin et al., 2016; Atay, 2017; Uysal & Atay, 2021). Nevertheless, Lutovinovas et al. (2018) published a list of 139 tachinid species from southern Türkiye, 52 of which are new records for the country.

Manisa Province has specific attributes in terms of soil and climate characteristics. The fact that the mountain ranges minimize the effect of the sea leads to the intermixing of Mediterranean and continental climate plant species. This increases insect and plant biodiversity. A detailed study of the Tachinidae has not been conducted in Manisa and only two species in the family have been reported (Kara, 2001a; Karsavuran & Kara, 2003). This paper reports an investigation of the Tachinidae fauna of Manisa Province, Türkiye.

Materials and Methods

Tachinid specimens were collected arbitrarily from a range of agricultural crops, weeds, forest trees and ornamental plants in districts of Manisa Province (Salihli, Sarıgöl, Selendi, Soma and Şehzadeler) from 2016 to 2020. Samples were collected an insect net and the latitude and altitude of the field was recorded by GPS. After collecting the flies were killed in ethyl acetate and taken to the laboratory, where they were processed according to museum standards.

The keys of Mesnil (1944-1965), Zimin (1966), Herting (1983), Tschorsnig & Herting (1994), Tschorsnig & Richter (1998) and Gilasian et al. (2013) were used to identify the tachinids. Nomenclature and arrangement of tachinids are based on Herting & Dely-Draskovits (1993). Confirmation of some species were made by Dr. Hans-Peter Tschorsnig (Staatliches Museum fur Naturkunde, Stuttgart, Germany). Images of the newly registered species were taken using a Leica M205 C stereoscopic microscope integrated with a Leica MC 170 digital camera and using the Leica Application Suite Software v4.13.0. The tachinid specimens are deposited at the Plant Protection Museum in Tokat Gaziosmanpaşa University, Agricultural Faculty, Tokat, Türkiye.

Results and Discussion

Thirty-six specimens were determined with three species being new to the Turkish fauna.

Subfamily: Exoristinae

Tribe: Eryciini

Erycia fasciata Villeneuve, 1924

Material examined. Selendi (Yıldız), 38°44'53" N, 28°53'06" E, 434 m, 22.05.2017, ♂.

Distribution in Türkiye. Ankara (Bayram & Kara, 1998), Eskişehir (Aksu, 2005), Kastamonu (Atay, 2017) and Muğla (Lutovinovas et al., 2018).

Hosts in Türkiye. *Melitaea didyma* (Esper, 1778) (Lepidoptera: Nymphalidae) (Bayram & Kara, 1998).

Tribe: Goniini

Pales processioneae Ratzeburg, 1840

Material examined. Şehzadeler (Ayvacık), 38°34'14" N, 27°26'59" E, 1421 m, 09.06.2019, ♀.

Distribution in Türkiye. Locality information is not provided (Herting & Dely-Draskowits, 1993), Isparta (Avcı & Kara, 2002), Kırklareli (Cerretti, 2005) and Çorum (Uysal, 2018; Uysal & Atay, 2021).

Hosts in Türkiye. *Lymantria dispar* (L., 1758) (Lepidoptera: Lymantriidae) (Dikyar, 1981) and *Thaumetopoea ispartaensis* Doğanlar & Avcı, 2001 (Lepidoptera: Notodontidae) (Kara & Tschorsnig, 2003).

Gonia bimaculata Widemann, 1819

Material examined. Şehzadeler (Ayvacık), 38°34'23" N, 27°26'55" E, 1415 m, 08.09.2017, ♂.

Distribution in Türkiye. Burdur (Tuatay et al., 1972), Balıkesir, Denizli, İzmir (Kavut et al., 1974), Ardahan, Erzurum, Kars (Doğanlar, 1982a), Şanlıurfa (Gözüaçık & Mart, 2009), Southeast Anatolia Region (Gözüaçık et al., 2009) and Muğla (Lutovinovas et al., 2018).

Host in Türkiye. *Agrotis ipsilon* (Hufnagel, 1766) (Lepidoptera: Noctuidae) (Kavut et al., 1974; Gözüaçık et al., 2009), *Agrotis* sp. (Tuatay et al., 1972; Gözüaçık et al., 2007) and *A. segetum* (Denis & Schiffermüller, 1775) (Gözüaçık & Mart, 2009).

Spallanzania hebes (Fallén, 1820)

Material examined. Selendi (Kurtuluş), 38°43'51" N, 28°51'21" E, 431 m, 12.07.2017, ♂; and 38°43'50" N, E 28°51'16", 427 m, 15.06.2019, ♂.

Distribution in Türkiye. Erzurum (Doğanlar, 1982a), Sakarya (Balkan, 2014; Balkan et al., 2015) and Burdur (Lutovinovas et al., 2018).

Hosts in Türkiye. *Agrotis* sp. (Lepidoptera: Noctuidae) (Tschorsnig, 2017).

Spallanzania griseiventris Herting, 1967

Material examined. Salihli (Allahdiyen), 38°24'24" N, 28°04'53" E, 1007 m, 01.08.2016, ♀; and Şehzadeler (Ayvacık), 38°34'23" N, 27°26'55" E, 1415 m, 08.09.2017, ♀.

Distribution in Türkiye. Eskişehir (Kara & Aksu, 2007).

Subfamily: Tachininae

Tribe: Tachinini

Tachina fera (L., 1761)

Material examined. Sarıgöl (Alemşahlı), N 38°06'56", E 28°40'08", 619m, 19.06.2017, ♂.

Distribution in Türkiye. Bingöl, Erzurum (Doğanlar, 1982b), Tokat (Kara, 1999b; Lekin, 2014; Lekin et al., 2016), Kastamonu (Korkmaz, 2007; Atay, 2017), Bolu, (Atay, 2017), Muğla (Lutovinovas et al., 2018) and Çorum (Uysal, 2018; Uysal & Atay, 2021).

Tachina magnicornis (Zetterstetd, 1844)

Material examined. Soma (Küçükgüney), 39°15'05" N, 27°38'11" E, 332 m, 21.06.2017, ♀.

Distribution in Türkiye. Erzurum (Doğanlar, 1975), Balıkesir (Kavut et al., 1974), Bingöl, Hakkari (Doğanlar, 1982b), Tokat (Kara, 1999a; Gürkan, 2010; Lekin, 2014; Lekin et al., 2016), Ankara (Kara & Özdemir, 2000), Bursa (Kaya & Kovancı, 2000), Kastamonu (Korkmaz, 2007; Atay, 2017), Sakarya (Balkan, 2014; Balkan et al., 2015), Bartın, Bolu (Atay, 2017) and Muğla (Lutovinovas et al., 2018).

Hosts in Türkiye. *Spodoptera exigua* (Hübner, 1808) (Steiner, 1937), *Malacosoma castrensis* (L., 1758) (Lepidoptera: Lasiocampidae) (Doğanlar, 1975), *Agrotis segetum* (Dennis & Schiffermüller, 1775) (Lepidoptera: Noctuidae) (Kavut et al., 1974; Gürkan, 2010), *Agrotis* sp. (Kara & Özdemir, 2000) and *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: Noctuidae) (Kaya & Kovancı, 2000).

Tachina danilewskyi (Portschinsky, 1882)

Material examined. Sarıgöl (Alemşahlı), 38°06'48" N, 28°40'05" E, 614 m, 24.06.2016, ♀; and 38°06'52" N, 28°40'05" E, 609 m, 08.09.2016, ♀.

Distribution in Türkiye. Bursa (Herting & Dely-Draskovits, 1993) and Eskişehir (Kara & Aksu, 2007).

Peleteria rubescens (Robineau-Desvoidy, 1830)

Material examined. Salihli (Allahdiyen), 38°24'28" N, 28°04'55" E, 1004 m, 04.07.2016, 2 ♀♀; 38°24'44" N, 28°05'05" E, 970 m, 08.08.2016, ♀; 38°24'46" N, 28°05'08" E, 936 m, 18.06.2017, 2 ♀♀; 38°24'24" N, 28°04'53" E, 1007 m, 05.09.2017, 2 ♀♀; 38°24'48" N, 28°05'02" E, 971 m, 08.06.2019, ♀; 38°24'54" N, 28°05'03" E, 937 m, 06.07.2019, ♀; Sarıgöl (Alemşahlı), 38°06'52" N, 28°40'05" E, 609 m, 08.09.2016, ♀; 38°06'37" N, 28°40'16" E, 623 m, 19.08.2017, 2 ♀♀; Selendi (Yıldız), 38°44'55" N, 28°53'12" E, 435 m, 12.06.2017, 2 ♀♀; Selendi (Kurtuluş), 38°43'47" N, 28°49'54" E, 413 m, 11.08.2017, ♀; 38°43'47" N, 28°51'15" E, 431 m, 10.09.2017, 2 ♀♀; Soma (Küçükgüney), 39°15'01" N, 27°38'10" E, 324 m, 10.09.2016, 3 ♀♀; 39°15'03" N, 27°38'10" E, 328 m, 27.05.2017, ♀; Soma (Zafer), 39°12'19" N, 27°41'13" E, 328 m, 22.07.2017, ♀; Soma (Yırca), 39°12'15", 27°41'10", 335 m, 11.08.2017, 4 ♀♀; 39°12'12" N, 27°41'08" E, 333 m, 15.07.2019, ♀; Şehzadeler (Ayvacık), 38°34'16" N, 27°26'58" E, 1416 m, 13.09.2016, ♀; 38°33'31" N, 27°26'56" E, 1255 m, 22.07.2016, ♀; 38°33'42" N, 27°26'51" E, 1290 m, 10.06.2017, ♀ and 38°33'28" N, 27°26'52" E, 1244 m, 25.08.2017, 2 ♀♀.

Distribution in Türkiye. Erzurum (Doğanlar, 1975), Tokat (Kara, 1999a; Lekin et al., 2016), Ankara (Khan & Özer, 1984; Kansu et al., 1986; Kara & Özdemir, 2000), Zonguldak (Korkmaz, 2007), Sakarya (Balkan, 2014; Balkan et al., 2015) and Çorum (Uysal, 2018; Uysal & Atay, 2021).

Hosts in Türkiye. *Malacosoma castrensis* (L., 1758) (Lepidoptera: Lasiocampidae) (Doğanlar, 1975) and *Agrotis* sp. (Lepidoptera: Noctuidae) (Khan & Özer, 1984; Kansu et al., 1986; Kara & Özdemir, 2000).

Peleteria iavana (Wiedemann, 1819)

Material examined. Sarıgöl (Alemşahlı), 38°06'53" N, 28°40'09" E, 617 m, 19.08.2018, ♀.

Distribution in Türkiye. Amasya (Kara, 2001b), Tokat (Lekin, 2014; Lekin et al., 2016) and Çorum (Uysal, 2018; Uysal & Atay, 2021).

Tribe: Macquartiini

Macquartia chalconota (Meigen, 1824)

Material examined. Şehzadeler (Ayvacık), 38°33'23" N, 27°26'44" E, 1242 m, 10.07.2017, ♀.

Distribution in Türkiye. Amasya (Kara, 2001b), Kayseri (Sahebari et al., 2013), Tokat (Lekin, 2014; Lekin et al., 2016) and Kastamonu (Atay, 2017).

Tribe: Siphonini

Siphona pauciseta Rondani, 1865

Material examined. Sarıgöl (Afşar), 38°13'54" N, 28°38'19" E, 488 m, 10.10.2020, ♀.

Distribution in Türkiye. Aydın, Muğla (Lutovinovas et al., 2018).

Tribe: Leskiini

Bithia immaculata (Herting, 1971)

Material examined. Şehzadeler (Ayvacık), 38°33'21" N, 27°26'40" E, 1243 m, 13.09.2016, ♀.

Distribution in Türkiye. Erzurum (Doğanlar, 1982b), Tokat (Kara, 1999a) and Zonguldak (Korkmaz, 2007).

Subfamily: Dexiinae

Tribe: Dexiini

Estheria cristata (Meigen, 1826)

Material examined. Selendi (Kurtuluş), 38°43'51" N, 28°51'21" E, 431 m, 12.07.2017, ♀.

Distribution in Türkiye. Recorded for the first time from Türkiye.

Identification. Parafacial with five short hair-like setae just below the first frontal seta. There are four bristles on the humeral callus, the strongest three of which are arranged more or less in a straight line. Three dorsocentral hairs are located behind the suture on the thorax. The petiole of the R5 vein is shorter than the r-m vein and at most 0.13 times as long as postangular section of M. The scutellum is reddish. Lower calypter has long hairs only at the base, and the remaining marginal hairs are shorter or at most as long as the marginal hairs of the upper calypter (Figure 1).

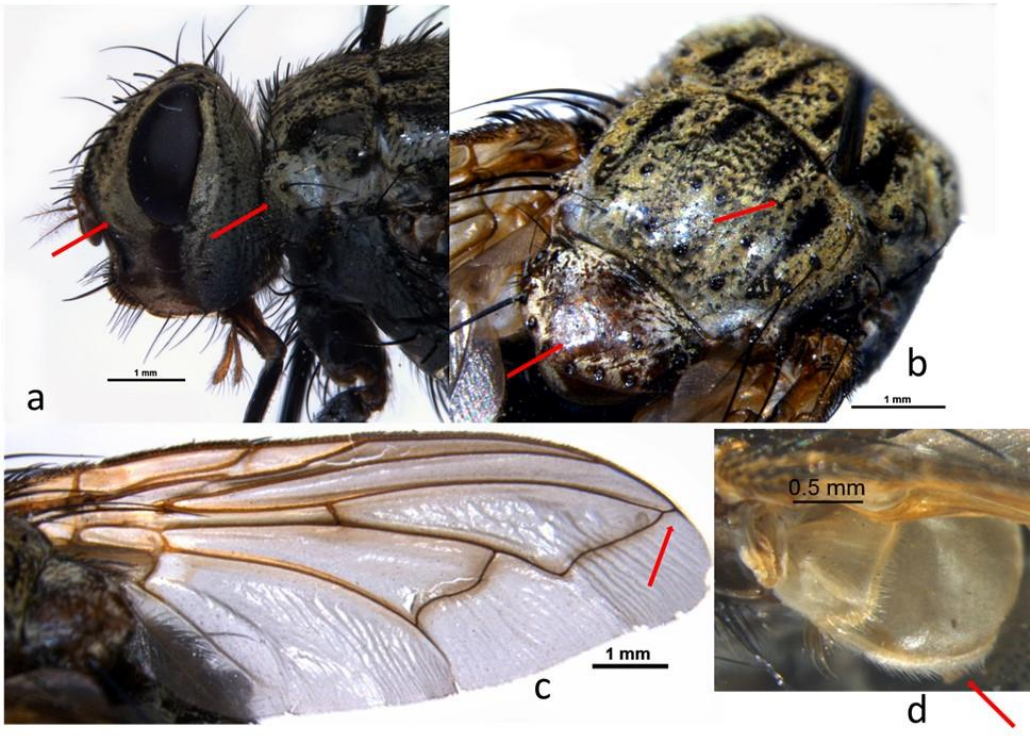

Figure 1. *Estheria cristata* ♀, a) head (lateral view), b) thorax (dorsal view), c) wing, and d) calypter.

Zeuxia cinerea Meigen, 1826

Material examined. Soma (Zafer), 39°12'19" N, 27°41'13" E, 328 m, 22.07.2017, ♂; Şehzadeler (Ayvacık), 38°33'23" N, 27°26'52" E, 1243 m, 19.05.2019, ♂; and N38°33'27" N, 27°26'48" E, 1243 m, 12.08.2019, ♀.

Distribution in Türkiye. Tokat (Kara, 1998; 1999b; Lekin, 2014; Lekin et al., 2016), Erzurum (Richter et al., 2002), Eskişehir (Kara & Aksu, 2007), Kastamonu (Korkmaz, 2007; Atay, 2017), Muğla (Lutovinovas et al., 2018) and Iğdır (Gültekin et al., 2020).

Hosts in Türkiye. *Larinus aeruginosus* (Hochhuth, 1851), *L. jaceae* (Fabricius, 1775), *Larinus* sp., *Rhinocyllus conicus* (Frölich, 1792) (Coleoptera: Curculionidae) (Richter et al., 2002), *Temnorhinus hololeucus* (Pallas, 1781) and *Maximus strabus* (Gyllenhal, 1834) (Coleoptera: Curculionidae) (Gültekin et al., 2020).

Zeuxia tricolor (Portschinsky, 1881)

Material examined. Salihli (Allahdiyen), 38°24'24" N, 28°04'53" E, 1007 m, 01.06.2019, 2 ♂♂; Sarıgöl (Alemşahlı), 38°06'41" N, 28°40'18" E, 634 m, 04.08.2016, 2 ♂♂; 38°06'37" N, 28°40'16" E, 623 m, 019.08.2017, ♂; Selendi (Yıldız), 38°44'51" N, 28°53'05" E, 434 m, 15.08.2016, ♂; 38°44'55" N, 28°53'12" E, 435 m, 12.06.2017, ♂; Selendi (Kurtuluş), 38°43'47" N, 28°49'54" E, 413 m, 11.08.2017, ♂; Selendi (Karabeyler), 38°45'06" N, 28°54'17" E, 489 m, 14.08.2019, ♀; Soma (Beyce), N 39°15'25", E 27°37'18", 326 m, 28.08.2016, 3 ♂♂; 39°15'30" N, 27°37'21" E, 331 m, 14.09.2017, 3 ♂♂, ♀; Şehzadeler (Ayvacık), 38°33'31" N, 27°26'56" E, 1255 m, 22.06.2016, 2 ♂♂, 2 ♀♀; and 38°33'31" N, 27°26'56" E, 1255 m 22.08.2016 , 2 ♂♂, ♀.

Distribution in Türkiye. Konya (Herting, 1984), Tokat (Kara, 1999b; Lekin, 2014; Lekin et al., 2016) Amasya (Kara, 2001b) and Eskişehir (Kara & Aksu, 2007).

Tribe: Voriini

Voria ruralis (Fallén, 1810)

Material examined. Salihli (Allahdiyen), 38°24'51" N, 28°05'05" E, 942 m, 18.08.2017, ♀.

Distribution in Türkiye. İzmir (Kavut et al., 1974), Erzurum (Avcı & Özbek, 1990), Tokat (Kara, 1999b), Adana (Anay, 2000), Niğde (Kara & Özdemir, 2000), Amasya (Kara, 2001b), Karabük (Korkmaz, 2007), Hatay (Kaya & Kornoşor, 2008), Tokat (Lekin, 2014; Lekin et al., 2016), Çorum (Uysal, 2018; Uysal & Atay, 2021), Aydın and Muğla (Lutovinovas et al., 2018).

Hosts in Türkiye. *Spodoptera exigua* (Hübner, 1808) (Lepidoptera: Noctuidae) (Steiner, 1937), *Autographa gamma* (L., 1758) (Lepidoptera: Noctuidae) (Kavut et al., 1974; Avcı & Özbek, 1990; Anay, 2000; Kara & Özdemir, 2000), *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: Noctuidae) (Anay, 2000), and Plusiinae (Lepidoptera: Noctuidae) species (Kaya & Kornoşor, 2008).

Subfamily: Phasiinae

Tribe: Phasiini

Clytiomya dupuisi Kugler, 1971

Material examined. Salihli (Allahdiyen), 38°24'59" N, 28°05'12" E, 869 m, 18.05.2019, ♂; Sarıgöl (Alemşahlı), 38°06'48" N, 28°40'05" E, 614 m, 12.05.2019, ♂; and Selendi (Kurtuluş), 38°43'50" N, 28°51'16" E, 427 m, 15.06.2019, ♂.

Distribution in Türkiye. Tokat (Kara, 1998; Kara & Alaoğlu, 1999), Aydın, İzmir (Karsavuran & Kara, 2003), Burdur and Muğla (Lutovinovas et al., 2018).

Hosts in Türkiye. *Ancyrosoma leucogrammes* (Gmelin, 1790) (Hemiptera: Pentatomidae) (Karsavuran & Kara, 2003).

Clytiomya sola (Rondani, 1861)

Material examined. Salihli (Allahdiyen), 38°24'59" N, 28°5'12" E, 869 m, 18.05.2019, ♂; Selendi (Kurtuluş), 38°43'45" N, 28°50'54" E, 425 m, 11.05.2019, ♂; and Soma (Yırca), 39°12'12" N, 27°41'08" E, 333 m, 15.07.2019, ♂.

Distribution in Türkiye. Konya (Tuatay et al., 1972), Manisa, İzmir (Karsavuran & Kara, 2003), Çorum (Uysal, 2018; Uysal & Atay, 2021) and Muğla (Lutovinovas et al., 2018).

Hosts in Türkiye. *Ancyrosoma leucogrammes* (Gmelin, 1790) (Hemiptera: Pentatomidae) (Karsavuran & Kara, 2003), *Carpocoris* sp. (Hemiptera: Pentatomidae) (Tuatay et al., 1972) and *Graphosoma lineatum* (L., 1758) (Hemiptera: Pentatomidae) (Kara & Tschorsnig, 2003).

Ectophasia crassipennis (Fabricius, 1794)

Material examined. Salihli (Allahdiyen), 38°26'04" N, 28°05'34" E, 721 m, 18.05.2017, ♀; 38°26'04" N, 28°05'34" E, 721 m, 18.08.2017, 2 ♂♂; 38°26'09" N, 28°05'36" E, 723 m, 18.08.2018, ♂; 38°24'48" N, 28°05'02" E, 971 m, 08.06.2019, 2 ♂♂; 38°24'54" N, 28°05'03" E, 937 m, 06.07.2019, ♂; 38°26'48" N, 28°06'36" E, 320 m, 11.08.2019, ♂, ♀; 38°26'42" N, 28°06'34" E, 354 m, 07.09.2019, ♂; 38°26'47" N, 28°06'34" E, 331 m, 14.09.2016, ♀; Sarıgöl (Alemşahlı), 38°06'59" N, 28°40'07" E, 611 m, 04.07.2016, ♀; Sarıgöl (Afşar), 38°13'40" N, 28°38'24 E, 299 m, 13.07.2019, 3 ♂♂, 2 ♀♀; Selendi (Yıldız), 38°44'53" N, 28°53'06" E, 434 m, 22.05.2017, ♀; 38°44'55" N, 28°53'12" E, 435 m, 12.06.2017, ♂; Selendi (Kurtuluş), 38°43'51" N, 28°51'21" E, 431 m, 12.07.2017, ♂, 2 ♀♀; Selendi (Eskicami), 38°44'45" N, 28°53'01" E, 435 m, 13.07.2019, ♂, ♀; Selendi (Karabeyler), 38°45'05" N, 28°54'13" E, 486 m, 13.08.2019, ♀; 38°45'06" N, 28°54'17" E, 489 m, 14.08.2019, ♀; Şehzadeler (Ayvacık), 38°33'28" N, 27°26'52" E, 1244 m, 20.05.2017, ♀; 38°33'27" N, 27°26'48" E, 1243 m, 12.08.2019, ♀; 38°33'21" N, 27°26'56" E, 1239 m, 08.09.2019, 2 ♂♂; Soma (Küçükgüney), 39°15'03" N, 27°38'10" E, 328 m, 27.05.2017, ♀; 39°15'05" N, 27°38'11" E, 332 m, 21.06.2017, 2 ♂♂; Soma (Beyce), 39°15'28" N, 27°37'23" E, 316 m, 27.06.2017, ♂; Soma (Zafer), 39°12'19" N, 27°41'13" E, 328 m, 22.07.2017, ♀; Soma (Beyce), 39°15'30" N, 27°37'21" E, 331 m, 14.09.2017, ♀; and Soma (Heciz), 39°15'35" N, 27°37'22" E, 343 m, 15.08.2019, ♀.

Distribution in Türkiye. Kilis (Zwölfer, 1932); South and Southeast Anatolia Region (Yüksel, 1968), Diyarbakır (Lodos, 1953, 1961; Duman & Sertkaya, 2015, 2016), Adana (Şimşek et al., 1994), Tokat (Atay, 2011; Atay & Kara, 2014; Lekin, 2014; Lekin et al., 2016), Şanlıurfa (Duman et al., 2015), Bartın, Karabük, Kastamonu (Atay, 2017), Çorum (Uysal, 2018; Uysal & Atay, 2021) and Muğla (Lutovinovas et al., 2018).

Hosts in Türkiye. *Eurygaster integriceps* Puton, 1881 (Hemiptera: Scutelleridae), (Zwölfer, 1932; Lodos, 1953, 1961, 1986; Şimşek et al., 1994; Duman & Sertkaya, 2015, 2016; Duman et al., 2015), *Eurydema ornata* (L., 1758), *Carpocoris pudicus* (Poda, 1761) (Hemiptera: Pentatomidae) and *Coreus marginatus* (L., 1758) (Hemiptera: Coreidae) (Atay & Kara, 2014).

Ectophasia oblonga (Robineau-Desvoidy, 1830)

Material examined. Sarıgöl (Alemşahlı), 38°06'59" N, 28°40'07" E, 611 m, 04.07.2016, ♂; and 38°06’37" N, 28°40’16" E, 623 m, 19.08.2017, ♂.

Distribution in Türkiye. Diyarbakır (Dupuis, 1963), Adana (Herting & Tschorsnig, 1993), Ankara (Memişoğlu & Özer, 1994), Tekirdağ (Öncüer & Kıvan, 1995; Kıvan, 1996), Tokat (Kara, 1998; Atay, 2011; Atay & Kara, 2014; Lekin, 2014; Lekin et al., 2016), Gaziantep, Kahramanmaraş, Kilis (İslamoğlu & Kornoşor, 2003, 2007), Eskişehir (Aksu, 2005), Bartın, Karabük, Kastamonu, Zonguldak (Korkmaz, 2007), Adıyaman, Batman, Diyarbakır, Mardin, Siirt, Şanlıurfa (Gözüaçık et al., 2010), Kastamonu (Atay, 2017), Çorum (Uysal, 2018; Uysal & Atay, 2021), Burdur and Muğla (Lutovinovas et al., 2018).

Hosts in Türkiye. *Eurygaster integriceps* Puton, 1881 (Hemiptera: Scutelleridae), (Dupuis, 1963; Yüksel, 1968; Öncüer & Kıvan, 1995; Kıvan, 1996; İslamoğlu & Kornoşor, 2003, 2007; Gözüaçık et al., 2010; Herting & Tschorsnig, 1993), *Eurygaster maura* (L., 1758) (Memişoğlu & Özer, 1994), *Lygaeus equestris* (L., 1758) (Hemiptera: Lygaedidae) (Kara & Alaoğlu, 1999), *Aelia* sp., *Dolycoris baccarum* (L., 1758) (Kara & Tschorsnig, 2003) and *E. ornata* (Kara & Tschorsnig, 2003; Atay & Kara, 2014).

Ectophasia leucoptera (Rondani, 1865)

Material examined. Sarıgöl (Alemşahlı), N 38°06'41", E 28°40'18", 634 m, 04.08.2016, ♀.

Distribution in Türkiye. Locality information is not provided (Herting & Dely-Draskovits, 1993).

Gymnosoma clavata (Rohdendorf, 1947)

Material examined. Salihli (Allahdiyen), 38°24'24" N, 28°04'53" E, 1007 m, 01.06.2016, ♂; 38°24'44" N, 28°05'05" E, 970 m, 08.08.2016, ♂; 38°26'04" N, 28°05'34" E, 721 m, 18.05.2017, ♂; 38°26'48" N, 28°06'36" E, 320 m, 11.08.2019, ♂; Sarıgöl (Alemşahlı), 38°06'42" N, 28°40'10" E, 620 m, 25.05.2017, ♂; 38°06'50" N, 28°40'03" E, 611 m, 12.09.2017, ♀; 38°06'48" N, 28°40'05" E, 614 m, 12.05.2019, ♂; Selendi (Yıldız), 38°44'51" N, 28°53'05" E, 434 m, 22.05.2017, ♂, ♀; Selendi (Karabeyler), 38°45'06" N, 28°54'17" E, 489 m, 14.08.2019, ♂; Şehzadeler (Ayvacık), 38°33'31" N, 27°26'56" E, 1255 m, 22.06.2016, ♀; 38°33'28" N, 27°26'52" E, 1244 m, 20.05.2017, ♂; Soma (Küçükgüney), 39°15'03" N, 27°38'10" E, 328 m, 27.05.2017, ♂; and Soma (Yırca), 39°12'12" N, 27°41'08" E, 333 m, 15.07.2019, ♀.

Distribution in Türkiye. Erzurum (Doğanlar 1982b), İzmir (Karsavuran, 1986; Karsavuran & Kara, 2003; Herting & Tschorsnig, 1993), Tokat (Kara, 1998; Atay, 2011; Atay & Kara, 2014; Lekin, 2014; Lekin et al., 2016), Eskişehir (Aksu, 2005), Antalya, Burdur (Keçeci et al., 2007), Karabük (Korkmaz, 2007; Atay, 2017), Kastamonu (Atay, 2017), Sakarya (Balkan, 2014; Balkan et al., 2015), Çorum (Uysal, 2018; Uysal & Atay, 2021) and Muğla (Lutovinovas et al., 2018).

Hosts in Türkiye. *Dolycoris baccarum* (L., 1758) (Karsavuran, 1986; Herting & Tschorsnig, 1993; Kara & Tschorsnig, 2003; Keçeci et al., 2007), *Carpocoris* sp. (Herting & Tschorsnig, 1993), *Ancyrosoma leucogrammes* (Gmelin, 1790) (Karsavuran & Kara, 2003) and *Carpocoris fuscispinus* (Boheman, 1850) (Hemiptera: Pentatomidae) (Atay, 2011; Atay & Kara, 2014).

Gymnosoma rotundata (L., 1758)

Material examined. Salihli (Allahdiyen), 38°26'04" N, 28°05'34" E, 721 m, 18.05.2017, ♀, Sarıgöl (Alemşahlı), 38°06'50" N, 28°40'03" E, 611 m, 12.09.2017, ♂; Soma (Heciz), 39°15'34" N, 27°37'21" E, 343 m, 22.06.2019, ♂; and Soma (Beyce) 39°15'30" N, 27°37'21" E, 331 m, 14.09.2017, ♀.

Distribution in Türkiye. Eastern Black Sea Region (Kurt, 1975), Tokat (Kara, 1998; Lekin, 2014; Lekin et al., 2016), Karabük, Kastamonu, Zonguldak (Korkmaz, 2007; Atay, 2017), Sakarya (Balkan, 2014; Balkan et al., 2015) and Çorum (Uysal, 2018; Atay & Uysal, 2021).

Hosts in Türkiye. *Aelia rostrata* Boheman, 1852 (Dikyar, 1981) and *Palomena prasina* (L., 1761) (Hemiptera: Pentatomidae) (Kurt, 1975).

Cistogaster globosa (Fabricius, 1775)

Material examined. Şehzadeler (Ayvacık), 38°33'28" N, 27°26'52" E, 1244 m, 25.08.2017, ♀.

Distribution in Türkiye. Recorded for the first time from Türkiye.

Identification. The antennae are brownish-black and half the length of the face. The length of the third antennal segment is about the same as its width. The cerci are triangular in shape. The light pruinescence part on the humeral callus protrudes a quadrate macula into the prescutum. The shiny black part on the parafrontal area starts at the back of the head and ends in a straight-cut form before reaching the anterior frontal line. The scutellum and abdomen are completely black and the ventral side of the abdomen is yellowish. Body length is 4 mm (Figure 2).

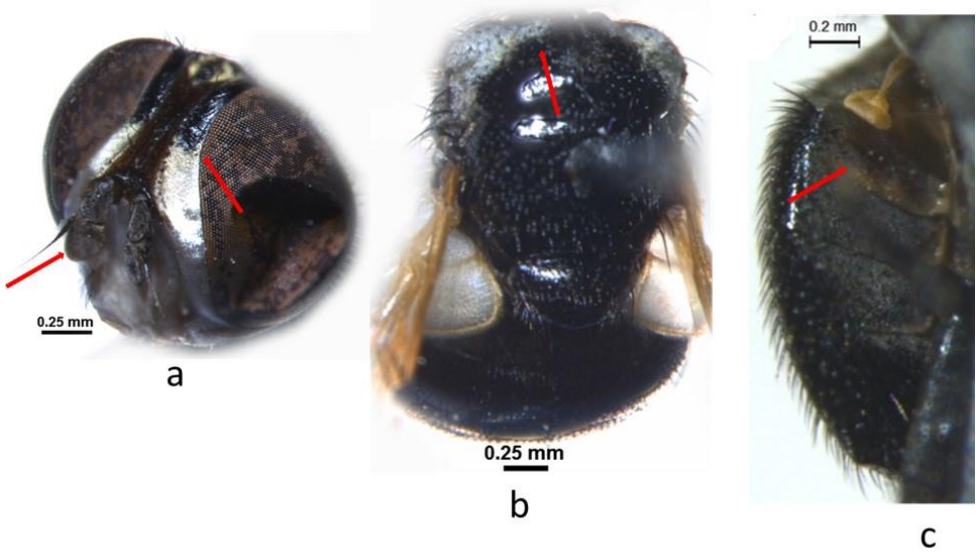

Figure 2. *Cistogaster globosa* ♀, a) head (lateral and dorsal view), b) thorax and abdomen (dorsal view), and c) abdomen (ventral view).

Phasia obesa (Fabricius, 1798)

Material examined. Şehzadeler (Ayvacık), 38°33'21" N, 27°26'40" E, 1243 m, 13.09.2016, ♂.

Distribution in Türkiye. Tokat (Kara, 1998; Kara & Alaoğlu, 1999; Lekin, 2014; Lekin et al., 2016), Bolu, Kastamonu (Atay, 2017) and Muğla (Lutovinovas et al., 2018).

Tribe: Leucostomatini

Leucostoma abbreviatum Herting, 1971

Material examined. Salihli (Allahdiyen), 38°26'42" N, 28°06'34" E, 354 m, 07.09.2019, ♀.

Distribution in Türkiye. Tokat (Kara, 1998; Kara & Alaoğlu, 1999), Kastamonu (Korkmaz, 2007), and Sakarya (Balkan, 2014; Balkan et al., 2015).

