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Original article

A novel capillary gel electrophoresis based fragment analysis method for the rapid detection of important thrips species on alfalfa in Turkey

Türkiye'de yoncada önemli thrips türlerinin hızlı tespiti için yeni bir kapiler jel elektroforez tabanlı fragment analiz yöntemi

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ABSTRACT

Thrips cause significant yield reduction in several industrial crops. Since these pests are also included in the quarantine organisms of Turkey, the rapid detection of agents is important to prevent their spread to new areas. Mitochondrial cytochrome oxidase I (COI) barcoding gene assay; one of the molecular methods is widely used in thrips identification. However, as the COI gene has a very short fragment length, it is very difficult to distinguish fragment sizes on agarose gel after PCR. In this study, a new identification method was developed by integrating the Capillary Gel Electrophoresis (CGE) system for *Thrips tabaci Lideman, Frankliniella occidentalis* (Pergande) and *Frankliniella intonsa* (Trybom) species, using primer pairs previously used by different researchers. The assay produces strong signals obtained by minimizing the margin of error in the separation of fragment lengths close to each other, especially in the short fragment length COI gene. Therefore, by eliminating the gel electrophoresis step, reliable detections could be obtained without exposure to hazardous chemicals. The novel method shortened the detection time and minimized the process mistakes on the detection of a single thrips with a low DNA concentration. Total 83 thrips individual (52 *F. intonsa*, 31 *F. occidentalis*) were able to be detected with this capillary gel electrophoresis based fragment analysis. The novel method is evaluated as unique, specific and quick for the detection of three different thrips species. It is also thought to be able to utilize for identification of different thrips species with short fragment sizes in the foreseeable future.

INTRODUCTION

There are many harmful organisms in alfalfa cultivation areas. Clover proboscis beetle [*Hypera variabilis* Hebst. (Coleoptera: Curculionidae)], 24-spot ladybird with main pest [*Subcoccinella vigintiquatuorpunctata* (Linnaeus 1758) (Coleoptera: Coccinellidae)], the Clover leaf beetle [(*Gonioctena fornicata* (Brüggem) (Coleoptera, Chrysomelidae) and thrips come primary pests on causing yield losses in alfalfa production. *Thrips palmi* Karny, 1925 and *Frankliniella occidentalis* (Pergande, 1895) are quarantine pests for many countries. It is essential to detect these pests quickly and accurately. It has been emphasized in different studies

that these pests need rapid detection to understand their biology and prevalence for developing a strategy in pest management (Danks 1988). Identification of thrips at genus level requires expertise and in most cases can only be conducted only by experienced people (Przybylska et al. 2015). However, although there are reports that thrips have been successfully identified at the larval stage (Skarlinsky and Funderburk 2016), this identification method is still not preferred (Mound 2013). As there are not enough diagnostic keys to identify at the level of eggs, larvae or pupae by morphological examination, adult individual thrips are generally preferred for identification (Mound 2013, Skarlinsky and Funderburk 2016). Besides identification studies of some thrips species such as *F. occidentalis* may cause doubtful results due to the high morphological diversity among species. Therefore, the use of molecular techniques is beneficial in reliable identification (Bravo-Pérez et al. 2018, Rugman-Jones et al. 2006). The use of molecular techniques gives more reliable results, especially in identification of the quarantine pests as mentioned above.

Hebert et al. (2003a, 2003b) designed a primer set to amplify the 648-nucleotide region of the mitochondrial cytochrome-c oxidase subunit I (COI) gene for rapid and accurate identification of a wide variety of biological samples. The method called "DNA barcoding" has several advantages compared to other methods. The standard DNA barcode region of the COI gene is very effective for species identification and discrimination. This region has good discrimination against most animal groups. These universal primers, originally designed for marine invertebrates, can be applied to all animal phylums (Folmer et al. 1994, Hebert et al. 2003a, 2003b, 2004). The 648-nucleotide fragment has sufficient information and can be sequenced directly. The alignment process is not difficult because it is a protein-coding region. Because of these advantages, the COI region is used for standard DNA barcoding studies. DNA barcoding is a simple and powerful method for routine identification by researchers, especially for large numbers of samples (Jinbo et al. 2011). DNA barcoding using sequence data obtained from a standard region of the COI gene offers important solutions for the identification of thrips species (Glover et al. 2010). The main reason for the use of this particular gene region is to obtain more phylogenetic signals with the presence of robust universal primers covering many animal branches (phyla) (Folmer et al. 1994, Glover et al. 2010, Zhang and Hewitt 1997). Mitochondrial DNA (mtDNA) is abundant, has a relatively fast evolution rate, and amplification of degraded mtDNA samples is possible. In contrast, nuclear genes have relatively slow evolution rates and are usually single copies (Glover et al. 2010). As a result of the analysis of the COI gene, three of *T. tabaci* (Brunner et al. 2004, Toda and Murai 2007) and two of *T. palmi* (Glover et al. 2010, Karimi et al. 2010) were identified. Similarly, two species of *F. occidentalis* were successfully determined (Iftikhar et al. 2016, Rugman-Jones et al. 2010). It has also been reported that the COI provides sufficient variation to be used in future DNA barcoding studies within the thrips species (Glover et al. 2010). Identifying economically important thrips species at the larval stage can be difficult and usually needs culturing (Jinbo et al. 2011). Especially in invasive quarantine organisms, the COI gene is very advantageous in terms of rapid pest identification (Glover et al. 2010). However, PCR products obtained from COI gene are usually required sequence analysis (Bravo-Pérez et al. 2018). In a study in 2017, *Thrips tabaci, T. palmi, F. occidentalis* and *Frankliniella intonsa* were identified by multiplex PCR in a single reaction using specific primers (Sabahi et al. 2017). As a result of the study, the researchers proved that the primers could detect these four species. The method was used for thrips identification at different developmental stages and reliable results were obtained for all samples examined. They reported that this method is simple to be applied by non-expert taxonomists and can also be detected quickly and reliably without sequence analysis (Sabahi et al. 2017). As interest in biodiversity has increased in the fields of ecological evolutionary biology, agriculture and economics, reliable identification of the organisms has been rising in importance (Jinbo et al. 2011). On the other hand, the number of taxonomists has decreased significantly, especially in the identification area of quarantine thrips pests. As a result, the necessity of alternative and reliable identification methods is getting increase day by day especially in absence of experts. Sabahi et al. (2017) could detect four different thrips quickly and reliably. However, it was determined in the study that the electrophoretic separation of *T. palmi, F. occidentalis* and *F. intonsa* fragments on agarose gel is quite difficult due to closely sized fragment lengths. The main reason of confusion is that 30-40 nucleotide differences are not clearly separated in the gel electrophoresis.

In this study, the amplicons obtained as a result of PCR analysis performed using primers developed by Sabahi et al. (2017) were analyzed using a high-efficiency DNA Fragment Analyzer instead of agarose gel. The closely sized fragments are separated with a DNA fragment analyzer and the analysis time is considerably shortened.

MATERIALS AND METHODS

Samples and DNA extraction

A total of 123 individual thrips were collected with a mouth aspirator from an alfalfa cultivation area in Aksaray province of Turkey. While 40 thrips were used for morphological identification, the rest of them were used for molecular identification. Collected samples were stored in ethyl alcohol before use. Positive controls DNA of *T. tabaci, F. occidentalis* and *F. intonsa* used in the molecular study were provided by the Plant Protection Central Research Institute, Ankara. Morphological identification was realized by Prof. Dr. Ekrem Atakan at Çukurova University. The samples were examined under a stereoscopic microscope and placed in AGA (9 parts of 60% ethyl alcohol, 1 part of glacial acetic acid, 1 part of glycerine) for identification. Thrips samples kept in this solution for one or two days were then taken and labelled in small plastic tubes containing 60% ethyl alcohol. To facilitate the preparation, the thrips samples, which were kept in AGA fluid for 2 days and then taken into alcohol (60% ethyl alcohol), were kept in a 5% NAOH fluid until individuals had a slight color change and the body content was cleaned by allowing this fluid to enter the body. After the samples were kept in 96% ethyl alcohol for 5 minutes, their preparations were made by taking them into Hoyer medium. The preparations (microscope slides) were kept in the oven at 45 °C for about 3 weeks to dry. Thysanoptera species were identified by the author using identification keys of Zur-Strassen (2003) and Balou et al. (2012).

Thrips samples were used in molecular studies for quick and economical DNA extraction as described in Sabahi et al. (2017). For this purpose, thrips individuals were ground with the help of a drill attached to the tip with a needle. The concentration and purity of the obtained total DNA was measured in an electrospectrophotometer (Nanodrop 2000-Thermo) and kept at -20oC for later use in the PCR stage.

Multiplex PCR

For the detection of *T. tabaci, F. occidentalis* and *F. intonsa* species in multiplex PCR, one general forward primer and three reverse primers (given in Table 1) specific to Cytochrome Oxidase I (COI) region were used. For PCR optimization studies, first, gradient PCR was performed with primers separately and optimal annealing degrees were determined.

Table 1. Primers and fragment sizes used in the detection of thrips species by multiplex PCR (Sabahi et al. 2017)

Name	Specificity	Sequence	Product length
tabR (reverse)	*Thrips tabaci*	5'-TGTGAT-AGCTCCCGCTAAC-3'	360 bp
occiR (reverse)	*Frankliniella occidentalis*	5'-GGTCCAGAGTGA-TAAAAAGTTGAC-3'	163 bp
intR (reverse)	*Frankliniella intonsa*	5'-AGGTATTTAAGT-TTCGATCTGTAAG-3'	390 bp
Common forward		5'-YTWGGAGCHCCH-GAYATAG -3	

In 25 µl total volume for multiplex PCR; 5 µl 5X GoTaq Buffer (Green), 1.25 µl MgCl2 (25 mM), 0.7 µl dNTPs (10 mM), 1 µl each of the specific reverse primers (10 µM), 2.5 µl common forward primer (10 µM), 0.5 µl DSMO (2%), 0.25 µl GoTaq Flexi DNA Polymerase (5 µl), 2.5 µl DNA template (20 ng/µl) and finally 8.3 µl nuclease free water were added. After 3 minutes of pre-denaturation at 94 oC, PCR was performed at 94 °C for 30 seconds, at 56 °C for 30 seconds, at 72 °C for 1 minute (35 cycles) and then at 72 °C for 1 minute. First amplicons were visualized on agarose gel before fragment analysis. PCR products were analyzed at 80 V for 60 minutes on a 1.5% agarose gel prepared with Pronosafe (Conda, Madrid, Spain) DNA dye and visualized under UV transilluminator.

Fragment analysis

After the gel electrophoresis process, 2 µl of the PCR products were mixed with 20 µl of dilution buffer for dilution of samples. The diluted PCR products were placed on the reading plate of the capillary gel electrophoresis (CGE) based analyzer (Qsep-100 ™, Bioptic, Taiwan). A high resolution cartridge with a capacity of 200 samples was placed to start the reading process on the device, and appropriate markers (marker created with quantitative markers and DNA amplicons determined for this study) and other buffer solutions (distilled water, separation buffer) were added. The reading procedures of 83 individuals were completed after the sample injection protocol at 8 kv for 10 seconds with a high resolution cartridge, following a 300 s separation process at 5 kv.

RESULTS AND DISCUSSION

Many of the thrips species are polyphagous pests and have similar host ranges. Although there are morphologically diagnostic keys (Glover et al. 2010, Rebijith et al. 2014), it is known that there are not sufficient criteria for the identification from egg, larva or pupa (Kadirvel et al. 2013).

This study morphological identification that 28 individuals (22♂ and 6♀) of 40 examined thrips were *F. intonsa* and the other 12 individuals (4♂ and 8♀) were *F. occidentalis*. In molecular studies, 11 larvae and 72 adults were used.

The economical DNA extraction method (20 µl of nuclease-free water) was performed for all individuals, including larvae. Approximately 5-25 ng/µl DNA concentration from each of the individuals was obtained. Multiplex PCR produced 163 bp and 390 bp fragments for *F. occidentalis* and *F. intonsa*, respectively (Figure 1). However, fragments of T. tabaci (360 bp) were not detected in the tests.

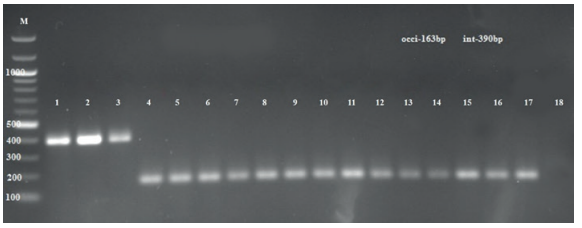

Figure 1. Agarose gel electrophoresis of the multiplex PCR products. (M: DNA 1-kb Ladder; line 1-3 *Frankliniella intonsa*; line 4-17, *Frankliniella occidentalis*; line 18, water control)

As the 360 bp and 390 bp fragments were found very closely to each other, capillary gel electrophoresis (Qsep-100 ™) was applied for these two primer pairs for quick and reliable results. It was observed that detection range of the commercial quantitative marker (20-1000 bp) in the range of 300-400 bp (*marked with) is quite wide (Figure 2). This marker was not used in the study due to the detection thresholds were in this range and deviated ± 10 bp instead of this marker, a new reference marker was created with previously determined DNA amplicons. The extreme signal values were obtained from the readings with this reference marker and they were recorded in the system. The markers used in the study showed that they are rather suitable for the identification of thrips.