Leucostoma anthracinum (Meigen, 1824)

Material examined. Salihli (Allahdiyen), 38°24'54" N, 28°05'03" E, 937 m, 06.07.2019, ♂.

Distribution in Türkiye. Tokat (Kara, 1998), Kastamonu, Karabük (Atay, 2017) and Muğla (Lutovinovas et al., 2018).

Clairvilla biguttata (Meigen, 1824)

Material examined. Salihli (Allahdiyen), 38°26'04" N, 28°05'34" E, 721 m, 18.05.2017, ♀.

Distribution in Türkiye. Tokat (Kara, 1998; Kara & Alaoğlu, 1999), Eskişehir (Aksu, 2005) and Muğla (Lutovinovas et al., 2018).

Labigastera nitidula (Meigen, 1824)

Material examined. Salihli (Allahdiyen), 38°26'04" N, 28°05'34" E, 721 m, 18.05.2017, ♂; Sarıgöl (Afşar), 38°13'47" N, 28°38'17" E, 287 m, 25.05.2017, ♂; and Şehzadeler (Ayvacık), 38°33'28" E, 27°26'52" E, 1244 m, 20.05.2017, ♂.

Distribution in Türkiye. Tokat (Kara, 2002).

Tribe: Cylindromyiini

Cylindromyia gemma (Richter, 1972)

Material examined. Sarıgöl (Afşar), 38°13'56" N, 28°38'14" E, 264 m, 24.06.2016, ♂; Sarıgöl (Alemşahlı), 38°06'41" N, 28°40'18" E, 634 m, 04.08.2016, ♂; 38°06'52" N, 28°40'05" E, 609 m, 08.09.2016, ♂; Selendi (Yıldız), 38°44'51" N, 28°53'05" E, 434 m, 15.08.2016, 2 ♂♂; and Selendi (Karabeyler), 38°45'07" N, 28°54'19" E, 492 m, 12.09.2016, ♂.

Distribution in Türkiye. Recorded for the first time from Türkiye.

Identification. Antenna is as long as the face, postpedicel is 5.0−5.7 times as long as its width. There are 8-10 pairs of black setae on the posterodorsal part of the head. The vibrissa is 0.35-0.4 times the length of the face. Frontal vitta black. Palpus 3−5 times as long as their width and easily visible. The hairs on the edge of the lower calypter at most as long as width of its thickened margin. Proepisternum bare. Posteroventral setae is absent on the hind tibia. The middle tibia has two anterodorsal setae. Scutellum black. Apical scutellar setae are 0.5 times the length of the subapical scutellar setae, posterior supra-alar seta present. The wing is widely infuscated, and the basicosta is black. Tergite 3 has a median longitudinal black stripe on its dorsal part. Syntergite 1+2 has median submarginal setae. Male sternite 5 is as in Figure 3.

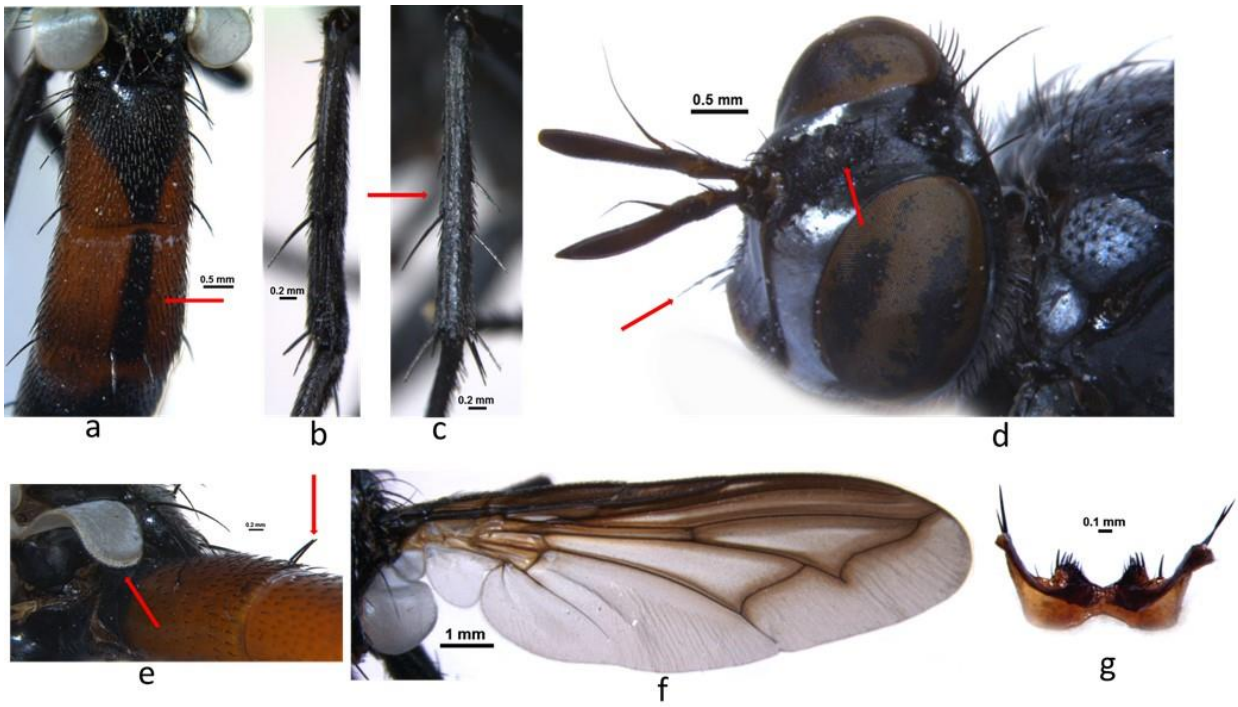

Figure 3. *Cylindromyia gemma* ♂, a) abdomen (dorsal view), b) hind tibia c) middle tibia, d) head (lateral view), e) syntergite 1+2, f) wing, and g) sternit 5.

Cylindromyia bicolor (Oliver, 1812)

Material examined. Selendi (Karabeyler), 38°45'07" N, 28°54'19" E, 492 m, 12.09.2016, ♂; 38°45'08" N, 28°54'19" E, 492 m, 10.09.2017, ♂; and Şehzadeler (Ayvacık), 38°34'14" N, 27°26'59" E, 1421 m, 09.06.2019, ♀.

Distribution in Türkiye. Samsun (Herting, 1983), Karadeniz Bölgesi (Işık et al., 1987), Tokat (Kara, 1998; Kara & Alaoğlu, 1999; Lekin, 2014; Lekin et al., 2016), Zonguldak (Korkmaz, 2007), Bartın, Karabük (Atay, 2017), Çorum (Uysal, 2018; Uysal & Atay, 2021), Aydın and Muğla (Lutovinovas et al., 2018).

Hosts in Türkiye. *Rhaphigaster nebulosa* (Poda, 1761) (Hemiptera: Pentatomidae) (Herting, 1983).

Cylindromyia brassicaria (Fabricius, 1775)

Material examined. Salihli (Allahdiyen), 38°24'54" N, 28°05'03" E, 937 m, 06.07.2019, ♂; Selendi (Halılar), 38°45'05" N, 28°54'14" E, 482 m, 12.07.2015, ♂; and Soma (Beyce), 39°15'30" N, 27°37'21" E, 331 m, 14.09.2017, ♂.

Distribution in Türkiye. Erzurum (Doğanlar, 1982b), İzmir (Karsavuran, 1986), Tokat (Kara, 1998; Kara & Alaoğlu, 1999; Atay, 2011; Atay & Kara, 2014; Lekin, 2014; Lekin et al., 2016), Eskişehir (Aksu, 2005), Antalya, Burdur (Keçeci et al., 2007); Kastamonu (Atay, 2017); Çorum (Uysal, 2018; Uysal & Atay, 2021); Aydın and Muğla (Lutovinovas et al., 2018).

Hosts in Türkiye. *Dolycoris baccarum* (L., 1758) (Hemiptera: Pentatomidae) (Karsavuran, 1986; Kara & Tschorsnig, 2003; Keçeci et al., 2007; Atay, 2011; Atay & Kara, 2014) and *Holcostethus vernalis* (Wolff, 1804) (Hemiptera: Pentatomidae) (Kara & Alaoğlu, 1999).

Cylindromyia pusilla (Meigen, 1824)

Material examined. Soma (Yırca), 39°12'12" N, 27°41'08" E, 333 m, 15.07.2019, ♂.

Distribution in Türkiye. Locality information is not provided (Herting & Dely-Draskovits, 1993), Zonguldak (Korkmaz, 2007), Karabük (Atay, 2017) and Muğla (Lutovinovas et al., 2018).

Cylindromyia intermedia (Meigen, 1824)

Material examined. Salihli (Allahdiyen), N 38°26'48", E 28°06'36", 320 m, 11.08.2019, ♂.

Distribution in Türkiye. Erzurum (Doğanlar, 1982b) and Kastamonu (Atay, 2017).

Cylindromyia auriceps (Meigen, 1838)

Material examined. Salihli (Allahdiyen), 38°24'44" N, 28°05'05", 970 m, 08.08.2016, ♂; Selendi (Kurtuluş), 38°43'45" N, 28°50'54" E, 425 m, 02.06.2016, 2 ♂♂; Selendi (Halılar), 38°45'05" N, 28°54'14" E, 482 m, 12.07.2016, 2 ♂♂; 38°45'05" N, 28°54'13" E, 486 m, 13.08.2019, ♂; Selendi (Karabeyler), 38°45'07" N, 28°54'19" E, 492 m, 12.09.2016, ♂; 38°45'06" N, 28°54'17" E, 489 m, 14.08.2019, ♂; Soma (Küçükgüney), 39°15'05" N, 27°38'11" E, 332 m, 21.07.2017, ♀; and Soma (Heciz), 39°15'35" N, 27°37'22" E, 343 m, 15.08.2019, ♂.

Distribution in Türkiye. Tokat (Kara, 1998; Kara & Alaoğlu, 1999; Lekin, 2014; Lekin et al., 2016), Eskişehir (Aksu, 2005), Kastamonu (Korkmaz, 2007; Atay, 2017), Zonguldak (Korkmaz, 2007), Sakarya (Balkan, 2014; Balkan et al., 2015), Aydın and Muğla (Lutovinovas et al., 2018).

Hosts in Türkiye. *Aelia acuminata* (L., 1758) (Hemiptera: Pentatomidae) (Kara & Tschorsnig, 2003).

Thirty-six species in the Tachinidae were determined in this study of the Tachinidae fauna of Manisa Province. Three of the identified species are new records for Türkiye and 35 for Manisa fauna. Information on the distribution of the majority of the determined species in Türkiye is quite limited. Considering the occurrences in Manisa, Phasiinae is most common, followed by Tachininae, Exoristinae and Dexiinae. However, for the country as a whole, the order is Exoristinae, Tachininae, Phasiinae and Dexiinae (Kara et al., 2020). The number of known species in the Tachinidae in Manisa has increased to 37 as a result of this study.

Tachinids parasitize a wide variety of hosts, the majority of which are plant pests, and thus these insects are important in natural regulation of pests in agriculture and forest areas. Therefore, it is essential to determine species diversity, habitat and host complexes of these beneficial insects so as to understand and protect their populations in nature.

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References

Aksu, S., 2005. Eskişehir ve Çevresinde Saptanan Exoristinae ve Phasiinae (Diptera: Tachinidae) Türleri. Osmangazi Üniversitesi, Fen Bilimleri Enstitüsü, (Unpublished) Yüksek Lisans Tezi, Eskişehir, 129 s (in Turkish with abstract in English).

Anay, A., 2000. Çukurova Koşullarında Yonca (*Medicago sativa* L.)'da Zararlı ve Yararlı Böcek Faunasının Saptanması. Çukurova Üniversitesi, Fen Bilimleri Enstitüsü, (Unpublished) Yüksek Lisans Tezi, Adana, 57 s (in Turkish with abstract in English).

Atay, T., 2011. Amasya, Sivas ve Tokat İllerinin Kelkit Havzasındaki Farklı Böcek Takımlarında Bulunan Tachinidae (Diptera) Türleri Üzerinde Çalışmalar. Tokat Gaziosmanpaşa Üniversitesi, Fen Bilimleri Enstitüsü, (Unpublished) Doktora Tezi, Tokat, 218 s (in Turkish with abstract in English).

Atay, T., 2017. Contributions to the knowledge of the Tachinidae (Diptera) fauna of Turkey from Western Blacksea region of Turkey with one new record. Journal of Agricultural Faculty of Gaziosmanpasa University, 34 (1): 137-145.

Atay, T. & K. Kara, 2014. Tachinids (Diptera: Tachinidae) reared from lepidopterous and heteropterous hosts from some localities in the Kelkit Valley (Amasya, Tokat, Sivas) of Turkey. Turkish Journal of Zoology, 38 (4): 500-507.

Avcı, M. & K. Kara, 2002. Tachinidae parasitoids of *Traumatocampa ispartaensis* from Turkey. Phytoparasitica, 30 (4): 361-364.

Avcı, Ü. & H. Özbek, 1990. "Erzurum'da lahana zararlısı lepidopter türleri ve parazitoidleri üzerinde bir araştırma, 319-330". Türkiye II. Biyolojik Mücadele Kongresi (26-29 Eylül 1990, İzmir, Türkiye), 330 s (in Turkish).

Balkan, T., 2014. Sakarya İlinde Tachinidae (Hexapoda: Diptera) Türleri Üzerinde Faunistik ve Sistematik Çalışmalar. Tokat Gaziosmanpaşa Üniversitesi, Fen Bilimleri Enstitüsü, (Unpublished) Yüksek Lisans Tezi, Tokat, 144 s (in Turkish with abstract in English).

Balkan, T., K. Kara & T. Atay, 2015. Tachinidae (Diptera) species of the Sakarya (Turkey) province with two new records. Turkish Journal of Zoology, 39 (6): 1050-1055.

Bayram, Ş. & K. Kara, 1998. Türkiye Tachinidae (Diptera) familyası için yeni bir kayıt *Erycia fasciata* Villeneuve 1924. Turkish Journal of Entomology, 22 (3): 217-224 (in Turkish with abstract in English).

Cerretti, P., 2005. Revision of the West Palaearctic species of the genus *Pales* Robineau-Desvoidy (Diptera: Tachinidae). Zootaxa, 885: 1-36.

Dikyar, R., 1981. Biology and control of *Aelia rostrata* in central Anatolia. European and Mediterranean Plant Protection Organisation Bulletin, 11 (2): 39-41.

Doğanlar, M., 1975. Erzurum Bölgesinde Önemli Lepidopter Tırtıllarında Bulunan Tachinidae Sinekleri ve Bunların Kısa Biyolojileri. Atatürk Üniversitesi Yayınları, Erzurum, 136 s (in Turkish).

Doğanlar, M., 1982a. Doğu Anadolu'da saptanan bazı parazit sinekler I. Exoristinae (Diptera: Tachinidae). Türkiye Bitki Koruma Dergisi, 6 (2): 75-79 (in Turkish with abstract in English).

Doğanlar, M., 1982b. Doğu Anadolu'da saptanan bazı parazit sinekler II. Echinomyiinae, Dexiinae, Phasiinae (Diptera: Tachinidae). Türkiye Bitki Koruma Dergisi, 6 (4): 209-220 (in Turkish with abstract in English).

Duman M., N. Güz & E. Sertkaya, 2015. DNA barcoding of sunn pest adult parasitoids using cytochrome coxidase subunit I (COI). Biochemical Systematics and Ecology, 59: 70-77.

Duman, M. & E. Sertkaya, 2015. Adult parasitoid species and parasitization rates of Sunn pest *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae) and fecundity of the parasitised females in Karacadağ overwintering region and Diyarbakır cereal fields. Türkiye Entomoloji Bülteni, 5 (2): 85-94 (in Turkish with abstract in English).

Duman, M. & E. Sertkaya, 2016. Karacadağ kışlak alanı ve Diyarbakır ili hububat alanlarında Süne, *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae) ergin parazitoitleri (Diptera: Tachinidae)'nin konukçu bitkileri ve Süne parazitleme oranları. Türkiye Entomoloji Bülteni, 6 (1): 61-73 (in Turkish with abstract in English).

Dupuis, C., 1963. Essai monographique sur les Phasiinae (Diptères Tachinaires parasites d' Hétéroptères). Mémoires du Muséum National d' Histoire Naturelle, Paris. Série A (Zoologie), 26 (1): 1-461 (in French).

Gilasian, E., A. A. Talebi, J. Ziegler, S. Manzari & M. P. Araghia, 2013. A review of the genus *Cylindromyia* Meigen (Diptera: Tachinidae) in Iran, with the description of two new species and the newly discovered male of *C. persica* Tschorsnig. Studia Dipterologica, 20 (2): 299-324.

Gözüaçık, C. & C. Mart, 2009. Güneydoğu Anadolu Bölgesi'nde mısırda (*Zea mays* L.) zararlı bazı Lepidoptera larvalarının doğal parazitlenme oranlarının belirlenmesi. Bitki Koruma Bülteni, 49 (3): 107-116 (in Turkish with abstract in English).

Gözüaçık, C., C. Mart & K. Kara, 2007. "Natural enemies of lepidopterous pests and their natural parasitization rates in maize plantations in the Southeast Anatolian region, 8". Türkiye II. Bitki Koruma Kongresi (27-29 August, Isparta), 342 s (in Turkish).

Gözüaçık, C., C. Mart & K. Kara, 2009. Parasitoids of several lepidopterous pests in maize plantations in the Southeast Anatolian Region of Turkey. Turkish Journal of Zoology, 33 (4): 475-477.

Gözüaçık, C., K. Kara, V. Karaca, M. Duman, Ç. Mutlu & K. Melan, 2010. Adult parasitoids of sunn pest, *Eurygaster integriceps* Put. (Het.: Scutelleridae) and their effectiveness in the Southeast Anatolia. Harran Üniversitesi Ziraat Fakültesi Dergisi,14 (1): 1-8 (in Turkish with abstract in English).

Grenier, S., 1988. Applied biological control with tachinid flies (Diptera, Tachinidae). Anzeiger für Schädlingskunde Pflanzenschutz Umweltschutz, 61 (3): 49-56.

Gültekin, N., C. Gözüaçık, K. Kara & T. Atay, 2020. New host records for *Zeuxia cinerea* Meigen, 1826 (Diptera: Tachinidae) from Turkey. Journal of the Institute of Science and Technology, 10 (4): 2378-2382.

Gürkan, İ., 2010. Tokat Kazova Domates Ekim Alanlarında Görülen Zararlılar ve Bunlar Üzerinde Yaşayan Doğal Düşmanların Tespiti Üzerinde Araştırmalar. Tokat Gaziosmanpaşa Üniversitesi, Fen Bilimleri Enstitüsü, (Unpublished) Yüksek Lisans Tezi, Tokat, 35 s (in Turkish with abstract in English).

Herting, B., 1983. "Phasiinae, 1-88". In: Die Fliegen der Paläarktischen Region (Ed. E. Lindner), Lieferung Stuttgart: Schweizerbarth, 64c, Lieferung 329, Germany, 88 pp (in German).

Herting, B. & Á. Dely-Draskovits, 1993. "Family Tachinidae, 118-458". In: Catalogue of Palaearctic Diptera. Anthomyiidae-Tachinidae, (Eds. A. Soós & L. Papp). Budapest, Hungary, 624 pp.

Herting, B. & H.-P. Tschorsnig, 1993. Determination list of entomophagous insects Nr. 12. International Organization for Biological and Integrated Control of Noxious Animals and Plants/West Palaearctic Regional Section Bulletin, 16 (3): 56.

Işık, M., O. Ecevit, A. Kurt & T. Yücetin, 1987. Doğu Karadeniz Bölgesi Fındık Bahçelerinde Entegre Savaş Olanakları Üzerinde Araştırmalar. Ondokuz Mayıs Üniversitesi Yayınları, Samsun, 95 s (in Turkish with abstract in English).

İslamoğlu, M. & S. Kornoşor, 2003. Gaziantep - Kilis illerinde kışlak ve buğday tarlalarındaki Süne ergin parazitoitleri (Diptera, Tachinidae) üzerinde araştırmalar. Bitki Koruma Bülteni, 43 (1-4): 99-110 (in Turkish with abstract in English).

İslamoğlu, M. & S. Kornoşor, 2007. Investigations on the adult parasitoids (Diptera, Tachinidae) of the sunn pest in overwintering site and wheat fields in Kahramanmaras. Süleyman Demirel Üniversitesi, Ziraat Fakültesi Dergisi, 2: 53-61 (in Turkish with abstract in English).

Kansu, A., N. Kılınçer, N. Uğur & O. Gürkan, 1986. "Ankara, Kırşehir, Nevşehir ve Niğde illerinde kültür bitkilerinde zararlı lepidopterlerin larva ve pupa asalakları, 146-161". Türkiye I. Biyolojik Mücadele Kongresi (12-14 Şubat Adana, Türkiye), 476 s (in Turkish).

Kara, K., 1998. Tokat ve Çevresinde Saptanan Exoristinae ve Phasiinae (Diptera: Tachinidae) Alt Familyalarına Ait Sinekler Üzerinde Sistematik Çalışmalar. Tokat Gaziosmanpaşa Üniversitesi, Fen Bilimleri Enstitüsü, (Unpublished) Doktora Tezi, Tokat, 248 s (in Turkish with abstract in English).

Kara, K., 1999a. Tachininae (Diptera: Tachinidae) species of the Tokat province. Türkiye Entomoloji Dergisi, 23 (2): 121-134 (in Turkish with abstract in English).

Kara, K., 1999b. Dexiinae (Diptera: Tachinidae) species of the Tokat province. Türkiye Entomoloji Dergisi, 23 (3): 203-210 (in Turkish with abstract in English).

Kara, K., 2001a. Additions to the fauna of Turkish Tachinidae (Insecta, Diptera). Zoology in the Middle East, 23 (1): 85-88.

Kara, K., 2001b. Amasya İlinde saptanan bazı Exoristinae, Tachininae ve Dexiinae (Diptera: Tachinidae) türleri. Türkiye Entomoloji Dergisi, 25 (3): 217-222 (in Turkish with abstract in English).

Kara, K. & O. Alaoğlu, 1999. "Tokat ve çevresinde saptanan Phasiinae (Diptera: Tachinidae) altfamilyasına ait sinekler üzerinde sistematik çalışmalar, 563-586". Türkiye IV. Biyolojik Mücadele Kongresi, (26-29 Ocak, Adana, Türkiye), 588 s (in Turkish).

Kara, K. & S. Aksu, 2007. "Eskişehir ve çevresinde belirlenen bazı Tachinidae (Insecta: Diptera) türleri, 166". Türkiye II. Bitki Koruma Kongresi, (27-29 Ağustos, Isparta, Türkiye), 342 s (in Turkish).

Kara, K. & Y. Özdemir, 2000. Tachinid flies (Diptera, Tachinidae) reared from lepidopterous larvae in Central Anatolia (Turkey). Zoology in the Middle East, 20 (1): 117-120.

Kara, K. & H.-P. Tschorsnig, 2003. Host catalogue for the Turkish Tachinidae (Diptera). Journal of Applied Entomology, 127 (8): 465-476.

Kara, K, H.-P. Tschorsnig & T. Atay, 2020. Checklist of Turkish Tachinidae (Insecta, Diptera) with new records. Journal of the Entomological Research Society, 22 (2): 163-190.

Karsavuran, Y., 1986. Bornova (İzmir) koşullarında çeşitli kültür bitkilerinde zarar yapan *Dolycoris baccarum* (L.) (Heteroptera: Pentatomidae)'un biyolojisi ve ekolojisi üzerinde araştırmalar. Türkiye Bitki Koruma Dergisi, 10 (4): 213-230 (in Turkish with abstract in English).

Karsavuran, Y. & K. Kara, 2003. Tachinid parasitoids of *Ancyrosoma leucogrammes* and notes on parasitization rates of *Clytiomya dupuisi*. Phytoparasitica, 31 (4): 371-372.

Kavut, N., J. Dinçer & M. Karman, 1974. Ege Bölgesi pamuk zararlılarının predatör ve parazitleri üzerinde ön çalışmalar. Bitki Koruma Bülteni, 14 (1): 19-28 (in Turkish with abstract in English).

Kaya, K. & S. Kornoşor, 2008. The lepidopterous pest species, their parasitoids and population dynamics of the important ones in winter vegetables areas in Hatay province. Turkish Journal of Entomology, 32 (3): 195-209 (in Turkish with abstract in English).

Kaya, M. & B. Kovancı, 2000. Bursa ilinde Yeşilkurt, *Helicoverpa armigera* (Hübn.) (Lepidoptera: Noctuidae)'nın Biyolojisi Üzerinde Araştırmalar. Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi, 10 (1): 37-43 (in Turkish with abstract in English).

Keçeci, M., İ. Tekşam, E. Topuz & A. Öztop, 2007. "Determination of adult parasitoid species (Dip.: Tachinidae) of Sunn Pests (*Eurygaster integriceps* Put.) (Het.: Scutelleridae) and their parasitoid ratios in Antalya and Burdur Provinces, 27-29". Türkiye II. Bitki Koruma Kongresi, (27-29 Ağustos, Isparta, Türkiye), 342 s (in Turkish).

Khan, S. M. & M. Özer, 1984. *Agrotis* spp. (Lepidoptera: Noctuidae) Parazitlerinin Saptanması ve Önemli Görülenlerin Konukçuları ile Biyolojik İlişkileri. Ankara Üniversitesi Fen Bilimleri Enstitüsü Yayınları, No: BK 7, Ankara, Türkiye, 19 s (in Turkish with abstract in English).

Kıvan, M., 1996. Tekirdağ ilinde *Eurygaster integriceps* Put. (Heteroptera, Scutelleridae)' in endoparazitleri ve etkinlikleri üzerinde araştırmalar. Türkiye Entomoloji Dergisi, 20 (3): 211-216 (in Turkish with abstract in English).

Korkmaz, Y., 2007. Batı Karadeniz Bölgesi Tachinidae (Hexapoda: Diptera) Türleri Üzerinde Faunistik Çalışmalar. Tokat Gaziosmanpaşa Üniversitesi, Fen Bilimleri Enstitüsü, (Unpublished) Yüksek Lisans Tezi, Tokat, 54 s (in Turkish with abstract in English).

Kurt, A. M., 1975. Doğu Karadeniz Fındıklarında Zarar Yapan *Palomena prasina* L. (Hemiptera: Pentatomidae) 'nın Biyo-Ekolojisi Üzerinde Araştırmalar. Samsun Bölge Zirai Mücadele Araştırma Enstitüsü Yayınları, Samsun, 57 s (in Turkish).

Lekin N., K. Kara & T. Atay, 2016. Tachinidae (Diptera) species from some uplands in Tokat province (Turkey). Journal of Agricultural Faculty of Gaziosmanpaşa University, 33 (1): 56-63.

Lekin, N., 2014. Tokat'taki Bazı Yaylalarda Tespit Edilen Tachinidae (Hexapoda: Diptera) Türleri Üzerinde Faunistik Çalışmalar. Tokat Gaziosmanpaşa Üniversitesi, Fen Bilimleri Enstitüsü, (Unpublished) Yüksek Lisans Tezi, Tokat, 52 s (in Turkish with abstract in English).

Lodos, N., 1953. The Bionomics and Control of *Eurygaster integriceps* Put. Türkiye Yüksek Ziraat Muhendisleri Birliği Neşriyatı, Ankara, 57 s (in Turkish).

Lodos, N., 1961. The Problem of the Sunn Pest (*Eurygaster integriceps* Put.) in Turkey, Iraq, Iran and Syria (Distribution, Damage, Biology, Parasites, and Fighting Tools). Ege Üniversitesi Ziraat Fakültesi Yayınları, İzmir, 115 pp (in Turkish).

Lodos, N., 1986. Türkiye Entomolojisi II: Genel Uygulamalı ve Faunistik. Ege Üniversitesi Ziraat Fakültesi Yayınları, İzmir, Türkiye, 580 pp (in Turkish).

Lutovinovas E., H.-P. Tschorsnig, M. Barták, Š. Kubík, O. Dursun, H. S. Civelek & K. Kara, 2018. Contribution to the tachinid fauna of southwestern Turkey (Diptera: Tachinidae). Annales de la Société Entomologique de France (N.S.), 54 (4): 335-366.

Memişoğlu, H. & M. Özer, 1994. "Ankara ilinde Avrupa sünesi (*Eurygaster maura* L., Hemiptera: Scutelleridae)'nin doğal düşmanları ve etkinlikleri, 175-186". Türkiye 3. Biyolojik Mücadele Kongresi, (25-28 Ocak, İzmir, Türkiye), 575 s (in Turkish).

Mesnil, L. P., 1944-1965. "Larvaevorinae (Tachininae), 370-751". In: Die Fliegen der Paläarktischen Region (Ed. E. Lindner). Schweizerbart'sche Verlagsbuchhandlung, Stuttgart, Germany, 1168 pp (in German).

O'Hara, J. E., S. J. Henderson & D. M. Wood, 2021. Preliminary checklist of the Tachinidae of the world. Version 2.1. PDF document, 1039p. (Web page: http://www.nadsdiptera.org/Tach/WorldTachs/Checklist/Worldchecklist.html) (Date accessed: 14.01.2022).

Öncüer, C. & M. Kıvan, 1995. Tekirdağ ve çevresinde *Eurygaster* (Heteroptera: Scutelleridae) türleri, tanımları, yayılışları ve bunlardan *Eurygaster integriceps* Put. 'in biyolojisi ve doğal düşmanları üzerinde araştırmalar. Turkish Journal of Agriculture and Forestry, 19 (4): 223-230 (in Turkish with abstract in English).

Richter, V. A., L. Gültekin & B. A. Korotyaev, 2002. *Zeuxia cinerea* Meigen new to the fauna of Northeastern Turkey (Diptera: Tachinidae). Zoosystematica Rossica, 11 (1): 234.

Sahebari, F. S., S. Khaghaninia & J. Ziegler, 2013. Contribution to the knowledge of the tachinid flies of the subfamily Tachininae (Diptera, Tachinidae) in northwestern Iran. Studia Dipterologica, 20 (2): 285-295.

Şimşek, N., M. Güllü & M. Yaşarbaş, 1994. "Akdeniz Bölgesinde Süne (*Eurygaster integriceps* Put.)' nin doğal düşmanları ve etkinlikleri üzerinde araştırmalar, 155-164". Türkiye 3. Biyolojik Mücadele Kongresi, (25-28 Ocak, İzmir, Türkiye), 575 s (in Turkish).

Steiner, P., 1937. Beiträge zur Kenntnis der Schädlingsfauna Kleinasiens III. Laphygma exigua Hb., ein Groß-Schädling der Zuckerrübe in Anatolien. Zeitschrift für Angewandte Entomologie, 23 (2): 177-222.

Stireman, J. O., J. E. O' Hara & D. M. Wood, 2006. Tachinidae: Evolution, behavior and ecology. Annual Review of Entomology, 51 (1): 525-555.

Tschorsnig, H.-P., 2017. Preliminary host catalogue of Palaearctic Tachinidae (Diptera), 480pp. (Web page: http://www.nadsdiptera.org/Tach/WorldTachs/CatPalHosts/Cat_Pal_tach_hosts_Ver1.pdf) (Date accessed 14.08.2020).

Tschorsnig, H.-P. & B. Herting, 1994. Die Raupenfliegen (Diptera: Tachinidae) Mitteleuropas: Bestimmungstabellen und angaben zur verbreitung und ökologie der einzelnen arten. Stuttgarter Beiträge zur Naturkunde, Serie A (Biologie), 506: 1-170 (in German).

Tschorsnig, H.-P. & V. A. Richter, 1998. "Family Tachinidae, 691-827". In: Contributions to a Manual of Palaearctic Diptera (Eds. L. Papp & B. Darvas), Science Herald Budapest, Hungary, 880 pp.

Tuatay N., A. Kalkandelen & N. Aysev Çağatay, 1972. Nebat Koruma Müzesi Böcek Kataloğu (1961-1971). Zirai Mücadele ve Zirai Karantina Genel Müdürlüğü, Ankara, 119s (in Turkish).

Uysal, İ. & T. Atay, 2021. A contribution to the Tachinidae (Diptera) fauna of Çorum Province in Turkey, with new records. Türkiye Biyolojik Mücadele Dergisi, 12 (1): 25-45.

Uysal, İ., 2018. Çorum İlinde Belirlenen Tachinidae (Hexapoda: Diptera) Türleri Üzerinde Faunistik Çalışmalar. Tokat Gaziosmanpaşa Üniversitesi, Fen Bilimleri Enstitüsü, (Unpublished) Yüksek Lisans Tezi, Tokat, 64s. (in Turkish with abstract in English).

Yüksel, M., 1968. Güney ve Güneydoğu Anadolu'da Süne *Eurygaster integriceps* Put.'un Yayılışı, Biyolojisi, Ekolojisi, Epidemiyolojisi ve Zararı Üzerinde Araştırmalar. TC Tarım Bakanlığı Zirai Mücadele ve Zirai Karantina Genel Müdürlüğü Yayınları, Ankara, 255 s (in Turkish).

Zimin, L. S., 1966. A review of the tribe Gymnosomatini (Diptera, Tachinidae) of the fauna of the USSR, parasitizing phytophagous bugs. Entomologicheskoe Obozrenie, 45 (2): 424-456.

Zwölfer W., 1932. Beiträge zur kenntnis der schädlingsfauna kleinasiens II. uber die beziehungen der getreidewanze *Eurygaster integriceps* Put. zu biotischen umweltfaktoren. Zeitschrift für Angewandte Entomologie, 19 (2): 161-187 (in German).

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Original article (Orijinal araştırma)

Behavioral responses of *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae) to hydrolyzed yeast and different types of sugars[1]

Ceratitis capitata (Wiedemann, 1824) (Diptera: Tephritidae)'nın hidrolize maya ve farklı şeker türlerine davranışsal tepkileri

Gülsevim TİRİNG[2*] **Serdar SATAR[2,3]**

Abstract

Ceratitis capitata (Wiedemann, 1824) (Diptera: Tephritidae) is a major pest of fruits and vegetables worldwide. This study was conducted at Çukurova University (Türkiye) in 2021. In this study, different types of sugar and hydrolyzed yeast were evaluated to determine the behavioral response of adult *C. capitata* using a four-arm olfactometer and wind tunnel. Some of the most attractive sugars to *C. capitata* were combined with hydrolyzed yeast, to check whether their attractiveness could be further improved. The sugars used in the study were alpha glucose, arabinose, fructose, galactose, maltose, melibiose, ribose, sucrose and trehalose. The results showed that *C. capitata* had a significantly higher attraction to arabinose, fructose, melibiose, ribose and trehalose than the other four sugars. The number of adults that responded to trehalose was higher than the other sugars, so behavioral responses of *C. capitata* to hydrolyzed yeast, trehalose and hydrolyzed yeast + trehalose were tested in comparison to a control group. This study demonstrated that *C. capitata* was more attracted to the combination of hydrolyzed yeast + trehalose than to each of these alone or to the control.

Keywords: Attractant, medfly, olfactometer, wind tunnel

Öz

Ceratitis capitata (Wiedemann, 1824) (Diptera: Tephritidae) dünya çapında meyve ve sebzelerin önemli bir zararlısıdır. Bu çalışma 2021 yılında Çukurova Üniversitesi'nde (Türkiye) yürütülmüştür. Bu çalışmada, dört kollu olfaktometre ve rüzgâr tüneli kullanılarak *C. capitat*a erginlerinin davranışsal tepkilerini belirlemek amacıyla farklı tipte şekerler ve hidrolize maya değerlendirilmiştir. İleriye yönelik bir adım olarak, cezbediciliğin daha da gelişip-gelişmediğini kontrol etmek için en çok yönelimin görüldüğü şekerlerden biri hidrolize maya ile kombine edilmiştir. Çalışmada kullanılan şekerler alfa glikoz, arabinoz, fruktoz, galaktoz, maltoz, melibioz, riboz, sakkaroz ve trehalozdur. Sonuçlar, arabinoz, fruktoz, melibioz, riboz ve trehalozun *C. capitata* için diğer dört şekerden önemli ölçüde daha yüksek bir çekiciliğe sahip olduğunu göstermiştir. Trehaloza tepki veren ergin sayısı diğer şekerlerden daha fazla olduğu için *C. capitata'*nın hidrolize maya, trehaloz, hidrolize maya + trehaloza karşı davranışsal tepkileri kontrol grubuna göre test edilmiştir. Bu çalışma*, C. capitata*'nın hidrolize maya + trehaloz kombinasyonun, bunların her birine veya kontrole göre daha fazla çekici olduğunu göstermiştir.

Anahtar sözcükler: Cezbedici, Akdeniz meyve sineği, olfaktometre, rüzgâr tüneli

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Introduction

Tephritids (Diptera: Tephritidae) comprise some of the most destructive pests of fruit and vegetable crops worldwide (Tiring & Satar, 2021). Crop losses due to fruit flies have been predicted to cause annual economic damage of 1 billion USD worldwide. The most noxious species belong to the genera *Anastrepha, Bactrocera, Ceratitis, Dacus, Rhagoletis* and *Zeugodacus* (White & Elson-Harris, 1992).

The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae) is one of the most devastating and economically significant pests worldwide (Elekçioğlu, 2013; Tiring & Satar, 2017, 2021). Feeding on more than 300 different hosts and having a cosmopolitan geographic distribution that is ever-expanding, it exerts a direct economic loss to growers such as dramatically affects national and international vegetable-fruit commerce (Liquido et al., 1990, 1991). If *C. capitata* populations are not managed, the percentage of damage often exceeds 50% of the total fruit production, and the infestation may reach 80-100% in highly susceptible hosts such as persimmon (Tiring & Satar, 2017, 2021; Kouloussis et al., 2022). Growers are very concerned about the high reproductive potential and adaptability of *C. capitata*, combined with the low effectivity of natural enemies and their wide range of hosts (Castillo et al., 2000).

New techniques for medfly control are being developed to replace the commonly-used organophosphate insecticide applications. Recently, insecticidal bait sprays have been used against *C. capitat*a. Mass trapping with liquid or dry food-based baits offers promising medfly control within integrated pest management (IPM) programs (Navarro-Llopis et al., 2011). The new attractants are very significant for food-based baits, so the studies related to lures need to continue.

Recently, some control of *C. capitata* has been achieved by mass trapping without bait spray application. However, protein hydrolysates and commercially fermented compounds are generally used as attractants to lure medfly and other fruit flies for monitoring and mass trapping. These attractants contain significant food resources required by the adults for egg development and sexual maturation and frequently consist of compounds such as sugar baits and yeast (Heath et al., 1997; Plácido-Silva et al., 2005; Epsky et al., 2014).

The high cost of commercial mass trapping products, especially attractants has prevented their use in medfly control in Türkiye. As alternatives, some farmers have used monitoring practices that depend on materials including fermented products, sugars, vinegar and diluted molasses as lures. However, the efficiency of these techniques has not been evaluated. Therefore, olfactometer and wind tunnel experiments were conducted to evaluate new and low-cost lures as tools to support sustainable *C. capitata* IPM.

Materials and Methods

Insects

Infested fruits were collected from a mixed fruit orchard at Çukurova University Research and Application Farm located in the southeast Mediterranean Region of Türkiye. Adults of *C. capitata* were cultured under laboratory conditions (25 ± 2°C, 60-70% RH and 12 h photoperiod). Adults were provided a solid diet consisting of sucrose and hydrolyzed yeast (Condolab, Laboratorios Conda S.A., Madrid, Spain) (3:1 w/w). Adults were kept in plexiglass cages. Eggs of *C. capitata* were collected through a fine-meshed tulle on the front wall of a cage into a trough of water. The larvae were reared on a wheat bran diet (wheat bran 65 g, sugar 30 g, yeast 20 g, hydrochloric acid (37%) 4 ml, sodium benzoate 1 g and tap water 127 ml). Individuals of the last larval stage were then placed in cages containing moist perlite to pupate.

Test insects were sexed and kept separate until use in olfactometer and wind tunnel studies. Virgin adults were used in the experiment. Bioassay studies were conducted between 10:00 and 16:00.

Compounds

Alpha glucose, arabinose, fructose, galactose, hydrolyzed yeast, maltose, melibiose, ribose, sucrose and trehalose were purchased from Sigma-Aldrich (Adana, Türkiye).

Four-arm olfactometer bioassay

Attraction of *C. capitata* to sugars and yeast was tested in a four-arm olfactometer. The olfactometer consisted of a central glass area (20 x 20 cm) with four arms. Each arm was connected via silicon tubing to gas-washing bottles that contained the odor source. Silicon tubes were used to connect the vacuum pump, activated carbon filter bottle, flow meter and gas-washing bottle containing water and compounds, respectively. To prevent visual disturbance, a 20 W light was placed above the olfactometer in a room at 25 ± 2°C and 70% RH. The bioassay studies were conducted using three-day-old adults. Test insects were unfed for 24 h before the bioassays. A piece of filter paper containing samples diluted to 5% (20 µl) or the control (fresh air) was placed into each of the gas-washing bottles. For each assay, one group of 10 adults (5 females and 5 males) was introduced into the release portion and observed for 10 min using a stopwatch. These assays were replicated four times. Flies entering an arm within this time were deemed to be responders. Olfactometer was cleaned thoroughly with 70% ethanol and distilled water before each use. Also, arms were rotated (90°) to minimize positional effects.

Wind tunnel bioassays

This study was conducted in a wind tunnel (45 × 80 × 220 cm). Charcoal-filtered air was passed through the chamber at 0.20 cm/s^{-1} with at 24 ± 1°C and 70% ± 5% RH. To avoid bias caused by light, the wind tunnel was lit from above by LED lights set to 10 lux. Test insects were unfed for 24 h prior to use in the assays. Samples for odor delivery were prepared at a concentration of 5% and transferred to a 20 ml polypropylene vial before testing. This vial was placed on the tripod in front of a 15 cm fan. For each treatment, we tested the landing rate of 10 separately released *C. capitata* that were given 10 min to respond to the volatile chemicals. These assays were repeated four times. If the adult did not take off, we terminated the test and deemed it to be a non-responder. Each adult was tested only once. At the end of each treatment, the wind tunnel was cleaned with 70% alcohol and distilled water.

Statistical analysis

All statistical tests were performed on IBM SPSS 23. Data were checked for homogeneity of variance (Levene test) and the normal distribution of all data (Shapiro-Wilk test; P = 0.05) before analysis. Data were transformed using log10(x + 1) to satisfy normality assumptions prior to analysis of variance (ANOVA). Olfactometer bioassays were conducted as completely randomized designs with the 4 test dates as replicates. For olfactometer assays, significant differences in the number of *C. capitata* were analyzed using a two-way (sex and chemicals as factors) ANOVA followed by Tukey's multiple comparison test at P = 0.05. Also, to further understand the effect of chemicals, data from females, males and both were subjected to separate a one-way ANOVA (chemicals as factors). Significant ANOVAs were followed by Tukey's test at P = 0.05. Also, the behavior of the adults in the wind tunnel were analyzed using the Chi-square goodness-of-fit test. Multiple comparisons were performed using Chi-squared tests with a Bonferroni correction. All data in this study are given as mean ± standard error.

Results

Fructose and galactose attracted significantly more females than alpha glucose and the control, but males were not significantly different (Figure 1) (female, F = 4.00; df = 3, 15; P = 0.035; male, F = 1.73; df = 3, 15; P = 0.214). Also, two-way analysis of the data showed that there was no significant interaction between sex and sugars in bioassay studies (Table 1).

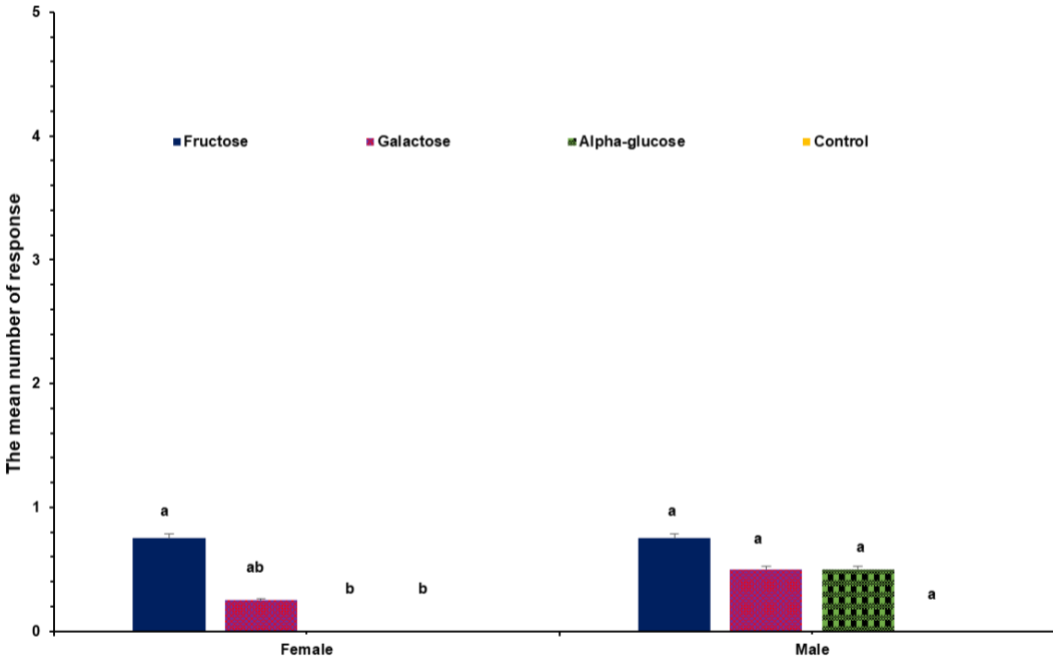

Figure 1. Mean number of *Ceratitis capitata* attracted to different types of sugar in a four-arm olfactometer. The data shows the attraction of *Ceratitis capitata* to sugars for each of the female and male adults listed on the x-axis. Means with the same letter are not significantly different (Tukey's test, P: 0.05).

Table 1. The result of the two-way analysis of the variance test for the number of behavioral responses in a four-arm olfactometer

			Fructose, galactose, alpha glucose and control		Ribose, arabinose, sucrose, and control		Maltose, melibiose, trehalose and control		Trehalose, yeast, trehalose+ yeast, and control	
		df	F	P	F	P	F	P	F	P
Attractant	Compounds	3	4.750	0.010	143.597	0.000	143.597	0.000	114.535	0.000
	Sex	1	0.750	0.395	1.013	1.000	1.331	0.285	0.016	0.900
	Compounds *Sex	3	0.750	0.533	1.000	0.287	1.333	0.048	1.749	0.184

Two-way analysis of variance did not indicate a significant effect of interaction between the sugars and sex for the following test compounds: arabinose, ribose, sucrose and the control (Table 1). Both females and males were significantly more attracted to the olfactometer arm containing sugars with arabinose and ribose in comparison to those containing sucrose and the control (female, F = 72.5; df = 3, 15; P = 0.000; male, F = 72.5; df = 3, 15; P = 0.000) (Figure 2).

Two-way analysis of variance indicated a significant interaction between the sugars and sex on the following test compounds: maltose, melibiose, trehalose and the control (Table 1).

Olfactometer experiments showed that adults were significantly more attracted to the sugars melibiose and trehalose compared to maltose and control (female, F = 9.33; df = 3, 15; P = 0.002; male, F = 11.3; df = 3, 15; P = 0.001) (Figure 3).

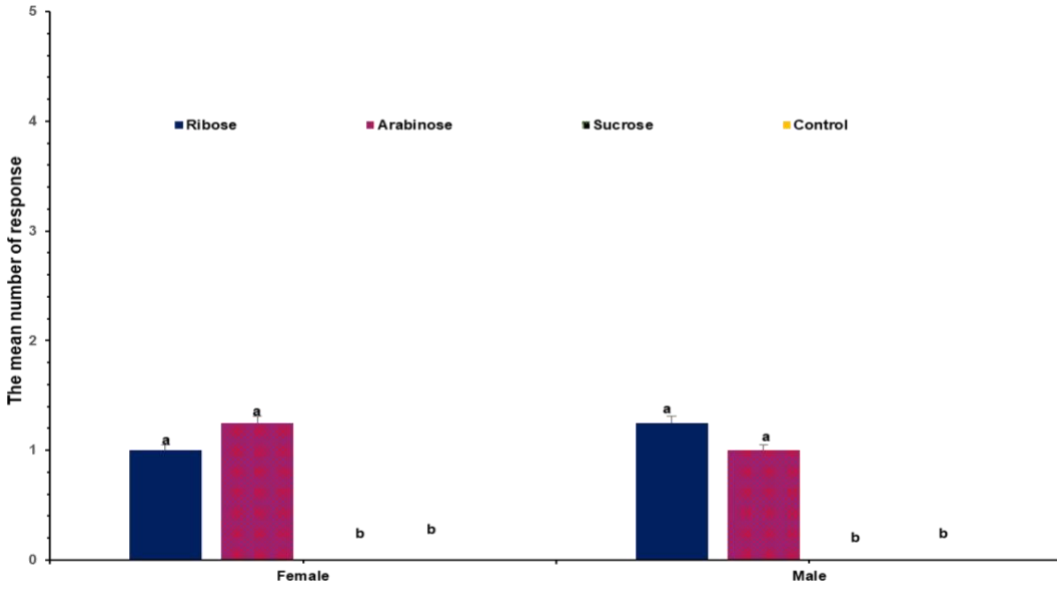

Figure 2. Mean number of *Ceratitis capitata* attracted to different types of sugar in a four-arm olfactometer. The data shows the attraction of *Ceratitis capitata* to sugars for each of the female and male adults listed on the x-axis. Means with the same letter are not significantly different (Tukey's test, P: 0.05).

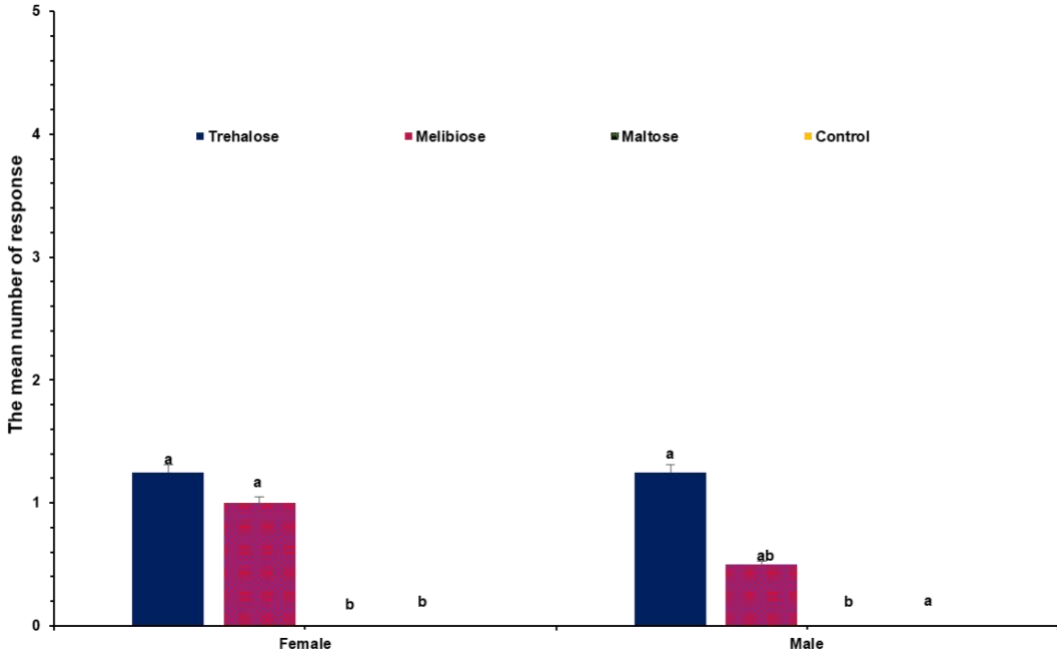

Figure 3. The mean number of *Ceratitis capitata* attracted to different types of sugars in a four-arm olfactometer. The data shows the attraction of *Ceratitis capitata* to sugars for each of the female and male adults listed on the x-axis. Means with the same letter are not significantly different (Tukey's test, P: 0.05).

Trehalose attracted more *C. capitata* than other sugars. Therefore, the response to trehalose, yeast, and the combination of both was also tested in the four-arm olfactometer (Figure 4). Adults showed significantly different responses to the treatments with trehalose, yeast, yeast + trehalose, and control (female, F = 92.5; df = 3, 15; P = 0.000; male, F = 39.7; df = 3, 15; P = 0.000). In addition, two-way analysis of the data showed that there was no significant interaction between sex and sugars (Table 1).

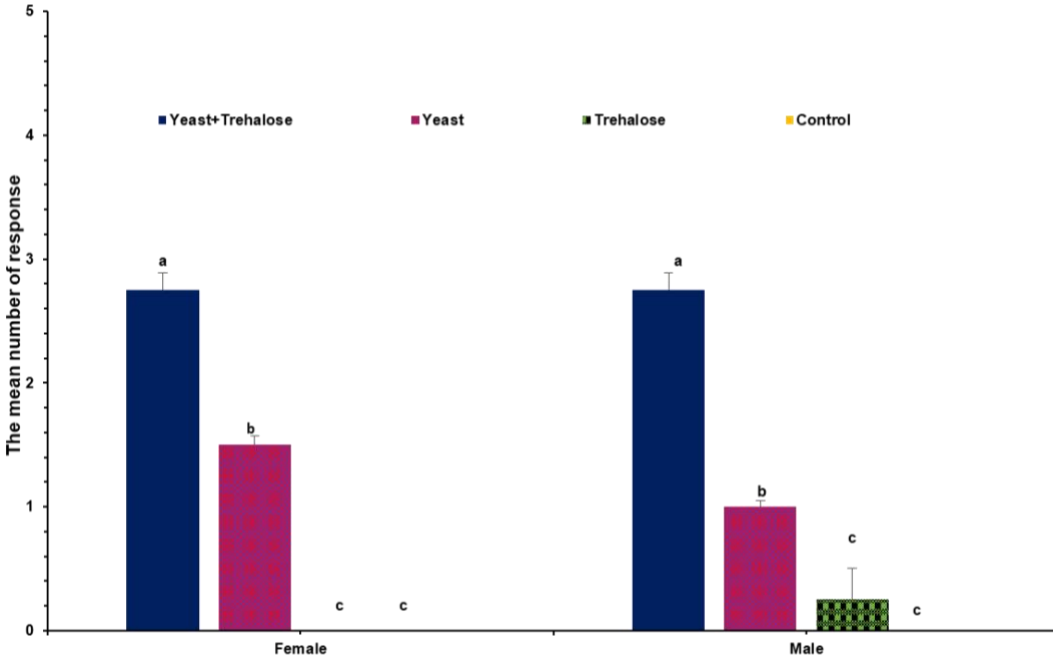

Figure 4. Mean number of *Ceratitis capitata* attracted to yeast, trehalose, and both in a four-arm olfactometer. The data shows the attraction of *Ceratitis capitata* to yeast, trehalose, and both for each of the female and male adults listed on the x-axis. Means with the same letter are not significantly different (Tukey's test, P: 0.05).

The percentage of upwind-oriented flights differed among different types of sugar and yeast (x^2 = 3.68; P = 0.000). Wind tunnel experiments confirmed that trehalose was more attractive than the other sugars. Also, the percentage of *C. capitata* attracted to yeast + trehalose was consistently higher than the others (Figure 5).

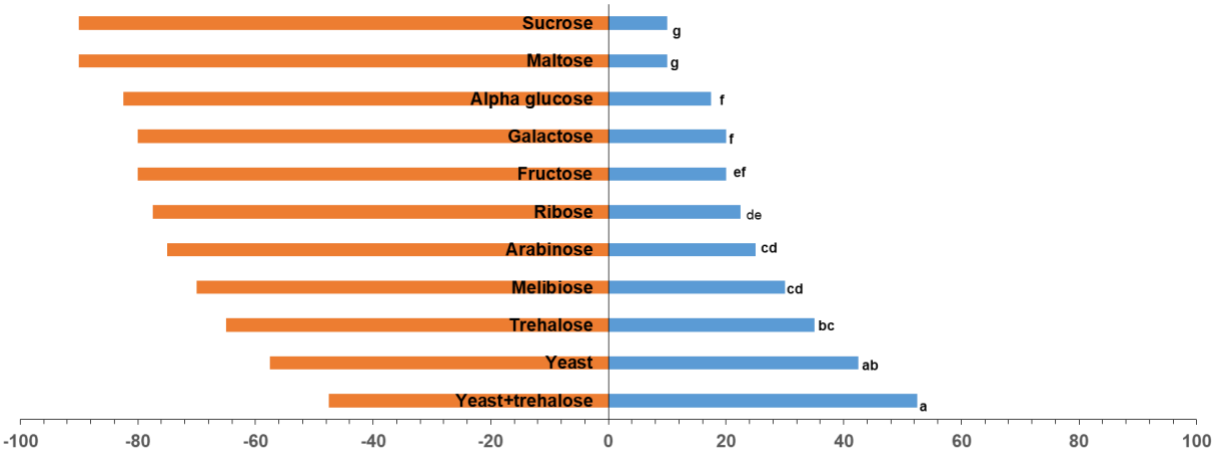

Figure 5. Response of *Ceratitis capitata* to different types of sugar and yeast in a wind tunnel. Horizontal bar plots with positive values represent the percentage of flies responding (take off) to the compounds. If the adult did not take off, we considered it a non-responder. Means with the same letter are not significantly different according to Chi-squared tests (P = 0.05).

Discussion

The results of the study revealed that trehalose is more attractive to *C. capitata* than the other sugars tested. Also, hydrolyzed yeast + trehalose is strongly attractive to medfly adults. The strong olfactometer and wind tunnel response suggest that the compounds contained in this mixture could influence the behavior of the insect in the field.

Food-based attractants are similar with nitrogen sources that provide the protein required by adults to reach sexual maturity. The female-biased attractants are generally food lures. The reason for this is that females have higher needs for protein acquisition than males for egg development (Christenson & Foote, 1960; Kouloussis et al., 2017). Hydrolyzed yeasts contain high protein (San Martin et al., 2020). The results of the present study were consistent with hydrolyzed yeast is more attractive to significantly more females than the others.

By the mid-1990s, an aqueous solution of torula yeast borax (TYB) pellets (Lopez et al., 1971) was a food lure used in fruit fly mass trapping systems worldwide (Heath et al., 1995) and is widely used still (Enkerlin & Reyes-Flores, 2018). For example, five TYB-baited traps per 2.59 km^2 are used as a component of a fruit fly detection network that covers ca. 64,750 km^2 in California (Vargas et al., 2013). The present study supported that hydrolyzed yeast is more attractive to *C. capitata* than the tested sugars.

With attractants for *C. capitata* now including both protein and sugar, different formulations of protein hydrolysates are commercially available for *C. capitata* control. Biodelear, a patented, female-specific attractant, produced by the Maillard reaction of fructose, urea and water at a ratio of 3:1:1 (Kouloussis et al., 2022). In the present study, fructose attracted significantly more females than alpha glucose and the control in a four-arm olfactometer. However, the adults responded significantly more to trehalose than fructose in the wind tunnel experiments. Also, wind tunnel experiments showed that arabinose, melibiose and ribose were more attractive to *C. capitata* than fructose.

Various formulations of protein hydrolysates are commercially available for *C. capitata* control. GF-120 Naturalyte is a formulated mixture that contains spinosad (0.02%) in a non-toxic bait (including water, different types of sugar and maize protein). The M3 bait station comprises a protein attractant and insecticide housed in a plastic device. The flies feed on the bait and die soon afterward (Ware et al., 2003).

In a mass trapping control of *Ceratitis* spp. in Türkiye (Başpınar et al., 2013) and Nigeria (Ekesi & Tanga, 2016), and *Bactrocera dorsalis* Hendel (Diptera: Tephritidae) in Kenya and Uganda (Umeh & Garcia, 2008) baits based on brewers-waste are used as a commercial hydrolyzed protein bait (e.g. NuLure).

Our study confirms that the attraction of different types of sugars and yeast can be used in mass trapping and insecticide bait sprays to manage *C. capitata*. The present study demonstrates that trehalose is more attractive to *C. capitata* than other sugars. Also, this study found that yeast + trehalose was more attractive to *C. capitata* than the others test substances.

The present study confirmed that the attraction of *C. capitata* to some sugars and hydrolyzed yeast particularly trehalose and yeast. This combination, therefore, has potential as a novel monitoring tool. Finally, further research is needed to determine whether a combination of sugar, yeast and ammonium odors is a more effective and species-specific novel monitoring tool than these types of odor alone.

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References

Başpınar, H., Y. Karsavuran, N. Başpınar, T. Koçlu, F. K. Apak & P. Güneyi, 2013. Determination of an adequate attractant and its concentration in mass-trapping of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) by a hand-made trap. IOBC-WPRS Bulletin, 95: 33-40.

Castillo, M., P. Moya, E. Hernandez & E. Primo-Yufera, 2000. Susceptibity of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) to entomophatogenic fungi and their extracts. Biological Control, 19 (3): 274-282.

Christenson, L. D. & R. H. Foote, 1960. Biology of fruit flies. Annual Review of Entomology, 5 (1): 171-192.

Ekesi, S. & C. M. Tanga, 2016. "Waste Brewer's Yeast as An Alternative Source of Protein for Use as A Bait in the Management of Tephritid Fruit Flies, 293-306". In: Fruit Fly Research and Development in Africa-Towards a Sustainable Management Strategy to Improve Horticulture (Eds. S. Ekesi & S. Mohamed). Springer, Cham, 693 pp.

Elekçioğlu, N. Z., 2013. Fruit flies of economic importance in Turkey, with special reference to Mediterranean fruit fly, *Ceratitis capitata (*Wied.). Türk Bilimsel Derlemeler Dergisi, 6 (2): 33-37.

Enkerlin, W. R. & J. Reyes-Flores, 2018. Trapping guidelines for area-wide fruit fly programmes. FAO/IAEA, 65 pp.

Epsky, N. D., P. E. Kendra & E. Q. Schnell, 2014. "History and Development of Food-Based Attractants, 75-118". In: Trapping and the Detection, Control and Regulation of Tephritid Fruit Flies (Eds. T. Shelly, N. Epsky, E. B. Jang, J. Reyes-Flores & R. Vargas). Springer, Dordrecht, 654 pp.

Heath, R. R., N. D. Epsky, B. D. Dueben, J. Rizzo & F. Jeronimo, 1997. Adding methyl-substituted ammonia derivatives to a food-based synthetic attractant on capture of the Mediterranean and Mexican fruit flies (Diptera: Tephritidae). Journal of Economic Entomology, 90 (6): 1584-1589.

Heath, R. R., N. D. Epsky, A. Guzman, B. D. Dueben, A. Manukian & W. L. Meyer, 1995. Development of a dry plastic insect trap with food-based synthetic attractant for the Mediterranean and Mexican fruit flies (Diptera: Tephritidae). Journal of Economic Entomology, 88 (5): 1307-1315.

Kouloussis, N. A., P. T. Damos, C. S. Ioannou, C. Tsitsoulas, N. T. Papadopoulos, D. Nestel & D. S. Koveos, 2017. Age related assessment of sugar and protein intake of *Ceratitis capitata* in ad libitum conditions and modeling its relation to reproduction. Frontiers in Physiology, 8: 271.

Kouloussis, N. A., V. G. Mavraganis, P. Damos, C. S. Ioannou, E. Bempelou, D. S. Koveos & N.T. Papadopoulos, 2022. Trapping of *Ceratitis capitata* using the low-cost and non-toxic attractant biodelear. Agronomy, 12 (2): 525-536.

Liquido, N. J., R. T. Cunningham & S. Nakagawa, 1990. Host plants of Mediterranean fruit fly (Diptera: Tephritidae) on the Island of Hawaii (1949-1985 survey). Journal of Economic Entomology, 83 (5): 1863-1878.

Liquido, N. J., R. T. Cunningham & L. A. Shinoda 1991. Host plants of the Mediterranean fruit fly, Diptera: Tephritidae, an annotated world review. Miscellaneous Publications of the Entomological Society of America, 77: 1-52.

Lopez, D. F., L. F. Steiner & F. R. Holbrook, 1971. A new yeast hydrolysate-borax bait for trapping the Caribbean fruit fly. Journal of Economic Entomology, 64 (6): 1541-1543.

Navarro-Llopis, V., S. Vacas, J. Sanchis, J. Primo & C. Alfaro, 2011. Chemosterilant bait stations coupled with sterile insect technique: an integrated strategy to control the Mediterranean fruit fly (Diptera: Tephritidae). Journal of Economic Entomology, 104 (5): 1647-1655.

Plácido-Silva, M. D. C., F. S., Zucoloto & I. S. Joachim-Bravo, 2005. Influence of protein on feeding behavior of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae): comparison between immature males and females. Neotropical Entomology, 34 (4): 539-545.

San Martin, D., M. Orive, B. Iñarra, J. Castelo, A. Estévez, J. Nazzaro, I. Iloro, F. Elortza & J. Zufia, 2020. Brewers' spent yeast and grain protein hydrolysates as second-generation feedstuff for aquaculture feed. Waste and Biomass Valorization, 11 (10): 5307-5320.

Tiring, G. & S. Satar, 2017. Population fluctuation of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) in some fruit orchards. Turkish Bulletin of Entomology, 7 (3): 29-37.

Tiring, G. & S. Satar, 2021. Annual population fluctuations of Mediterranean fruit fly in the Eastern Mediterranean Region of Turkey; Problem of non-marketing fruit. Phytoparasitica, 49 (5): 807-817.

Umeh, V. C. & L. E. Garcia, 2008. Monitoring and managing *Ceratitis* spp. complex of sweet orange varieties using locally made protein bait of brewery waste. Fruits, 63 (4): 209-217.

Vargas, R. I., D. Haviland, B. Faber, J. Kabashima, B. Grafton-Cardwell & J. Morse, 2013. Improving trapping systems for early detection and eradication of fruit flies (Diptera: Tephritidae) in California. Citrograph, 4 (4): 28-34.

Ware, T., G. Richards & J. H. Daneel, 2003. The M3 bait station. A novel method of fruit fly control. SA Fruit Journal, 2 (1): 44-47.

White, I. M. & M. M. Elson-Harris, 1992. Fruit Flies of Economic Significance: Their Identification and Bionomics. CAB International, Wallingford, 601 pp.

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Original article (Orijinal araştırma)

Distribution and identification of important plant parasitic nematodes in anise growing areas[1]

Anason yetiştirilen alanlarda önemli bitki paraziti nematodlarının dağılımı ve tanımlanması

İbrahim MISTANOĞLU[2*] **Gülsüm UYSAL[3]** **Zübeyir DEVRAN[4]**

Abstract

Anise, *Pimpinella anisum* L. (Apiales: Apiaceae) is an important medicinal aromatic plant and can be attacked by different pests and pathogens. Plant parasitic nematodes are important pests that can be confused with nutrient deficiency or symptoms of various diseases or pests. Therefore, rapid and accurate identification of these pests is essential for integrated nematode management and rotation. In 2021, a survey was conducted in Bolvadin District of Afyonkarahisar Province, which is one of the most important anise production areas of Türkiye. Forty-two soil samples were collected from the anise growing areas in the district and 16 species-specific primers were used for molecular identification of plant parasitic nematodes. In the samples, *Meloidogyne hapla* Chitwood, 1949 (Tylenchida: Heteroderidae), *Pratylenchus neglectus* (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941, *Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida: Pratylenchidae) and *Aphelenchoides besseyi* Christie, 1942 (Aphelenchida: Aphelenchoididae), were detected at the rates of 57% (24), 52% (22), 36% (15) and 7% (3), respectively. Plant parasitic nematodes were found in both single and mixed populations. In addition, *A. besseyi* was found for the first time in anise growing areas.