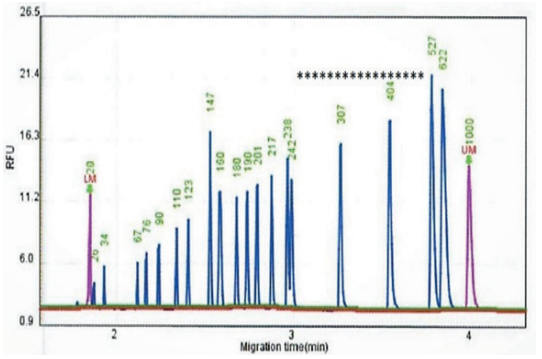

Figure 2. The image of the signal values of the commercial quantitative marker was used first in the study

Effective signal values in expected sizes were obtained with a deviation of approximately ± 2 bp in each reading with the values of the reference marker and commercial quantitative marker recorded in the Qsep-100 system. As a result of the signal values the samples were separated and identified in a more detailed form for three thrips species (Figure 3).

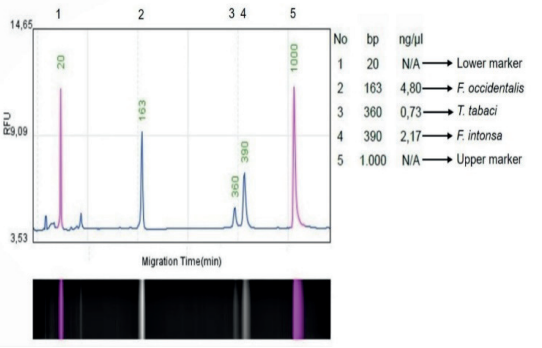

Figure 3. Capillary gel electrophoresis-based fragment analysis of multiplex PCR amplicons of thrips species (No 2, *Frankliniella occidentalis*; No 3, *Thrips tabaci* and No 4, *Frankliniella intonsa* positive control DNA)

COI was used as a universal DNA barcode (Hebert et al. 2003a, 2003b). The DNA barcode was considered as the official protocol for the identification of insects, not only as a competitor to traditional taxonomy, but also as a powerful tool to assist in detection and identification of new species (Leite 2012). Herein, some thrips individuals could not be morphologically identified by experts. The study brought out that the CGE method was highly beneficial in cases of difficulties during morphological identification.

Molecular methods support morphological identification and both methods yield consistent results in many studies (Marullo et al. 2020, Xie et al. 2019). Meanwhile, the number of morphological identification experts in quarantine laboratories is getting decrease.

Thrips are significant pests in plant quarantine (Fekrat et al. 2015, Haung et al. 2009, Sabahi et al. 2017). So, quick identification of these harmful organisms in trade is very important (Danks 1988). The combination of different detection methods gets more reliable results. Combined molecular methods for identifying thrips species are a valuable alternative when morphological identification is difficult or almost impossible. Modern methods used together with morphological keys to identify thrips species are highly useful in identifying species in all life cycles of thrips (Mehle and Trdan 2012). So far, many studies have been performed by using different molecular techniques for eliminate of the morphological identification problems (Fekrat et al. 2015, Gariepy et al. 2005, Huang et al. 2010, Mehle and Trdan 2012, Przybylska et al. 2016, Sabahi et

al. 2017, Saccaggi et al. 2008, Toda et al. 2013, Zhang et al. 2012). In these studies, it was aimed to establish faster and more accurate identification criteria with the primer pairs developed by Sabahi et al. (2017) for the rapid detection of thrips. In this novel method presents a different diagnostic assay with a new fragment analysis method without the need for sequence analysis and gel electrophoresis. Different primer sets were used to amplify the 648-base pair (bp) region of the mitochondrial cytochrome-c oxidase subunit 1 (COI) gene identified by Hebert et al. (2003a, 2003b). However, either this gene region is subjected to sequence analysis or it is used in identification by using specific primers.

Specific primer pairs can lead to misleading results on agarose gel, due to amplification of a 648-base region. Przybylska et al. (2016) showed that close amplicon length leads to unreliable results. With the CGE method, these misleading results can be eliminated and identification studies can be performed with ± 2 bp deviation values for each thrips species. In identification studies, it has been observed that very successful results have been obtained with CGE assay. Similar results have also been obtained in Kerékgyártó et al. (2013). The methods used in molecular analysis are still in progress. Especially, the CGE technology (Qsep100) method is very advantageous in terms of time, cost, convenience and accuracy. With this novel method developed for thrips, it has become the primary tool, especially in quarantine and research studies. Many advantages of CGE technology have been mentioned in different studies. Agarose gel electrophoresis is a widely used method because it is cheap and simple, but the deterioration during electrophoresis significantly affects the analysis. In some cases, it may not be possible to obtain the correct amplicon sizes on visual inspection (Yokoyama et al. 2006). It has been reported that it is possible to determine the length of the pieces without bioinformatics experience and to share the obtained data with other laboratories thanks to CGE. Similarly, it has been reported that many steps of the current method, including gel electrophoresis, can be carried out reliably without the need for steps with harmful chemicals (Karakuş et al. 2017). In different studies, it has been reported that the signals obtained from electropherograms in CGE technology can be detected as low as 0.01 ng/µl and 0.002 ng/µl after the injection of samples with different concentrations (Kerékgyártó et al. 2013). In this study, successful results were obtained from nucleic acids with low concentration obtained from a single individual (larva) regardless of their developmental level. The samples even with a concentration of 0.73 ng/µl had a Relative Fluorescence Units (RFU) value of 5>. One of the strongest aspects of the method is that the cost is reduced by significantly reducing the total volume in PCR.

This study revealed production of each thrips species effective signals at very low concentrations and likewise, the bands were quite clear in the agarose gel. Fragment analysis results showed that 52 individuals were *F. intonsa* and 31 individuals were *F. occidentalis*. Based on morphological and molecular analyses, their results were found compatible. In the results of both analyses, all collected samples were *F. intonsa* and *F. occidentalis*.

CGE technology is used in many areas of molecular studies (genotyping, PCR, RFLP, SNP, SSR, etc.) it provides benefits in terms of sensitivity, reliability, convenience, cost and time. In this new capillary gel electrophoresis analysis method, *T. tabaci*, *F. instonsa* and *F. occidentalis* the rapid identification of species without the use of any commercial extraction kit is described. This method can be easily applied by taxonomists who are not experts in quarantine analysis. Also, provide a reliable diagnosis without exposure to carcinogenic chemicals used in gel electrophoresis.

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ÖZET

Thripsler, birçok endüstriyel üründe önemli verim kayıplarına neden olur. Bu zararlılar Türkiye'deki karantina organizmaları arasında yer aldığından, etmenlerin hızlı tespiti yeni alanlara yayılmalarını önlemek için önemlidir. Mitokondriyal sitokrom oksidaz I (COI) barkodlama geninin analizleri; moleküler yöntemlerden biri olarak thrips teşhislerinde yaygın olarak kullanılmaktadır. Ancak COI geninin fragman uzunluğu çok kısa olduğundan, PCR sonrası agaroz jel üzerinde fragman boyutlarını ayırt etmek çok zordur. Bu çalışmada, daha önce farklı araştırmacılar tarafından kullanılan primer çiftleri kullanılarak *Thrips tabaci Lideman, Frankliniella occidentalis* (Pergande) ve *Frankliniella intonsa* (Trybom) türleri için Kapiler Jel Elektroforez (CGE) sistemi entegre edilerek yeni bir tanımlama yöntemi geliştirilmiştir. Analiz, özellikle kısa parça uzunluklu COI geninde birbirine yakın parça uzunluklarının ayrılmasında hata payını en aza indirerek, elde edilmiş güçlü sinyaller üretir. Bu sebeple, jel elektroforezi adımı ortadan kaldırılarak, tehlikeli kimyasallara maruz kalmadan güvenilir tespitler elde edilmiştir. Yeni yöntem, tespit süresini kısaltmış ve düşük DNA konsantrasyonuna sahip tek bir thripsin saptanmasındaki işlem hatalarını da en aza indirmiştir. Kapiler jel elektroforezi tabanlı fragman analizi ile toplam 83 thrips bireyi (52 *F. intonsa*, 31 *F. occidentalis*) tespit edilebilmiştir. Yeni yöntem, üç farklı thrips türünün tespiti için benzersiz, spesifik ve hızlı olarak

değerlendirilmektedir. Ayrıca yakın gelecekte kısa fragman boyutlarına sahip farklı thrips türlerinin tanımlanmasında da kullanılabileceği düşünülmektedir.

Anahtar kelimeler: *Frankliniella intonsa, Frankliniella occidentalis*, mitokondriyal sitokrom oksidaz I, multipleks polimeraz zincir reaksiyonu, Thysanoptera

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Original article

Occurrence of fungal strawberry diseases in Central Anatolia Region of Turkey and reactions of some varieties grown widely against the important pathogens

Orta Anadolu Bölgesinde çilek ekiliş alanlarında görülen fungal hastalıkların tespiti, yaygın olarak yetiştirilen çeşitlerin önemli patojenlere karşı çeşit reaksiyonlarının belirlenmesi

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ABSTRACT

Strawberry diseases, occurring in Central Anatolia region of Turkey was studied in the years 2013-2016 in the strawberry growing areas of Bartın, Kayseri, Konya, and Zonguldak provinces. Totally 515.5 da strawberry fields in the above mentioned provinces were visited and plants showing disease symptoms were counted and their percentages were determined. Foliage and root diseases were determined by using 383 samples collected from various fields. Seventeen fungal pathogens: namely *Alternaria alternata* (Fr.) Keissler, *Boeremia exigua* (Desmazières) Aveskamp, Gruyter & Verkley, *Mycosphaerella fragariae* (Tul.) Lindau., *Colletotrichum acutatum* J.H. Simmonds, *Colletotrichum gleosporoides* (Penz.) Penz., *Botrytis cinerea* (de Bary) Whetzel, *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., *Diaporthe actinidae* N.F. Sommer & Beraha., *Diaporthe eres Nitschke*, *Didymella americana* Saccardo ex Saccardo, *Didymella pomorum* (Thümen) Aveskamp, Gruyter & Verkley, *Diplodia seriata* de Notaris, *Macrophomina phaseolina* (Tassi) Goidanich, *Peyronellae prosopidis*, *Phytophthora cactorum* (Lebert & Cohn) J.Schröter, *Phytophthora plurivora* Jung & Burgess., *Phytophthora kelmania* were identified. *A. alternata*, was the most frequently isolated fungus and was obtained from 41.94% of the plant samples while root rot diseases caused by various fungi occurred on 38.13% of the fields. Reactions of twelve strawberry cultivars; (Festival, Kabarla, Sweetann, Alison, Osmanlı, Kara çilek, Tüylü, Sabrina, Rubigem, San Andreas, Albion, Festival, Monterey) were tested against the three most widespread pathogen; *A. alternata, M. phaseolina* and *L. theobromae*. With the 86% disease severity, Kabarla was found the most susceptible cv. against *A. alternata* while Tüylü was the most susceptible cv. against *M. phaseolina* and *L. theobromae*. Only one variety, Osmanlı, showed low disease intensity against three of the pathogens.

INTRODUCTION

Strawberry (*Fragaria* × *ananassa*) is widely cultivated worldwide for its fruit which is consumed in large quantities, either fresh or in various prepared foods. With 486.705 tons of production, Turkey is the fourth largest producer in the world after China, USA, and Mexico (Faostat 2021). Strawberry production in Turkey is mainly focused in

Mediterranean region and it is followed by Aegean, Thrace and Central Anatolia regions respectively. A lot of fungal, bacterial, and viral diseases occur on strawberries and cause considerable damage not only on the field grown strawberries but also on nursery grown plants (Averre et al. 1992).

Fungal diseases cause economically important crop losses on strawberries grown in the field and under cover in many parts of the world as well as Turkey. The main fungal foliage diseases of strawberries are gray mold (*Botrytis cinerea*), powdery mildew (*Sphaerotheca macularis f.sp. fragariae*), leaf spots (*Mycosphaerella fragariae, Alternaria tenuissima, Alternaria alternata*), anthracnose (*Colletotrichum acutatum, C. dematium*), Cercospora leaf spot (*Cercospora fragariae Lobik*) and downy mildew (*Peronospora potentillae* de Bary). Among the pathogens causing root rots are *Phytophthora cactorum, Pythium* spp., *Rosellinia necatrix* Prill., *Rhizoctonia fragariae* Hussain & W.E. McKeen, *M. phaseolina* (Tassi) Goidanich, *Armillaria mellea* (Vahl:Fr.) P. Kumm. (Maas 1998).