Keywords: Anise, identification, PCR, plant parasitic nematodes

Öz

Anason, *Pimpinella anisum* L. (Apiales: Apiaceae) önemli bir tıbbi aromatik bitkidir ve farklı zararlılar ve patojenler tarafından saldırıya uğrayabilmektedir. Bitki paraziti nematodlar, zararları besin eksikliği veya çeşitli hastalık veya zararlıların semptomları ile karıştırılabilen önemli zararlılardır. Bu nedenle, entegre nematod mücadele programları ve ürün rotasyonu için bu organizmaların hızlı ve doğru tanımlanması esastır. 2021 yılında Türkiye'nin en önemli anason üretim alanlarından biri olan Afyonkarahisar İli Bolvadin İlçesi'nde sürvey çalışması yapılmıştır. Bölgedeki anason alanlarından 42 toprak örneği alınmış ve bitki paraziti nematod türlerinin moleküler tanımlanmasında türe özgü 16 primer kullanılmıştır. Örneklerde *Meloidogyne hapla* Chitwood, 1949 (Tylenchida: Heteroderidae), *Pratylenchus neglectus* (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941, *Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida: Pratylenchidae) ve *Aphelenchoides besseyi* Christie, 1942 (Aphelenchida: Aphelenchoididae) sırasıyla %57 (24), %52 (22), %36 (15) ve %7 (3) oranlarında belirlenmiştir. Bitki paraziti nematodlar hem tek hemde karışık popülasyonlar halinde bulunmuştur. Ayrıca *A. besseyi*, anason üretim alanlarında ilk kez belirlenmiştir.

Anahtar sözcükler: Anason, tanımlama, PCR, bitki paraziti nematodlar

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Introduction

Anise, *Pimpinella anisum* L. (Apiales: Apiaceae) is an annual and aromatic herb belong to the Apiaceae family (Ghorbanpour et al., 2017; ITIS, 2021). The flowers of the plant are umbrella-shaped and white color, while the fruits are greenish yellow and hairy (Orav et al., 2008; Shojaii & Abdollahi, 2012; Karık, 2020). Plants belonging to the order Apiales are generally rich in essential oil. In addition, this oil is extremely valuable in terms of vitamins and minerals (Keskin & Baydar, 2016). Many are of pharmaceutical importance in making herbal medicines. Anise which is one of the most important plants in this family as an example cumin [*Carum carvi* L. (Apiales: Apiaceae)], coriander [*Coriandrum sativum* L. (Apiales: Apiaceae)] and fennel [*Foeniculum vulgare* Miller (Apiales: Apiaceae)], is an ancient cultivar originated from the eastern Mediterranean Basin. Anise is cultivated in countries such as China, Egypt, Greece, India, Russia, Spain, Syria and Türkiye (Demirayak, 2002). In 2019, about 2 Mt of fennel, star anise and coriander was harvested from about 2 Mha globally, with an average yield of 0.95 t ha^{-1} (FAOSTAT, 2019). The countries with the highest production areas were India, Syria, Türkiye, Russia and China (in decreasing order of production) (FAOSTAT, 2019). In Türkiye, 10.7 kt of anise was produced on 15 kha, according to 2020 data, and the average yield is 0.69 t ha^{-1} (TUIK, 2021). Afyonkarahisar is one of the main anise growing provinces of Türkiye (TUIK, 2021).

In the production of anise, different diseases, organisms, nematodes and various weeds can cause significant losses (Anonymous, 2008). However, among these factors plant parasitic nematodes (PPNs) are very important pests but their damage is mostly confused with the symptoms of other factors or can be misidentified as nutrient deficiencies (Singh & Phulera, 2015). Globally, studies on anise have been limited. *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Tylenchida: Heteroderidae) has only reported in anise production areas in Nepal (Bhardwaj & Hogger, 1984). There are various studies on other medicinal aromatic plants such as cumin, fennel and coriander. In the cumin production areas of India, it was found that these areas were infested with *Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida: Pratylenchidae), *Hoplolaimus indicus* Sher, 1963 (Tylenchida: Hoplolaimidae) and *Meloidogyne incognita* (Kofoid & White, 1919) (Tylenchida: Heteroderidae) (Kant et al., 2017). In another study, *M. incognita* was found in fennel production areas in Egypt (Ibrahim & Mokbel, 2009). Similarly, fennel production areas in Iran, PPNs in the genera *Meloidogyne*, *Helicotylenchus*, *Tylenchorhynchus*, *Tylenchus* and *Xiphinema* was reported (Nasresfahani et al., 2015). In a study conducted on the coriander in Pakistan, *Tylenchorhynchus annulatus* (Cassidy, 1930) Golden, 1971 (Tylenchida: Belonolaimidae), *Hoplolaimus pararobustus* (Schuurmans Stekhoven & Teunissen, 1938) Sher, 1963 (Tylenchida: Hoplolaimidae) and *Xiphinema* sp. were detected (Khan et al., 2021).

In Türkiye there is only one report of PPNs in anise production areas and 15 plant parasitic nematode species, including *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936 (Tylenchida: Anguinidae), *Helicotylenchus dihystera* (Cobb, 1893) Sher, 1961 (Tylenchida: Hoplolaimidae), *M. arenaria* and *Pratylenchus zeae* Graham, 1951 (Tylenchida: Pratylenchidae) were identified (Kepenekci, 2003). Also, in fennel, another important plant of the Apiaceae family, 10 PPNs have been identified, including *P. thornei*, *P. zeae* and *H. dihystera* (Evlice & Kepenekci, 2006). Crop rotation is extensively practiced in anise production in Türkiye and cereals are generally used in the rotational crop. *Pratylenchoides alkani* Yüksel, 1977, *Pratylenchus crenatus* Loof (1960) (Tylenchida: Pratylenchidae), *Pratylenchus neglectus* (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941 (Tylenchida: Pratylenchidae), *P. thornei* and *Pratylenchus vulnus* Allen & Jensen, 1951 (Tylenchida: Pratylenchidae) have been reported in cereal growing fields of Türkiye (Söğüt et al., 2011; Yavuzaslanoğlu et al., 2012; Kasapoğlu Uludamar et al., 2018; Dababat et al., 2019; Yavuzaslanoğlu et al., 2020; Göze Özdemir et al., 2021). Therefore, identification of PPNs is needed for anise fields. This study was conducted in Bolvadin District of Afyonkarahisar Province which has an important province in anise growing in Türkiye.

Materials and Methods

Sampling

To detect harmful PPNs in anise, sampling was done from anise areas in Bolvadin District, where production is made within the scope of organic or good agriculture in June 2021 (Figures 1 & 2). The samples were taken with a shovel along zigzag transects in the fields. Each sample consisted of 5-10 spade slices (3 cm thick, 15-20 cm deep and 15 cm wide). Forty-two samples were taken in total. Global positioning system coordinates of the sampled sites are given in Table 1.

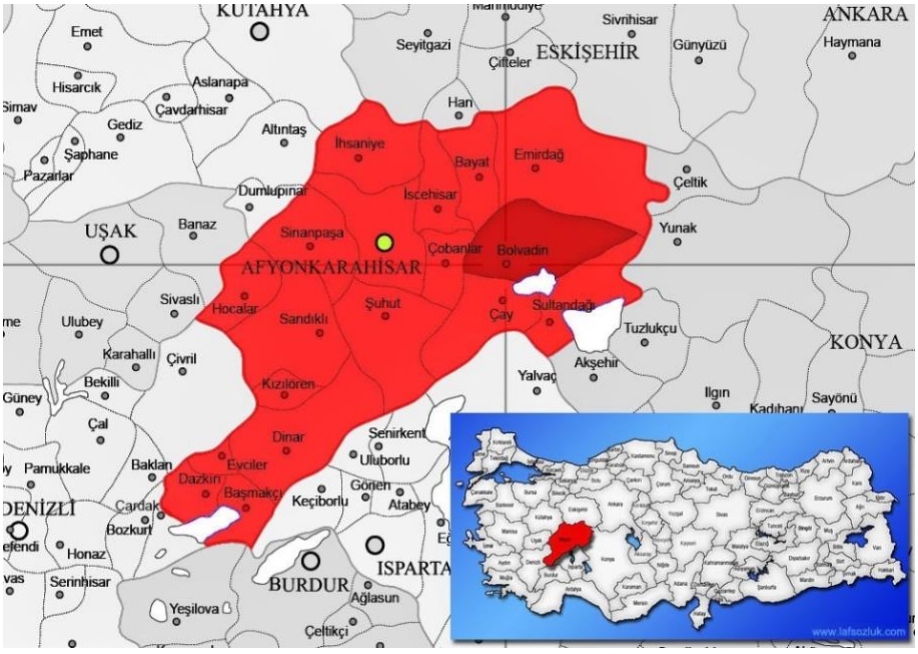

Figure 1. Location of the Bolvadin District where the samples were collected for this study (Anonymous, 2021).

Figure 2. A typical anise crop in Bolvadin District.

Table 1. Morphological-molecular analysis results and location information of samples obtained from anise growing areas in Bolvadin, Türkiye

Sample codes	Coordinates	Morphologic results *	Mi **(MincF/R)	Mi** (INK14F/R)	Mj** (Fjav/Rjav)	Ma** (Far/Rar)	Mh** (JMV1/JMV2/JMVHapla)	Pn**(PNEG/D3B)	Pn** (PNEGF1/D3B5)	Pp** (PPENA/A28)	Pt** (PTHO/D3B)	Pt** (18sInt/26sInt)	Hf**(HfITS-F1HfITS-R1)	Ha** (HalTS-F6/ R4)	Hl** (Hlat-actF/R)	Ab** (AbF5/AbR5)	Af** (AfragF1/R1)	Ar** (BSF/ArtR)
A-1	38º45'50" / 31º16'01"	M, P, A	-	-	-	-	+	-	+	-	-	+	-	-	-	+	-	-
A-2	38º45'47" / 31º16'02"	M, P, A	-	-	-	-	+	-	+	-	-	+	-	-	-	+	-	-
A-3	38º45'46" / 31º16'00"	M, P	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-
A-5	38º47'39" / 31º16'27"	No PPN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A-6	38º47'52" / 31º17'21"	M, P, A	-	-	-	-	+	-	+	-	-	+	-	-	-	+	-	-
A-7	38º47'53" / 31º17'19"	No PPN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A-8	38º47'52" / 31º17'16"	No PPN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A-9	38º47'54" / 31º17'15"	M	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
A-10	38º47'56" / 31º17'15"	M, P	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-
A-11	38º44'29" / 31º13'33"	P	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
A-12	38º44'28" / 31º13'32"	M, P	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
A-13	38º47'34" / 31º16'43"	M, P	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
A-14	38º47'38" / 31º16'44"	P	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
A-15	38º45'46" / 31º16'00"	M, P	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-
A-16	38º47'41" / 31º18'02"	P	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
A-17	38º47'46" / 31º18'04"	M, P	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
A-18	38º47'44" / 31º18'07"	M, A	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-
A-19	38º48'08" / 31º20'00"	No PPN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A-20	38º48'05" / 31º20'03"	No PPN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A-21	38º48'03" / 31º20'05"	No PPN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A-22	38º47'08" / 31º16'26"	P	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
A-23	38º48'43" / 31º20'59"	P	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
A-24	38º48'41" / 31º20'56"	M, P	-	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-
A-25	38º48'43" / 31º20'55"	P	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-
A-26	38º48'44" / 31º21'00"	M, P	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
A-27	38º49'00" / 31º21'17"	M, P	-	-	-	-	+	-	+	-	+	+	-	-	-	-	-	-
A-28	38º48'57" / 31º21'20"	M	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
A-30	38º49'24" / 31º21'43"	M	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
A-31	38º49'21" / 31º21'47"	M, P	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
A-32	38º49'16" / 31º21'50"	M, P	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
A-33	38º47'52" / 31º17'16"	M, P	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
A-34	38º49'14" / 31º21'49"	M, P	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-
A-35	38º49'19" / 31º21'55"	No PPN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A-36	38º49'21" / 31º21'57"	M, P	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-
A-37	38º49'23" / 31º21'53"	M, P	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
A-38	38º49'45" / 31º22'06"	P	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-

Table 1. Continued

Sample codes	Coordinates	Morphologic results *	Mi **(MincF/R)	Mi** (INK14F/R)	Mj** (Fjav/Rjav)	Ma** (Far/Rar)	Mh** (JMV1/JMV2/JMVHapla)	Pn**(PNEG/D3B)	Pn** (PNEGF1/D3B5)	Pp** (PPENA/A28)	Pt** (PTHO/D3B)	Pt** (18sInt/26sInt)	Hf**(HfITS-F1HfITS-R1)	Ha** (HaITS-F6/ R4)	Hl** (Hlat-actF/R)	Ab** (AbF5/AbR5)	Af** (AfragF1/R1)	Ar** (BSF/ArtR)
A-41	38º49'58" / 31º22'14"	No PPN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A-42	38º50'00" / 31º22'17"	P	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
A-43	38º50'00" / 31º22'19"	P	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
A-46	38º49'55" / 31º22'22"	M	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
A-47	38º49'54" / 31º22'25"	M	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
A-50	38º49'52" / 31º22'32"	No PPN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mi[1]	-	*M. incognita*	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mj[1]	-	*M. javanica*	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Ma[1]	-	*M. arenaria*	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Mh[2]	-	*M. hapla*	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Pn[2]	-	*P. neglectus*	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
Pp[2]	-	*P. penetrans*	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Pt[2]	-	*P. thornei*	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
Hf[3]	-	*H. filipjevi*	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Ha[3]	-	*H. avenae*	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Hl[3]	-	*H. latipons*	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Ab[4]	-	*A. besseyi*	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Af[5]	-	*A. fragariae*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Ar[5]	-	*A. ritzemabosi*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

* Morphological identification indicates M: *Meloidogyne* spp. P: *Pratylenchus* spp. A: *Aphelenchoides* spp., PPN: Plant parasitic nematodes;

** Mi: *Meloidogyne incognita*; Mj: *M. javanica;* Ma: *M. arenaria*; Mh: *M. hapla*; Pn: *Pratylenchus neglectus*; Pp: *P. penetrans*; Pt: *P. thornei*; Hf: *Heterodera filipjevi*; Ha: *H. avenae*; Hl: *H. latipons*; Ab: *Aphelenchoides besseyi*; Af: *A. fragariae*; Ar: *A. ritzemabosi*;

[1] Positive control from laboratory culture (Devran & Söğüt, 2009);

[2] Positive control from previous study (Sert Celik et al., 2019);

[3] Positive control from Bolu Abant Izzet Baysal University, Türkiye (by Mustafa Imren);

[4] Positive control from previous study (Devran et al., 2017);

[5] Positive control from National Plant Protection Organization, Netherlands (by Gerrit Karssen).

Nematode extraction

Nematodes in the soil samples were extracted by using the modified Baermann funnel technique (Hooper, 1986).

Morphological identification

Plant parasitic nematode species were checked as morphologically in genus level with the stereo binocular microscope according to Jepson (1987), Handoo & Golden (1989) and EPPO (2021).

Molecular identification

DNA isolation

Isolation of total genomic DNAs from nematodes was performed using a High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) following the manufacturer's instructions.

PCR amplification

PPNs were determined using species-specific primers (Table 2). The PCR reactions were carried out on the SimpliAmp™ (Applied Biosystems, San Francisco, CA, USA) using the reaction conditions specified in former studies for different plant parasitic nematode species (Waeyenberge et al., 2000; Zijlstra et al., 2000; Wishart et al., 2002; Al-Banna et al., 2004; Gleason et al., 2008; Troccoli et al., 2008; Yan et al., 2008; Devran et al., 2018). PCR outcomes were analyzed on a 1.5% agarose gel in 1X TAE and visualized with Xpert Green DNA Stain using the Gel iX Imager (Intas Science, Göttingen, Germany).

Results

Morphological identification

Morphological analysis indicated PPNs belonging to the genera *Aphelenchoides, Meloidogyne* and *Pratylenchus* were present in the samples. PPNs were not found in nine samples (Table 1).

Molecular identification

Molecular analysis of the samples were performed with 16 species-specific primer sets that could identify PPNs belonging to *P. thornei*, *P. neglectus*, *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941 (Tylenchida: Pratylenchidae), *Heterodera avenae* Wollenweber, 1924, *Heterodera filipjevi* (Madzhidov, 1981), *Heterodera latipons* Franklin, 1969 (Tylenchida: Heteroderidae), *Aphelenchoides besseyi* Christie, 1942, *Aphelenchoides fragariae* (Ritzema-Bos, 1891) Christie, 1932, *Aphelenchoides ritzemabosi* (Schwartz, 1911) Steiner & Buhrer, 1932 (Aphelenchida: Aphelenchoididae), *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, *M. incognita*, *M. arenaria* and *Meloidogyne hapla* Chitwood, 1949 (Tylenchida: Heteroderidae) (Table 2).

Both PTHO/D3B and 18sInt/26sInt primer sets were used to identify *P. thornei*. DNA fragments were obtained of about 288 bp and 828 bp, respectively (Table 2). *Pratylenchus thornei* was detected in 15 samples in total (Table 1). DNA fragments of about 150 bp and 300 bp were obtained with primer sets PNEGF1/D3B5 and PNEG/D3B, respectively, which were used for identification of *P. neglectus,* which was found in 22 samples (Table 1). The PPENA/AB28 primer sets (Table 2) were used for the detection of *P. penetrans*, however, no DNA band was obtained in the samples analyzed.

In all identification of samples species specific primer sets were used, HaITS-F6/R4, Hlat-act F/R, Hf ITS-F1/R1 that identified *H. avenae*, *H. latipons* and *H. filipjevi* positive controls respectively; however, primers did not give any DNA fragments from the samples assayed (Table 1).

To detect foliar nematodes, primer sets AbF5/AbR5, BSF/ArtR and AfragF1/AfragR1 were used to identify *A. besseyi*, *A. ritzemabosi* and *A. fragariae*, respectively (Table 2), with only *A. besseyi* detected in three samples (Table 1).

Species-specific primers MincF/MincR-Inck14F/Inck14R, Far/Rar and Fjav/Rjav were used for identification of *M. incognita*, *M. arenaria* and *M. javanica,* respectively. However, these root-knot nematodes (RKNs) were not detected in the samples assayed (Table 1). Samples were assayed with JMV1, JMV2 and JMV hapla primer sets (Table 2). *Meloidogyne hapla* was detected in 24 of the 42 samples (Tables 1 & 2).

Distribution of PPNs

Meloidogyne hapla, *P. neglectus*, *P. thornei* and *A. besseyi* were detected at the rates of 57, 52, 36 and 7%, respectively, in the anise production areas in Bolvadin District. *Meloidogyne hapla* was the most prevalent species and *A. besseyi* was the least common in sampled areas. Similarly, *P. thornei* and *P. neglectus* were also determined a moderate number of sampling sites. In addition, these species were found as mixed populations in the fields. PPNs were mixed in 50% of the samples analyzed (Table 1).

Table 2. Species-specific primer pairs used in molecular analyses of samples obtained from anise production areas in Bolvadin, Türkiye

Species	Primer name	Amplicon (s) size (bp)	References
Meloidogyne incognita	IncK14-F / IncK14-R	~399	Randig et al., 2002
	MincF / MincR	~150	Devran et al., 2018
Meloidogyne javanica	Fjav / Rjav	~670	Zijlstra et al., 2000
Meloidogyne arenaria	Far / Rar	~420	Zijlstra et al., 2000
Meloidogyne hapla	JMV1 / JMV2 / JMVhapla	~440	Wishart et al., 2002
Aphelenchoides besseyi	AbF5 / AbR5	~340	Devran et al., 2017
Aphelenchoides fragariae	AFragF1 / AFragR1	~169	McCuiston et al., 2007
Aphelenchoides ritzemabosi	BSF / ArtR	~208	Cui et al., 2010
Heterodera filipjevi	HfITS-F1 / HfITS-R1	~170	Yan et al., 2013
Heterodera avenae	HaITS-F6 / HaITS-R4	~242	Yan et al., 2013
Heterodera latipons	Hlat-actF / Hlat-actR	~204	Toumi et al., 2013
Pratylenchus neglectus	PNEG / D3B	~290	Al-Banna et al., 2004
	PNEGF1 / D3B5	~144	Yan et al., 2008
Pratylenchus penetrans	PPENA / AB28	~660	Waeyenberge et al., 2000
Pratylenchus thornei	PTHO / D3B	~288	Al-Banna et al., 2004
	18sInt / 26sInt	~828	Troccoli et al., 2008

Discussion

Medicinal aromatic plants are unique plants that have many uses such as food, spice, medicine and cosmetics and are known to have been used for similar purposes since the beginning of humanity (Ullah et al., 2015). Some of these plants can be collected directly from nature, while others are cultivated professionally. Anise is known as the most important medicinal and aromatic plants cultivated for using agricultural and cosmetic industries (Demirayak, 2002). However, many pests and diseases can cause yield losses in anise including PPNs, the damage of which can be confused with nutrient deficiency (Anonymous, 2008; Singh & Phulera, 2015). Morphological and morphometric identifications of PPNs are time-consuming and require expertise. In addition, mixed plant parasitic nematode species can be found in agricultural production areas. Therefore, rapid, correct and easier identification of plant parasitic nematode species in production areas is important for the management of these pests. For these reasons, molecular identification techniques can be used intensively to identify the pests in question. In this study, species-specific primer sets were used to detect economically important RKNs, only *M. hapla* was detected in anise production areas. Adam et al. (2007) reported that *M. incognita*, *M. arenaria* and *M. javanica* are common in tropical regions, while *Meloidogyne chitwoodi* Golden, O'Bannon, Santo & Finley, 1980 (Tylenchida: Heteroderidae), *M. hapla* and *Meloidogyne fallax* Karssen, 1996 (Tylenchida: Heteroderidae) are mostly distributed in cooler areas. Our sampling area has an elevation of 995-1100 m and is a relatively cooler area. Therefore, our results are consistent with previous reports on the geographical distribution of *Meloidogyne* spp. However, *M. arenaria* was reported in anise production areas in Nepal (Bhardwaj & Hogger, 1984). Also, in a study conducted in Pakistan, *M. javanica* and *M. incognita* were determined in

about 12% of the production areas of coriander from the same family as anise (Anwar & McKenry, 2010). Similarly, Singh & Gupta (2011) show that RKNs (*M. incognita* and *M. javanica*) were detected in coriander and ginger production areas in India. The reason for the differences may be climatic conditions.

In Türkiye, no previous work has been done on the identification of PPNs in the areas surveyed. However, a study was conducted in anise production areas in Burdur, which is also located in the Lakes Region of Türkiye, 15 PPN species were morphologically determined (Kepenekci, 2003). However, cereals are used for rotation in anise fields in Türkiye. Some studies were conducted to identify PPNs in cereal growing areas. *Pratylenchus thornei* and *P. neglectus* were reported as mixed populations (Sahin et al., 2008; Yavuzaslanoğlu et al., 2012; Göze Özdemir et al., 2021). In the present study, *P. neglectus* and *P. thornei* were found as mix populations in the area sampled. Our results were consistent with previous studies. The population densities and prevalence of PPNs may be due to variations in crop rotation and soil conditions (such as humidity and temperature) (Wallace et al., 1993; Yavuzaslanoğlu et al., 2020). In cereal production fields of Türkiye, *H. filipjevi*, *H. latipons* and *H. avenae* can cause significant crop damage (Imren et al., 2012; Yavuzaslanoğlu et al., 2012, Dababat et al., 2015). However, these species were not found in the anise growing areas surveyed. It is thought that the reason why *Heterodera* spp. could not be found in the samples examined may be due to the sampling time. *Aphelenchoides besseyi* is reported to occur in rice fields of Türkiye (Oztürk & Enneli, 1997; Tülek & Cobanoğlu, 2010). Recently, studies were conducted on molecular identification of *A. besseyi* and estimation of the number of it in paddy rice (Devran et al. 2017; Sert Celik & Devran, 2019; Sert Celik et al., 2020). In this study, *A. besseyi* was identified for the first time in anise production areas of Türkiye. However, there is no published information about the suitability of anise as a host for foliar nematodes (*Aphelenchoides* spp.) (CABI, 2021; Nemaplex, 2021; EPPO, 2021). However, *Avena sativa* L. (Poales: Poaceae) is a known host for *A. besseyi* (Nemaplex, 2021). In Türkiye, oats can be grown in rotation with anise in the area surveyed. Therefore, this nematode may have originated from oats.

In conclusion, this is the first study on the identity of PPNs in anise growing areas in Afyonkarahisar Province of Türkiye. These results could prove useful for integrated pest management practices and crop rotation to decrease the yield loses and increase the quality in anise growing areas.

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References

Adam, M. A. M., M. S. Phillips & V. C. Block, 2007. Molecular diagnostic key for identification of single juveniles of seven common and economically important species of root-knot nematode (*Meloidogyne* spp.). Plant Pathology, 56 (1): 190-197.

Al-Banna, L., A. T. Ploeg, W. M. Williamson & I. Kaloshian, 2004. Discrimination of six *Pratylenchus* species using PCR and species specific primers. Journal of Nematology, 36 (2): 142-146.

Anonymous, 2008. Zirai Mücadele Teknik Talimatları Cilt 2. Gıda, Tarım ve Hayvancılık Bakanlığı Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü, Ankara, Türkiye, 175 pp (in Turkish).

Anonymous, 2021. Bolvadin neredadir nereye bağlıdır? Bolvadin hangi ilin ilçesidir? (Web page: https: //www.lafsozluk.com/2009/01/bolvadin-neredadir-nereye-baglidir.html) (Date accessed: March 2021) (in Turkish).

Anwar, S. A. & M. V. McKenry, 2010. Incidence and reproduction of *Meloidogyne incognita* on vegetable crop genotypes. Pakistan Journal of Zoology, 42 (2): 135-141.

Bhardwaj, L. N & C. H. Hogger, 1984. Root-knot nematodes of Chitwan district of Nepal. Nematologia Mediterranea, 12 (1): 155-158.

CABI, 2021. Invasive species compendium, *Aphelenchoides besseyi* (rice leaf nematode). (Web page: www.cabi.org/isc/datasheet/6378#tohostPlants) (Date accessed: March 2021).

Cui, R. Q., L. R. Zhao & G. Q. Zhong, 2010. A rapid method to detect *Aphelenchoides ritzemabosi* by PCR. Acta Agriculturae Universitatis Jiangxiensis, 32 (4): 714-717.

Dababat, A. A., M. Imren, G. Erginbas-Orakci, S. Ashrafi, E. Yavuzaslanoglu, H. Toktay, S. Pariyar, H. I. Elekcioglu, A. Morgounov & T. Mekete, 2015. The importance and management strategies of cereal cyst nematodes, *Heterodera* spp., in Turkey. Euphytica, 202 (2): 173-188.

Dababat, A. A., F. Mokrini, S. E. Laasli, S. Yildiz, G. Erginbas-Orakci, N. Duman & M. Imren, 2019. Host suitability of different wheat lines to *Pratylenchus thornei* under naturally infested field conditions in Turkey. Nematology, 21 (6): 557-571.

Demirayak, Ş., 2002. Bazı Anason (*Pimpinella anisum* L.) Populasyonlarında Farklı Ekim Zamanlarının Verim ve Verim Öğeleri Üzerine Etkileri. Ankara Üniversitesi, Fen Bilimleri Enstitüsü, (Unpublished) Yüksek Lisans Tezi, Ankara, Türkiye, 45 s (in Turkish with abstract in English).

Devran, Z., I. Polat, I. Mıstanoğlu & O. Baysal, 2018. A novel multiplex PCR tool for simultaneous detection of three root-knot nematodes. Australasian Plant Pathology, 47 (4): 1-4.

Devran, Z. & M. A. Söğüt, 2009. Distribution and identification of root-knot nematodes from Turkey. Journal of Nematology, 41 (2): 128-133.

Devran, Z., A. Tülek, I. Mıstanoğlu, T. H. Çiftçigil & T. Ozalp, 2017. A rapid molecular detection method for *Aphelenchoides besseyi* from rice tissue. Australasian Plant Pathology, 46 (1): 43-48.

EPPO, 2021. EPPO Global Database, *Aphelenchoides besseyi*. (Web page: gd.eppo.int/taxon/APLOBE/hosts) (Date accessed: February 2021).

Evlice, E. & I. Kepenekci, 2006. Preliminary list of Tylenchida (Nematoda) associated with fennel flower (*Nigella* L.) in Turkey. Pakistan Journal of Nematology, 24 (1): 121-123.

FAOSTAT, 2019. Crops and livestock products. (Web page: www.fao.org/faostat/en/#data/QCL) (Date accessed: February 2019).

Ghorbanpour, M., J. Hadian, S. Nikabadi & A. Varma, 2017. "Importance of Medicinal and Aromatic Plants in Human Life, 1-23". In: Medicinal Plants and Environmental Challenges (Eds. M. Ghorbanpour & A. Varma). Switzerland, Springer International Publishing, 413 pp.

Gleason, C. A., Q. L. Liu & V. M. Williamson, 2008. Silencing a candidate nematode effector gene corresponding to the tomato resistance gene *Mi-1* leads to acquisition of virulence. Molecular Plant-Microbe Interactions, 21 (5): 576-585.

Göze Özdemir, F. G., B. Yaşar & I. H. Elekcioğlu, 2021. Distribution and population density of plant parasitic nematodes on cereal production areas of Isparta and Burdur provinces of Turkey. Turkish Journal of Entomology, 45 (1): 53-64.

Handoo, Z. A. & A. M. Golden, 1989. A key and diagnostic compendium to the species of the genus *Pratylenchus* Filipjev 1936 (Lesion Nematodes). Journal of Nematology, 21 (2): 202-218.

Hooper, D. J., 1986. "Extraction of Free-Living Stages from Soil, 5-30". In: Laboratory Methods for Work with Plant and Soil Nematodes (Eds. J. F. Southey). Her Majesty's Stationery Office, London, 202 pp.

Ibrahim, I. K. A. & A. A. Mokbel, 2009. Occurrence and distribution of the root-knot nematodes *Meloidogyne* spp. and their host plants in northern Egypt. The Egyptian Journal of Experimental Biology (Botany), 5: 125-129.

Imren, M., L. Waeyenberge, N. Viaene, H. Toktay, A. Dababat & I. H. Elekcioglu, 2012. Molecular characterization of cereal cyst nematodes from South Anatolian Region in Turkey using ITS-rDNA sequences. Turkish Journal of Entomology, 36 (4): 491-499.

ITIS, 2021. Integrated Taxonomic Information System-Report. (Web page: www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=29822) (Date accessed: June 2021).

Jepson, S. B., 1987. Identification of Root-knot nematodes (*Meloidogyne* species), 1st Edition. CAB International, Wallingford, 265 pp.

Kant, K., B. Singh, Y. K. Sharma, S. R. Meena, R. K. Solanki, B. K. Mishra, N. K. Meena & A. U. Siddiqui, 2017. Diversity of nematodes population in cumin (*Cuminum cyminum*) crop in semi-Arid regions of Rajasthan, India. Indian Journal of Agricultural Sciences, 87 (12): 1664-1667.

Karık, U., 2020. Türkiye anason (*Pimpinella anisum* L.) genetik kaynaklari ve yabanci anason genotiplerinin uçucu yağ bileşenleri. Anadolu Journal of Aegean Agricultural Research Institute, 30 (2): 163-178 (in Turkish with abstract in English).

Kasapoğlu Uludamar, E. B., S. Yıldız, M. Imren, A. Ocal & I. H. Elekçioğlu, 2018. Occurrence of plant parasitic nematode species in important crops in the Southeast Anatolia Region of Turkey. Turkish Journal of Entomology, 42 (1): 63-74.

Kepenekci, I., 2003. Preliminary list of Tylenchida (Nematoda) associated with anise (*Pimpinella anisum* L.) in Turkey. Pakistan Journal of Nematology, 21 (1): 37-40.

Keskin, S. & H. Baydar, 2016. Umbelliferae familyasından bazı önemli kültür türlerinin Isparta ekolojik koşullarında tarımsal ve teknolojik özelliklerinin belirlenmesi. Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 20 (1): 133-141 (in Turkish with abstract in English).

Khan, A., K. A. Khanzada, S. A. Sheikh, S. S. Shaukat & J. Akhtar, 2021. Nematodes of coriander (*Coriandrum sativum* L.) and their management using a newly developed plant-based nematicide. International Journal of Biology and Biotechnology, 18 (1): 119-122.

McCuiston, J. L., L. C. Hudson, S. A. Subbotin, E. L. Davis & C. Y. Warfield, 2007. Conventional and PCR detection of *Aphelenchoides fragariae* in diverse ornamental host plant species. Journal of Nematology, 39 (4): 343-355.

Nasresfahani, M., A. Mohsenzadeh Kermani, M. Zargani & M. Alizadeh, 2015. Evaluation nematode of some medicinal plants in Isfahan. Indian Journal of Fundamental and Applied Life Sciences, 5 (S1): 2665-2674.

Nemaplex, 2021. Host range of a genus and species of plant-feeding nematodes. (Web page: http: //nemaplex.ucdavis.edu/Nemabase2010/NematodePageHostRangeResults.aspx?NgenusNspec=Aphelenchoides%20besseyi) (Date accessed: March 2021).

Orav, A., A. Raal & E. Arak, 2008. Essential oil composition of *Pimpinella anisum* L. fruits from various European countries. Natural Product Research, 22 (3): 227-232.

Oztürk, G. & S. Enneli, 1997. Determination of *Aphelenchoides besseyi* (Aphelenchida: Aphelenchoididae). The white tip nematode, harmful on rice for the first time in Turkey. Turkish Journal of Entomology, 21 (2): 129-132.

Randig, O., M. Bongiovanni, R. M. D. G. Carneiro & P. Castagnone-Sereno, 2002. Genetic diversity of root-knot nematodes from Brazil and development of SCAR markers specific for the coffee damaging species. Genome, 45 (5): 862-870.

Sahin, E., J. M. Nicol, A. Yorgancilar, I. H. Elekcioglu, A. Tülek, A. F. Yıldırım & N. Bolat, 2008. Seasonal variation of field population of *Heterodera filipjevi*, *Pratylenchus thornei* and *P. neglectus* on winter wheat in Turkey. Nematologia Mediterranea, 36 (1): 51-56.

Sert Celik, E. & Z. Devran, 2019. Identification and quantification of *Aphelenchoides besseyi* from rice using qPCR. European Journal of Plant Pathology, 154 (3): 691-703.

Sert Celik, E., T. Özalp, I. Mıstanoğlu & Z. Devran, 2019. Identification of plant-parasitic nematodes associated with cut flowers. Journal of Plant Diseases & Protection, 126 (5): 409-420.

Sert Celik, E., A. Tülek & Z. Devran, 2020. Development of a novel scale based on qPCR for rapid and accurate prediction of the number of *Aphelenchoides besseyi* in paddy rice. Crop Protection, 127: 104975.

Shojaii, A. & F. M. Abdollahi, 2012. Review of pharmacological properties and chemical constituents of *Pimpinella anisum*. Journal of Medicinal Plants, 11 (41): 22-33.

Singh, V. K. & R. K. Gupta, 2011. First report of *Meloidogyne javanica* on ginger and *Meloidogyne incognita* on coriander in Jammu and Kashmir (India). Journal of Horticultural Sciences, 6 (1): 74-75.

Singh, R. & S. Phulera, 2015. “Plant Parasitic Nematodes: The Hidden Enemies of Farmers, 68-81”. In: Environmental Issues for Socio-Ecological Development (Eds. S. Yadav & R. Singh). Excel India Publishers, India, 115 pp.

Söğüt, M. A., T. Yılmaz, F. G. Göze, Z. Devran & I. H. Elekcioğlu, 2011. “Migratory endoparasitic nematodes of wheat cultivation in Lakes Region, 496”. IV. Plant Protection Congress of Turkey (28-30 June 2011; Kahramanmaraş, Türkiye) (in Turkish with abstract in English).

Toumi, F., L. Waeyenberge, N. Viaene, A. Dababat, J. M. Nicol, F. Ogbonnaya & M. Moens, 2013. Development of a species-specific PCR to detect the cereal cyst nematode, *Heterodera latipons*. Nematology, 15 (6): 709-717.

Troccoli, A., F. De Luca, Z. A. Hando & M. Di Vito, 2008. Morphological and molecular characterization of *Pratylenchus lentis* n. sp. (Nematoda: Pratylenchidae) from Sicily. Journal of Nematology, 40 (3): 190-196.

TUIK, 2021. Crop production statistics. (Web page: https: //biruni.tuik.gov.tr/medas/?kn=92&locale=tr) (Date accessed: June 2021).

Tülek, A. & S. Cobanoğlu, 2010. Distribution of the rice white tip nematode, *Aphelenchoides besseyi*, in rice growing areas in Thrace region of Turkey. Nematologica Mediteranea, 38 (2): 215-217.

Ullah, H., A. Mahmood, M. I. Awan & B. Honermeier, 2015. Effect of row spacing and rate on fruit yield, essential oil and composition of anise (*Pimpinella anisum* L.). Pakistan Journal of Agricultural Sciences, 52 (2): 349-357.

Waeyenberge, L., A. Ryss, M. Moens, J. Pinochet & T. Vrain, 2000. Molecular characterization of 18 *Pratylenchus* species using rDNA restriction fragment length polymorphism. Nematology, 2 (2): 135-142.

Wallace, M. K., R. H. Rust, D. M. Hawkins & D. H. Macdonald, 1993. Correlation of edaphic factors with plant-parasitic nematode population densities in forage fields. Journal of Nematology, 25 (4): 642-653.

Wishart, J., M. S. Phillips & V. C. Blok, 2002. Ribosomal Intergenic Spacer: a polymerase chain reaction diagnostic for *Meloidogyne chitwoodi*, *M. fallax*, and *M. hapla*. Nematology, 92 (8): 884-892.

Yan, G. P., R. W. Smiley, P. A. Okubara & A. M. Skantar, 2013. Species-specific PCR assays for differentiating *Heterodera filipjevi* and *H. avenae*. Plant Disease, 97 (12): 1611-1619.

Yan, G. P., R. W. Smiley, P. A. Okubara, A. Skantar, S. A. Easley, J. G. Sheedy & A. L. Thompson, 2008. Detection and discrimination of *Pratylenchus neglectus* and *P. thornei* in DNA extracts from soil. Plant Disease, 92 (11): 1480-1487.

Yavuzaslanoğlu, E., I. H. Elekcioğlu, M. Nicol, O. Yorgancılar & D. Hodson, 2012. Distribution, frequency and occurrence of cereal nematodes on the Central Anatolian Plateau in Turkey and their relationship with soil physicochemical properties. Nematology, 14 (7): 839-854.

Yavuzaslanoğlu, E., M. S. Karaca, A. Sönmezoğlu, A, Ocal, I. H. Elekcioğlu & M. Aydoğdu, 2020. Occurrence and abundance of cereal nematodes in Konya and Karaman province in Turkey. Turkish Journal of Entomology, 44 (2): 223-236.

Zijlstra, C., D. T. H. M. Donkers-Venne & M. Fargette, 2000. Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterized amplified region (SCAR) based PCR assays. Nematology, 2 (8): 847-853.

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Original article (Orijinal araştırma)

A rare and endemic species distributed in the Black Sea Region of Türkiye with first description of its female: *Agatharchus ponticus* Belousova, 1999 (Hemiptera: Heteroptera: Pentatomidae)

Türkiye'nin Karadeniz Bölgesi'nde yayılış gösteren nadir ve endemik bir tür ve dişinin ilk tanımı: *Agatharchus ponticus* Belousova, 1999 (Hemiptera: Heteroptera: Pentatomidae)

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Abstract

During a study conducted in Amasya and Çorum Provinces of Türkiye between 2020-2021, the endemic species *Agatharchus ponticus* Belousova, 1999 (Hemiptera: Heteroptera: Pentatomidae) was recorded for the first time in the Black Sea Region. The female of the species, whose original description was based on a male specimen, is described here for the first time. In addition, new locality information has been added to the distribution area of the species, which have previously been known to be rare in Anatolia, and male and female genitalia with photographs are given to verify the identification of the species.

Keywords: *Agatharchus ponticus,* endemic, female description, Türkiye

Öz

Amasya ve Çorum illerinde 2020-2021 yılları arasında yapılan çalışmada, endemik bir tür olan *Agatharchus ponticus* Belousova, 1999 (Hemiptera: Heteroptera: Pentatomidae), Karadeniz Bölgesi'nde ilk kez kaydedilmiştir. Tek erkek örneğe dayanılarak orijinal tanımı yapılmış olan türün dişisine ait ilk tanımlama bu çalışmada verilmiştir. Ayrıca daha önce Anadolu'da nadir olduğu bilinen türün yayılış alanına yeni lokalite bilgileri eklenmiş, erkek ve dişiye ait genital organ fotoğrafları verilerek türün ayırt edici karakterleri ortaya konmuştur.

Anahtar sözcükler: *Agatharchus ponticus,* endemik, dişi tanımı, Türkiye

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Introduction

The suborder Heteroptera (Hemiptera) is currently known to be represented by more than 45,000 described species in more than 10 subfamilies globally, and more than 9,365 described species belonging to 1,632 genera are distributed in the Palaearctic Region (Aukema et al., 2013; Henry, 2017; Rider et al., 2018). The species of family Pentatomidae are known from all terrestrial biomes except Antarctica and it has 940 genera and 4,949 species belonging to 10 subfamilies (Rider et al., 2018). Pentatomidae is the third largest family of the suborder Heteroptera includes four subfamilies, 219 genera, 841 species and 19 subspecies in the Palaearctic and 61 genera and 174 species/subspecies in Türkiye (Henry, 2017; Fent & Dursun, 2022). However, Roca-Cusachs et al. (2021) reported that some tribes belonging to the subfamilies Podopinae and Pentatominae are not monophyletic. The recent studies in Türkiye revealed presence of 14 species from nine genera of Asopinae, 125 species from 39 genera of Pentatominae, one species from one genus of Phyllocephalinae and 34 species from 12 genera of Podopinae. Among these species, the type localities of 37 species, of which 15 are endemic, from 16 genera of Pentatomidae were given in Türkiye (Lodos et al., 1978, 1998; Önder et al., 1981, 1984, 2006; Lodos & Önder, 1983; Ahmad & Önder, 1990; Belousova, 1999; Fent & Aktaç, 1999; Tezcan & Önder, 1999, 2003; Awad, 2000; Awad & Pehlivan, 2001; Beyaz & Tezcan, 2002; Kıvan, 2004; Kıyak et al., 2004, 2019; Kment & Jindra, 2005; Özgen et al., 2005a,b; Bolu et al., 2006; Fent & Aktaç, 2007; Külekçi et al., 2009; Dursun & Fent, 2010, 2013, 2015, 2017, 2018; Fent, 2010; Fent et al., 2010; Matocq et al., 2014; Yazıcı et al., 2014; Çerçi & Koçak, 2017; Çerçi & Gözüaçık, 2019; Çerçi, 2021; Çerçi & Özgen, 2021; Fent & Dursun, 2022).

The Carpocorini Mulsant & Rey, 1866 one of largest tribes of family Pentatomidae are distributed worldwide and 120 species belonging to 29 genera have been identified in the Palaearctic Region and 39 species belonging to 16 genera in Türkiye (Aukema et al., 2013; Fent & Dursun, 2022). The genus *Agatharchus* Stål, 1876 belonging to tribe Carpocorini has two subgenera (*Agatharchus* s. str., and *Afghanotharchus* Belousova, 1999) and 12 species are currently recognized within the genus, all limited to the Palaearctic Region. *Agatharchus* s. str. contains eleven species and *Afghanotharchus* have a single species. A detailed study of the genus *Agatharchus* in Türkiye without *A. ponticus* was given by Awad (2000). Five species of the genus *Agatharchus* have been reported from Türkiye. Of these species, the type localities of *Agatharchus tritaenia* Horváth, 1897, *Agatharchus escalerae* Horváth, 1901 and *Agatharchus ponticus* Belousova, 1999 are located in Türkiye, the latter two species being endemic (Rider, 2006).

Agatharchus ponticus Belousova, 1999 was described from Erzurum-Pazaryolu (Belousova, 1999) based on a male specimen and since then, a male specimen was recorded in Elazığ-Haroğlu by Çerçi & Özgen (2021). One female, from Çorum is described below with the aim of presenting new information about *A. ponticus.*

Material and Methods

The study material was collected between 2020-2021 under *Astragalus* sp. (Fabaceae) in provinces Amasya and Çorum. The male genitalia (pygophore, paramere and aedegus) were used for the species identification. For the preparation of genital organs, the sample was softened in hot water and the genitalia were extracted. Genitalia of male and female were examined using a Leica SZX stereoscopic microscope and body Canon 70D, ring flash, 69 mm. Macrotube, Canon 100 mm. IS USM 2.8L (Figures 1-12). Belousova (1999) and Çerçi & Özgen (2021) were followed in identification of the specimens. The material is deposited in the collection of Amasya University, Faculty of Science and Arts, Department of Biology. In addition, the localities where *A. ponticus* was detected in previous studies and in this study are shown on the map (Figure 13).

Results and Discussion

Pentatomidae Leach, 1815

Pentatominae Leach, 1815

Carpocorini Mulsant & Rey, 1866

***Agatharchus* Stål, 1876**

***Agatharchus (Agatharchus) ponticus* Belousova, 1999**

Material examined. Amasya: Gümüşhacıköy-Maden, 40°52’14" N 35°12’50" E, 810 m, 7.IX.2021, 2♂♂, leg. A. Dursun; Çorum: Osmancık-Sarpunkavak, 40°56’47" N 34°41’47" E, 640 m, 19.X.2020, ♂, N. Akman; Yaylabaşı Bahçeler, 41°02’47" N 35°00’08" E, 1065 m, 8.III.2020, ♀, ♂, leg. N. Akman (det. A. Dursun and M. Fent).

Distribution in Türkiye. Erzurum-Pazaryolu (Belousova, 1999) and Elazığ-Haroğlu (Çerçi & Özgen 2021).

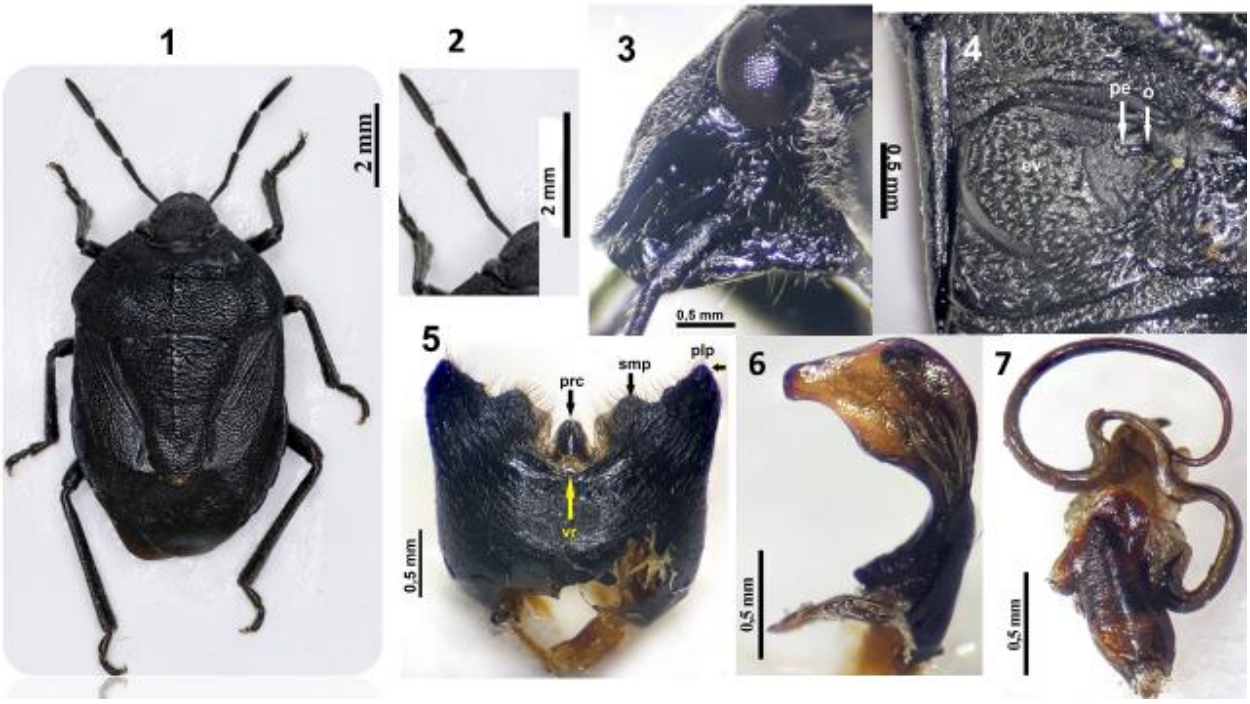
Distribution in Palaearctic Region. Türkiye (Rider, 2006).

Host plant. The specimens were collected under *Astragalus* sp. (Fabaceae).

Redescription of male (Figures 1-7). Surface of body black, rugose and punctures (Figure 1). Clypeus shorter than mandibular plates. Antennae black with short and long yellowish hairs. Lengths of antennomeres I-V (mm): 0.70, 1.0, 0.83, 1.08 and 1.25 (Figure 2). Labium blackish brown, with yellowish hairy and extends to metacoxa. Posterior edge of bucculae protrusive (Figure 3). Median of pronotum with intermittent yellowish carina. Pronotum posteriorly with transverse impression, sublaterally with roundish impression. Scutellum black, posterior area with yellowish callosity. Hemelytra, connexivum and abdominal dorsum black. Thoracic pleuron and sternum blackish brown, abdominal venter black with sparse, short, yellowish hairs. Peritreme of scent gland ostiole short and rounded apically, evaporatorium large, matte and rugose (Figure 4). Legs black, with short yellowish hairs, tibia with both short and sparse long hairs.

Pygophore black with yellowish hairs, the ventral rim (infolding) of pygophore is deeply incised medially, the rounded incision is limited by pair of submedial rectangular projections. Posterolateral projection of pygophore prominent, triangular in outline, distinctly projecting over the submedial projections (Figure 5). Basal plate large. Blade of paramere widely rounded dorsally, towards tip nearly straight; tip of paramere subquadrangular; ventral outline bisinuate. Outer surface of hypophysis with several setae (Figure 6). Apex of the ventro-lateral lobes of the conjunctiva narrowly hooked, vesica appearing as a rather long and curved (Figure 7).

Description of female (Figures 8-12). Surface of body black, rugose and punctured (Figure 8). Clypeus shorter than mandibular plates. Head with gray short hairs. Posterior part of head with yellowish callosity, lateral margins of anteocular part slightly upturned. Antennae black with short and long yellowish hairs. Lengths of antennomeres I-V (mm): 0.80, 1.15, 0.92, 1.28 and 1.22 (Figure 9). Labium blackish brown, labiomere II, yellowish brown with yellowish hairs and extending to metacoxae. Lengths of labiomeres I-IV (mm): 1.70, 1.90, 0.90 and 1.0. Bucculae yellowish brown with short yellowish hairs and posterior edge only slightly protrusive (Figure 10). Surface of pronotum, scutellum, clavus, corium and exocorium with very shallow black punctured. Pronotum medially with intermittent yellowish carina. Pronotal surface posteriorly with transverse impression and with one roundish impression sublaterally on each side. Pronotum with sparse, short gray hairs. Anterior and posterior parts of scutellum with yellowish callosity. Membrane blackish brown, abdominal dorsum black, connexivum blackish brown.



Figures 1-7. *Agatharchus ponticus* male: 1) Dorsal view; 2) Antennae; 3) Bucculae; 4) Evaporatorium surface; 5) Pygophore (ventral view); 6) Paramere; 7) Aedegus (ev: evaporatorium of metathoracic scent gland; o: ostiole; pe: peritreme; prc: proctiger of genital capsule; plp: posterolateral lobes of genital capsule; smp: submedial projection; vr: ventral rim).

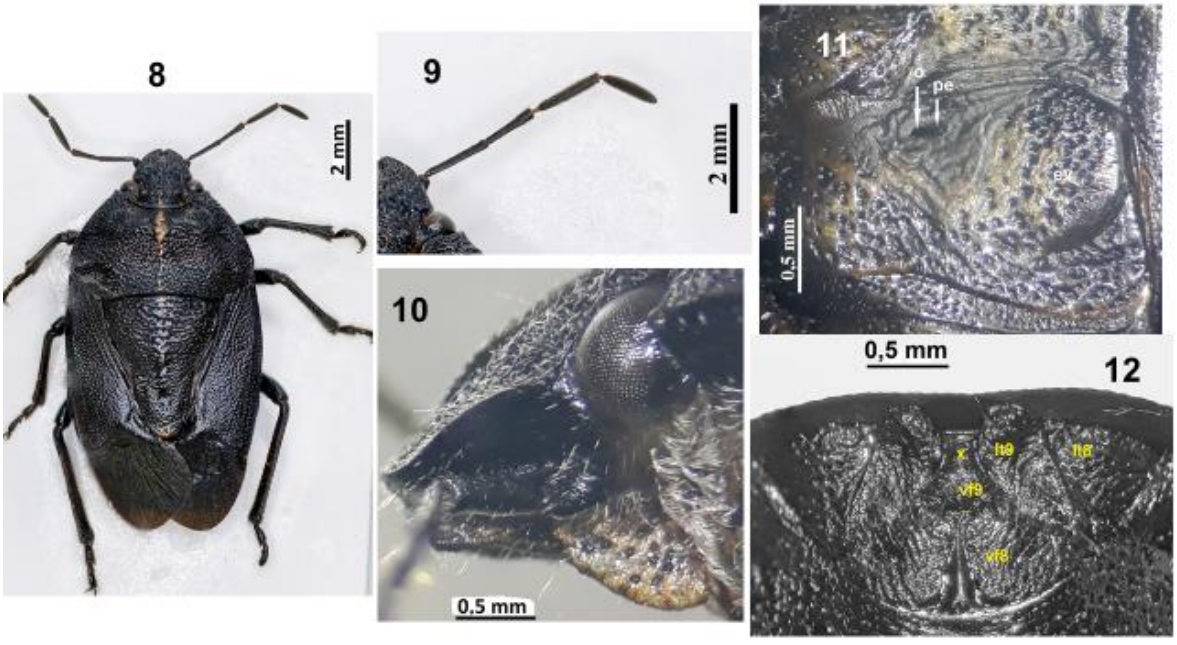
Thoracic pleuron and sternum and abdominal venter blackish brown with very shallow black punctures. Peritreme of scent gland ostiole short and rounded apically, evaporatorium large and folds with prominent, fold-like gyrification, laterally narrowed (Figure 11).

Legs blackish brown with yellowish hairs. Tibia black with yellowish and black hairs, inner surface densely covered with short spines. Tarsus blackish brown with dense hairs. Surface of laterotergites IX and valvifers VIII black, rugose with black hairs. Lateral and posterior margins of valvifers VIII convex, laterotergites IX in apical half parabolic, broadly rounded of external genitalia of female (Figure 12).

Measurements (mm). Total length 11. Length of head 3, width of head 2.9, intraocular width 1.5. Length of pronotum 2.8, width of pronotum 6. Length of scutellum 4.3 and width of scutellum 3.9.

The type locality as well as the second record of *Agatharchus ponticus* were given from the Eastern Anatolia Region from Türkiye (Belousova, 1999; Çerçi & Özgen, 2021).). In the present study, new faunistic record of *A. ponticus* from Black Sea Region are given and the previously unknown female is described. *Agatharchus ponticus* is a rarely distributed and endemic species in Anatolia. It is characterized by the second antennomere 1.2 times as long as the third and by clypeus shorter than mandibular plates in males. In the female, the clypeus is shorter than mandibular plates, but the second antennomere is 1.25 times longer than third. Evaporatorium of metathoracic scent gland of male and female are large, with large fold-like gyrification, laterally narrowed. As reported in the original description of the species based on a single male specimen (holotype) by Belousova (1999), there is no yellowish-white medial longitudinal stripe on pronotum and scutellum (dorsum entirely black). In the second male record of Çerçi & Özgen (2021) from Elazığ, on the other hand, the medial part of pronotum and scutellum bear a continuous yellowish-white stripe. Males reported in the present study lack the median stripe in accordance with Belousova (1999), while only a small yellowish-white callose spot is present apically on scutellum. In the female specimen, anterior half of pronotum bears a distinct pale median stripe (less distinct in posterior part) and scutellum

is both anteriorly and apically bearing yellowish-white callose spot (Figures 1 & 8). Morphological characters of pygophore are given for the first time in this study (Figure 5). The morphological characters of bucculae, vesica and parameres fit with the description of holotype by Belousova (1999).



Figures 8-12. *Agatharchus ponticus* female: 8) Dorsal view; 9) Antennae; 10) Bucculae; 11) Evaporatorium surface; 12) Genitalia. (ev: evaporatorium of metathoracic scent gland; lt8-9, laterotergites 8-9; o: ostiole; pe: peritreme; t8: tergite 8; vf 8-9: valvifers 8-9; x: segment X).

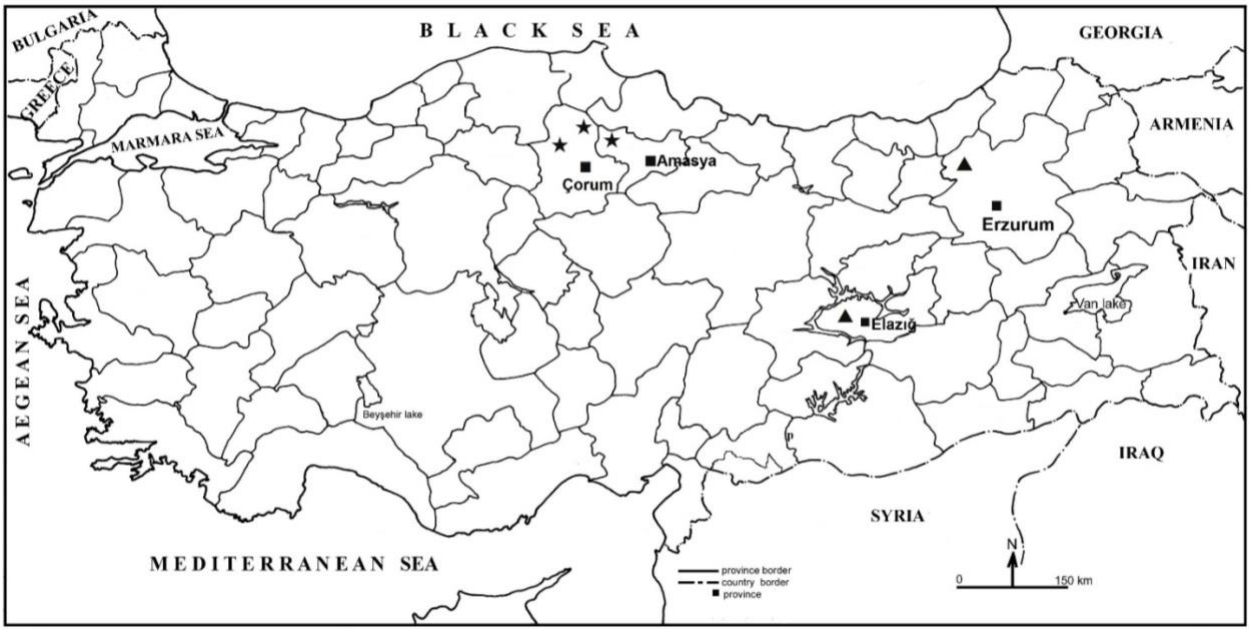


Figure 13. Distribution of *Agatharchus ponticus* in Türkiye (▲, records of previous studies; and ★, this study).

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References

Ahmad, I. & F. Önder, 1990. A revision of the genus *Picromerus* Amyot and Serville (Heteroptera: Pentatomidae: Pentatominae: Asopini) from western Palaearctic with description of the new species from Turkey. Türkiye Entomoloji Dergisi, 14 (2): 75-84.

Aukema, B., Ch. Rieger & W. Rabitsch, 2013. Catalogue of the Heteroptera of the Palaearctic Region. VI. Supplement. The Netherlands Entomological Society, Amsterdam, 629+ xxiii pp.

Awad, T. I., 2000. Türkiye Carpocorini (Heteroptera: Pentatomidae: Pentatominae) Türleri Üzerinde Sistematik ve Faunistik Araştırmalar. Ege University, (Unpublished) PhD Thesis, İzmir, Türkiye, 171 s (in Turkish with abstract in English).

Awad, T. & E. Pehlivan, 2001. Systematic and faunistic study of the species of the tribe Carpocorini (Heteroptera: Pentatomidae: Pentatominae) in Turkey part I: *Holcogaster* FB., *Staria* D. and *Cnephosa* JAK. Türkiye Entomoloji Dergisi, 25 (3): 163-174.

Belousova, E. N., 1999. Revision of shield bugs of the genus *Agatharchus* Stål (Heteroptera, Pentatomidae). Entomologicheskoe Obozrenie, 75 (4): 836-856.

Beyaz, G. & S. Tezcan, 2002. Kültür kekiği (*Origanum* spp.) (Lamiaceae)'ndeki Heteroptera takımına bağlı böcek faunasının belirlenmesi üzerinde çalışmalar. Türkiye Entomoloji Dergisi, 26 (1): 3-10 (in Turkish with abstract in English).

Bolu, H., I. Özgen & M. Fent, 2006. Diyarbakır, Elazığ ve Mardin illeri badem ağaçlarında bulunan Pentatomidae (Heteroptera) türleri. Yüzüncü Yıl Üniversitesi Ziraat Fakültesi Tarım Bilimleri Dergisi, 16 (1): 25-28 (in Turkish with abstract in English).

Çerçi, B., 2021. First record of *Halyomorpha halys* (Stål, 1855) (Pentatomidae: Heteroptera) in Aegean Region of Turkey. Acta Biologica Turcica, 34 (1): 35-37.

Çerçi, B. & G. Gözüaçık, 2019. Contribution to Pentatomoidea (Heteroptera) Fauna of Iğdır and Istanbul with three new records for Turkish fauna. Journal of the Heteroptera of Turkey, 1 (1-2): 33-40.

Çerçi, B. & Ö. Koçak, 2017. Further contribution to the Heteroptera (Hemiptera) fauna of Turkey with a new synonymy. Acta Biologica Turcica, 30 (4): 121-127.

Çerçi, B. & İ. Özgen, 2021. Contribution to the knowledge of Heteroptera (Hemiptera) fauna of Elazığ Province with a new record for the fauna of Turkey. Journal of the Heteroptera of Turkey, 3 (1): 50-75.

Dursun, A. & M. Fent, 2010. Systematische und faunistische Untersuchungen über die Überfamilie Pentatomoidea (Insecta: Heteroptera) aus dem Kelkit-Tal der Türkei. Linzer biologische Beiträge, 42 (1): 587-598 (in German with abstract in English).

Dursun, A. & M. Fent, 2013. Overview of the subgenus *Ventocoris* s. str. (Hemiptera: Heteroptera: Pentatomidae) with new records and a revised key to the Ventocoris species of Turkey. Zootaxa, 3681 (1): 151-177.

Dursun, A. & M. Fent, 2015. Notes on some little known species of Heteroptera from Turkey with new records for the fauna of Europe and the Turkish Thrace. North-Western Journal of Zoology, 11 (1): 92-96.

Dursun, A. & M. Fent, 2017. Type Localities of Heteroptera (Insecta: Hemiptera) from Turkey. Zootaxa, 4227 (4): 451-494.

Dursun, A. & M. Fent, 2018. Erstnachweis von *Perillus bioculatus* (Fabricius, 1775) (Hemiptera: Heteroptera: Pentatomidae) für Anatolien (Türkei). Heteropteron, 53: 18-20 (in German with abstract in English).

Fent, M., 2010. Contributions to Pentatomoidea (Heteroptera) fauna of Western Black Sea Region with a new record for Anatolian fauna: *Neottiglossa lineolata* (Mulsant and Rey, 1852). Journal of the Entomological Research Society,12 (1): 53-65.

Fent, M. & N. Aktaç, 1999. Edirne yöresi Pentatomidae (Heteroptera) faunası üzerine taksonomik ve faunistik araştırmalar. Turkish Journal of Zoology, 23 (Ek Sayı 2): 377-395 (in Turkish with abstract in English).

Fent, M. & N. Aktaç, 2007. Die Verbreitung des *Perillus bioculatus* (Fab.) (Heteroptera: Pentatomidae: Asopinae) im türkischen Teil Thrakiens. Heteropteron, 25: 7-10 (in German with abstract in English).

Fent, M. & A. Dursun, 2022. An up-to-date checklist of Turkish Pentatomidae (Hemiptera: Heteroptera) with additional records. Trakya University Journal of Natural Sciences, 23 (Special Issue): 65-111.

Fent, M., A. Dursun, Y. Karsavuran, S. Tezcan & O. Demirözer, 2010. A review of the tribe Halyini in Turkey (Hemiptera: Heteroptera: Pentatomidae) with two new records: *Apodiphus integriceps* and *Mustha vicina*. Journal of the Entomological Research Society, 12 (2): 1-13.

Henry, T. J., 2017. Biodiversity of Heteropterai, 279-335. In: Insect Biodiversity (Eds. R. G. Foottit & P. H. Adler). Science and Society, Vol. I, Wiley-Blackwell, Oxford, UK, 904 pp.

Kıvan, M., 2004. Some observations on *Perillus bioculatus* (F.) (Heteroptera: Pentatomidae) a new record for the entomofauna of Turkey. Turkish Journal of Entomology, 28 (2): 95-98.

Kıyak, S., P. Alacapunar & H. Özdamar, 2019. The second record of *Perillus bioculatus* (Fabricius, 1775) (Hemiptera: Heteroptera: Pentatomidae), Invasive Alien Species (IAS) from Anatolia. Journal of the Heteroptera of Turkey, 1 (1-2): 4-6.

Kıyak, S., Ö. Özsaraç & A. Salur, 2004. Additional notes on the Heteroptera fauna of Nevşehir province (Turkey). Gazi University Journal of Science, 17 (1): 21-29.

Kment, P. & Z. Jindra, 2005. New and interesting records of true bugs (Heteroptera) from Turkey, southeastern Europe, Near and Middle East. Acta Entomologica Musei Nationalis Pragae, 45: 3-16.

Külekçi, G., E. Yıldırım & S. Tezcan, 2009. Contribution to the knowledge of the Pentatomidae (Heteroptera) fauna of Turkey. Linzer Biologische Beiträge, 41 (1): 697-708.

Lodos, N. & F. Önder, 1983. Contribution to the study on the Turkish Pentatomoidea (Heteroptera). VI. Asopinae (Amyot & Serville, 1843). Türkiye Bitki Koruma Dergisi, 7 (4): 221-230.

Lodos, N., F. Önder, E. Pehlivan & R. Atalay, 1978. Ege ve Marmara Bölgesinin Zararlı Bölge Faunasının Tespiti Üzerine Çalışmalar. T.C. Gıda-Tarım ve Hayvancılık Bakanlığı Zirai Mücadele ve Zirai Karantina Genel Müdürlüğü, Ankara, 301 pp (in Turkish).

Lodos, N., F. Önder, E. Pehlivan, R. Atalay, E. Erkin, Y. Karsavuran, S. Tezcan & S. Aksoy, 1998. Faunistic studies on Pentatomoidea (Plataspidae, Acanthosomatidae, Cydnidae, Scutelleridae, Pentatomidae) of Western Black Sea, Central Anatolia and Mediterranean Regions of Turkey. Department of Plant Protection Faculty of Agriculture University of Ege, Bornova, İzmir, 75 pp.

Matocq, A., D. Pluot-Sigwalt & I. Özgen, 2014. Terrestrial Hemiptera (Heteroptera) collected in South-East Anatolia (Diyarbakır, Mardin and Elazığ provinces) (Turkey): second list. Munis Entomology & Zoology, 9 (2): 884-930.

Önder, F., Y. Karsavuran, S. Tezcan & M. Fent, 2006. Türkiye Heteroptera (Insecta) Kataloğu. Meta Basım Matbaacılık Hizmetleri, İzmir, 164 s (in Turkish).