The main crop loss attributed to fungal diseases is root and crown rots caused by *P. cactorum* which is reported in Norway (Eikomo et al. 2000), in Poland (Irzykowska et al. 2005), and in Spain (Porras et al. 2007). Along with *P. cactorum*, another *Phytophthora* sp., *Phytophthora fragaria* was also reported to cause root rot on strawberries, but this species were not found widespread as *P. cactorum* (Eikemo et al. 2003). Both of the species mentioned above are known to survive in soil for long periods because of their resting spores (oospores). Eikemo et al. (2000) pointed out the rapid spread of *P. cactorum* in and between countries by mainly infected seedlings and also mentioned that the disease also spread locally by contaminated irrigation and drainage water and soil cultivation machinery. *P. cactorum* also causes leathery fruit rot on strawberries (Irzykowska et al. 2005).

Maas (1998) and De los Santos et al. (2003) also pointed out the importance of soilborne fungal diseases of strawberries and listed *Verticillium dahliae, P. cactorum, P. fragariae, Pythium* spp., *Rhizoctonia solani*, and *Fusarium* spp. as the most important ones.

Some of the soil borne pathogens such as *Rhizoctonia* spp., *Pythium* spp., *Cylindrocarpon* spp., and *Fusarium* spp. inciting black root rot are also considered important diseases causing significant losses in the strawberry growing countries the former two being the most important (Manici et al. 2005, Martin 2000, Martin and Bull 2002).

Fang et al. (2008) found many pathogens affecting strawberry, including *Fusarium oxysporum, R. solani* (AG-A, AG-C, AG-I, AG-K), *Cylindrocarpon destructans, Phoma exigua, Gnomonia fructicola, P. cactorum, Pythium ultimum, Macrophomina phaseolina, F. oxysporum,* and *R. solani* being the most widespread. *Fusarium oxysporum* f. sp. *fragariae* Winks and Williams was reported to cause about 30% crop loss in South Korea (Nam et al. 2005). Other than these pathogens, *V. dahliae* was also reported to cause about 80% crop loss (Kurze et al. 2001). Due to wide host range and long survival, effective control of this disease is not practicable (Ellis 2008, Gordon et al. 2006).

Strawberry is grown in various regions of Turkey and early production especially comes from the warmer parts, from the low tunnels, mainly from the Mediterranean and Aegean regions. Occurrence of diseases and their control on the production regions were studied by various researchers and more of them were done in the Aegean region especially in Aydın province which is an important strawberry producing area of Turkey. With the study done by Benlioglu et al. (2004) in this region, many of soil-borne pathogens affecting strawberry, such as *R. solani, Fusarium* spp., *Macrophomina* spp., *Phoma* spp., *P. cactorum, Pythium* spp., *V. dahliae* were also found as important pathogens in the order of frequency respectively.

Occurrence of *Lasiodiplodia theobromae* was first reported in 2014 in this region and found highly aggressive (Yıldız et al. 2014). Later on in the same region, Dinler et al. (2015) investigated fungal diseases of strawberry seedlings (plantlets) and found *Fusarium* spp. on 291 seedlings, *Rhizoctonia* spp. on 53, *Cylindrocarpon* sp. on 13, *Macrophomina* sp. on 4 seedling samples out of 2248 strawberry seedlings of Camarosa, Sweetcharlie, Rubygem and Festival cvs. The first study on fungal diseases in this region, on the other hand, was carried out in 1978 as a MSc thesis (Kapkın 1978).

The first study to reveal root rot pathogens of strawberry in the Mediterranean region was done in 1986 (Yürüten et al. 1986). Following this study a PhD study was completed on the pathogens causing root rot in this region and *R. solani* was found as a primary pathogen (Pala 1987).

Phytopathological problems of strawberry was also investigated in Erzurum province of Turkey and gray mold (*B. cinerea*), leaf spot (*M. fragariae*), and soil borne diseases (*Rhizoctonia* spp., *Fusarium* spp., *Pythium* spp., and *Verticillium* sp.) were the main problems (Eken 2008).

There has been an increase on the acreage of strawberry production in Central Anatolia recently and fungal diseases have not been investigated in the region with the only exception of the study of Gürer and Coşkun (1993), carried out in Bartın and Zonguldak provinces.

With another study, carried out in Düzce province of Turkey, in 2012; *Alternaria* spp., *B. cinerea*, *Hainesia lythri*, *M. fragariae, Phoma* sp., *Phomopsis* sp., *R. solani* were isolated from the leaves and petioles; *B. cinerae* from the fruits and *Alternaria* spp., *F. oxysporum, M. phaseolina*, *Phytophthora*

spp. and *R. solani* were isolated from the roots. Pathogenicity of these fungi was proven on detached leaf assay (Sarıgül Ertek et al. 2018).

The aim of this work is to identify fungal diseases in Central Anatolia strawberry growing fields and to search for the reactions of some extensively grown varieties against the most important pathogens.

MATERIALS AND METHODS

Collection of disease samples

About 5% (51.5 ha) of the strawberry growing fields of Kayseri, Bartın, Konya, and Zonguldak were visited and 383 disease samples showing leaf spots, leaf scorch, powdery and downy mildews and wilt symptoms were collected and brought to the laboratory in a cool box. Total number of the samples collected from these provinces were 157, 80, 135, and 30 from Konya, Bartın, Kayseri, and Zonguldak, respectively.

Isolation of the fungi

Samples having root and crown rot first were washed under tap water thoroughly, blot dried and subsamples were taken from the adjacent parts of the intact and necrotized tissues and disinfected by immersing 0.1% NaOCl for 1 or 3 min depending of the tenderness of the tissues. After disinfection, samples were blot dried and small parts about 2-3 mm dissected and placed on the appropriate media. Leaf spots and scorches, fruit rots were treated the same way except duplicate subsamples of some leaf spots were incubated on a humid chamber in addition to plating isolation media. For isolation of *Phytophthora* spp. samples were directly plated on the semi selective medium (CMA-PARP) of Jeffers and Martin (1986). For the isolation of other pathogens Potato Dextrose Agar (PDA) and 2% Water Agar (WA) were used. The Petri plates containing PDA and WA were incubated at 24±1 °C for a week then subcultures were prepared by removing mycelial tips from the edge of the growing colonies under a stereomicroscope. The semi selective Phytophthora medium was incubated at 18-20 °C in darkness and the growing colonies were examined under a microscope for 2-3 days. Subcultures were prepared by removing mycelial tips from the right angle branching mycelia and plating them on Carrot Agar (CA) (40 g thinly grated carrot, 20 g agar and 1000 ml water) as described above. The isolates obtained were transferred to PDA or for Phytophthora spp. Corn Meal Agar (CMA) slants in tubes.

Pathogenicity of the Phytophthora spp. isolates obtained from the roots

Pathogenicity of the *Phytophthora* spp. isolates was tested by soil inoculation method. The isolates were grown on wheat seeds sterilized twice daily at 121 °C by inoculating with culture discs and incubated at 24 °C in darkness for two weeks. This inoculum was added to torf + perlite mixture at 5% (Latorre et. al. 2001). The inoculum was placed in 4 l pots and 5 healthy strawberry seedlings were planted in this inoculum. Five control seedlings were also planted in uninoculated soils. To maintain the sufficient humidity, the pots were regularly watered. The strawberry plants were uprooted after seven weeks and rated for the root rot index. Re-isolations were also done.

Pathogenicity of the Phytophthora spp. isolates obtained from the fruits

Healthy strawberry fruits were used for this test. The fruits were sterilized by immersing them in 10% NaOCl for 15 min and they were rinsed three times by sterile water. After that, the fruits were transferred to sterile Petri plates. Fruit inoculation was done in a sterile cabin by placing mycelial discs of 2 mm diameter cultures on slightly punctured fruit surfaces. Only plane agar discs were used as controls. Inoculated fruits, ten for each isolate were incubated at 24±1 °C and re-isolations were done from the fruits showing diseases symptoms.

Pathogenicity of the other fungi

Pathogenicity of the other soil-borne pathogens was performed by using Toothpicks method described by Benlioglu et al. (2014). For this aim, ten healthy looking runners (stolons) for each isolate about 8-8.5 cm long, not contacted to the soil were dipped into 70% alcohol for 5 min and rinsed by sterile water and each fungus was inoculated as described. Inoculated stolons were kept at 28±2 °C in a humid chamber in Petri dishes. Five to seven days after inoculation, lesion sizes were compared by the controls.

For the pathogenicity of leaf spot and blight pathogens, detached leaf inoculation method was used (Dolar et al. 1994, Sarıgül Ertek et al. 2018). Healthy looking trifoliate strawberry leaves were removed from the plants and disinfected in 1% NaOCl for three min, blot dried and placed into Petri dishes having 2 fold humid blotter papers. Fungal discs of 0.5 cm in diameter, taken from the peripheries of young cultures were placed on the trifoliate leaflets wounded by piercing from 3 to 4 points by a needle. Stipes of the leaves were wrapped by cotton wool and wetted by sterile water in order to keep the turgor of the leaves. Lesion formation and fungal growth was observed until the symptoms onset. Each pathogen was inoculated on ten leaves and disease severity was calculated by the following formula:

$$\text{Percent disease severity} = \frac{\Sigma \text{ (value of each scale x number of samples at each scale)}}{\text{the highest scale value x total no of the samples}} \times 100$$

Scale for root pathogens: 0, no lesion; 1, 1-25% of the stolon have lesion; 2, 26-50% of the stolon have lesions; 3, 51-75% have lesions, 4, 76-100% have lesions.

Scale for foliage pathogens: 0, no disease on the trifoliate leaf; 1, 1/5 of the trifoliate leaf have lesion; 2, 2/5 of the trifoliate leaf infected; 3, 3/5 of the trifoliate leaf infected; 4, 4/5 of the leaf infected; 5, whole leaf infected (Dolar et al. 1994).

Determination the reactions of the cultivars

Reactions of 12 widely grown strawberry cultivars; Festival, Sweetann, Kabarla, Alison, Osmanlı, Kara çilek, Tüylü, Sabrina, Rubigem, San Andreas, Albion, Monterey were tested against the most virulent isolates of three most widespread pathogens; *A. alternata*, *M. phaseoli* and *Lasiodiplodia theobromae*. Detached leaf assay and toothpick assay as described above were used for the reactions of the cultivars for *A. alternata* and *M. phaseoli* and *L. theobromae,* respectively.

Identification of the fungal pathogens of strawberry

The obtained fungi, first were identified at genus level by using published textbooks (Barnett and Hunter 1998, Ellis 1976, Gerlach and Nirenberg 1982, Samson et al. 1996, Tousson 1995). Identification at species level was done by comparing DNA sequences of ITS-4 and ITS-5 regions of the isolates with the sequences deposited in GenBank. For this study; the isolates were grown on PDA and a small amount of mycelia was macerated in liquid nitrogen and DNA isolation was performed by using DNeasy Blood & Tissue Kit (Qiagen). PCR reaction was performed as described by Cobos and Martin (2008) by using 50 µl PCR mixture of the following amounts; 5 µl DNA (about 10 ng), 5 µl 10x buffer (75 mM Tris HCl, pH 9.0, 50 mM KCl, 20 mM (NH4)2SO4) (Biotools), 2 µM each of primers (MWG-Biotech), 5 mM MgCl2, 2 mM dNTPs, 2 U Taq polimerase (Biotools), 5 µl bovine serum albumin (BSA) (10 mg/ml; Sigma Aldrich). The primers used were; ITS-4 (5'-TCC TCC GCT TAT TGA TAT GC-3') and ITS-5 (5'-GGA AGT AAA AGT CGT AAC AAG G-3'). The PCR mixture was pre-denatured at 96 °C for 5 min; followed by 35 cycles of denaturing at 94 °C for 30 s, annealing at 52 °C for 30 s and 72 °C for 90 s, followed by final hold at 72 °C for 7 min. PCR products were run 1.5% agarose gel in 1X TBE (40 mM Tris-borate, 1 mM EDTA, pH: 8.0). PCR products were sent to BM Laboratories (Ankara, Turkey) for sequencing facility.

RESULTS

Identification of the fungi

The following genera and species were identified from 402 samples collected from 47 fields of Kayseri, Bartın, Konya, and Zonguldak provinces about 515.5 ha; *A. alternata, Boeremia exigua, M. fragariae, C. acutatum, Colletotrichum gleosporoides, B. cinerea, L. theobromae, Diaporthe actinidae, Diaporthe eres, Didymella americana, Didymella pomorum, Diplodia seriata, F. oxysporum, M. phaseolina, Peyronellae prosopidis, P. cactorum, Phytophthora plurivora, Phytophthora kelmania.*

Occurrence of the diseases

Three pathogens; *A. alternata, M. phaseoli* and *L. theobromae* were the most widespread diseases in strawberry fields of four provinces of Central Anatolia and the former was obtained from 41.94%, and the latter two root pathogens were obtained 38.13% of the fields, evaluated the at two growth stages Table 1.