Önder, F., A. Ünal & E. Ünal, 1981. Heteroptera fauna collected by light traps in some districts of northwestern part of Anatolia. Türkiye Bitki Koruma Dergisi, 5 (3): 151-169.

Önder, F., E. Ünal, & A. Ünal, 1984. Heteropterous insects collected by light traps in Edirne (Turkey). Türkiye Bitki Koruma Dergisi, 8: 215-224.

Özgen, İ., C. Gözüaçık, Y. Karsavuran & M. Fent, 2005a. Güneydoğu Anadolu Bölgesi buğday alanlarında bulunan Pentatomidae (Heteroptera) familyasına ait türler üzerinde araştırmalar. Türkiye Entomoloji Dergisi, 29 (1): 61-68 (in Turkish with abstract in English).

Özgen, İ., C. Gözüaçık, Y. Karsavuran & M. Fent, 2005b. Doğu ve Güneydoğu Anadolu Bölgesinde antepfıstığı, kayısı, kiraz ve zeytin ağaçlarında bulunan Pentatomidae (Heteroptera) familyasına ait türlerin saptanması üzerinde çalışmalar. Ege Üniversitesi Ziraat Fakültesi Dergisi, 42 (2): 35-43 (in Turkish with abstract in English).

Rider, D. A., 2006. Family Pentatomidae Leach, 1815, 233-415. In: Catalogue of Heteroptera of the Palaearctic Region Vol. 5. Pentatomomorpha II (Eds. B. Aukema & Ch. Rieger) Netherlands Entomological Society, Amsterdam, 550+xiv pp.

Rider, D. A., C. F. Schwertner, J. Vilímová, D. Rédei, P. Kment & D. B. Thomas, 2018. "Higher Systematics of the Pentatomoidea, 25-201". In: Invasive Stink Bugs and Related Species (Pentatomoidea): Biology, Higher Systematics, Semiochemistry, and Management (Ed. J. E. McPherson). American Entomologist, 819 pp.

Roca-Cusachs, M., J. G. Kim, C. F. Schwerner, J. Grazia, J. Eger & S. Jung, 2021. Opening Pandora's box: molecular phylogeny of the stink bugs (Hemiptera: Heteroptera: Pentatomidae) reveals great incongruences in the current classification. Systematic Entomology, 47: 36-51.

Tezcan, S. & F. Önder, 1999. Heteropterous insects associated with cherry trees in Kemalpaşa district of Izmir, Turkey. Ege Üniversitesi Ziraat Fakültesi Dergisi, 36 (1-3): 119-124.

Tezcan, S. & F. Önder, 2003. İzmir ve Manisa İlleri ekolojik kiraz bahçelerinin faunası üzerinde araştırmalar: Heteroptera takımına bağlı türler üzerinde bir değerlendirme. ANADOLU Ege Tarımsal Araştırma Enstitüsü Dergisi, 13 (1): 124-131 (in Turkish with abstract in English).

Yazıcı, G., E. Yıldırım & P. Moulet, 2014. Contribution to the knowledge of the Pentatomidae and Plataspidae (Hemiptera, Heteroptera, Pentatomomorpha) fauna of Turkey. Linzer Biologische Beiträge, 46 (2): 1819-1842.

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Original article (Orijinal araştırma)

Monitoring and distribution of *kdr* and *ace-1* mutation variations in *Culex pipiens* L., 1758 (Diptera: Culicidae) in artificial sites and agricultural fields in the central and eastern Black Sea Region of Türkiye[1]

Türkiye'nin Orta ve Doğu Karadeniz Bölgesi tarımsal ve yapay alanlarda yayılım gösteren *Culex pipiens* L., 1758 (Diptera: Culicidae)'te *kdr* ve *ace-1* mutasyon varyasyonlarının izlenmesi ve dağılımı

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Abstract

Culex pipiens L., 1758 (Diptera: Culicidae) is one of the most important pests and disease vectors in the world. It is of major importance to monitor the development of insecticide resistance in order to effectively control. This study investigated the presence of mutations in specific loci of the *Vgsc* (*kdr* L1014F/C) and *ace-1* (G119S, F290V) gene, associated with insecticide resistance in *Culex pipiens* collected from nine provinces in central and eastern Black Sea Region of Türkiye in the 2020 active season. For *kdr*, L1014F mutation was determined for each region with three different silent mutations for wild and resistant type alleles, while L1014C was not recorded in any of the analyzed populations. For *ace-1*, substitution F290V was detected at a low frequency in heterozygosity, while G119S was more widespread, in the analyzed populations. For *ace-1*, G119I (6 populations) and G119A (5 populations) substitution was firstly described. Types of mutations differences related to the resistance between artificial sites and agricultural fields were not significantly different.

Keywords: *ace-1* resistance, common house mosquito, insecticide resistance, *kdr* resistance

Öz

Culex pipiens L., 1758 (Diptera: Culicidae) dünyadaki en önemli ve hastalık vektörü olan türlerden biridir. Efektif bir kontrol yapılabilmesi için insektisitlere karşı gelişen direnci takip etmek büyük öneme sahiptir. Bu çalışmada Türkiye Orta ve Doğu Karadeniz Bölgesi'nde 2020 aktif sezonunda dokuz ilden toplanan *Cx. pipiens* örneklerinde *vgsc* (*kdr* L1014F/C) ve *ace-1* (G119S, F290V) spesifik bölgelerinde direnç ile ilgili mutasyonların varlığı araştırılmıştır. *kdr* için, her bölgede L1014F mutasyonu belirlenirken, yabanıl ve dirençli tip aleller için üç farklı sessiz mutasyon tespit edilirken çalışılan popülasyonların hiçbirinde L1014C mutasyonu saptanmamıştır. *ace-1* bölgesi için, çalışılan popülasyonlarda F290V değişimi heterozigot ve düşük oranlarda saptanırken, G119S değişimi daha yaygın bulunmuştur. *ace-1* bölgesi için G119I (6 popülasyon) ve G119A (5 popülasyon) değişimleri ilk defa tespit edilmiştir. Dirence neden olan mutasyon tiplerinde yapay ve tarımsal alanlar arasında anlamlı fark bulunamamıştır.

Anahtar sözcükler: *ace-1* direnci, ev sivrisineği, insektisit direnci, *kdr* direnci

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Introduction

Mosquito-borne diseases pose a significant threat to public health, as they affect half of the population around the globe, leading to millions of fatal outcomes. Those caused by arboviruses are increasingly emerging or re-emerging in Europe. For example, West Nile virus disease cases have been reported from southeastern Europe and the Mediterranean Basin since the first large epidemic in 1996 in Romania (Ceianu et al., 2001; Rezza, 2014; Martinet et al., 2019). The epidemic potential of such diseases has been enhanced in the Palearctic Region by the spread of invasive mosquitoes (Marshall, 2000). *Culex pipiens* L., 1758 (Diptera: Culicidae) species complex, commonly known the house mosquito, are a pest and can also serve as vector for several arboviruses like West Nile virus (WNV), Rift Valley fever, and *Dirofilaria immitis* (Leidy, 1856) (Spirurida: Onchocercidae) (Diaz-Badillo et al., 2011; Akıner & Eksi, 2015; Grigoraki et al., 2018; Zakhia et al., 2018). WNV was first detected in Türkiye in the 1970s and has since spread to different areas of the country (Ari, 1972; Ozkul et al., 2006; Kalaycioglu et al., 2012; Ergunay et al., 2014; Akıner et al., 2019). There is no cure or efficacious vaccine for most vector-borne diseases. Therefore, the main control method to prevent these diseases is vector control. In the past organochlorine insecticides were used, but now pyrethroids, organophosphates and carbamates insecticides are most commonly used for mosquito control in Türkiye. However, the overuse of chemical insecticides imposes selection pressure for resistance genes, leading to mosquitoes becoming resistant to insecticides over time.

Two main insecticide resistance mechanisms are important in mosquitoes: (1) metabolic resistance arising from an increase in detoxification activity of enzyme families, namely glutathione S-transferases, mixed-function oxygenases, and carboxyl-esterase (Kasai et al., 1998; Hemingway et al., 2004; Whalon et al., 2008; Akıner & Ekşi, 2015); and (2) target-site insensitivity deriving from point mutations related to the nervous system proteins (Hemingway et al., 2004; Shi et al., 2015).

Pyrethroid and DDT insecticides affect the voltage-gated sodium channels *(vgsc)* of insects (Donnely et al., 2009). However, single nucleotide polymorphisms [SNP] or multiple substitutions occurring in the *Vgsc* genes reduce or eliminate the binding affinity of these insecticides to the sodium channel protein. There are more than 30 resistance-associated single nucleotide polymorphisms in sodium channel protein encoding genes (Wang et al., 2012; Lol et al., 2013). Knockdown resistance (*kdr*) resistance, the most important and well-known single nucleotide polymorphisms, involves the replacement of leucine (TTA) to phenylalanine (TTT) (L-to-F) at codon 1014 in domain II (Shi et al., 2015). This genetic locus in homozygosity (1014F/1014F) combined with the P450 metabolic resistance could produce highly resistant phenotype (Edi et al., 2012). In addition, *kdr*-type resistance mutations, such as L1014H/C/S/W have been identified in previous studies (Rinkevich et al., 2013; Scott et al., 2015; Taskin et al., 2016).

Organophosphates and carbamates target the acetylcholinesterase (AChE) which hydrolyzes the neurotransmitter acetylcholine (ACh) to acyl-enzyme and free choline to terminate nerve impulses of insects (Colovic et al., 2013). These insecticide groups are structurally similar to ACh, which is the substrate of the AChE enzyme, so inhibit AChE irreversibly by competing with ACh (Alout et al., 2008). Three different point mutations in the *ace-1* gene are responsible for resistance to organophosphates and carbamates. However, in *ace-1*, only Gly-to-Ser at codon 119 (G119S) and Phe to Val (F290V) at codon 290 were detected in *Cx. pipiens* species complex (Alout et al., 2008).

This study investigated of the target site mutations of *Cx. pipiens* species complex, which are widespread in parts of the Black Sea Region. For this purpose, L1014F, G119S and F290V mutations related to the insecticide resistance were screened in *Cx. pipiens* species complex and the variation of the mutation types in artificial sites and agricultural fields were also investigated.

Materials and Method

Mosquito collection and field classification

Thirty-three locations from nine provinces in the Black Sea Region were selected as the study areas. Mosquito collection was performed according to field sampling methods for mosquitoes described by European Center of Disease Control (Medlock et al., 2018). Briefly, larval mosquito samples were collected using 250 ml standard larval dipper and adult mosquito samples were collected using EVS trap with CO_2. The collections were performed in the active season of 2020 (May to October). Collection sites were classified artificial (man-made containers, inside usage tires, discarded metal and plastic containers, buckets, basement water puddles, marble, irrigation canals and ponds) and agricultural fields. Coordinates of sampling areas were recorded in decimal degrees with the help of GPS device (eTrex Vista HCx, Garmin, Olathe, KS, USA).

Sampling sites classification was performed by embedding the coordinates obtained in the field studies into CORINE (coordinated information on the environment) land cover (obtained from EEA, 2018) with a resolution of 1 km, and the CORINE equivalents of the coordinates were determined on the ArcGIS 10.5. The first level CORINE was used as the class of the samples (Table 1).

Table 1. Mosquito collection locations and features

Province	Location	Latitude (°N)	Longitude (°E)	CORINE code (class level 1)	Stage	Habitat
Amasya	Amasya	40.6674	35.8462	112 (artificial)	Larvae	Used tires
	Merzifon	40.8710	35.4639	111 (artificial)	Larvae	Puddle
	Saluca	40.7841	35.6817	242 (agricultural)	Larvae	Irrigation canal
Artvin	Arhavi	41.3586	41.3184	112 (artificial)	Larvae	Roadside puddle
	Artvin	41.1810	41.8308	112 (artificial)	Larvae	Used tires
	Borçka	41.3832	41.6909	222 (agricultural)	Larvae	Used tires
	Hopa	41.3876	41.4378	121 (artificial)	Larvae and adults	Used tires
Çorum	Osmancık	40.9691	34.8042	112 (artificial)	Larvae	Pond
Giresun	Görele	41.0374	38.9839	222 (agricultural)	Larvae	Metal container
Ordu	Gülyalı	40.9668	38.0572	112 (artificial)	Larvae	Roadside puddle
	Turnasuyu	40.9803	38.0019	222 (agricultural)	Larvae	Puddle
	Ünye	41.1229	37.2947	111 (artificial)	Larvae	Used tires
Rize	Ardeşen	41.1893	40.9701	112 (artificial)	Larvae	Used tires
	Çayeli	41.0720	40.7152	121 (artificial)	Larvae	Roadside puddle
	Fındıklı	41.2801	41.1527	112 (artificial)	Larvae	Used tires
	Hamidiye	41.1832	40.9535	222 (agricultural)	Larvae	Marble
	Ikizdere	40.7740	40.5577	242 (agricultural)	Larvae	Used tires
	Iyidere	40.9880	40.3309	112 (artificial)	Larvae	Used tires
	Pazar	41.1820	40.8932	112 (artificial)	Larvae and adults	Used tires. Near the larval habitat
	Rize	41.0416	40.5771	121 (artificial)	Larvae	Puddle

Table 1. Continued

Province	Location	Latitude (°N)	Longitude (°E)	CORINE code (class level 1)	Stage	Habitat
Samsun	Bafra	41.6177	35.8746	212 (agricultural)	Larvae and adults	Irrigation canal
	Çarşamba	41.2052	36.7417	242 (agricultural)	Larvae and adults	Puddle and tunnel
	Engiz	41.4941	36.0854	212 (agricultural)	Larvae	Irrigation canal
Sinop	Boyabat	41.4654	34.8217	213 (agricultural)	Larvae	Irrigation canal
	Dikmen	41.6508	35.2678	242 (agricultural)	Larvae	Irrigation canal
	Laçin	40.7751	34.8870	112 (artificial)	Larvae	Puddle
Trabzon	Akçaabat	41.0122	39.5935	111 (artificial)	Larvae	Used tires
	Arsin	40.9631	39.9889	112 (artificial)	Larvae	Metal container
	Çarşıbaşı	41.0877	39.3859	112 (artificial)	Larvae	Used tires
	Sümela	40.7307	39.6374	243 (agricultural)	Larvae	Plastic container
	Sürmene	40.9086	40.1078	112 (artificial)	Larvae	Roadside puddle
	Trabzon	40.9766	39.7480	121 (artificial)	Larvae	Used tires
	Vakfıkebir	41.0402	39.2802	112 (artificial)	Larvae and adults	Marble. Near the larval habitat

Species identification

Identification of *Cx. pipiens* species complex specimens was conducted using a computer-assisted Leica Microsystem EZ4 stereomicroscope (Leica Microsystems, Wetzlar, Germany) and a mosquito identification key prepared by Schaffner et al. (2001).

Molecular studies

DNA isolation

DNA isolation from *Cx. pipiens* samples individually was performed with the Gene JET genomic DNA isolation Kit (Thermo Fisher Scientific Baltics UAB, Vilnius, Lithuania). Isolation was performed according to the manufacturer's instructions. Obtained DNA was labeled and stored at -20°C until the PCR was performed.

Identification of species complex members

The *ace-2* gene second intron region was amplified according to Smith & Fonseca (2004) to detect *Cx. pipiens*. The primers of ACEquin (5'-CCTTCTTGAATGGCTGTGGCA-3'), ACEpip (5'-GGAAACAACGACGTATGTACT-3'), ACEtorr (5′-TGCCTGTGCTACCAGTGATGTT-3′) and B1246 (5'-TGGAGCCTCCTCTTCACGGC-3') were used for species complex identification. The PCR conditions were as described by Smith and Fonseca (2004). Amplified DNA regions were visualized using 1.5% agarose gel.

Molecular assays

***kdr* mutations (L1014) diagnostic assays**

kdr mutation assays were performed according to the PCR method described by Martinez-Torres et al. (1998). PCR process was performed in two parallel reactions; the first reaction included forward-Cgd1 (5'-GTGGAACTTCACCGACTTC-3') reverse-Cgd2 (5'-GCAAGGCTAAGAAAAGGTTAAG-3') and forward-Cgd3 (5'-CCACCGTAGTGATAGGAAATTTA-3') primers, and the second reaction included forward-Cgd1, reverse-Cgd2 and forward-Cgd4 (5'-CCACCGTAGTGATAGGAAATTTT-3') primers. Amplified PCR products were run on the 1.5% agarose. The samples were classified according to base size using PCR

amplification of specific alleles (PASA). The sample subset consisting of 330 samples was sequenced using primers Cgd1 and Cgd2 (Macrogen Europe, Amsterdam, Netherlands).

Ace-1 mutations (F290V and G119S) diagnostic assays

Ace-1 mutations assays were performed using two different primer sets. For F290V mutation, Valdir 5'-ACGCTGGGGATCTGCGAGG-3', Valrev 5'-TCCACAACCGGAACGAACGGAAA-3', CxEx5dir 5'-GTCTGGCCGAGGCCGTCA-3', CxKrev2 5'-TGCTTCTGTGCGTGTACAGG-3' primers described by Weill et al. (2004) were used. PCR was performed according to the Weill et al. (2004). Amplified PCR products were run on 1.5% agarose gel, the samples were classified according to base size using PCR PASA method. Three hundred and thirty-three samples arbitrarily selected from the sample subset were amplified and sequenced using the CxEx5 and CxKrev2 primers covering the entire region (Macrogen).

For G119S mutation, molecular assays were performed using CxEx3dir (5'-CGACTCGGACCCACT GGT-3') and CxEx3rev (5'-GTTCTGATCAAACAGCCCCGC-3') primer set described by Weill et al. (2004). PCR was performed according to the Weill et al. (2004). The amplicons were sequenced (Macrogen). After the obtained sequences were aligned, each complementary sequence was cut virtually from the Alu-1 restriction site by using ClustalX2 program.

Data analysis

The frequencies were determined by the PASA method (L1014F and F290V) and sequencing (G119S) results were compared with Hardy-Weinberg expectations in the GenAlEx (ver 6.5) software. Differences between frequencies from the artificial sites and agricultural fields were examined using the AMOVA test (calculated in the Arlequin program using resistance codons and obtained frequencies). Raw sequence data were processed with Mega 7 (Kumar et al., 2016). Single nucleotide polymorphism points in the gene regions were determined according to the methods determined by Martinez-Torres et al. (1998), Alout et al. (2007a), and Weill et al. (2004), and the frequencies of the SNPs were calculated. Discrimination of species belonging to the *Cx. pipiens* species complex was based on different bands size upon PCR amplification of an *ace-2* region.

Results

Species identification

One thousand six hundred and fifty *Cx. pipiens* species complex field samples were analyzed. *Culex pipiens* species complex specimens were collected as larvae from all sampling points and as adults in some areas. The majority of the larvae were collected from the insides of used tires (~39%).

After morphological and molecular identification, the samples were determined to belong to the *Cx. pipiens* species complex. All samples produced approximately 600 bp bands on the agarose gel.

DDT and Pyrethroids resistance mutations

The most common L1014F mutation in *Cx. pipiens* species complex were screened using the PASA method. The scans included different frequencies for all three alleles, wild-type, heterozygous, and homozygous, of 33 populations from nine provinces. The frequency of the *kdr* wild-type allele (1014L) in the populations ranged up to 0.7 and the highest frequency denoted in Arsin population. Dikmen population was showed lowest degree of the wild-type allele frequency. The heterozygous frequency ranged up to 0.75, while the Iyidere population had the highest heterozygous allele frequency. Heterozygous genotypes were not observed in the Fındıklı population. The resistance allele frequency was zero in some populations (i.e., Borçka, Arhavi, Ardeşen, Pazar, Iyidere, Ikizdere, Arsin, Vakıfkebir, Boyabat and Laçin populations). Most of the population genotype frequencies were not suitable for Hardy-Weinberg equilibrium ($P < 0.05$) (Table 2).

Table 2. Genotype and variant allele frequencies (VAF) Hardy-Weinberg equilibrium ($\chi 2$) and P-values for L1014 *kdr* mutation in *Culex pipiens* according to PASA method (n = 50)

Provinces	Location	Genotype frequency				$\chi 2$	P
		L1014L	L1014L/F	L1014F	VAF		
Amasya	Amasya	0.545	0.364	0.091	0.273	0.347	0.556
	Merzifon	0.411	0.214	0.375	0.482	16.3	0.000*
	Saluca	0.333	0.222	0.444	0.556	15.1	0.000*
Artvin	Artvin	0.368	0.474	0.158	0.395	0.004	0.951
	Arhavi	0.471	0.529	0.000	0.265	6.48	0.011*
	Borçka	0.385	0.615	0.000	0.308	9.88	0.002*
	Hopa	0.264	0.415	0.321	0.528	1.40	0.237
Çorum	Osmancık	0.250	0.250	0.500	0.625	10.9	0.001*
Giresun	Görele	0.625	0.250	0.125	0.250	5.56	0.018*
Ordu	Gülyalı	0.275	0.275	0.450	0.588	9.36	0.002*
	Turnasuyu	0.448	0.172	0.379	0.466	21.4	0.000*
	Ünye	0.308	0.500	0.192	0.442	0.009	0.924
Rize	Ardeşen	0.556	0.444	0.000	0.222	4.08	0.043*
	Çayeli	0.176	0.765	0.059	0.441	15.2	0.000*
	Fındıklı	0.286	0.000	0.714	0.714	50.0	0.000*
	Hamidiye	0.333	0.375	0.292	0.479	3.09	0.079
	Ikizdere	0.667	0.333	0.000	0.167	2.00	0.157
	Iyidere	0.250	0.750	0.000	0.375	18.0	0.000*
	Pazar	0.600	0.400	0.000	0.200	3.12	0.077
	Rize	0.275	0.319	0.406	0.565	6.17	0.013*
Samsun	Bafra	0.484	0.226	0.290	0.403	14.1	0.000*
	Çarşamba	0.111	0.111	0.778	0.833	18.0	0.000*
	Engiz	0.286	0.429	0.286	0.500	1.02	0.312
Sinop	Boyabat	0.500	0.500	0.000	0.250	5.56	0.018*
	Dikmen	0.000	0.250	0.750	0.875	1.02	0.312
	Laçin	0.600	0.400	0.000	0.200	3.12	0.077
Trabzon	Akçaabat	0.417	0.333	0.250	0.417	4.94	0.026*
	Arsin	0.700	0.300	0.000	0.150	1.56	0.212
	Çarşıbaşı	0.444	0.333	0.222	0.389	4.46	0.035*
	Sümela	0.250	0.500	0.250	0.500	0.00	1.000
	Sürmene	0.514	0.257	0.229	0.357	9.68	0.002*
	Trabzon	0.556	0.222	0.222	0.333	12.5	0.000*
	Vakfıkebir	0.333	0.667	0.000	0.333	12.5	0.000*

* significant at $P < 0.05$.

Organophosphate/carbamate resistance mutations

Screening for the F290V mutation, which causes organophosphate/carbamate resistance, was performed using the PASA method. The frequency of the wild-type genotype frequency was high, while the frequencies of the resistant alleles were quite low. Wild-type genotype frequencies varied between 0.6 and 1 (except Amasya, Fındıklı, Sürmene populations) and the highest allele frequency was observed for four populations (Ardeşen, Çayeli, Ünye and Merzifon). The resistant allele frequency ranged up to 0.05 and the highest allele frequency was observed in the Arsin population. The variant allele was found in most of the populations except in Ardeşen, Çayeli, Ünye and Merzifon populations. All population genotype frequencies were suitable for Hardy-Weinberg equilibrium except for Fındıklı, Arsin and Sürmene populations (Table 3).

Table 3. Genotype and variant allele frequencies (VAF) Hardy-Weinberg equilibrium (χ2) and P-values for F290 a*ce-1* mutation in *Culex pipiens* according to PASA method (n = 50)

Provinces	Location	Genotype frequency				χ2	P
		F290F	F290F/V	F290V	VAF		
Amasya	Amasya	0.660	0.300	0.040	0.190	0.032	0.858
	Merzifon	1.000	0.000	0.000	0.000	-	-
	Saluca	0.840	0.160	0.000	0.080	0.378	0.539
Artvin	Arhavi	0.840	0.160	0.000	0.080	0.378	0.539
	Artvin	0.700	0.280	0.020	0.160	0.087	0.768
	Borçka	0.640	0.340	0.020	0.190	0.547	0.459
	Hopa	0.860	0.140	0.000	0.070	0.283	0.595
Çorum	Dikmen	0.780	0.200	0.020	0.120	0.140	0.708
	Osmancık	0.820	0.160	0.020	0.100	0.617	0.432
Giresun	Görele	0.800	0.180	0.020	0.110	0.326	0.568
Ordu	Gülyalı	0.840	0.140	0.020	0.090	1.05	0.304
	Turnasuyu	0.860	0.140	0.000	0.070	0.283	0.595
	Ünye	1.000	0.000	0.000	0.000	-	-
Rize	Ardeşen	1.000	0.000	0.000	0.000	-	-
	Çayeli	1.000	0.000	0.000	0.000	-	-
	Fındıklı	0.020	0.960	0.020	0.500	42.3	0.000*
	Hamidiye	0.94	0.060	0.000	0.030	0.047	0.827
	Ikizdere	0.920	0.080	0.000	0.040	0.087	0.768
	Iyidere	0.660	0.340	0.000	0.170	2.10	0.148
	Pazar	0.920	0.080	0.000	0.040	0.087	0.768
	Rize	0.860	0.140	0.000	0.070	0.283	0.595
Samsun	Bafra	0.920	0.080	0.000	0.040	0.086	0.768
	Çarşamba	0.620	0.340	0.040	0.210	0.030	0.861
	Engiz	0.820	0.160	0.020	0.100	0.617	0.432

Table 3. Continued

Provinces	Location	Genotype frequency				χ2	P
		F290F	F290F/V	F290V	VAF		
Sinop	Boyabat	0.760	0.200	0.040	0.140	1.44	0.231
	Laçin	0.720	0.260	0.020	0.150	0.019	0.890
Trabzon	Akçaabat	0.720	0.260	0.020	0.150	0.019	0.890
	Arsin	0.800	0.150	0.050	0.130	7.26	0.007*
	Çarşıbaşı	0.780	0.200	0.020	0.120	0.140	0.708
	Sümela	0.680	0.280	0.040	0.180	0.132	0.716
	Sürmene	0.380	0.620	0.000	0.310	10.1	0.001*
	Trabzon	0.86	0.14	0.000	0.070	0.283	0.595
	Vakfıkebir	0.840	0.160	0.000	0.080	0.378	0.539

* significant at P < 0.05.

The G119S mutation screening was performed by sequence analysis. The frequency of the wild-type genotype was high, while the frequencies of the resistant alleles were quite low. Wild-type genotype frequencies varied between 0.3 and 0.8 and the highest allele frequency was observed in Rize, Iyidere, Arsin, Çarşıbaşı, Ünye. The resistant genotype frequency ranged up to 0.5 and the highest allele frequency was observed in the Merzifon population. The variant allele was found in all of the populations. Population genotype frequencies were suitable for Hardy-Weinberg equilibrium except for Bafra, Dikmen, Osmancık and Amasya populations (Table 4).

Table 4. Genotype and variant allele frequencies (VAF) Hardy-Weinberg equilibrium (χ2) and P-values for G119 *ace-1* mutation in *Culex pipiens* according to sequence data (n = 10 unless noted)

Province	Location	Genotype frequency				χ2	P
		G119G	G119G/S	G119S	VAF:		
Amasya	Amasya	0.600	0.100	0.300	0.350	6.09	0.014*
	Merzifon	0.300	0.200	0.500	0.600	3.40	0.065
	Saluca	0.600	0.200	0.200	0.300	2.74	0.098
Artvin	Arhavi	0.500	0.300	0.200	0.350	1.16	0.281
	Artvin	0.700	0.300	0.000	0.150	0.311	0.577
	Borçka	0.600	0.400	0.000	0.200	0.625	0.429
	Hopa	0.600	0.400	0.000	0.200	0.625	0.429
Çorum	Osmancık	0.700	0.000	0.300	0.300	10.0	0.002*
Giresun	Görele	0.600	0.300	0.100	0.250	0.400	0.527
Ordu	Gülyalı	0.600	0.200	0.200	0.300	2.74	0.098
	Turnasuyu	0.600	0.400	0.000	0.200	0.625	0.429
	Ünye	0.800	0.200	0.000	0.100	0.123	0.725
Rize	Ardeşen	0.500	0.300	0.200	0.350	1.16	0.281
	Çayeli	0.600	0.300	0.100	0.250	0.400	0.527
	Fındıklı	0.600	0.200	0.200	0.300	2.74	0.098

Table 4. Continued

Province	Location	Genotype frequency			VAF:	χ2	P
		G119G	G119G/S	G119S			
	Hamidiye	0.600	0.200	0.200	0.300	2.74	0.098
	Ikizdere	0.700	0.300	0.000	0.150	0.311	0.577
Rize	Iyidere	0.800	0.200	0.000	0.100	0.123	0.725
	Pazar	0.500	0.400	0.100	0.300	0.023	0.880
	Rize	0.800	0.200	0.000	0.100	0.123	0.725
	Bafra	0.700	0.000	0.300	0.300	10.0	0.002*
Samsun	Çarşamba	0.600	0.200	0.200	0.300	2.74	0.098
	Engiz	0.500	0.300	0.200	0.350	1.16	0.281
	Boyabat (n = 9)	0.667	0.222	0.000	0.222	1.15	0.284
Sinop	Dikmen	0.700	0.100	0.200	0.250	5.38	0.020*
	Laçin	0.500	0.400	0.100	0.300	0.023	0.880
	Akçaabat	0.700	0.200	0.100	0.200	1.41	0.236
	Arsin	0.800	0.200	0.000	0.100	0.123	0.725
	Çarşıbaşı	0.800	0.200	0.000	0.100	0.123	0.725
Trabzon	Sümela	0.600	0.200	0.200	0.300	2.74	0.098
	Sürmene (n = 9)	0.667	0.333	0.000	0.167	0.360	0.549
	Trabzon	0.600	0.200	0.200	0.300	2.74	0.098
	Vakfıkebir	0.600	0.200	0.200	0.300	2.74	0.098

* significant at P < 0.05.

Mutation combinations

We examined the different codon combinations in the loci of interest associated with insecticide resistance, in particular *kdr* L1014 and *ace-1* G119, F290. We identified six different genotype combinations at the L1014F mutation point. For all locations, the TTA (leucine) codon had a highest frequency (0.679). Frequencies of TTA/C (leucine/phenylalanine), TTG (leucine), TTT/G (phenylalanine/leucine) codons were quite low and their values were 0.009, 0.006, and 0.009, respectively. In addition, the TTG codon encoding the amino acid leucine was a silent mutation. The frequencies of the determined gene combinations are given in Table 5.

We identified four different codons in the *ace-1* gene locus G119S. Among all the sequences, the GGC (glycine) codon and AGC (serine) codon frequencies followed, and their values were 0.709 and 0.079, respectively. Heterozygote frequency of the point mutation (RGC) was 0.176. The frequencies of ATC and ARC mutations were quite low in the population and their values were 0.018 and 0.015 respectively. Glycine/isoleucine (6 populations) and glycine/asparagine (5 populations) substitutions frequencies quite low and found around 0.1 for all determined populations. These types of substitutions were firstly described in G119 locus. TTT (phenylalanine) and G/TTT (valine/phenylalanine) codon combinations were determined at the F290V mutation point, which is the other *ace-1* resistance mutation point, and the frequency values were 0.842 and 0.158, respectively (Table 5).

Table 5. Codon combinations in *kdr* L1014F and *ace-1* G119S, F290V mutation sites and their frequencies (W:A or T, M:A or C, K:G or T, and R:A or G; n = 10 per location)

Location	L1014F						G119S					F290V	
	TTA	TTW	TTT	TTM	TTG	TTK	GGC	AGC	RGC	ATC	ARC	TTT	KTT
Amasya	0.8	0.1	0.1	-	-	-	0.6	0.3	0.1	-	-	0.9	0.1
Merzifon	1.0	-	-	-	-	-	0.5	0.3	0.1	0.1	-	1.0	-
Saluca	0.9	-	0.1	-	-	-	0.6	0.1	0.2	0.1	-	1.0	-
Arhavi	0.7	0.3	-	-	-	-	0.6	0.1	0.2	0.1	-	0.9	0.1
Artvin	0.4	0.3	0.1	-	0.1	0.1	0.8	-	0.1	0.1	-	0.8	0.2
Borçka	0.5	0.3	0.1	-	-	0.1	0.8	-	0.2	-	-	0.9	0.1
Hopa	0.7	0.3	-	-	-	-	0.7	-	0.2	-	0.1	0.9	0.1
Osmancık	0.5	0.3	0.2	-	-	-	0.8	0.2	-	-	-	0.9	0.1
Görele	0.7	0.3	-	-	-	-	0.8	-	0.2	-	-	0.7	0.3
Gülyalı	0.7	0.2	0.1	-	-	-	0.7	-	0.3	-	-	0.9	0.1
Turnasuyu	0.7	0.2	0.1	-	-	-	0.8	-	0.1	-	0.1	0.8	0.2
Ünye	0.9	0.1	-	-	-	-	0.8	-	0.2	-	-	1.0	-
Ardeşen	0.9	0.1	-	-	-	-	0.5	0.2	0.3	-	-	1.0	-
Çayeli	0.9	0.1	-	-	-	-	0.6	0.1	0.3	-	-	1.0	-
Fındıklı	0.2	0.7	0.1	-	-	-	0.8	-	0.2	-	-	0.8	0.2
Hamidiye	0.8	0.1	0.1	-	-	-	0.7	0.1	0.2	-	-	0.8	0.2
Ikizdere	1.0	-	-	-	-	-	0.8	-	0.2	-	-	0.8	0.2
Iyidere	0.5	0.2	0.2	0.1	-	-	0.8	-	0.2	-	-	0.7	0.3
Pazar	0.8	0.2	-	-	-	-	0.6	0.1	0.3	-	-	0.9	0.1
Rize	0.7	0.2	-	0.1	-	-	0.8	-	0.2	-	-	0.8	0.2
Bafra	0.6	0.2	-	-	0.1	0.1	0.8	0.1	-	-	0.1	1.0	-
Çarşamba	0.5	0.4	0.1	-	-	-	0.6	0.2	0.2	-	-	0.7	0.3
Engiz	0.7	0.2	0.1	-	-	-	0.6	0.1	0.3	-	-	0.9	0.1
Boyabat	0.5	0.4	0.1	-	-	-	0.8	-	0.1	-	-	0.7	0.3
Dikmen	0.6	0.2	0.2	-	-	-	0.7	0.2	0.1	-	-	0.8	0.2
Laçin	0.6	0.3	0.1	-	-	-	0.5	0.1	0.3	0.1	-	0.8	0.2
Akçaabat	0.6	0.3	0.1	-	-	-	0.8	-	0.1	-	0.1	0.7	0.3
Arsin	0.8	0.1	0.1	-	-	-	0.8	-	0.2	-	-	0.9	0.1
Çarşıbaşı	0.7	0.2	0.1	-	-	-	0.9	-	0.1	-	-	0.8	0.2
Sümela	0.6	0.2	0.2	-	-	-	0.7	0.1	0.1	0.1	-	0.7	0.3
Sürmene	0.4	0.5	-	0.1	-	-	0.7	-	0.2	-	0.1	0.6	0.4
Trabzon	0.7	0.2	0.1	-	-	-	0.7	0.2	0.1	-	-	0.9	0.1
Vakfıkebir	0.8	0.2	-	-	-	-	0.7	0.1	0.2	-	-	0.8	0.2
Total	0.679	0.224	0.073	0.009	0.006	0.009	0.709	0.079	0.176	0.018	0.015	0.842	0.158

Amova analyses

We conducted AMOVA analysis to determine the *kdr* and *ace-1* resistance among the CORINE land cover in level 1. The analysis of the *kdr* resistance variance component among groups was found to be low (0.61%) and FCT distance was 0.242 (P > 0.05). The difference between the populations was high and the FST value revealed a low distance of 75.3% (P < 0.005). The AMOVA analysis by *ace-1* F290V region resistance among the CORINE land cover in level 1 results was similar to *kdr* resistance analysis. Variance between groups -0.98% and FCT value was 0.218 (P > 0.05). Variance component among populations within groups and within populations was 0.003 (21.0%) and 0.012 (79.0%) respectively and FSC and FST values was 0.210 and -0.010, respectively (P > 0.05). For G119S region analysis did not revealed any significant differences among groups and populations. FCT, FSC and FST statistics were the lowest of the tested locations and no significant differences (Table 6).