Table 1. Percentages of infested areas by three pathogens

Provinces	Pathogens		
	Alternaria alternata	*Macrophomina phaseolina*	*Lasiodiplodia theobromae*
Zonguldak, Çaycuma	25	25	25
Zonguldak, Ereğli	38.7	12.9	0.0
Bartın, Merkez	31.8	37.8	9.1
Kayseri, Tomarza	19.6	35.3	29.4
Kayseri, Merkez	33.3	19.6	29.4
Konya, Akşehir	19.3	0.0	0.0

Alternaria leaf spot (*A. alternata*) occurred in about half of the fields, producing intensive yellowing and reddening on leaves (Figure 1a). First symptoms leaf spots were irregular in shape, 3 to 6 mm in diameter, and blackish brown. Later on, yellowing occurred around the leaf spots (Figure 1b). *A. alternata* shows characteristic concentric growth in PDA media (Figure 1c). Conidial chain can be seen easily under 10x microscope magnificent (Figure. 1d).

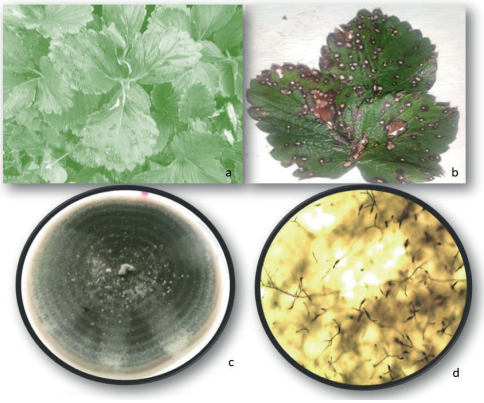

Figure 1. Various aspects of *Alternaria alternata* a) a general view from an infected field showing yellowing and reddening b) leaf spots with purple margins and whitish centres, c) consantric colonial growth d) conidia of *Alternaria alternata* under 10x microscope magnicifient

Two pathogens; *M. phaseoli* and *L. theobromae* were isolated the most frequently from the plants showing decline and

necrotic roots. The plants infected by *M. phaseoli* lost their vigour and dried (Figure 2a). This pathogen caused reddish brown root discoloration (Figure 2b) and produced grayish black growth with microsclerotia of average 105 × 74 µm (Figure 2c,d) on PDA medium.

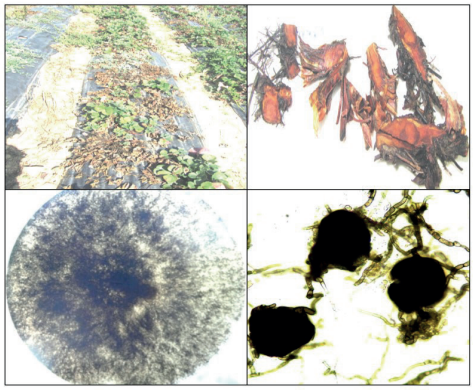

Figure 2. a) a field showing severe root rot caused by *Macrophomina phaseoli,* b) sections of infected roots having reddish discoloration, c) dark colour growth on PDA d) microsclerotia of *Macrophomina phaseoli*

L. theobromae was also isolated from the wilted and dead plants having necrosis on roots. This pathogen first produced a whitish growth on PDA, later on the growth turned to olive colour to dark brown (Figure 3a). The pathogen produced pycnidia having one celled, hyaline or two celled, dark coloured conidia of 25.42 ± 2.12 × 12.87 ±1.08 µm when incubated about 20-30 days (Figure 3b).

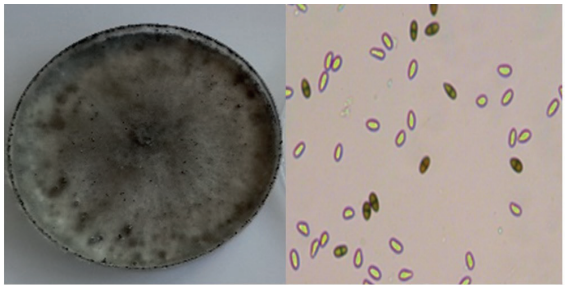

Figure 3. a) olive coloured, 5 days old growth of *Lasiodiplodia theobromae* on PDA, b) hyaline and dark coloured conidia

Reactions of common strawberries against three of the most widespread diseases

Disease intensities produced by the three widespread pathogens on eleven strawberry varieties are given in Table 2. Strawberry varieties showed different reactions against the three pathogens. The varieties Festival, Alison, Tüylü, Osmanlı, and Sabrina had the lowest percentages of diseases against *A. alternata*, while the varieties Karaçilek, Osmanlı, Rubigem, Sabrina, Monterey, and Albion against *M. phaseoli*; Karaçilek, Alison, Osmanlı, were against *L. theobromae.* Only one variety, Osmanlı, showed low disease intensity against three of the pathogen Table 2.

Table 2. Disease intensities produced by three most widespread strawberry pathogens on eleven strawberry varieties grown widely in Central Anatolia region

Variety/ pathogens	*Alternaria alternata*	*Macrophomina phaseolina*	*Lasiodiplodia theobromae*
Festival	31.33± 12.05de	48.75± 8.82 bcd B[2]	67.50± 6.23 cd
Kara çilek	84.67± 10.79ab	17.50± 6.23 ef A	25.00± 5.89 e
Alison	24.00± 8.32e	75.00± 5.33 f A	22.50± 9.82 e
Tüylü	41.33± 8.24de	100± 0 a A	100± 0 a
Osmanlı	26.67± 8.94e	16.25± 8.34 ef B	35± 5.20 e
Rubigem	51.33±12.57cde	28.75±7.22 def B	60± 1.247 cd
Sabrina	40.67± 13.38de	20.0± 3.33 ef B	70.0± 8.16 bcd
San Andreas	54.67± 12.87bcde	63.75± 6.83 b B	80± 8.16 abc
Monterey	76.67± 82.70abc	32.5± 1.16 de B	100± 0 a
Albion	50.67± 13.84cde	37.5± 6.71 cde B	90± 6.66 ab
Kabarla	96.67± 2.27cde	57.5± 6.77 bc A	56.25± 6.52 d
Sweetann	64.00± 4.23 bcd	61.25± 3.46 b A	59.72± 3.47 cd

Molecular identification of the strawberry pathogens

The internal transcribed spacer (ITS) regions of ITS-4 and ITS-5, of the whole pathogenic isolates were amplified and the obtained gene sequences were compared with the ones deposited in GenBank.

Identification of the pathogenic fungi obtained from strawberry plants were done by comparing DNA sequences of ITS-4 and ITS-5 gene regions of all the fungi with the sequences deposited in GenBank. Many pathogens were identified by species level (Table 3).

DISCUSSION AND CONCLUSION

In order to determine diseases on strawberry surveys were done in fields of Kayseri, Bartın, Konya, and Zonguldak provinces in Central Anatolia in 2013-2016. The most widespread disease was Alternaria leaf spot, determined at 41.94% of the fields, and the second widespread disease was root rots caused by *M. phaseoli* and *L. theobromae* which occurred in 38.13% of the fields.

Alternaria leaf spot is frequently occurring disease of strawberries worldwide (Cho et al. 1980, Fu et al. 2019, Mehmood et al. 2018, Wassenear and Scheer 1989) and it is also known in Turkey (Sarıgül Ertek et al. 2018).

Wilting and root rot symptoms, especially reddish necrotic areas, were also observed in many fields and various pathogenic fungi were isolated from the necrotic root tissues, *M. phaseoli* and *L. theobromae* being the most frequent. Along with these two pathogens; *Fusarium* spp,

Table 3. Molecular identification of pathogenic fungi obtained from strawberry production areas of Central Anatolia region

Species identified	Percent similarity to GenBank records	Accession number of most similar GenBank records	GenBank accession number of isolates from this study
Alternaria alternata	100	MT126620.1 MH384939.1 MK578900.1	MK571621
Boeremia exigua	100	MN077426.1 MK907733.1 MK541618.1	MK571625
Botrytis cinerea	100	MT573470.1 MN589849.1 MN589847.1	MK554490
Colletotrichum acutatum	99.82	MH532959.1	MK571604
Colletotrichum gleosporoides	100	MK474924.1	MK571633.1
Diaporthe actinidae	100	MK541622.1 KT163360.1	MK541622
Diaporthe eres	100	MT561403.1 MK431122.1 MK571633.1	MK571606
Didymella americana	99.80	KY070283.1 KY070282.1 KY070281.1	MK571615
Didymella pomorum	100	MH861278.1 AY904062.1	MK541633
Diplodia seriata	100	KJ921854.1 KJ921853.1 KJ921852.1	MK571617
Fusarium oxysporum	100	MT530269.1 MT530243.1 MT530242.1	MK554485
Lasiodiplodia theobromae	100	MT123030.1 MN909160.1 MH793584.1	MK571624
Macrophomina phaseolina	100	MT735239.1 MT183520.1 MT127393.1	MK571619
Neofusicoccum parvum	100 99 99	MT012295.1 MH057199.1 MH623075.1	MK554486
Peyronellae prosopidis	100	MH866094.1 KF777181.1	MK571630
Phytophthora cactorum	99	EU045748.1 MT558729.1 MT280033.1	KJ603452
Phytophthora plurivora	100	KT306852.1 KF682435.1 JQ730714.1	KJ603449 KJ603450
Phytophthora kelmania	100	KU053244.1 KU053242.1 KU053234.1	KJ603451

Rhizoctonia spp., *Phytophthora* spp. were also obtained from root rots. *M. phaseolina* was also reported from the main strawberry production areas of the world (Freeman and Zveibil 2009, Mertely et al. 2005). Martin (2000) and Manici et al. (2005) on the other hand, reported that soil borne *Rhizoctonia* spp., *Pythium* spp., *Cylindrocarpon* sp. and *Fusarium* spp. are also important soil borne pathogens affecting strawberry production in the world. Occurrence of Fusarium and Rhizoctonia root rots was also reported by other researchers (Freeman and Zveibil 2009, Mertely et al. 2005). Root rots on strawberry have also been determined in different strawberry fields. Yürüten et al. (1986) found out that root rots caused by soil borne pathogens caused serious problems and produced about 40-60% yield loss in the Mediterranean region of Turkey. The same situation is seen in our study area and this situation is attributed to use of infected strawberry plantlets (seedlings) extensively.

Another root rot pathogen of strawberry, *L. theobromae*, has only been encountered at strawberry growing areas in Aydın province in western Turkey so far (Benlioglu et al. 2014) and in our study area, which was found so aggressive on strawberry killing the whole plants in seven days. Our findings suggest that more time should be spent on the disease.

To find a solution for the difficulty of finding disease free seedlings, different plant structures were used for testing pathogenicity. Detached leaf assay as used for other host plants and for strawberry by various authors (Argun et al. 2008, Sarıgül Ertek et al. 2018, Sezer and Dolar 2012) was also found suitable for strawberry since the detached leaves remained intact about 3-4 weeks. Toothpicks method, tested by Benlioglu et al., (2014) for root rot pathogens on the stolons was also applied successfully in our study. In our study, for the pathogenicity of *Phytophthora* spp, especially for *P. cactorum*, strawberry fruits and soil infestation methods were also used successfully.

Testing the susceptibility of twelve strawberry cultivars against three of the most common fungal diseases formed another aspect of our study, which will help growers to choose suitable varieties for their regions. Most of the varieties, except Festival, Alison and Osmanlı, had more than 40% infection when inoculated by *A. alternata*, in other words they were found susceptible (having infections between 40-60%) and highly susceptible (having disease ratios over 60%). The cultivars "Karaçilek" and "Osmanlı" had lower disease intensities against the two of the root rot pathogens while some of the remaining varieties like "Alison", "Rubigem", "Sabrina", and "Monterey" showed resistance to only one of the root rot pathogens. Some varieties; such as "Festival", "Tüylü", "San Andreas", "Kabarla" and "Sweetann" were susceptible against both of the root rot pathogens (Table 2).

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ÖZET

2013-2016 yılları arasında Bartın, Kayseri, Konya ve Zonguldak illeri çilek üretim alanlarında sorun olan çilek fungal hastalıklarının tespiti ve yaygın olan fungal patojene karşı mücadele olanaklarının araştırılması hedeflenmiştir. Bu amaçlar doğrultusunda Bartın, Kayseri, Konya ve Zonguldak illerine ait 515.5 da çilek alanından alınan hastalıklı bitki örnekleri toplanarak laboratuvara getirilmiş ve bu örnekler uygun besi ortamlarına ekilerek etmenler izole edilmiştir. Çeşitli arazilerden toplanan 383 adet yaprak ve kök örneklerinden fungal etmenler izole edilmiştir. 17 fungal patojen: *Alternaria alternata* (Fr.) Keissler, Boeremia exigua (Desmazières) Aveskamp, Gruyter & Verkley, *Mycosphaerella fragariae* (Tul.) Lindau., *Colletotrichum acutatum* J.H. Simmonds, *Colletotrichum gleosporoides* (Penz.) Penz., *Botrytis cinerea* (de Bary) Whetzel, *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., *Diaporthe actinidae* N.F. Sommer & Beraha., *Diaporthe eres* Nitschke, *Didymella americana* Saccardo ex Saccardo, *Didymella pomorum* (Thümen) Aveskamp, Gruyter & Verkley, *Diplodia seriata* de Notaris, *Macrophomina phaseolina* (Tassi) Goidanich, *Peyronellae prosopidis, Phytophthora cactorum* (Lebert & Cohn) J.Schröter, *Phytophthora plurivora* Jung & Burgess., *Phytophthora* kelmania olarak tespit edilmiştir. Yaprak ve köklerden izole edilen bu etmenler uygun metotlarla çilek bitkilerine inokule edilerek patojenisiteleri gerçekleştirilmiştir. Patojenisitesi yapılan etmenlerden en yaygın olarak tespit edilen etmen yaprak lekesi hastalığı (*A. alternata*), %41.94 oranında; çeşitli fungusların sebep olduğu kök hastalıkları ise %38.13 oranında tespit edilmiştir. En yaygın bulunan 3 patojenle (*A. alternata, M. phaseolina* ve *L. theobromae*) çeşit-reaksiyon çalışmaları 12 çilek çeşidi (Festival, Kabarla, Sweetann, Alison, Osmanlı, Kara çilek, Tüylü, Sabrina, Rubigem, San Andreas, Albion, Festival,

Monterey) üzerinde gerçekleştirilmiştir. Buna göre *A. alternata* yaprak lekesine en hassas çeşit %86 hastalık şiddetiyle Kabarla, *M. phaseolina* ve *L. theobromae*'ye karşı hassas olan çeşit ise Tüylü çilek çeşididir. Osmanlı çilek çeşidi her 3 hastalık etmenine karşı diğer çeşitlere göre daha dayanıklı bulunmuştur.

Anahtar kelimeler: çilek fungal hastalıkları, *Alternaria alternata*, *Macrophomina phaseolina, Lasiodiplodia theobromae*

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Original article

Contributions to the knowledge of the vernal butterflies of East Mediterranean region in Turkey

Doğu Akdeniz Bölgesi (Türkiye) bahar kelebeklerinin bilgisine katkılar

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ABSTRACT

The present paper is a continuation of the previously published spring butterflies of the Eastern Mediterranean Region. The information of collection station of the previously listed species of 68 stations was included in this study. The stations were divided into 8 groups according to their vertical distribution. Habitat types at various altitudes have been defined. The communities of Lepidoptera detected in these habitats are given. The status of the *Pontia daplidice/edusa* species group in the region is discussed.

INTRODUCTION

Biodiversity in Turkey is generally higher in plants and animals, not only in butterflies and moths but also in other insect species compared to European and the Near East countries (Wagener 2006). The Mediterranean Region is one of the richest regions of Turkey in terms of endemism. Çukurova valley, located in the Mediterranean region, has the most fertile agricultural soils in Turkey. This region also includes the Seyhan and Ceyhan deltas, which were declared as important natural areas (Eken et al. 2006). Intensive agricultural practices, animal husbandry, tourism, and urbanization pressure in the coastal areas of the region are some adverse impacts on butterfly diversity.

Although there are many studies on the butterflies in Turkey, the number of the studies containing spring butterflies is limited. Some of these contain information about the early developmental stages of spring species (Koçak 1982, Koçak and Seven 1990, 1991, Torun and Seven 2016). Some studies on Turkey's spring butterflies as follows: Kemal and Seven 2008, Koçak 1993, Kemal and Koçak 2017a, 2017b, Kemal and Koçak 2018a, 2018b.

This article includes supplementary notes to the previous article on spring butterflies in the Eastern Mediterranean Region of Turkey (Seven and Bozacı 2020). In the study, information was given about the labels of butterfly collection stations. Collection stations were divided into 8 groups according to their vertical distribution and habitat definitions of them were recorded. In addition, some identified species were discussed.

MATERIALS AND METHODS

Butterfly species were collected in daylight within the borders of the Eastern Mediterranean region between April and June

2008. The species list is given by Seven and Bozacı (2020) and the stations are shown on Figure 1. The open addresses of the collection stations are presented in Table 1. Photographs of some species recorded from the area are shown in Table 2. The field research of this study was conducted within the scope of the Anatolian Cross Biodiversity Project of The Nature Conservation Center.

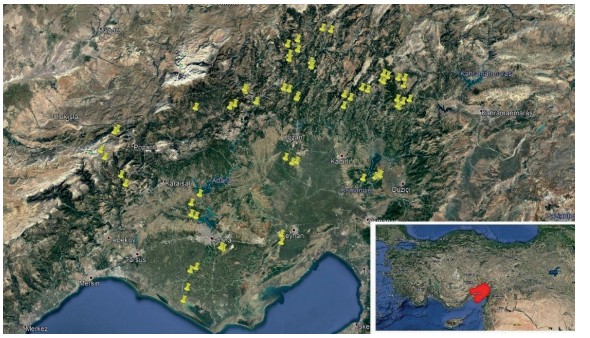

Figure 1. The stations collected butterfly species within the borders of the Eastern Mediterranean region (Seven & Bozacı, 2020)

Table 1. Numbers, full names, altitudes, dates, and coordinates of collecting stations

1. Adana,İnnapli,0002m, 27.04.2008, 36S 0692570D, 4060939K	35. Adana,Yaylapınar,1068m,29.05.2008,36S 748996D,4197468K
2. Adana,Çavuşlu,0001m, 27.04.2008, 36S 0694466D, 4067412K	36. Adana,Yerebakan,0846m, 29.05.2008,36S 749207D,4192245K
3. Adana,Irmakbaşı,0002m, 27.04.2008,36S 0698865D, 4078187K	37. Adana,Değirmenuşağı,1373m, 29.05.2008,36S 744851D,4189564K
4. Adana,Karaahmetli,0003m, 27.04.2008, 36S 0695920D, 4076610K	38.Adana,Değirmenuşağı-Kayadibi,1516m, 29.05.2008, 36S 744848D,4187265K
5. Adana,Yukarıçiçekli,0007m, 27.04.2008, 36S 0711573D, 4089131K	39. Adana,Hıdıruşağı,0975m, 29.05.2008,36S 743776D,4194785K
6. Adana,Yukarıçiçekli,0016m, 27.04.2008, 36S 0712757D, 4088409K	40. Adana,Akkaya,0519m, 30.05.2008,36S 756105D,4186073K
7. Adana,Kabasakal, 0211m, 28.04.2008, 36S 0695681D, 4105747K	41. Adana,Cıvıklı,1112m, 30.05.2008,36S 761673D,4203496K
8. Adana,Kabasakal, 0089m, 28.04.2008, 36S 0698216D, 4104657K	42. Adana,Cumhurlu,0974m, 30.05.2008,37S 238864D,4202615K
9. Adana,Kaşoba,0124m, 28.04.2008, 36S 0695584D, 4111436K	43. Adana, Darılık, 1045m , 04.05.2008, 36S 0717102D,4163265K
10. Adana,Kırıklı,0078m, 28.04.2008, 36S 0699850D, 4116310K	44. Adana,Darılık,0690m, 04.05.2008, 36S 0714635D,4161954K
11. Adana,Ağaçpınar-İsalı,0051m, 29.04.2008, 36S 0742954D, 4092499K	45. Adana,Kökez-Kıçak,1017m, 04.05.2008, 36S 0696750D,4161015K
12. Adana,Çokçapınar,0125m, 29.04.2008, 36S 0742928D, 4096317K	46.Kahramanmaraş,1297m, 01.05.2008, 37S 0276016D,4176177K
13. Adana,Hamam,0072m, 29.04.2008, 36S 0749969D, 4134457K	47. Kahramanmaraş,1384m, 1.05.2008, 37S 0275310D,4172684K
14. Adana,Aslanlı-Hamam,0055m, 29.04.2008, 36S 0747637D, 4133812K	48.Kahramanmaraş,Torlar,1181m, 01.05.2008, 37S 0271974D,4162395K
15. Adana,Ufacıkören,0073m, 29.04.2008, 36S 0744124D, 4136272K	49. Kahramanmaraş, Yeniköy, Torlar, 1044m, 01.05.2008, 37S 0271760D,4165511K
16. Adana,Olukluçunur, 0431m, 30.04.2008, 37S 0260755D, 4127254K	50.Kahramanmaraş,Gökgedik,1029m,01.05.2008, 37S 0278092D,4165112K

17. Adana,Olukluçunur,0387m, 30.04.2008, 37S 0261452D, 4126656K	51. Kahramanmaraş,Kargaçayırı,1298m, 31.05.2008, 37S 275950D,4175989K
18. Adana,Olukluçunur-Karagedik, 0313m, 30.04.2008, 37S 0260540D, 4126014K	52. Kahramanmaraş,Kargaçayırı/ Kaleboynu,1445m, 31.05.2008, 37S 272648D,4176416K
19. Adana,Karagedik,0144m, 30.04.2008, 37S 0258860D, 4125779K	53. Kahramanmaraş,Gökgedik,0933m, 31.05.2008, 37S 274746D,4164152K
20. Adana,Karatepe,0286m, 30.04.2008, 37S 0252444D, 4125294K	54. Kahramanmaraş,Torlar,1132m,31.05.2008, 37S 272609D,4161559K
21. Mersin, Çukurbağ, 0960m, 24.05.2008, 36S 0661282D, 4120694K	55. Osmaniye,Yeşilyayla, 0785m, 02.05.2008, 37S 0243826D,4163082K
22. Adana,Horoz,1371m,25.05.2008, 36S 655205D, 4147528K	56. Osmaniye,Esenli,1321m, 02.05.2008, 37S 0247508D,4167150K
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Table 2. Some vernal butterflies of the East Mediterranean Region

Aricia anteros

Glaucopsyche lessei

Chilades galba

Polyommatus antiochenus

Zizeeria karsandra

Tomares nesimachus

Tarucus balkanicus

Callophrys paulae

Zerynthia cerisy

Elphinstonia penia

Euchloe ausonia

Zegris eupheme

Polygonia egea

Thaleropis ionia

Ypthima asterope

RESULTS

The collection stations were divided into 8 groups according to their 250 m altitude ranges. The habitat definitions of the stations where the collection is made at the altitude ranges in question and the identified species are as follows.

A (0-250 m): Habitat types seen at between 0-250 m are mostly plantation areas (citrus groves, ruderal areas in the vicinity of the residential area, road edges with acacia and eucalyptus trees beside them) and natural areas (dried river beds and maquis areas where kermes oak and Ramhnus are densely seen).

Butterfly species: Pieridae: *Colias crocea* (Fourcroy, 1785), *Euchloe ausonia* (Hübner, [1804]), *Pieris rapae* (L., 1758), *P. brassicae* (L., 1758), *Pontia edusa* (Fabricius, 1777). Nymphalidae: *Melitaea fascelis* (Esper, 1783), *Polygonia egea* (Cramer, [1775]), *Vanessa cardui* (L., 1758), *V.atalanta* (L., 1758), Satyrinae: *Kirinia roxelana* (Cramer, [1777]), *Lasiommata megera* (L., 1767), *Maniola telmessia* (Zeller, 1847), *Ypthima asterope* (Klug, 1832), Lycaenidae: *Aricia agestis* ([D. and Sch.], 1775), *Chilades trochylus* (Freyer, [1843]), *C. galba* (Lederer, 1855), *Glaucopsyche alexis* (Poda, 1761), *Lampides boeticus* (L., 1767), *Lycaena phlaeas* (L., 1761), *Plebejus sephirus* (Frivaldszky, 1835), *Polyommatus icarus* (Rottemburg, 1775), *Satyrium ilicis* (Esper, [1779]), *S. spini* ([D. and Sch.], 1775), *Tarucus balkanicus* (Freyer, [1844]), *Zizeeria karsandra* (Moore, 1865), Hesperiidae: *Carcharodus orientalis* Reverdin, 1913, *C. alceae* (Esper, [1780]), *Gegenes pumilio* (Hoffmannsegg, 1804), *G. nostrodamus* (Fabricius, 1793), *Muschampia tessellum* (Hübner, [1802]), *Pyrgus melotis* (Duponchel, [1834]), *Pelopidas thrax* (Hübner, [1821]), *Spialia orbifer* (Hübner, 1823), *Thymelicus sylvestris* (Tutt, [1905]).

B (250-500 m): Types of habitat at their heights, mostly edges of pine plantation, open fields in the forest, Cistus dominated by olive trees, olive groves, roadside in forests, and areas with low vegetation due to maquis groves.

Butterfly species: Pieridae: *Colias crocea* (Fourcroy, 1785), *Euchloe ausonia* (Hübner, [1804]), *Pieris rapae* (L.,1758), *P. brassicae* (L.,1758). Nymphalidae: *Melitaea phoebe* ([D. and Sch.], 1775), *M. fascelis* (Esper, 1783), *Vanessa cardui* (L., 1758), *V. atalanta* (L., 1758), Satyrinae: *Lasiommata megera* (L., 1767), *Maniola telmessia* (Zeller, 1847), *Pararge aegeria* (L., 1758), *Ypthima asterope* (Klug, 1832), Lycaenidae: *Aricia agestis* ([D. and Sch.], 1775), *Glaucopsyche lessei* (Bourgogne, 1954), *G. alexis* (Poda, 1761), *Lycaena phlaeas* (L., 1761), *Polyommatus icarus* (Rottemburg, 1775), Hesperiidae: *Carcharodus alceae* (Esper, [1780]), *Gegenes pumilio* (Hoffmannsegg, 1804).