Table 6. Analysis of Molecular Variance (AMOVA) of the two groups (artificial sites and agricultural fields) of the *Culex pipiens* L1014F and F290V, G119S mutations

Cross comparison	Variance components (% of variation)			F-statistics		
	Among groups	Among populations within groups	Within populations	FCT	FSC	FST
L1014F	0.00057 (0.61%)	0.02269 (24.9%)	0.07090 (75.3%)	0.24242	0.24705*	0.00610*
F290V	-0.00015 (-0.98%)	0.00343 (21.0%)	0.01229 (79.0%)	0.21798	0.21032*	-0.00980*
G119S	-0.00053 (-0.64%)	0.00024 (0.29%)	0.08260 (100%)	-0.00650	0.00294	-0.00354

* significant at P < 0.05.

Discussion

Culex pipiens are biting pests and are vectors of many pathogens important to human and animal health. Therefore, vector control studies generally target this species in many areas. However, vector control studies restrict to the development of insecticide resistance. Rapid identification of target-site resistance mutations in *Cx. pipiens* wild populations can improve control operations through effective resistance management. In this study, mutations in the *vgsc* and *ace-1* genes, related to insecticide resistance, were monitored in *Cx. pipiens* populations in the central and eastern Black Sea Region of Türkiye. Insecticide application related to the agricultural purposes supported the selection pressure in many agricultural areas for mosquito species (Awolola et al., 2007; Akıner et al., 2013). Therefore, different areas *vgsc* and *ace-1* genes frequencies may be different according to the insecticide selection pressure from different areas. Secondly, we investigated frequencies of different alleles that are related to insecticide resistance and possible differences in artificial (constructed or changed by human) sites and agricultural fields. In addition, we screened the presence of new mutation types in the genetic loci related to the insecticide resistance.

Voltage-gated sodium channels are an important for membrane exitability and are responsible for the depolarization phase of action potential in all types of exitable cells (Yu & Catterall, 2003). It is important to get basic data about frequencies of different allele combinations related to the pyrethroid and DDT resistance on local and regional scales (Wang et al., 2012). The L1014F mutation was firstly discovered in *Musca domestica* L related to the Pyrethroid group insecticides and DDT (Chandrasiri et al., 2020). The *Vgsc* L1014 (TTA) codon which encodes leucine and is known as a wild type, was present in all populations, and varied various degrees. The TTT codon encodes phenylalanine and has been reported by many sources to cause resistance (Martinez-Torres et al., 1998; Scott et al., 2015; Shi et al., 2015; Wang et al., 2015; Bkhache et al., 2016; Taskin et al., 2016). Our results revealed three different silent mutations for wild-type and resistant genotypes. While one of them encodes leucine, other genotypes displayed heterozygote

properties (L/F). Our study identified genotypes with TTA homozygote susceptible, TTW (A/T) heterozygote resistance, TTT homozygote resistance, TTM (A/C) heterozygote resistance with silent mutations, TTG homozygote susceptibility with silent mutations and TTK (G/T) heterozygote resistance with silent mutations. Ponce et al. (2016) reported several substitutions with cysteine, histidine, serine or tryptophan in *Culex quinquefasciatus* (Say, 1823) (Diptera: Culicidae) populations. Although our results revealed six mutation types, only one amino acid substitution was observed. The substitution of A to T and A to C and A to G was found in our study and incidence of the A to T was found at a high rate. Although Roberts & Andre (1994) reported the predominance A to C mutations in Sri Lankan *Cx. quinquefasciatus*, Chandrasiri et al. (2020) found A to T mutations predominance in *Cx. quinquefasciatus* populations of Sri Lanka in subsequent years. These results indicated that mutation frequencies can change over time and insecticide resistance dynamic process. A to T or A to C mutation types in third position of codon (1014) is also described in Turkish *Cx. pipiens* populations (Taskin et al., 2016). They also reported predominance of A to T mutations like our study. TTG codon mutation encoding leucine was determined as homozygosity and heterozygosity in some populations of the study (Artvin, Borcka and Bafra populations). It has also been detected in *Anopheles sinensis* (Wiedemann, 1828) (Diptera: Culicidae) in China in previous studies (Zhong et al., 2013; Wang et al., 2015). However, there has been no previous report of this mutation type in *Cx. pipiens* species complex. The average frequency of the wild-type, susceptible allele (L1014) was comparably high (0.2-0.9) in our study. Heterozygote and homozygote resistance type substitutions frequency may be supported moderate or low level of resistance in the middle and eastern Black Sea populations in Türkiye. Taskin et al. (2016) reported high frequencies of two types substitutions (L1014F and L1014C) in *Cx. pipiens* Aegean Region populations in Türkiye. Although they found high frequency of L1014C substitution, we did not observe this type of substitutions. They indicated the possibility of *kdr* as an important mechanism of insecticide resistance in Türkiye *Cx. pipiens* species complex (Taskin et al., 2016). Many factors affect pyrethroid and DDT resistance such as P450 mediated enhanced metabolism. Therefore, real resistance ratio should estimate all types of mechanisms together when using this type of insecticides.

The *kdr* allele frequencies in the studied populations were generally not suitable for Hardy-Weinberg expectation and the difference was statistically significant ($P < 0.05$). This situation can be associated with resistance selection pressure in these areas. In Borçka, Arhavi, Ardeşen, Iyidere, Vakfıkebir, Boyabat and Çayeli populations, homozygous resistance genotype frequencies were zero or quite low, and the frequencies of heterozygous genotypes were high. This may indicate that there is a balance selection in these locations. High resistant genotype frequencies (Rize, Gülyalı, Fındıklı, Çarşamba and Osmancık populations) may explain selection pressure continues in these areas. Low or moderate rate of L1014F mutations may explain long term use of DDT in Türkiye for malaria eradication campaign and agriculture in many areas. Taskin et al. (2016) reported the same situation in Aegean Region of Türkiye where they found another mutation, L1014C, in high frequency. They associated these results with permethrin and another novel insecticide (pyrethroid) usage in those areas. They additionally highlighted the prolonged and excessive use of pyrethroids and the imposing selection pressure against *Cx. pipiens* in that region.

Three distinct mutations in *ace-1* region were described related to the organophosphate and carbamate resistance (Massouli et al., 1992). Most common resistance mutation type in mosquitoes (including *Cx. pipiens*) was identified G119S in the *ace-1* gene region around the catalytic site (Weill et al., 2003). F290V was described in *Cx. pipiens* strain collected in Cyprus (Wirth & Georghiou, 1996) and has been found around in Mediterranean areas several times (Alout et al., 2009; Osta et al., 2012; Taskin et al., 2016) Another point mutation (F331W) related to the resistance was described in East Asian *Culex tritaeniorhynchus* (Giles, 1901) (Diptera: Culicidae) populations (Nabeshima et al., 2004; Alout et al., 2007a, b). Four combinations were found in *ace-1* G119 position in our study: GGC (glycine) homozygote susceptible, AGC (serine) homozygote resistant, RGC (GGC/AGC) heterozygote resistant, and different codon combinations encoding isoleucine and serine, asparagine ARC (AGC/AAC). Although four

combinations were found, homozygote susceptible frequencies were dominant (0.709). Homozygote resistant frequencies were found around half of the tested populations but frequencies were quite low (0.079). Heterozygote resistant genotypes were detected across all analyzed populations except Artvin, Hopa, Borçka, Bafra and Osmancık. The other types frequencies were quite low, encoding for isoleucine (ATC) or asparagine/serine (AAC/AGC). Many studies on *Cx. pipiens* and other mosquito species have reported that the frequency of the GGC codon is high, and the frequency of the AGC codon is low (Alout et al., 2007b; Dabire et al., 2014; Taskin et al., 2016; Bkhache at al., 2019; Major et al., 2020). Our results are consistent with the findings of studies conducted in Mediterranean countries (Osta et al., 2012; Kioulos et al., 2014). The high mutation frequencies found could reflect the history of insecticidal interventions around the Mediterranean Basin, possibly implying that selection pressure still occurs. Taskin et al. (2016) described the same situation in Aegean Region of Türkiye. In addition, no records were found for the ATC and AAC codons determined in other studies of *Cx. pipiens* and other mosquito species. The effect of the new codon type on the species needs to be determined. In this regard, their contribution to insecticide resistance should be investigated. Second mutation in the *ace-1* gene combination F290V was found in the study area. These mutation types were described in Cyprus *Cx. pipiens* populations (Alout et al., 2007b; Alout et al., 2009). Although G119S mutation were described around the world, F290V was rarely recorded in Mediterranean countries such as Greece, Morocco, Tunisia, and Türkiye (Alout et al., 2007b; Kioulous et al., 2014; Ben Cheikh et al., 2009; Taskin et al., 2016; Arich et al., 2021). Two different codon combinations were identified at the F290 mutation point causing organophosphate/carbamate resistance in this study, and these combinations were TTT codon (wild type) and GTT codon (encoding valine). The frequency of the TTT codon in all populations was high and its value was 0.842. The GTT codon was found only as heterozygous in the populations. The results obtained in the study correlated with the results obtained in the Aegean Region of Türkiye (Taskin et al., 2016). For all AChE mutations, studied populations showed tendencies towards an excess of heterozygotes. Similar results were obtained by some authors (Taskin et al., 2016; Arich et al., 2021).

Our results revealed different degrees of the target-site mutation related to the insecticide resistance. Target-site mutations showed heterozygosity in the field the degree of the mutations still low in the field. This situation will be problematic for the future control application in the field due to the nature of the insecticide resistance. In the AMOVA analyses made with the resistance frequencies obtained, the resistance differences between artificial sites and agricultural fields were determined to be low. However, there was substantial variation within populations. This situation shows that insecticide resistance did not differ in terms of area (constructed/modified or temporary growing areas by changing human and agricultural areas) and that insecticide resistance could be related to whether or not insecticide was used in the areas.

References

Akıner, M. M., S. S. Caglar & F. M. Simsek, 2013. Yearly changes of insecticide susceptiblity and possible insecticide resistance mechanisms of *Anopheles maculipennis* Meigen (Diptera: Culicidae) in Turkey. Acta Tropika, 126 (3): 280-285.

Akıner, M. M. & E. Ekşi, 2015. Evaluation of insecticide resistance and biochemical mechanisms of *Culex pipiens* L. in four localities of east and middle mediterranean basin in Turkey. International Journal of Mosquito Research, 2 (3): 39-44.

Akıner, M. M., M. Öztürk, A. B. Başer, F. Günay, S. Hacıoğlu, A. Brinkmann, N. Emanet, B. Alten, A. Ozkul, A. Nitsch, Y. M. Linton & K. Ergünay, 2019. Arboviral screening of invasive Aedes species in northeastern Turkey: West Nile virus circulation and detection of insect-only viruses. PLoS Neglected Tropical Diseases, 13 (5): e0007334.

Alout, H., A. Berthomieu, F. Cui, Y. Tan, C. Berticat, C. L. Qiao & M. Weill, 2007a. Different amino-acid substitutions confer insecticide resistance through acetylcholinesterase 1 insensitivity in *Culex vishnui* and *Culex tritaeniorhynchus* (Diptera: Culicidae) from China. Journal of Medical Entomology, 44 (1): 463e469.

Alout, H., A. Berthomieu, A. Hadjivassilis & M. Weill, 2007b. A new amino-acid substitution in acetylcholinesterase 1 confers insecticide resistance to *Culex pipiens* mosquitoes from Cyprus. Insect Biochemistry & Molecular Biology, 37 (1): 41-47.

Alout, H., L. Djogbénou, C. Berticat, F. Chandre & M. Weill, 2008. Comparison of *Anopheles gambiae* and *Culex pipiens* acetycholinesterase 1 biochemical properties. Biochemistry & Molecular Biology, 150 (3): 271-277.

Alout, H., P. Labbe, A. Berthomieu, N. Pasteur & M. Weill, 2009. Multiple duplications of the rare *ace-1* mutation F290V in *Culex pipiens* natural populations. Insect Biochemistry & Molecular Biology, 39 (1): 884-891.

Ari, A., 1972. Studies on activity and ecology of arboviruses in Turkey. Türk Hijyen ve Deneysel Biyoloji Dergisi, 32 (1): 134-143.

Arich, S., N. Assaid, H. Taki, M. Weill, P. Labbé & M. H. Sarih, 2021. Distribution of insecticide resistance and molecular mechanisms involved in the West Nile vector *Culex pipiens* in Morocco. Pest Management Science, 77 (3): 1178-1186.

Awolola, T. S., A. O. Oduola, I. O. Oyewole, J. B. Obansa, C. N. Amajoh, L. L. Koekemoer & M. Coetzee, 2007. Dynamics of knockdown pyrethroid insecticide resistance alleles in a field population of *Anopheles gambiae* ss in southwestern Nigeria. Journal of Vector Borne Diseases, 44 (3): 181-188.

Ben Cheikh, R., C. Berticat, A. Berthomieu, N. Pasteur, H. Ben Cheikh & M. Weill, 2014. Genes conferring resistance to organophosphorus insecticides in *Culex pipiens* (Diptera: Culicidae) from Tunisia. Journal of Medical Entomology, 46 (3): 523-530.

Bkhache, M., F. Z. Tmimi, O. Charafeddine, C. Faraj, A. B. Faillou & M. H. Sarih, 2016. First report of L1014F-*kdr* mutation in *Culex pipiens* complex from Morocco. Parasites & Vectors, 9 (1): 1-7.

Bkhache, M., F. Z. Tmimi, O. Charafeddine, O. B. Filali, M. Lemrani, P. Labbé & M. H. Sarih, 2019. G119S *ace-1* mutation conferring insecticide resistance detected in the *Culex pipiens* complex in Morocco. Pest Management Science, 75 (1): 286-291.

Ceianu, C. S., A. Unqureanu, G. Nikolescu, C. Cernescu, L. Nitescu, G. Tardei, A. Petrescu, D. Pitigoi, D. Martin & V. Ciulacu-Purcarea, 2001. West Nile virus surveillance in Romania: 1997-2000. Viral Immunology, 14 (1): 251-262.

Chandrasiri, P. G. K., S. D. Fernando & B. N. K. De Silva, 2020. Insecticide resistance and molecular characterization of knockdown resistance (*kdr*) in *Culex quinquefasciatus* mosquitoes in Sri Lanka. Journal of Vector Ecology, 45 (2): 204-210.

Colovic, M. B., D. Z. Krstic, T. D. Lazarevic-Pasti, A. M. Bondzic & V. M. Vasic, 2013. Acetylcholinesterase inhibitors: pharmacology and toxicology. Current Neuropharmacology, 11 (3): 315-335.

Dabire, R. K., M. Namountougou, A. Diabaté, D. D. Soma, J. Bado, H. K. Toé & P. Combary, 2014. Distribution and frequency of *kdr* mutations within *Anopheles gambiae* sl populations and first report of the *ace-1* G119S mutation in *Anopheles arabiensis* from Burkina Faso (West Africa). PloS One, 9 (7): e101484.

Diaz-Badillo, A., B. G. Bolling, G. Perez-Ramirez, C. G. Moore, J. P. Martinez-Munoz, A. A. Padilla-Viveros & M. De Lourdes Munoz, 2011. The distribution of potential West Nile virus vectors, *Culex pipiens pipiens* and *Culex pipiens quinquefasciatus* (Diptera: Culicidae), in Mexico City. Parasites & Vectors, 4 (1): 1-12.

Donnely, M. J., V. Corbel, D. Weetman, C. S. Wilding, M. S. Williamson & W. C. Black, 2009. Does *kdr* genotype predict insecticide-resistance phenotype in mosquitoes? Trends in Parasitology, 25 (5):213-219.

Edi, C. V., B. G. Koudou, C. M. Jones, D. Weetman & H. Ranson, 2012. Multiple-insecticide resistance in *Anopheles gambiae* mosquitoes, Southern Côte d'Ivoire. Emerging Infectious Diseases, 18 (1): 1508-1511.

EEA, 2018. CORINE Land Cover (CLC), Version 17. (Web page: https://land.copernicus.eu/pan-european/corine-land-cover/clc2018) (Date accessed: March 2022).

Ergunay, K., F. Gunay, O. E. Kasap, K. Oter, S. Gargari, T. Karaoglu & B. Alten, 2014. Serological, molecular and entomological surveillance demonstrates widespread circulation of West Nile virus in Turkey. PLoS Neglected Tropical Diseases, 8 (7): e3028.

Grigoraki, L., A. Puggioli, K. Mavridis, V. Douris, M. Montanari, R. Bellini & J. Vontas, 2018. Author correction: striking diflubenzuron resistance in *Culex pipiens*, the prime vector of West Nile Virus. Scientific Reports, 8 (1): 1-8.

Hemingway, J., N. J. Hawkes, L. McCarrol & H. Ranson, 2004. The molecular basis insecticide resistance in mosquitoes. Insect Biochemistry and Molecular Biology, 34 (7): 653-665.

Kalaycioglu, H., G. Korukluoglu, A. Ozkul, O. Oncul, S. Tosun, O. Karabay & A. B. Altas, 2012. Emergence of West Nile virus infections in humans in Turkey, 2010 to 2011. Euro surveillance, 17 (21): 20182.

Kasai, S., I. S. Weerashingle & T. Shono, 1998. P450 monooxygenase are an important mechanism of permethrin resistance in *Cx. quinquefasciatus* Say larvae. Archives Insect Biochemistry & Physiology, 37 (1): 47-56.

Kioulos, I., A. Kampouraki, E. Morou, G. Skavdis & J. Vontas, 2014. Insecticide resistance status in the major West Nile virus vector *Culex pipiens* from Greece. Pest Management Science, 70 (4): 623-627.

Kumar, S., G. Stecher & K. Tamura, 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology & Evolution, 33 (7): 1870-1874.

Lol, J. C., M. E. Castellanos, K. A. Liebman, A. Lenhart, P. M. Pennington & N. R. Padilla, 2013. Molecular evidence for historical presence of knock-down resistance in *Anopheles albimanus*, a key malaria vector in Latin America. Parasites & Vectors, 6 (1): 1-7.

Major, K. M., D. P. Weston, M. J. Lydy, K. E. Huff Hartz, G. A. Wellborn, A. R. Manny & H. C. Poynton, 2020. The G119S *ace-1* mutation confers adaptive organophosphate resistance in a nontarget amphipod. Evolutionary Applications, 13 (4): 620-635.

Marshall, E., 2000. A renewed assault on an old and deadly foe. Science, 290 (1): 428-430.

Martinet, J. P, H. Ferte, A. B. Faillox, F. Schafner & J. Depaquit, 2019. Mosquitoes of North-Western Europe as Potential Vectors of Arboviruses. A Review, 11 (11): 1059.

Martinez-Torres, D., F. Chandre, M. S. Williamson, F. Darriet, J. B. Bergé, A. L. Devonshire & D. Pauron, 1998. Molecular characterization of pyrethroid knockdown resistance (*kdr*) in the major malaria vector *Anopheles gambiae* ss. Insect Molecular Biology, 7 (2): 179-184.

Massouli, E. J., J. L. Sussman, B. P. Doctor, H. Soreq, B. Velan, M. Cygler, R. Rotundo, A. Shafferman, I. Silman & P. Taylor, 1992. "Recommendations for Nomenclature in Cholinesterases, 285-288". In: Multidisciplinary Approaches to Cholinesterase Functions (Eds. A. Shafferman & B. Velan). Plenum Press, New York, USA, 293 pp.

Medlock, J., T. Balenghien, B. Alten, V. Versteirt & F. Schaffner, 2018. Field sampling methods for mosquitoes, sandflies, biting midges and ticks: VectorNet project 2014-2018. EFSA Supporting Publications, 15 (6): 1435E.

Nabeshima, T., A. Mori, T. Kozaki, Y. Iwata, O. Hidoh, S. Harada, S. Kasai, D. W. Severson, Y. Kono & T. Tomita, 2004. An amino acid substitution attributable to insecticide-resistance in a Japanese encephalitis vector mosquito, *Culex tritaeniorhynchus*. Biochemical and Biophysical Research Communications, 313 (1): 794e801.

Osta, M. A., Z. J. Rizk & P. Labbé, 2012. Insecticide resistance to organophosphates in *Culex pipiens* complex from Lebanon. Parasites & Vectors, 5 (1): 1-6.

Ozkul A., Y. Yildirim, D. Pinar, A. Akcali, V. Yilmaz & D. Colak, 2006. Serological evidence of West Nile Virus (WNV) in mammalian species in Turkey. Epidemiology & Infection, 134 (4): 826-829.

Ponce, G., I. P. Sanchez, S. M. García, J. M. Torrado, S. Lozano-Fuentes, B. Lopez-Monroy & A. E. Flores, 2016. First report of L1014F *kdr* mutation in *Culex quinquefasciatus* in Mexico. Insect Science, 23 (1): 829- 834.

Rezza, G., 2014. West Nile virus infections in south-eastern Europe and in the Eastern Mediterranean area. Journal of Microbiology and Infectious Diseases Special Issue, 1 (1): 10-16.

Rinkevich, F. D., Y. Du & K. Dong, 2013. Diversity and convergence of sodium channel mutations involved in resistance to pyrethroids. Pesticide Biochemistry & Physiology, 106 (3): 93-100.

Roberts, D. R. & R. G. Andre, 1994. Insecticide resistance issues in vector-borne disease control. American Journal of Tropical Medicine & Hygiene, 5 (1): 21-34.

Schaffner, F., A. Guy, G. Bernard, H. Jean-Paul, A. Rhaiem & J. Brunhes, 2001. Les moustiques d'Europe: Logiciel d'identification et d'enseignement [The Mosquitoes of Europe: An Identification and Training Program]. Paris (FRA); Montpellier: IRD; EID, 1 CD ROM (Didactiques). ISBN 2-7099-1485-9.

Scott, J. G., M. H. Yoshimizu & S. Kasai, 2015. Pyrethroid resistance in *Culex pipiens* mosquitoes. Pesticide Biochemistry & Physiology, 120 (1): 68-76.

Shi, L., H. Hu, K. Ma, D. Zhou, J. Yu & D. Zhong, 2015. Development of resistance to pyrethroid in *Culex pipiens pallens* population under different insecticide selection pressures. PLOS Neglected Tropical Diseases, 9 (8): e0003928.

Smith, J. L. & D. M. Fonseca, 2004. Rapid assays for identification of members of the *Culex (Culex) pipiens* complex, their hybrids, and other sibling species (Diptera: Culicidae). The American Journal of Tropical Medicine & Hygiene, 70 (4): 339-345.

Taskin, B. G., T. Dogaroglu, S. Kilic, E. Dogac & V. Taskin, 2016. Seasonal dynamics of insecticide resistance, multiple resistance, and morphometric variation in field populations of *Culex pipiens*. Pesticide Biochemistry & Physiology, 129 (1): 14-27.

Wang, Z. M., C. X. Li, D. Xing, Y. H. Yu, N. Liu, R. D. Xue, Y. D. Dong & T. Y. Zhao, 2012. Detection and widespread distribution of sodium channel alleles characteristic of insecticide resistance in *Culex pipiens* complex mosquitoes in China. Medical & Veterinary Entomology, 26 (2): 228-232.

Wang, Y., W. Yu, H. Shi, Z. Yang & Y. Ma, 2015. Historical survey of the *kdr* mutations in the populations of *Anopheles sinensis* in China in 1996-2014. Malaria Journal, 14 (1): 1-10.

Weill, M. G., K. Lutfalla, F. Mogensen, A. Chandre & C. Berthomieu, 2003. Berticat Comparative genomics: insecticide resistance in mosquito vectors. Nature, 423 (6936): 136-137.

Weill, M., C. Malcolm, F. Chandre, K. Mogensen, A. Berthomieu & M. Marquine, 2004. The unique mutation in ace-1 giving high insecticide resistance is easily detectable in mosquito vectors. Insect Molecular Biology, 13 (1): 1-7.

Whalon, M. E., D. Mota-sanchez & R. M. Hollingworth, 2008. “Analysis of Global Pesticide Resistance in Arthropods, 5-31”. In: Global Pesticide Resistance in Arthropods (Eds. M. E. Whalon, D. Mota-sanchez & R. M. Hallington). CAB international, Cambridge, MA, UK, 166 pp.

Wirth, M. C. & G. P. Georghiou, 1996. Organophosphate resistance in *Culex pipiens* from Cyprus. Journal of the American Mosquito Control Association, 12 (1): 112e118.

Yu, F. H. & W. A. Catterall, 2003. Overview of the voltage-gated sodium channel family. Genome Biology, 4 (3): 1-7.

Zakhia, R., L. Mousson, M. Vazeille, N. Haddad & A. B. Failloux, 2018. Experimental transmission of West Nile virus and Rift Valley Fever virus by *Culex pipiens* from Lebanon. PLOS Neglected Tropical Diseases, 12 (1): e0005983.

Zhong, D., X. Chang, G. Zhou, Z. He, F. Fu, Z. Yan, G. Zhu & T. Xu, 2013. Relationship between knockdown resistance, metabolic detoxification and organismal resistance to pyrethroids in *Anopheles sinensis*. PLoS One, 8 (2): e55475.

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Original article (Orijinal araştırma)

Distribution and prevalence of root-knot nematode species in greenhouse vegetables in northern Iraq[1]

Kuzey Irak'taki sera sebzelerinde kök-ur nematodu türlerinin dağılımı ve yaygınlığı

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Abstract

The objective of the study was to determine the distribution and prevalence of root-knot nematodes (*Meloidogyne* spp.) in greenhouse vegetables in Sulaymaniyah, Erbil and Duhok Provinces of northern Iraq. One hundred and eighty-seven greenhouses were surveyed during November and December 2018. *Meloidogyne* spp. were identified by perineal patterns and esterase phenotype. *Meloidogyne* were detected in 37% of the greenhouses surveyed and the prevalence were 40% in Sulaymaniyah, 38% in Duhok and 34% in Erbil. *Meloidogyne javanica* Treub, 1885 and *Meloidogyne incognita* Kofoid & White, 1919 (Tylenchida: Meloidogynidae), were found in 64 and 36% of the greenhouses infested with *Meloidogyne*, respectively. By province surveyed, *M. incognita* and *M. javanica* were detected in 23 and 15% of greenhouses in Duhok, 12 and 22% of greenhouses in Erbil, 10 and 30% of surveyed greenhouses in Sulaymaniyah, respectively. *Meloidogyne* spp. were found in arugula, cauliflower, cucumber, eggplant, lettuce, tomato and zucchini. The highest prevalence of *Meloidogyne* spp. were in cucumber (58%) and tomato (33%), which are the most commonly grown vegetables in greenhouses in the study area.

Keywords: Esterase, greenhouse, identification, Iraq, *Meloidogyne*

Öz

Bu çalışmanın amacı, Kuzey Irak'ın Süleymaniye, Erbil ve Duhok illerindeki sera sebzelerinde kök-ur nematodlarının (*Meloidogyne* spp.) dağılımının ve yaygınlığının belirlenmesidir. Yüz seksen yedi serada 2018 yılı Kasım ve Aralık aylarında sürvey yapılmıştır. *Meloidogyne* spp., perineal patternler ve esteraz fenotipi kullanılarak teşhis edilmiştir. Sürvey yapılan seraların %37'sinde *Meloidogyne* varlığı tespit edilmiş ve yaygınlık Süleymaniye'de %40, Duhok'da %38 ve Erbil'de %34'dür. *Meloidogyne* ile bulaşık seraların %64'ünde *Meloidogyne javanica* (Treub, 1885) ve %36'sında *Meloidogyne incognita* (Kofoid & White, 1919) (Tylenchida: Meloidogynidae) bulunmuştur. Sürvey yapılan ile göre, *M. incognita* ve *M. javanica*, sırasıyla Duhok'da seraların %23 ve %15'de, Erbil'de seraların %12 ve %22'de, Süleymaniye'de seraların %10 ve %30'da tespit edilmiştir. *Meloidogyne* spp., roka, karnabahar, hıyar, patlıcan, marul, domates ve kabakta bulunmuştur. *Meloidogyne* spp.'nin en yüksek yaygınlığı, çalışma alanındaki seralarda en yoğun yetiştirilen sebzeler olan hıyar (%58) ve domates (%33)'de tespit edilmiştir.

Anahtar sözcükler: Esteraz, sera, teşhis, Irak, *Meloidogyne*

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Introduction

Agricultural production has made significant progress in Iraq over the past few years (Hilal et al., 2022). Especially, northern Iraq has shifted from being a smallholder-based, food-producing region that met its basic needs to being significant food importer (Jongerden et al., 2019). Greenhouses, which can increase productivity and profitability and extend crop production season, contribute valuable to agricultural production (Omer, 2016; Hilal et al., 2022). This vegetable production system is expanding in northern Iraq and total area of the greenhouses in 2021 reached ~112 ha in this region, where Sulaymaniyah is the leader province with 71%, followed with Erbil 18% and Dohuk 10% (MoAWR, 2022). However, one of the main obstacles to the continued expansion of greenhouse production of vegetables is the greater need for plant protection practices, since greenhouses have suitable conditions for the development of pests and diseases. In contrast to open fields, populations of plant-parasitic nematodes in greenhouse soil rapidly develop in the root zone due to stable microclimate, continuous plant cultivation, and the use of nematode-infested planting material by uniformed growers (Phani et al., 2021).

Root-knot nematodes (RKNs), *Meloidogyne* Göldi, 1887 (Tylenchida: Meloidogynidae), are considered to be some of the most harmful groups of plant-parasitic nematodes, and a limiting factor in the yield of greenhouse vegetable production globally (Sikora & Fernández, 2005). These obligate endoparasites feed within plant roots and induce root galls, which is the primary symptom of RKN infection on many plants. Due to the damaged root system, the capacity of the plant to absorb nutrients and water from the soil is reduced. In addition, nematode feeding sites, called giant cells, disrupt the plant metabolism and photosynthesis products are directed to these differentiated cells that provide nutrients for the nematode (Carneiro et al., 1999; Williamson & Gleason, 2003). As a result, the growth of infested plants is retarded and a reduction in crop yield and product quality occurs. In heavy nematode infestations, especially the seedlings, rapidly wilt and usually die (Sikora & Fernández, 2005).

Of more than 100 RKN species so far described (Ghaderi & Karssen, 2020), five have been found in Iraq (Hasan et al., 2020). Four of these species (*Meloidogyne arenaria* Neal, 1889, *Meloidogyne hapla* Chitwood, 1949, *Meloidogyne incognita* Kofoid & White, 1919 and *Meloidogyne javanica* Treub, 1885) have been present in this country for many years (Katcho, 1972; Katcho et al., 1976; Stephen et al., 1977, 1985; Al-Saaedy & Stephan, 1986; Stephan, 1997) whereas *Meloidogyne cruciani* Garcia-Martinez, Taylor & Smart, 1982 was only recently recorded as a new species for Iraq (Hasan et al., 2020). These species have been reported to infest vegetable crops in various regions of Iraq (Al-Saaedy & Stephan, 1986; Al-Sabie & Ami, 1990; Stephan, 1997; Al-Kubaicy & Al-Sabe'a, 2014; Ami et al., 2018; Kandouh et al., 2018; Hasan et al., 2020). Of these studies, only one was conducted in greenhouses (Ami et al., 2018), while other studies were in open fields. Consequently, there is a lack of information on the distribution and identification of RKNs in the greenhouses in Iraq. In most of these studies, which were conducted to detect *Meloidogyne* spp. in Iraq, species identification was only made by microscopic examination of perineal patterns of the females (Al-Saaedy & Stephan, 1986; Al-Sabie & Ami, 1990; Ali et al., 2014; Al-Kubaicy & Al-Sabe'a, 2014; Ami et al., 2018; Kandouh et al., 2018; Aljuboori & Al-Hakeem, 2020). More recently, a few reports indicated that molecular methods were used for species identification combined with the perineal patterns (Hasan & Abood, 2018; Hanoon et al., 2018; Hasan et al., 2020). However, no studies have used biochemical methods (isozyme analysis) for the identification of RKNs.