C (500-750 m): Heights at between 500-750 m, there are forest openings, kermes oak, dried river beds, and maquis groves dominated by Rhamnus.

Butterfly species: Pieridae: *Anthocharis cardamines* (L.,1758), *Colias crocea* (Fourcroy, 1785), *Euchloe ausonia* (Hübner, [1804]), *Euchloe (Elphinstonia) penia* (Freyer, 1851), *Pieris rapae* (L.,1758), *P. brassicae* (L.,1758), *Pontia edusa* (Fabricius, 1777), Nymphalidae: *Argynnis pandora* ([D. and Sch.], 1775), *Issoria lathonia* (L., 1758), *Limenitis reducta* Staudinger, 1901, *Melitaea phoebe* ([D. and Sch.], 1775), *M. telona* Fruhstorfer, 1908, *Vanessa cardui* (L., 1758), *V. atalanta* (L., 1758), Satyrinae: *Hipparchia mersina* (Staudinger, 1871), *Kirinia roxelana* (Cramer, [1777]), *Maniola telmessia* (Zeller, 1847), *Pararge aegeria* (L., 1758), Lycaenidae: *Aricia agestis* ([D. and Sch.], 1775), *Chilades trochylus* (Freyer, [1843]), *Glaucopsyche alexis* (Poda, 1761), *Lampides boeticus* (L., 1767), *Lycaena phlaeas* (L., 1761), *Plebejus sephirus* (Frivaldszky, 1835), *Polyommatus icarus* (Rottemburg, 1775), *Rubrapterus bavius* (Eversmann, 1832), *Satyrium ilicis* (Esper, [1779]), *S. spini* ([D. and Sch.], 1775), Hesperiidae: *Carcharodus alceae* (Esper, [1780]), *Muschampia tessellum* (Hübner, [1802]), *Pyrgus melotis* (Duponchel, [1834]), *P. armoricanus* (Oberthür, 1910), *Spialia orbifer* (Hübner, 1823), *Thymelicus sylvestris* (Tutt, [1905]).

D (750-1000 m): At these heights, there are fields where dryland farming (wheat) is practised and Riparian forest is dominated by poplar trees.

Butterfly species: Papilionidae: *Iphiclides podalirius* (L., 1758), *Papilio machaon* (L., 1758), *Zerynthia cerisy* (Godart, [1824]), Pieridae: *Anthocharis cardamines* (L.,1758), *Colias crocea* (Fourcroy, 1785), *Gonepteryx cleopatra* (L.,1767), *G. rhamni* (L.,1758), *Pieris napi* (L.,1758), *P. rapae* (L.,1758), *P. brassicae* (L.,1758), *Pontia edusa* (Fabricius, 1777), Nymphalidae: *Argynnis pandora* ([D. and Sch.], 1775), *Issoria lathonia* (L., 1758), *Limenitis reducta* Staudinger, 1901, *Melitaea phoebe* ([D. and Sch.], 1775), *M. telona* Fruhstorfer, 1908, *M. fascelis* (Esper, 1783), *Nymphalis polychloros* (L.,1758), *Vanessa cardui* (L., 1758), *V. atalanta* (L., 1758), Satyrinae: *Coenonympha pamphilus* (L.,1758), *Hipparchia mersina* (Staudinger, 1871), *Kirinia roxelana* (Cramer, [1777]), *Lasiommata maera* (L.,1758), *L. megera* (L., 1767), *Maniola telmessia* (Zeller, 1847), Lycaenidae: *Aricia agestis* ([D. and Sch.], 1775), *Lycaena phlaeas* (L., 1761), *Lampides boeticus* (L., 1767), *Polyommatus anteros* (Freyer, [1838]), *P. bellis* (Freyer, [1842]), *P. bellargus* (Rottemburg, 1775), *P. syriacus burak* Koçak, 1992, *P. amandus* (Schneider, 1792), *P. thersites* (Cantener, [1835]), *P. icarus* (Rottemburg, 1775), *Plebejus sephirus* (Frivaldszky, 1835), *Satyrium ilicis* (Esper, [1779]), *S. spini* ([D. and Sch.], 1775), *Tarucus balkanicus*

(Freyer, [1844]), Hesperiidae: *Carcharodus orientalis* Reverdin, 1913, *C. alceae* (Esper, [1780]), *Gegenes pumilio* (Hoffmannsegg, 1804), *Muschampia nomas* (Lederer, 1855), *M. proto* (Ochsenheimer, 1808), *M. tessellum* (Hübner, [1802]), *Pyrgus armoricanus* (Oberthür, 1910), *P. melotis* (Duponchel, [1834]), *Spialia orbifer* (Hübner, 1823), *Thymelicus sylvestris* (Tutt, [1905]).

E (1000-1250 m): In the residential areas, there are gardens, non-deep short valleys, outdoor areas with well-developed vegetation, and non-cultivated fields.

Butterfly species: Papilionidae: *Archon apollinus* (Herbst, 1798), *Parnassius mnemosyne* (L., 1758), *Zerynthia cerisy* (Godart, [1824]), Pieridae: *Colias crocea* (Fourcroy, 1785), *C. alfacariensis* Ribbe, 1905, *Euchloe ausonia* (Hübner, [1804]), *Leptidea sinapis* (L., 1758), *Pieris ergane* (Geyer, [1828]), *P. napi* (L., 1758), *P. rapae* (L., 1758), *P. brassicae* (L., 1758), *Pontia edusa* (Fabricius, 1777), Nymphalidae: *Argynnis pandora* ([D. and Sch.], 1775), *Issoria lathonia* (L., 1758), *Melitaea phoebe* ([D. and Sch.], 1775), *M. telona* Fruhstorfer, 1908, *M. didyma* (Esper, 1778), *M. fascelis* (Esper, 1783), *M. cinxia* (L., 1758), *M. collina* Lederer, 1861, *Polygonia egea* (Cramer, [1775]), *Thaleropis ionia* (Eversmann, 1851), *Vanessa cardui* (L., 1758), *V. atalanta* (L., 1758), *Limenitis reducta* Staudinger, 1901, Satyrinae: *Coenonympha pamphilus* (L., 1758), *Hipparchia mersina* (Staudinger, 1871), *Lasiommata maera* (L., 1758), *Maniola telmessia* (Zeller, 1847), Lycaenidae: *Aricia agestis* ([D. and Sch.], 1775), *Callophrys danchenkoi* Zhdanko, 1998, *C. rubi* (L., 1758), *Glaucopsyche alexis* (Poda, 1761), *G. lessei* (Bourgogne, 1954), *Lampides boeticus* (L., 1767), *Lycaena tityrus* (Poda, 1761), *L. asabinus* (Gerhard, [1850]), *L. ochimus* (Herrich- Schäffer, [1851]), *L. phlaeas* (L., 1761), *Polyommatus anteros* (Freyer, [1838]), *P. antiochenus, P. bellis* (Freyer, [1842]), *P. bellargus* (Rottemburg, 1775), *P. coelestinus ponticus* (Courvoisier, 1911), *P. amandus* (Schneider, 1792), *P. cornelia* (Freyer, [1850]), *P. thersites* (Cantener, [1835]), *P. icarus* (Rottemburg, 1775), *Plebejus sephirus* (Frivaldszky, 1835), *P. argus* (L., 1758), *Satyrium spini* ([D. and Sch.], 1775), *Tomares nesimachus* (Oberthür, 1893), *T. nogelii* (Herrich-Schäffer, 1851), Hesperiidae: *Carcharodus orientalis* Reverdin, 1913, *Erynnis tages* (L., 1758), *Pyrgus melotis* (Duponchel, [1834]), *P. armoricanus* (Oberthür, 1910), *Spialia orbifer* (Hübner, 1823), *Thymelicus sylvestris* (Tutt, [1905]), *T. acteon* (Rottemburg, 1775).

F (1250-1500 m): At these heights, the collection was mainly carried out in coniferous forests and forest openings, and stony rocky areas. In the spring season, overcast weather and rain negatively affected the species numbers. Hence, species seen were common and pioneer species.

Butterfly species: Papilionidae: *Iphiclides podalirius* (L., 1758), *Parnassius mnemosyne* (L., 1758), *Zerynthia cerisy* (Godart, [1824]), Pieridae: *Anthocharis cardamines* (L.,1758), *A. damone* Boisduval, 1836, *Colias crocea* (Fourcroy, 1785), *C. alfacariensis* Ribbe, 1905, *Gonepteryx rhamni* (L., 1758), *Leptidea sinapis* (L., 1758), *Pieris ergane* (Geyer, [1828]), *P. krueperi, P. rapae* (L., 1758), *P. brassicae* (L., 1758), *Pontia edusa* (Fabricius, 1777), Nymphalidae: *Aglais urtica* (L., 1758), *Argynnis niobe* (L., 1758), *A. pandora* ([D. and Sch.], 1775), *Issoria lathonia* (L., 1758), *Libythea celtis* (Laicharting, 1782), *Melitaea telona* Fruhstorfer, 1908, *M. didyma* (Esper, 1778), *M. arduinna* (Esper, [1783]), *M. cinxia* (L., 1758), *Polygonia egea* (Cramer, [1775]), *Vanessa cardui* (L., 1758), *V. atalanta* (L., 1758), Satyrinae: *Coenonympha pamphilus* (L., 1758), *Lasiommata maera* (L., 1758), *Maniola telmessia* (Zeller, 1847), Lycaenidae: *Aricia agestis* ([D. and Sch.], 1775), *Callophrys rubi* (L., 1758), *Cupido minimus* (Fuesslin, 1775), *C. osiris* (Meigen, [1829]), *Glaucopsyche alexis* (Poda, 1761), *G. lessei* (Bourgogne, 1954), *Lampides boeticus* (L., 1767), *Lycaena alciphron* (Rottemburg, 1775), *L. tityrus* (Poda, 1761), *L. ochimus* (Herrich-Schäffer, [1851]), *L. thersamon* (Esper, [1784]), *L. phlaeas* (L., 1761), *Polyommatus anteros* (Freyer, [1838]), *P. bellis* (Freyer, [1842]), *P. bellargus* (Rottemburg, 1775), *P. cornelia* (Freyer, [1850]), *P. thersites* (Cantener, [1835]), *P. icarus* (Rottemburg, 1775), *Plebejus sephirus* (Frivaldszky, 1835), *P. argus* (L., 1758), *Rubrapterus bavius* (Eversmann, 1832), *Tomares nogelii* (Herrich-Schäffer, 1851), Hesperiidae: *Carcharodus orientalis* Reverdin, 1913, *C. alceae* (Esper, [1780]), *Erynnis tages*, *Pyrgus melotis* (Duponchel, [1834]), *P. armoricanus* (Oberthür, 1910), *P. sidae* (Esper, [1784]), *Spialia orbifer* (Hübner, 1823).

G (1500-1750 m): At these heights, the anthropogenic effect is very low, and collection and observation were mostly performed in coniferous forest openings, rocky areas, and dried river beds.

Butterfly species: Papilionidae: *Parnassius mnemosyne* (L., 1758), *Zerynthia cerisy* (Godart, [1824]), Pieridae: *Leptidea duponcheli* (Staudinger, 1871), *Colias crocea* (Fourcroy, 1785), *C. alfacariensis* Ribbe, 1905, *Pieris ergane* (Geyer, [1828]), *P. brassicae* (L., 1758), *Zegris eupheme* (Esper, [1804]), Nymphalidae: *Issoria lathonia* (L., 1758), *Melitaea telona* Fruhstorfer, 1908, *M. didyma* (Esper, 1778), *M. cinxia* (L., 1758), *Vanessa cardui* (L., 1758), Satyrinae: *Coenonympha pamphilus* (L., 1758), *Lasiommata maera* (L., 1758), Lycaenidae: *Aricia agestis* ([D. and Sch.], 1775), *Callophrys rubi* (L., 1758), *Cupido osiris* (Meigen, [1829]), *Glaucopsyche alexis* (Poda, 1761), *G. astraea* (Freyer, [1851]), *Lycaena tityrus* (Poda, 1761), *L. asabinus* (Gerhard, [1850]), *Polyommatus antiochenus, P. bellis* (Freyer, [1842]),

P. bellargus (Rottemburg, 1775), *P. coelestinus ponticus* (Courvoisier, 1911), *P. amandus* (Schneider, 1792), *P. cornelia* (Freyer, [1850]), *P. thersites* (Cantener, [1835]), *P. icarus* (Rottemburg, 1775), *Plebejus sephirus* (Frivaldszky, 1835), *Pseudophilotes vicrama* (Moore, 1865), *Rubrapterus bavius* (Eversmann, 1832), *Tomares nesimachus* (Oberthür, 1893), Hesperiidae: *Carcharodus orientalis* Reverdin, 1913, *C. alceae* (Esper, [1780]), *Erynnis tages* (L., 1758), *Pyrgus melotis* (Duponchel, [1834]), *P. serratulae* (Rambur, 1839), *Spialia orbifer* (Hübner, 1823).

H (1750 m-): Above 1750 m: High mountain steppes. The weather is windy and rainy.

Butterfly species: Pieridae: *Pontia callidice* (Hübner, 1800), Nymphalidae: *Aglais urticae* (L., 1758), *Issoria lathonia* (L., 1758), *Melitaea cinxia* (L., 1758), *Vanessa cardui* (L., 1758), Lycaenidae: *Callophrys paulae* Pfeiffer, 1932, *Lycaena asabinus* (Gerhard, [1850]), *Polyommatus cornelia* (Freyer, [1850]), *Tomares nesimachus* (Oberthür, 1893), Hesperiidae: *Erynnis tages* (L., 1758).

DISCUSSION

The number of butterfly species was considerably high compared to previous studies on spring species in the southern parts of Turkey.

Indeed, Kemal and Koçak (2017b) recorded 95 taxa of vernal Lepidoptera from the Euphrates Region in Southern Turkey, with only 16 butterflies (3 Nymphalidae, 3 Hesperiidae, 5 Lycaenidae, and 5 Pieridae). Kemal and Koçak (2017a) recorded 59 taxa of vernal Lepidoptera in SE Turkey, but only 14 butterflies (Nymphalidae 2, Lycaenidae 5, Pieridae 6, Satyridae 1). Kemal and Koçak (2018a) recorded only 16 species from four families (Nymphalidae 1, Lycaenidae). The same authors listed 29 spring butterflies from 6 families in another study (Kemal and Koçak 2018b). 104 butterfly species were identified by Seven and Bozacı (2020). The reason for the high species diversity of spring butterfly species in the study is related to the Mediterranean climate. According to the results of the study, the highest diversity was at between 1250 and1500 m with 58 species. The increase in butterfly species numbers resulted from the decrease in the anthropogenic effects on habitats, and the plentifulness of favourable natural spaces at these heights. Favorable seasonal temperatures and weather conditions were another positive factors. It was seen that the species number at 1500 m was quite low. One of the reasons for this decrease was the food plants visited butterfly had not bloomed yet due to the weather conditions at these altitudes and climatic events such as rain, wind, and temperature. Naturally, with the increase in temperatures, species diversities and species compositions are expected to increase.

In the study area were identified the representatives of Mediterranean species *Luthrodes galba* (Lederer, 1855), *Ziezeria karsandra* (Moore, 1865), and *Ypthima asterope* (Klug, 1832). *Pontia callidice* (Hübner, [1800]), *Callophrys danchenkoi* Zhdanko, 1998 and *Callophrys paulae* Pfeiffer, 1932 are local-spreading species that lived at high mountain steppes, could only be detected from only one station in the area.

The taxonomic status of *Pontia daplidice/edusa* species group was unclear. Two seemingly identical species have been separated on the basis of biochemical and DNA data. Specimens from different populations could be unseparated on a morphological basis and the recent split of the species based on allozyme differences causes additional taxonomic problems with old taxa (Tshikolovets 2011). The Asian contact zone between *P. daplidice* and *P. edusa* has not well known and need further investigation. According to the available data and biogeographic considerations, it probably runs from Hatay in southern Turkey along the Turkish border with Syria and Iraq, and the border of Iraq and Iran to the Persian Gulf (John et al. 2013). Koçak and Kemal (2018) reported that *P. edusa* had spread in every region of Turkey and *P. daplidice* had spread only in Hatay and Şanlıurfa. According to the biogeographical data, collected specimens have been accepted as *P.edusa* clade.

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ÖZET

Bu makale, Doğu Akdeniz Bölgesi'nin daha önce yayınlanmış bahar kelebeklerinin bir devamı niteliğindedir. Daha önce 68 istasyondan listelenen türlerin toplama istasyonu bilgileri bu çalışmaya dahil edilmiştir. İstasyonlar dikey dağılımlarına göre 8 gruba ayrılmıştır. Farklı rakımlardaki habitat tipleri tanımlanmıştır. Bu habitatlarda tespit edilen Lepidoptera toplulukları verilmiştir. *Pontia daplidice/edusa* tür grubunun bölgedeki durumu tartışılmıştır.

Anahtar kelimeler: kelebek, dikey yayılış, yaşam alanı, Doğu Akdeniz Bölgesi

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Original article

Investigation of Cardinium endosymbiont in the micro-fauna of granaries and surroundings

Tahıl depoları ve çevresinin mikro faunasında Cardinium endosymbiontunun incelenmesi

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ABSTRACT

Cardinium is an endosymbiotic bacterium known as a reproductive manipulator in terrestrial ecosystem arthropods. Although *Cardinium* host species have been identified in recent studies, its prevalence is relatively low, perhaps due to the investigation of fewer taxonomic groups. This study was aimed to investigate *Cardinium* bacteria in micro-fauna samples in granaries of Turkey, which has suitable conditions for the distribution of arthropods. For this purpose, *Stratiolaelaps scimitus* (Womersley, 1956) (Acari: Laelapidae), *Entomobrya* sp. (Collembola: Entomobryidae), and *Balaustium* sp. (Acari: Actinotrichida) samples were investigated using the *Cardinium Clo* primer set and compared with the endosymbiont of *Bemisia tabaci* (Hemiptera: Aleyrodidae). It was determined that the *Cardinium* sequences from *S. scimitus* and *Entomobrya* sp., obtained from granaries and in close contact with each other, were quite similar and could be considered as a monophyletic group. This data can be considered as an indicator of horizontal transfer of *Cardinium* between these two taxa. *However, Balaustium* sp. and *B. tabaci* endosymbiont *Cardinium* are phylogenetically distant from them. It is thought that this study, which presents the first data on *Cardinium* endosymbiont in the granary microfauna, will contribute to studies on endosymbiotic bacteria, which are promising reproductive manipulators in the fight against harmful species, especially in agricultural production under pressure due to global warming, decreasing production, and increasing food demand. However, considering the diversity, distribution, and ecological niches of the studied species, more studies are needed to determine the prevalence of *Cardinium*.

INTRODUCTION

The population densities and distribution rates of arthropods, the most successful creatures in terrestrial habitats, are remarkable. Symbiotic, especially endosymbiotic, bacteria have an important role in this success. These bacteria can provide the essential nutrients needed by the host, take part in its defense or increase its resistance to environmental factors (Hedges et al. 2008, Kashkouli et al. 2021, Nakabachi et al. 2006, Oliver et al. 2003, Penz et al. 2012, Werren et al. 2008, Zug and Hammerstein 2012). In addition, these symbionts can be transported vertically and/or horizontally between their hosts, as well as contribute to biodiversity through gene transfer (Aikawa et al. 2014, Aikawa et al. 2022, Kashkouli et al. 2021, Telschow et al. 2005, Zug and Hammerstein 2012,). Of these bacteria, *Cardinium* (Cytophaga - Flavobacterium - Bacteroides) endosymbiont manipulates

the reproduction of its host and uses it to its advantage. Mating between males carrying this maternally inherited bacterium and uninfected females results in cytoplasmic incompatibility. After this situation, the offspring cannot develop and die. On the other hand, if the female carries the same symbiont, the offspring will survive. This feature of *Cardinium* can be used as a promising method to control arthropods that cause damage to humans, animals, and plants or are vectors of various diseases (Doremus et al. 2020, Gotoh et al. 2007, Nakamura et al. 2009, Penz et al. 2012, Werren et al. 2008, Zhao et al. 2018).

Cardinium affects and shapes the population structure, ecology, and evolution of the arthropods (Doremus et al. 2020, Telschow et al. 2005). However, the prevalence of *Cardinium* in arthropods is relatively low and is limited to less taxonomic groups (Zchori-Fein and Perlman 2004). However, although the number of studies has increased recently, the number of taxa examined/investigated is also low. As far as is known, *Cardinium* has been detected in micro-fauna members (Chaisiri et al. 2015, Chang et al. 2010, Gotoh et al. 2007). However, endosymbiont composition in arthropods may vary in populations of the same species in different geographies. This is explained by the host's nutritional regimen or by variables in environmental conditions (Gomard et al. 2021). In fact, arthropods are in constant motion for anthropogenic and/or natural reasons such as trade, migration, and climate changes (Inci et al. 2016). However, it is reported that the distribution area of especially harmful arthropods is expanding (advancing north) due to global warming (Bouchard et al. 2019). Insects associated with agricultural products (especially cereals) due to the increasing food demand are the current study (Ipekdal and Kaya 2020). One of the most suitable geographies for the examination of these dynamic processes is Anatolia. As a matter of fact, Anatolia is a geography with a climate and topography suitable for biological diversity and a high rate of species diversity and endemism. In addition, since it is a bridge between Asia, Africa and Europe, it is a route for both trade and wildlife, which accelerates diversity and diversification (Inci et al. 2016, Ipekdal and Kaya 2020, Özdikmen 2016). Biodiversity in the region, which includes Anatolia, is also seen in the studies carried out, and new records are reported (Hosseini et al. 2016, Noei et al. 2017, Noei et al. 2019, Yahyapour et al. 2018). Therefore, this variety can also be used as a resource for studies on bacterial symbionts such as *Cardinium*. Because endosymbiotic bacteria are important for biotechnological and/or integrated control methods for the protection of agricultural commodities, which have increased in importance due to global warming, drought and various geopolitical risks, which are among the most important problems of the last period (Bouchard et al. 2019, FAO 2021, Gomard et al. 2021, Ipekdal and Kaya 2020). Therefore, in the present study, it was aimed to investigate the micro-fauna components and endosymbiotic *Cardinium* bacteria in the granaries and their surroundings in Kırşehir, the center of Anatolia.

MATERIALS AND METHODS

Micro fauna sampling of granaries

In the study, samples obtained from granaries in Kırşehir Province (Turkey) and its surroundings were examined. Sampling was made from five different indoor granaries and the open area around them in the same location in November-December-2021 (coordinates: 38o10'48"N-34o18'70"E). Samples were taken from the granaries in two ways, directly from the wheat heaps and from the relatively humid parts. Samples found in the stony areas were taken from the open area. The samples were examined using a dissecting microscope in the laboratory. Micro-flora members grouped according to their morphological appearances were washed with 70% alcohol for 30 seconds, rinsed with sterile distilled water, and stored at -20 oC until they were taken into alcohol and worked (Ipekdal and Kaya

Table 1. List of primers used in this study for *Cardinium* endosymbiont and insects

Primer	Sequence (5'-3')	Target genus and gene region	PCR product (bp)	Annealing (°C)	Reference
*LCO*1490-F	GGTCAACAAATCATAAAGATATTGG	COI	710	52	Folmer et al. (1994)
*HCO*2198-R	TAAACTTCAGGGTGACCAAAAAATCA				
Clo-F	GCGGTGTAAAATGAGCGTG	16S rRNA	466	54	Weeks et al. (2003)
Clo-R	ACCTMTTCTTAACTCAAGCCT				

2020). Whiteflies from tomatoes were used as a comparison and positive control. Micro-flora samples and whitefly detection were performed by molecular methods.

DNA extraction and PCR screening

Total DNA was extracted from the samples using the CTAB method (Doyle and Doyle 1990). The mitochondrial cytochrome c oxidase I subunit (COI) primer pair LCO1490-F and HCO2198-R were used to identify the species (Table 1). *Clo*-F/R primer pair was used for screening and diagnosis of *Cardinium* bacteria (Table 1). PCR reactions were carried out in a 20 µl reaction medium (Ipekdal and Kaya 2020). PCR products including negative and positive controls were electrophoresed on 1% agarose gel. Total DNAs belonging to whiteflies were used as positive controls for *Cardinium* screenings. PCR products were electrophoresed and gels were screened on a UV Transilluminator (ThermoScientific). Samples that gave electrophoretic bands in the same position as the positive control were considered positive for the presence of *Cardinium*.

Sequence analysis

PCR products obtained from at least one individual from each sample group studied were sequenced. Reverse and forward sequencing of PCR products obtained with COI and Clo primers was performed in Macrogen Inc., The Netherlands. Dendrograms were created from the obtained sequence data to show the taxonomic data generated in the study. For this, consensus sequences were obtained using the Clustal W 2.0 algorithm (Thompson et al. 1994) in BioEdit (Hall 1999). Consensus sequences were identified in NCBI databases using BLAST analyses (Altschul et al. 1990). In addition, *Cardinium* consensus sequences were compared using dendrograms created by downloading additional sequences (GenBank accession numbers in Figure 1) from NCBI databases.

Dendrograms for *Cardinium* arrays were created using the Maximum Likelihood method. Model testing was performed to find the best substitution model for each sequence set (taking into account nucleotide-sequence divergences), and as a result, the Kimura 2 + G model (Kimura 1980) (1000 replicates) was used for *Cardinium*. All phylogenetic and molecular evolutionary analyzes were performed using MEGA version X (Kumar et al. 2018).