The objective of the study was to determine the prevalence of RKNs in the greenhouses of Sulaymaniyah, Erbil and Dohuk Provinces of northern Iraq, and to identify *Meloidogyne* species collected from infested greenhouses in this region using morphological (perineal pattern morphology) and biochemical (esterase phenotype) methods.

Materials and Methods

Survey and sample analyses

The survey was conducted during November and December 2018 in greenhouse vegetables in Sulaymaniyah, Erbil and Duhok Provinces of northern Iraq (Figure 1). A total of 187 greenhouses arbitrarily selected from 30 districts were surveyed at the end of the season, whenever plants were at least 3 months old post planting. In each greenhouse, 5 to 8 plants with the aboveground symptoms of RKN (yellowing, wilting and stunting) were sampled. Root zone soil and root samples were taken and combined to obtain a composite sample for each greenhouse. These samples were placed into plastic bags, labeled and taken to the laboratory for assessment where the samples were kept at 4°C and processed within 3 days.

In composite samples, the roots were washed with water, and rated on a scale of 0 to 5: 0, no galling; 1, trace infestation with some minor galls; 2, <25% galled roots; 3, 25-50% of galled roots; 4, 51-75%; and 5, >75% of galled roots (Hussey & Janssen, 2002). The RKN severity in each greenhouse was determined based on the roots with highest gall rating in each composite sample. The prevalence of RKN for each province was calculated as the number of greenhouses with RKN divided by total number of greenhouses surveyed × 100 (Carrillo-Fasio et al., 2021).

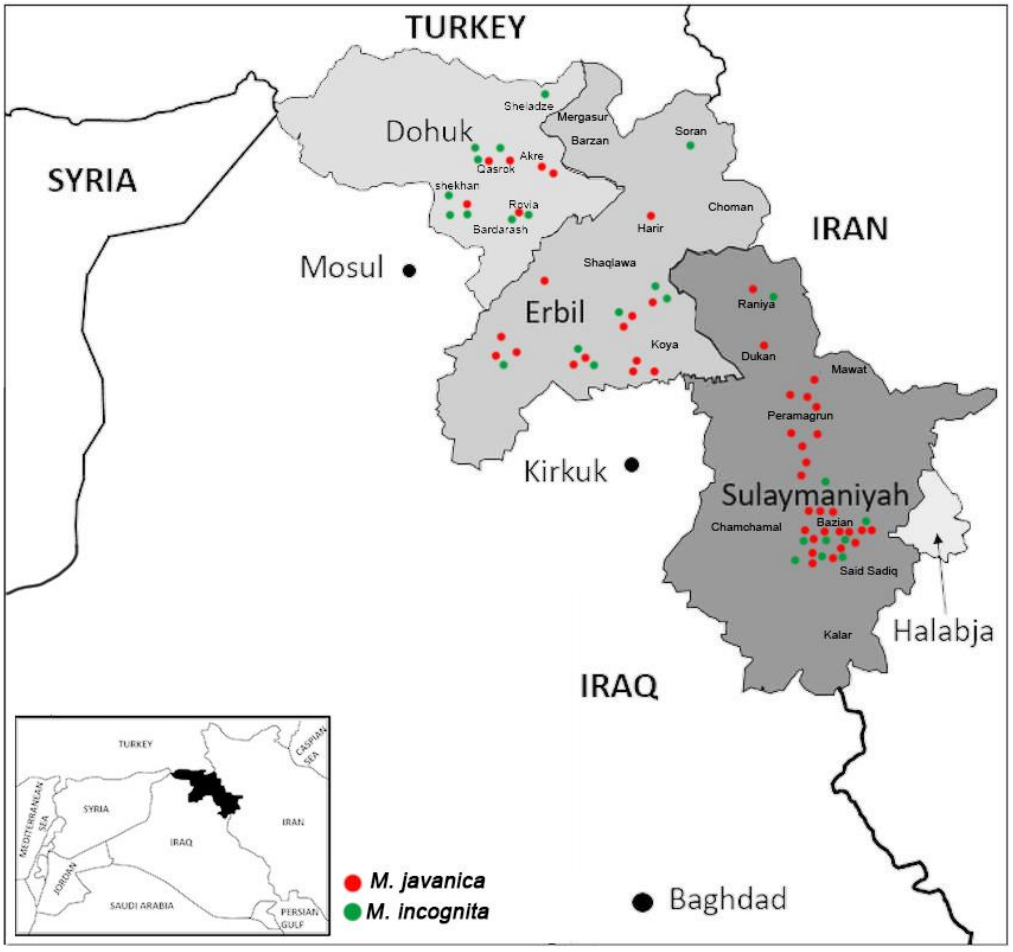

Figure 1. Distribution map of *Meloidogyne* spp. in vegetable greenhouses in northern Iraq. Each dot represents a single population.

Nematode extraction and identification

RKN populations were obtained by planting tomato (*Solanum lycopersicum* L.) cv. Falcon as individual seedlings into pots filled with 450 cm^3 of composite soil samples from each greenhouse (Aydınlı, 2018). Pots were maintained at 25 ± 2°C in the greenhouses and plants were uprooted after 60 days. Females were randomly picked from roots and used for morphological (perineal pattern) and biochemical (esterase phenotype) identification. Ten mature females from each population were arbitrarily selected for morphological identification. Females were transferred into 45% lactic acid and their perineal areas were cut and cleaned, then mounted in glycerine on glass slides (Hartman & Sasser, 1985). Perineal patterns were examined with a light microscope. Twenty-one young females from each population were used for biochemical identification. A single female was transferred to a bottom-sealed microhematocrit tube with 5 µL of extraction solution (20% sucrose with 1% Triton X-100) and crushed with a pestle. The specimens were stored at –20°C. Electrophoresis was performed according to Aydınlı & Mennan (2016). The polyacrylamide gels were stained for esterase activity with the substrate α-naphthyl acetate in the dark at 37°C for 20-30 min. Protein of females obtained from pure laboratory cultures of *M. javanica* was included in each gel as reference samples.

Results

RKNs was found in the three provinces surveyed (Figure 1). Of the 187 greenhouses, 70 (37%) were infested with RKN. The occurrence of RKN was greater in Sulaymaniyah (40%) than Duhok (38%) and Erbil (34%) Provinces (Table 1).

Eighty-eight greenhouses from 11 districts in Sulaymaniyah Province were surveyed and RKN was detected in eight districts. RKNs were not found in Tasluja, Tainal and Takia districts. The highest number of surveyed greenhouses was located to the Allai district (20 greenhouses) with RKN found in 55% (11 greenhouses), so in combination about one-third of greenhouses were infested with RKNs in this province. The prevalence of RKN in other districts varied from 25 to 50% (Table 1).

In Erbil Province, which ranks second in terms of greenhouse area after Sulaymaniyah in northern Iraq, the survey included 59 greenhouses from 11 districts. *Meloidogyne* was not detected in greenhouses in Choman, Gomaspan, Mamajalka and Grdarasha districts, while 20 to 63% of greenhouses in other districts of Erbil were found to be infested with RKNs (Table 1).

In Duhok Province, 40 greenhouses in eight districts were surveyed. RKNs were not found in Ble and Bardarash districts, but in the other districts the prevalence of RKN varied from 20 to 66.7% (Table 1).

When the perineal patterns of the females in 70 populations multiplied on tomatoes were examined, the morphology of perineal patterns was very similar to those of the original descriptions of *M. incognita* or *M. javanica*. Perineal patterns of females of 45 populations showed a district lateral field apparently separated from striae by parallel lines similar to that of *M. javanica* (Figure 2). Additionally, the patterns of these females were oval-shaped or rounded with a low dorsal arch. When the individual females of these populations were analyzed for their esterase phenotypes, *M. javanica* specific esterase phenotype (J3) was only detected (Figure 3). Perineal patterns of females in the remaining 25 populations had a high and squarish dorsal arch without lateral lines, representing *M. incognita* (Figure 2). The esterase phenotypes I1 and I2 observed in these populations confirmed the occurrence of *M. incognita* (Figure 3). In contrast to the phenotype I2 detected as the most common esterase phenotypes in these populations, phenotype I1 was only found in three populations from Erbil (ER6 and ER13) and Duhok (DU7) Provinces.

Table 1. Distribution and prevalence of root-knot nematodes (*Meloidogyne* spp.) in greenhouse vegetables in northern Iraq

Province	District	Greenhouses surveyed	Prevalence (%)*	Population code	Host plant	GI (0-5)**	Species
Duhok	Qasrok	6	50.0	DU1	Tomato	5	*M. incognita*
				DU2	Cucumber	2	*M. incognita*
				DU3	Cucumber	1	*M. javanica*
	Chammah	3	66.7	DU4	Lettuce	1	*M. javanica*
				DU5	Cucumber	3	*M. incognita*
	Shifazan	7	42.9	DU6	Tomato	5	*M. javanica*
				DU7	Tomato	4	*M. incognita*
				DU8	Cucumber	3	*M. incognita*
	Bjil	5	40.0	DU9	Cucumber	4	*M. javanica*
				DU10	Cucumber	4	*M. javanica*
	Shiladz	5	20.0	DU11	Zucchini	2	*M. incognita*
	Spimar	7	57.1	DU12	Cucumber	2	*M. incognita*
				DU13	Cucumber	1	*M. javanica*
				DU14	Tomato	2	*M. incognita*
				DU15	Tomato	5	*M. incognita*
	Ble	3	0	-	-	-	-
	Bardarash	4	0	-	-	-	-
Erbil	Soran	5	20.0	ER1	Cucumber	1	*M. incognita*
	Harir	3	33.3	ER2	Cucumber	2	*M. javanica*
	Qaryatakh	8	37.5	ER3	Cucumber	1	*M. incognita*
				ER4	Cucumber	5	*M. javanica*
				ER5	Tomato	1	*M. javanica*
	Bnberz	6	50.0	ER6	Tomato	3	*M. incognita*
				ER7	Lettuce	2	*M. incognita*
				ER8	Lettuce	3	*M. javanica*
	Mastawa	8	62.5	ER9	Cucumber	3	*M. javanica*
				ER10	Tomato	3	*M. javanica*
				ER11	Cucumber	5	*M. javanica*
				ER12	Cucumber	2	*M. javanica*
				ER13	Zucchini	3	*M. incognita*
	Qushtapa	7	57.1	ER14	Cucumber	1	*M. javanica*
				ER15	Lettuce	1	*M. javanica*
				ER16	Cucumber	4	*M. javanica*
				ER17	Cucumber	5	*M. incognita*
	Pirdawd	5	60.0	ER18	Cucumber	2	*M. incognita*
				ER19	Cucumber	2	*M. javanica*
				ER20	Cucumber	3	*M. javanica*
	Mamajalka	4	0	-	-	-	-
	Gomaspan	5	0	-	-	-	-
	Grdarasha	4	0	-	-	-	-
	Choman	4	0	-	-	-	-

Table 1. Continued

Province	District	Greenhouses surveyed	Prevalence (%)*	Population code	Host plant	GI (0-5)**	Species
Sulaymaniyah	Allai	20	55.0	SU1	Tomato	3	*M. javanica*
				SU2	Tomato	4	*M. incognita*
				SU3	Cucumber	5	*M. incognita*
				SU4	Cucumber	3	*M. javanica*
				SU5	Cucumber	4	*M. incognita*
				SU6	Zucchini	4	*M. javanica*
				SU7	Cucumber	2	*M. javanica*
				SU8	Cucumber	1	*M. javanica*
				SU9	Cucumber	2	*M. javanica*
				SU10	Cucumber	5	*M. incognita*
				SU11	Cucumber	4	*M. incognita*
	Mahmudia	8	37.5	SU12	Eggplant	2	*M. javanica*
				SU13	Cucumber	3	*M. incognita*
				SU14	Cucumber	4	*M. javanica*
	Qushqaya	10	50.0	SU15	Cucumber	5	*M. javanica*
				SU16	Tomato	4	*M. javanica*
				SU17	Cucumber	1	*M. javanica*
				SU18	Tomato	2	*M. incognita*
				SU19	Cucumber	1	*M. javanica*
	Halai	9	44.4	SU20	Cucumber	3	*M. javanica*
				SU21	Arugula	1	*M. javanica*
				SU22	Eggplant	5	*M. javanica*
				SU23	Cucumber	1	*M. javanica*
	Bazian	10	50.0	SU24	Cucumber	4	*M. javanica*
				SU25	Zucchini	5	*M. javanica*
				SU26	Cauliflower	5	*M. javanica*
				SU27	Cucumber	1	*M. incognita*
				SU28	Tomato	1	*M. javanica*
	Piramagron	9	44.4	SU29	Cucumber	5	*M. javanica*
				SU30	Cucumber	3	*M. javanica*
				SU31	Arugula	1	*M. javanica*
				SU32	Cucumber	5	*M. javanica*
	Dokan	4	25.0	SU33	Cucumber	1	*M. javanica*
	Rania	5	40.0	SU34	Tomato	5	*M. incognita*
				SU35	Tomato	3	*M. javanica*
	Tasluja	5	0	-	-	-	-
	Tainal	4	0	-	-	-	-
	Takia	4	0	-	-	-	-

* Number of greenhouses with *Meloidogyne* spp. divided by total number of greenhouses surveyed × 100. **Gall index: 0, no galling; 1, trace infestation with some minor galls; 2, <25% galled roots; 3, 25-50% of galled roots; 4, 51-75%; and 5, >75% of galled roots (Hussey & Janssen, 2002).

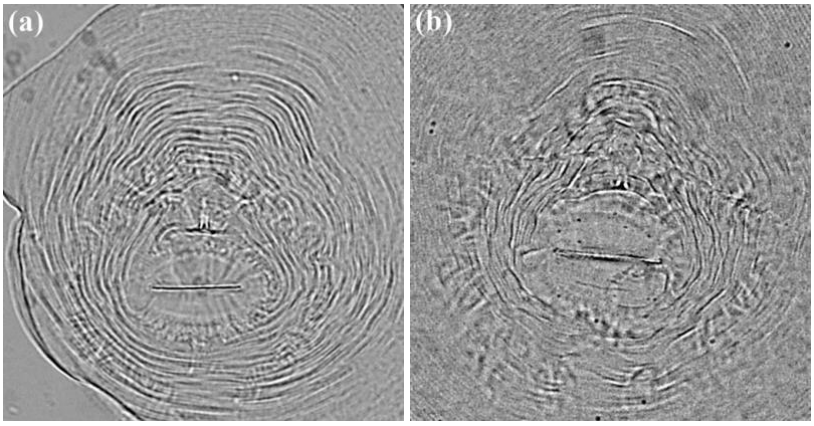

Figure 2. Perineal patterns of *Meloidogyne incognita* (a) and *Meloidogyne javanica* (b) from greenhouses in northern Iraq.

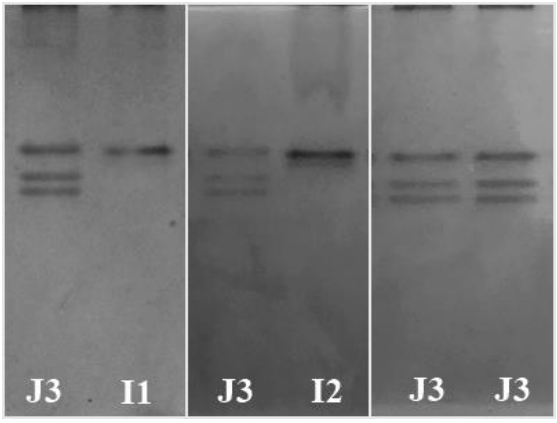

Figure 3. Esterase phenotypes of *Meloidogyne incognita* (I1 and I2) and *Meloidogyne javanica* (J3) from greenhouses in northern Iraq.

Based on the identification results obtained perineal pattern and esterase phenotypes of females, *M. javanica* and *M. incognita* were detected in 64 and 36% of the greenhouses infested with RKN, respectively. Considering the distribution of RKN species, both RKN species were found in the three provinces surveyed in northern Iraq (Table 1). In Duhok, *M. incognita* and *M. javanica* were detected in 23 and 15% of surveyed greenhouses, respectively. Both species occurred in all districts infested with RKN of this province, except in Bjil and Shiladz (Figure 1). In Erbil, *M. javanica* was the most common RKN species detected in 22% of the surveyed greenhouses, but *M. incognita* was found in 12% of the greenhouses. Both species were found in most districts infested with RKN in Erbil, except in Soran and Harir districts. In Sulaymaniyah, *M. javanica* was found in 30% of greenhouses surveyed and in all districts with RKNs. *Meloidogyne incognita* was found in 10% of surveyed greenhouses and was not detected in Halai, Piramagron and Dokan districts, where *M. javanica* occurred.

Eight vegetable species including cucumber (72 greenhouses), tomato (45 greenhouses), lettuce (20 greenhouses), zucchini (19 greenhouses), arugula (10 greenhouses), eggplant (8 greenhouses), cauliflower (7 greenhouses), broccoli (6 greenhouses) were sampled in the greenhouses surveyed and RKNs were found in all these vegetable species, except broccoli. The highest prevalence of RKNs was in cucumber (58%), which was cultivated in 39% of the greenhouses surveyed. The prevalence of *M. javanica* and *M. incognita* in cucumber were 39 and 19%, respectively. The prevalence in tomato was the second highest at 33%, of which 18 and 16% were *M. incognita* and *M. javanica*, respectively. RKN prevalence was 25% in eggplant, 20% in arugula and 14% in cauliflower with only *M. javanica* found in these species. *Meloidogyne javanica* and *M. incognita* were found in 15 and 5% on lettuces surveyed respectively, and both at 11% in zucchini.

Discussion

This study constitutes a comprehensive survey of RKN on vegetables in greenhouses in northern Iraq. Except for the study of Ami et al. (2018), the previous RKN surveys of vegetables in Iraq were conducted in open fields (Al-Saaedy & Stephan, 1986; Al-Sabie & Ami, 1990; Al-Kubaicy & Al-Sabe'a, 2014; Kandouh et al., 2018). Ami et al. (2018) reported the occurrence of *M. javanica* in cucumber in 16 greenhouses from four locations in Semel district of Duhok Province. RKN survey in open fields in various locations in northern Iraq found *M. javanica* and *M. incognita* in tomato in Duhok, *M. javanica* in eggplant in Erbil (Al-Sabie & Ami, 1990). In our study, *M. incognita* occurred more frequently than *M. javanica* in the greenhouses surveyed in Duhok Province than in Erbil and Sulaymaniyah. *Meloidogyne javanica* was the most common species found (24%) across all greenhouses surveyed, but *M. incognita* was found in 13% of greenhouses surveyed. Our results confirm earlier reports on the occurrence of RKN in several parts of Iraq (Al-Saaedy & Stephan, 1986; Al-Sabie & Ami, 1990; Al-Kubaicy & Al-Sabe'a, 2014; Kandouh et al., 2018). *Meloidogyne javanica*, which was detected in 80% of the eggplant fields surveyed in 17 provinces in Iraq, was the most abundant species, followed by *M. incognita* (Al-Saaedy & Stephan, 1986). Al-Kubaicy & Al-Sabe'a (2014) reported similar results on the occurrence and prevalence of both species in eggplant fields in Nineveh Province in northern Iraq. In these surveys of eggplant fields, *M. arenaria* was detected only in a few locations (Al-Saaedy & Stephan, 1986) or mixed with *M. javanica* (Al-Kubaicy & Al-Sabe'a, 2014). A similar pattern for these two species was also observed in okra fields of Najaf Province in southern Iraq, with 69% *M. javanica* and 31% *M. incognita* (Kandouh et al., 2018).

Globally, *M. arenaria, M. incognita* and *M. javanica*, with particularly wide host ranges, are the most prevalent RKN species. These species are mainly found in tropical and subtropical regions as well as in glasshouses in temperate regions (Zijlstra et al., 2000). According to the International *Meloidogyne* Project, which provides an overview of the global distribution of RKN species, *M. incognita* and *M. javanica* are more prevalent species than *M. arenaria* despite possessing similar temperature requirements (Van Gundy, 1985). Earlier reports, which indicate a rare occurrence of *M. arenaria* in Iraq, are in agreement with this global trend, and this species was only found in crops in open field (Katcho, 1972; Al-Saaedy & Stephan, 1986; Al-Sabie & Ami, 1990; Al-Kubaicy & Al-Sabe'a, 2014), so this is consistent with *M. arenaria* not being detected in the present study.

In the present study, RKNs were found in seven economically-important vegetable species (cucumber, tomato, lettuce, zucchini, arugula, eggplant and cauliflower), with prevalence was particularly high in cucumber and tomato, the most commonly grown vegetables in the study area. These results indicate that RKNs are a potential threat to greenhouse vegetable production in Iraq, and suitable control techniques should be developed and applied. Accordingly, accurate identification of RKNs is required to determine the most appropriate control methods (Coyne et al., 2009). In the past, perineal patterns have been frequently used for the identification of RKN in Iraq (Katcho, 1972; Al-Saaedy & Stephan, 1986; Al-Sabie & Ami, 1990; Al-Kubaicy & Al-Sabe'a, 2014; Ali et al., 2014; Ami et al., 2018; Kandouh et al., 2018; Aljuboori & Al-Hakeem, 2020). Although the identification of *Meloidogyne* spp. has relied on this morphology for many years, the value of this has decreased with the increasing number of RKN species described (Hunt & Handoo, 2009). Also, species-level identification is difficult due to variation in perineal patterns within and between populations (Garcia & Sanchez-Puerta, 2012). In our study, the perineal patterns of *M. javanica* had clear lateral lines separating the pattern into ventral and dorsal areas, this characteristic allowed for confident species determinations (Janati et al., 2018). The remaining populations had the typical perineal pattern of *M. incognita*. However, this does not provide reliable species-level identification because *incognita*-type perineal patterns have been observed in a considerable number of species, with some of them consequently being misidentified as *M. incognita* (Hunt & Handoo, 2009). The isozyme analyses, especially esterase phenotypes, have been widely used over many years as a reliable diagnostic technique for distinguishing RKN species from diverse geographical areas worldwide (Dickson et al., 1970; Esbenshade

& Triantaphyllou, 1985; Pais & Abrantes, 1989; Carneiro et al., 2000; Cofcewicz et al., 2004; Brito et al., 2008; Kolombia et al., 2017). In the present study, esterase enzyme phenotypes of females were also used for the diagnosis of *Meloidogyne* spp. Combining perineal pattern morphology and esterase phenotypes allowed for more reliable identification. Three esterase phenotypes, J3, I1 and I2, were obtained. The phenotype J3 is species-specific for *M. javanica*, and I1 and I2 for *M. incognita*. These phenotypes have consistently been associated with populations of these species from other parts of the world (Esbenshade & Triantaphyllou, 1985; Pais & Abrantes, 1989; Carneiro et al., 2001; Brito et al., 2008; Aydınlı & Mennan, 2016; Kolombia et al., 2017). To our knowledge, this exploration is the first study on esterase phenotypes of *Meloidogyne* populations from Iraq.

The occurrence of *M. javanica* and *M. incognita* has been commonly reported in studies conducted in open vegetable fields in Iraq (Al-Saaedy & Stephan, 1986; Al-Sabie & Ami, 1990; Al-Kubaicy & Al-Sabe'a, 2014; Kandouh et al., 2018). The prevalence of both RKN species in greenhouse vegetable production in northern Iraq confirms that these species are currently the dominant species of RKN in Iraq. This study provides evidence that these species are a significant threat in Iraq, with the potential considerable losses in both quality and quantity of vegetables. Consequently, further studies should focus on management approaches that will be needed to reduce this threat and the potential damage.

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References

Al-Kubaicy, L. M. & R. F. Al-Sabe'a, 2014. Survey of root-knot nematode *Meloidogyne* spp. association with eggplant plants in Ninevh province. Anbar Journal of Agricultural Sciences, 12 (2): 289-301.

Al-Saaedy, H. A. & Z. A. Stephan, 1986. Root-knot nematodes on eggplant in Iraq. Nematologia Mediterranea, 14 (2): 283-284.

Al-Sabie, R. F. & S. N. Ami, 1990. Identification of races of root-knot nematodes *Meloidogyne* spp. in northern Iraq. Arab Journal of Plant Protection, 8 (2): 83-87.

Ali, H. H., K. M. Fatah & A. I. Ahmed, 2014. First record of nematode root knot on *Zennia elegans* caused by *Meloidogyne javanica* plant in Erbil Governorate, Kurdistan Region, Iraq. Arab Journal of Plant Protection, 32 (3): 276-277.

Aljuboori, F. K. & A. M. Al-Hakeem, 2020. The first report of- root-knot nematode on *Cestrum nocturnum* in Ninawa, Iraq. Plant Archives, 20 (2): 6778-6780.

Ami, S. N., S. Ghaeib & A. Shingaly, 2018. Disease incidence, identification, and monthly fluctuations in the population density of root-knot nematodes *Meloidogyne javanica* on cucumber plants in Semel District, Duhok, Kurdistan Region, Iraq. Acta Universitatis Sapientiae, Agriculture and Environment, 10 (1): 52-65.

Aydınlı, G., 2018. Detection of the root-knot nematode *Meloidogyne luci* Carneiro et al., 2014 (Tylenchida: Meloidogynidae) in vegetable fields of Samsun Province. Turkish Journal of Entomology, 42 (3): 229-237.

Aydınlı, G. & S. Mennan, 2016. Identification of root-knot nematodes (*Meloidogyne* spp.) from greenhouses in the Middle Black Sea Region of Turkey. Turkish Journal of Zoology, 40 (5): 675-685.

Brito, J. A., R. Kaur, R. Cetintas, J. D. Stanley, M. L. Mendes, E. J. McAvoy, T. O. Powers & D. W. Dickson, 2008. Identification and isozyme characterization of *Meloidogyne* spp. infecting horticultural and agronomic crops, and weed plants in Florida. Nematology, 10 (5): 757-766.

Carneiro, R. M. D. G., M. R. A. Almeida & P. Queneherve, 2000. Enzyme phenotypes of *Meloidogyne* spp. populations. Nematology, 2 (6): 645-654.

Carneiro, R. G., P. Mazzafera & L. C. C. B. Ferraz, 1999. Carbon partitioning in soybean infected with *Meloidogyne incognita* and *M. javanica*. Journal of Nematology, 31 (3): 348-355.

Carrillo-Fasio, J. A., A. Angúlo-Castro, J. Á. Martínez-Gallardo, F. Ayala-Tafoya, M. G. Yáñez-Juárez & J. E. Retes-Manjarrez, 2021. Distribution and incidence of root-knot nematodes (*Meloidogyne* spp.) on pepper in Sinaloa, Mexico. Tropical Plant Pathology, 46 (2): 195-200.

Cofcewicz, E. T., R. M. D. Carneiro, P. Castagnone-Sereno & P. Quénéhervé, 2004. Enzyme phenotypes and genetic diversity of root-knot nematodes parasitising *Musa* in Brazil. Nematology, 6 (1): 85-95.

Coyne, D. L., H. H. Fourie & M. Moens, 2009. "Current and Future Management Strategies in Resource-Poor Farming, 444-475". In: Root-Knot Nematodes (Eds. R. N. Perry, M. Moens & J. L. Starr). CAB International, Wallingford, UK, 488 pp.

Dickson, D. W., J. N. Sasser & D. Huising, 1970. Comparative disc-electrophoretic protein analyses of selected *Meloidogyne, Ditylenchus, Heterodera*, and *Aphelenchus* spp. Journal of Nematology, 2 (4): 286-293.

Esbenshade, P. R. & A. C. Triantaphyllou, 1985. Use of enzyme phenotypes for identification of *Meloidogyne* species (Nematoda: Tylenchida). Journal of Nematology, 17 (1): 6-20.

Garcia, L. E. & M. V. Sanchez-Puerta, 2012. Characterization of a root-knot nematode population of *Meloidogyne arenaria* from Tupungato (Mendoza, Argentina). Journal of Nematology, 44 (3): 291-301.

Ghaderi, R. & G. Karssen, 2020. An updated checklist of *Meloidogyne* Göldi, 1887 species, with a diagnostic compendium for second-stage juveniles and males. Journal of Crop Protection, 9 (2): 183-193.

Hanoon, W. M., Z. A. Altememe & A. M. A. Rasool, 2018. Morphological and molecular diagnosis of root knot nematode species associated with olive seedling and trees in Baghdad, Babil and Karbala/ Iraq. Rafidain Journal of Science, 27 (4): 138-150.

Hartman, K. M. & J. N. Sasser, 1985. "Identification of *Meloidogyne* Species on the Basis of Differential Host Test and Perineal-Pattern Morphology, 69-77". In: An Advanced Treatise on *Meloidogyne*. Volume II: Methodology (Eds. K. R. Barker, C. C. Carter & J. N. Sasser). North Carolina State University Graphics, Raleigh, North Carolina, USA, 223 pp.

Hasan, S. T. & I. D. Abood, 2018. Identification of *Meloidogyne* spp. according to morphological characteristics, PCR-SCAR marker and 18S rDNA region in Iraq. IOSR Journal of Agriculture and Veterinary Science, 11 (1): 50-56.

Hasan, S. T., I. D. Abood & A. M. A. Rasoul, 2020. Diagnostic of new species of root knot nematode (*Meloidogyne cruciani*) associated with egg plant in Babylon Governorate/Iraq based on morphological characters and molecular methods. Ecology, Environment and Conservation, 26 (1): 69-74.

Hilal, Y. Y., M. K. Khessro, J. van Dam & K. Mahdi, 2022. Automatic water control system and environment sensors in a greenhouse. Water, 14 (7): 1166.

Hunt, D. J. & Z. A. Handoo, 2009. "Taxonomy, Identification and Principal Species, 55-97". In: Root-Knot Nematodes (Eds. R. N. Perry, M. Moens & J. L. Starr). CAB International, Wallingford, UK, 488 pp.

Hussey, R. S. & G. J. W. Janssen, 2002. "Root-Knot Nematodes: *Meloidogyne* Species, 43-70". In: Plant Resistance to Parasitic Nematodes (Eds. J. L. Starr, R. Cook & J. Bridge). CAB International, Wallingford, UK, 272 pp.

Janati, S., A. Houari, A. Wifaya, A. Essarioui, A. Mimouni, A. Hormatallah, M. Sbaghi, A. A. Dababat & F. Mokrini, 2018. Occurrence of the root-knot nematode species in vegetable crops in Souss Region of Morocco. The Plant Pathology Journal, 34 (4): 308-315.

Jongerden, J., W. Wolters, Y. Dijkxhoorn, F. Gür & M. Öztürk, 2019. The politics of agricultural development in Iraq and the Kurdistan Region in Iraq (KRI). Sustainability, 11 (21): 5874.

Kandouh, B. H., A. E. Hasan & A. M. A. Rasoul, 2018. Occurrence, prominence and severity of root-knot nematodes (*Meloidogyne* sp) associated with okra in Najaf province. Journal of Research in Ecology, 6 (2): 2130-2138.

Katcho, Z. A.,1972. First occurrence of certain root-knot nematode species in Iraq. Plant Disease Reporter, 56 (9): 824.

Katcho, Z. A., A. H. Alwan & A. H. Bandar, 1976. Root-knot nematodes and their hosts in Iraq. Bulletin of Natural History Research Centre, 7 (1): 38-41.

Kolombia, Y. A., G. Karssen, N. Viaene, P. L. Kumar, N. de Sutter, L. Joos, D. L. Coyne & W. Bert, 2017. Diversity of root-knot nematodes associated with tubers of yam (*Dioscorea* spp.) established using isozyme analysis and mitochondrial DNA-based identification. Journal of Nematology, 49 (2): 177-188.

MoAWR, 2022. The Data of Greenhouses in Kurdistan Region, Iraq. Ministry of Agriculture and Water Resources, KRG-Erbil, 5 pp.

Omer, A. M., 2016. Sustainable food production in greenhouses and its relations to the environment. Advances in Plants & Agriculture Research, 5 (3): 538-542.

Pais, C. S. & I. M. de O. Abrantes, 1989. Esterase and malate dehydrogenase phenotypes in Portuguese populations of *Meloidogyne* species. Journal of Nematology, 21 (3): 342-346.

Phani, V., M. R. Khan & T. K. Dutta, 2021. Plant-parasitic nematodes as a potential threat to protected agriculture: Current status and management options. Crop Protection, 144: 105573.

Sikora, R. A. & E. Fernández, 2005. "Nematode Parasites of Vegetables, 319-392". In: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture (Eds. M. Luc, R. A. Sikora & J. Bridge). CAB International, Oxfordshire, UK, 871 pp.

Stephan, Z. A., 1997. "Iraq". In: Plant Nematode Problems and Their Control in the Near East Region (Eds. M. A. Maqbool & B. Kerry), 320 pp. (Web page: https://www.fao.org/3/v9978e/v9978e0g.htm#iraq) (Date accessed: April, 2022).

Stephan, Z. A., A. H. Alwan & A. S. Al-Beldawi, 1977. General survey of root-knot nematode, *Meloidogyne* sp., on tobacco in Iraq. Yearbook of Plant Protection Research, 1: 284-294.

Stephan, Z. A., A. H. Alwan & B. G. Antone, 1985. Occurrence of plant parasitic nematodes in vineyard soils in Iraq. Nematologia Mediterranea, 13 (2): 261-264.

Van Gundy, S. D., 1985. "Ecology of *Meloidogyne* spp. - Emphasis on Environmental Factors Affecting Survival and Pathogenicity, 177-182". In: An Advance Treatise on *Meloidogyne*. Volume I: Biology and Control (Eds. J. N. Sasser & C. C. Carter). North Carolina State University Graphics, Raleigh, North Carolina, USA, 223 pp.

Williamson, V. M. & C. A. Gleason, 2003. Plant-nematode interactions. Current Opinion in Plant Biology, 6 (4): 327-333.

Zijlstra, C., D. T. H. M. Donkers-Venne & M. Fargette, 2000. Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterized amplified region (SCAR) based PCR assays. Nematology, 2 (8): 847-853.

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