RESULTS

Examples of micro-fauna studied

In the study, micro-fauna samples obtained from five different granaries where wheat is stored in Kırşehir (Turkey) city center and their surroundings were examined. Samples collected directly from heaps and relatively humid parts were used for sampling from inside the granaries. All samples were examined with a dissecting microscope. No sample findings were found in the samples taken from the direct piles. However, micro-fauna samples were detected in the humid parts of the granaries and the surrounding stony areas. Identification of micro-fauna members and whiteflies used as positive controls for *Cardinium* was based on matching consensus sequences from sequence data in GenBank databases. Based on BLAST similarities, those obtained from the granaries were *Stratiolaelaps scimitus* (*Hypoaspis miles*) (Acari: Laelapidae) (100%, MN781133) and *Entomobrya* sp. (Collembola: Entomobryidae) (96%, KY468324), while *Balaustium* sp. (98%, KY922407) (Acari: Actinotrichida). However, the whitefly was defined as *Bemisia tabaci* (Hemiptera: Aleyrodidae) (100%, LN829678) (Table 2).

Table 2. The examined granary micro-fauna samples, *Cardinium* screening results, and presence rate (pr: *Cardinium* positive individual/number of individuals screened) (n: number of individuals screened)

Collected area	Insect species (*n*)	Ecological niche	*Cardinium*		
			pr	GenBank	
				Similarity rate	Accession Number
In granaries	*S. scimitus* (15)	Predator	1.0	100	MN781133
In granaries	*Entomobrya* sp. (9)	Regulatory, Predator	1.0	96	KY468324
Out granaries	*Balaustium* sp. (12)	Predator, Pest agricultural	1.0	98	KY922407
In the field	*B. tabaci* (10)	Pest agricultural	1.0	100	LN829678

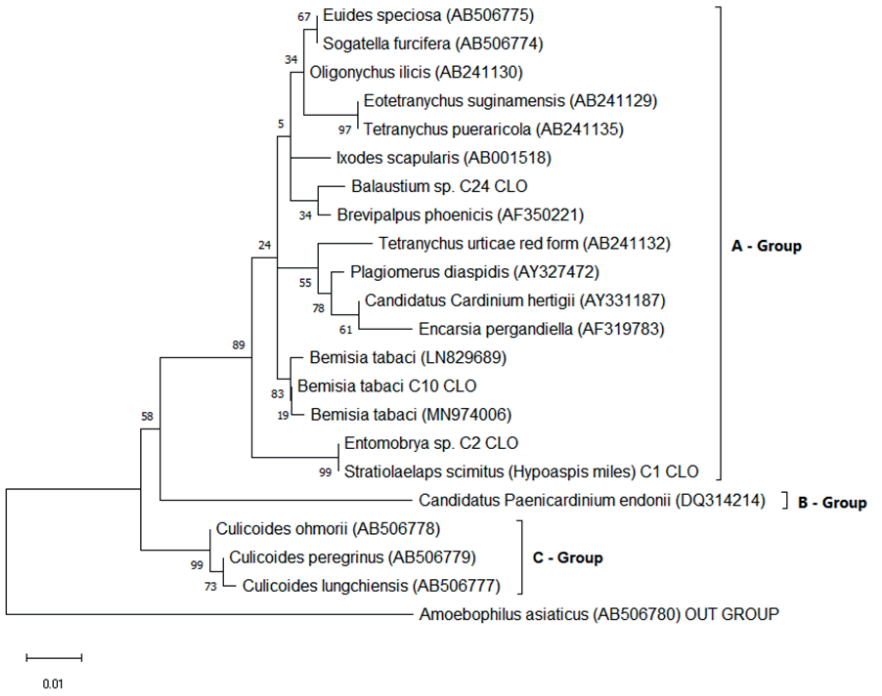

Figure 1. Maximum likelihood (ML) trees of the novel isolates based on *Clo*. Phylogenetic analysis was performed using the Kimura 2 + G parameter model and 1000 bootstraps, with four strains (*Stratiolaelaps scimitus* C1, *Entomobrya* sp. C2, *Balaustium* sp. C24, and *Bemisia tabaci* C10) from this study and additional sequences downloaded from NCBI databases (GenBank accession numbers in parentheses). The percentage of trees in which related taxa are clustered together is shown next to the branches. The display bracket and branch line display the *Cardinium* supergroup. *Amoebophilus asiaticus* was used as outgroup.

Cardinium in mites and its phylogeny

Cardinium bacteria were screened using Clo F/R primer pair in *S. scimitus, Entomobrya* sp., and *Balaustium* sp. and *B. tabaci* detected in and around the granary. The resulting PCR products were sequenced for diagnostic and confirmation purposes. Consensus sequences obtained from sequence data showed 100% similarity to *Cardinium* endosymbiont in the NCBI database (Table 2). It was determined that *Cardinium* infection had a widespread incidence in all individuals who were members of the micro-flora examined (Table 2).

Dendrograms were created using the Kimura 2-parameter model with the Maximum Likelihood method using *Cardinium* consensus sequences and DNA sequences downloaded from GenBank databases. Accordingly, the sequence data in the 16S rRNA region of 468 bp obtained from *S. scimitus, Entomobrya* sp. in the warehouses showed the presence of *Cardinium* with the same topology. In other words, they can be considered as a monophyletic group (Figure 1). On the other hand, *Balaustium* sp. outside the granaries has a different phylogeny (Figure 1). In addition, *B. tabaci*, which is used as a positive control, shows a more distant topology.

DISCUSSION

This study reports endosymbiotic *Cardinium* infection in three different mite species, *S scimitus, Entomobrya* sp., and *Balaustium* sp., which were detected in and around the granaries in Kırşehir Province (Turkey). Although *Cardinium* has been previously reported in arthropods (Chaisiri et al. 2015, Doremus et al. 2020), further studies are needed to elucidate its prevalence in populations and its effects on genotype and phenotype.

In the study, micro-fauna samples obtained from granaries in the same location and their surroundings were examined. It has been observed that *S. scimitus* and *Entomobrya* sp. species show fire spread in the parts of the warehouses where the humidity ratio is high for explainable reasons, and *Balaustium* sp. species are found around the granaries. These findings are not surprising. *S. scimitus* thrives especially in hot and humid environments. The most important feature of this mite is that it is a predatory species and is used as a biological control agent. Naturally found in the northern hemisphere, *S. scimitus* is a polyphagous mite and preys on thrips nymphs, nematodes, sciarid fly larvae, and various species of mites and many soil invertebrates (Navarro-Campos et al. 2016, Rondeau et al. 2018, Walter et al.

2003, Wright and Chambers 1994). *Entomobrya* sp., on the other hand, is a thin arc tail for which there is limited data. Although it has a cosmopolitan distribution, it is generally distributed in the northern hemisphere. It lives in a wide variety of biotopes such as soil, shrubs, bark, and canopy, and feeds on microorganisms and plant organic matter (Baquero and Jordana 2008, Hosseini et al. 2016, Kahrarian 2019). It is also stated that *Collembola* members are even soil conditioners and/or predatory species (Castaño-Meneses et al. 2004). The red mite *Balaustium* sp. is widespread and some species adapt well to man-made structures (Hiruta et al. 2018). Red mites live inside and outside crevices on bricks, stone structures, and tree bark (Halliday 2001). On the other hand, some members of *Balaustium* sp. are predators and feed on other insects. Some are known as grain pests (Halliday 2001). Notable for its diversity, *Balaustium* sp. was previously detected in Turkey (Noei et al. 2017, Noei et al. 2019). On the other hand, there is no data from Turkey on *S. scimitus* and *Entomobrya* sp. species, and this was reported for the first time in this study. However, further studies are needed on the distribution and prevalence of these micro-fauna members.

Cardinium (Cytophaga - Flavobacterium - Bacteroides) from endosymbiotic bacteria, which is a reproductive manipulator in *S. scimitus* and *Entomobrya* sp. and *Balaustium* sp., was screened and examined by this study. In addition, *B. tabaci* individuals, previously reported to have *Cardinium* (Zhao et al. 2018), were used as a comparison and positive control. As a result of the studies, the presence and common incidence of *Cardinium* were determined in all three micro-fauna members. The presence of *Cardinium* in the micro-fauna is a well-known phenomenon (Chaisiri et al. 2015, Chang et al. 2010, Gotoh et al. 2007, Nakamura et al. 2009). In addition, it is more common in *Cardinium* mites than other endosymbionts and is reported to be more important. Although the results obtained are in parallel with the previous studies, *Cardinium* was detected for the first time in the species studied here, for the first time to the best of our knowledge. However, the consensus sequences of *Cardinium*, the symbiont of *S. scimitus* and *Entomobrya* sp., are quite similar. This may be coincidental. However, it raises the question of whether *Cardinium* could have been transferred by horizontal transfer between these species, which are in close contact with each other (?). So, they can be considered a monophyletic group from a phylogenetic point of view (Figure 1). Endosymbionts can be transferred between their hosts horizontally, through feeding, predator-prey relationship, or injury, as well as maternal (vertical) transmission (Gomard et al. 2021). On the other hand, it is known that the same endosymbiotic bacteria strain can be found in different hosts. However, phylogenetic incompatibilities are also expected between hosts and endosymbiotic bacterial strains due to horizontal transfer (Tolley et al. 2019). Another view is that these bacteria can occur in distant host species (Gehrer and Vorburger 2012, Russell and Moran 2006). Another thing worth mentioning is the *Cardinium* variety. *Cardinium* has been evaluated in three upper groups in previous studies (Kageyama et al. 2009). According to the tree drawing based on this in this study, *S. scimitus* and *Entomobrya* sp. and *Balaustium* sp. endosymbiont *Cardinium* (including *B. tabaci*) clustered together with those in group A (Figure 1). However, *Cardinium* in *Balaustium* sp. is distinguished phylogenetically (Figure 1). The obtained data can be explained by the fact that the same symbionts can be found naturally in the same source host populations (Kageyama et al. 2009). However, although the first known determinations of the presence of *Cardinium* in micro-flora members in granaries and related areas are presented here, more studies are needed on the prevalence and phenotype effects of this endosymbiosis. As a matter of fact, as Tolley et al. (2019) stated, it is not clear whether this is the result of horizontal transfer of endosymbionts between distant taxa, and it would be appropriate to consider all these as hypotheses to be tested in future studies.

As a result, this study reports for the first time the presence of micro-flora members in and around granaries and the endosymbiotic *Cardinium* bacteria in them. Considering the diversity, distribution, and ecological niches of the studied species, it is thought that the obtained data will contribute to the studies of endosymbiotic bacteria, which are promising especially in the fight against harmful species.

ÖZET

Cardinium karasal ekosistem eklembacaklılarındaki üreme manipülatörü olarak bilinen endosimbiyotik bir bakteridir. Her ne kadar son dönemde çalışmalarla *Cardinium* konakçısı türler tespit edilse de, belki de daha az taksonomik grubun incelenmesinden dolayı prevalansı görece düşüktür. Bu çalışmada eklembacaklıların yayılışı için uygun koşullara sahip Türkiye'nin tahıl ambarlarındaki mikro-fauna örneklerinden *Cardinium* bakterisinin incelenmesi hedeflenmiştir. Bu amaçla tahıl ambarlarından ve çevresinden temin edilen *Stratiolaelaps scimitus* (Womersley, 1956) (Acari: Laelapidae), *Entomobrya* sp. (Collembola: Entomobryidae) ve *Balaustium* sp. (Acari: Actinotrichida) örneklerindeki *Cardinium Clo* primer seti kullanılarak incelenmiş ve *Bemisia tabaci* (Hemiptera: Aleyrodidae) endosimbiyontu ile mukayese edilmiştir. Sekans verilerine göre ambarlardan elde edilen ve yakın temasta bulunan *S. scimitus* ve *Entomobrya* sp.'teki *Cardinium* benzer homolojiye sahiptir. Bu veri bu iki takson arasında *Cardinium*'un yatay transferinin bir göstergesi olarak düşünülebilir. Ancak ne yazık ki elde

edilen veri seti bunu kesin olarak kanıtlayamaz. Ayrıca *Balaustium* sp. ve *B. tabaci* endosimbiyontu *Cardinium* ise filogenetik olarak bunlardan uzaktır. Tahıl ambarı mikrofaunasında *Cardinium* endosimbiyontuna ilişkin ilk verilerin sunulduğu bu çalışmanın; özellikle küresel ısınma, azalan üretim ve artan gıda talebi nedeniyle baskı altındaki tarımsal üretimde zararlı türlerle mücadelede umut vaat eden üreme manipülatörü endosimbiyotik bakterilere yönelik çalışmalara katkı sunacağı düşünülmektedir. Ancak incelenen türlerin çeşitliliği, yayılışı ve habitatları içerisindeki ekolojik nişleri dikkate alındığında *Cardinium* prevalansının belirlenmesi için daha fazla çalışmaya ihtiyaç vardır.

Anahtar kelimeler: *Balaustium, Cardinium*, endosimbiyotik bakteri, *Entomobrya, Stratiolaelaps scimitus*

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Original article

Survey of mite species of tea plantations in Rize

Rize ili çay alanlarındaki akar türlerinin sürveyi

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ABSTRACT

This study was carried out between 2017-2019 to determine the mite species found in the tea gardens of Rize province (Türkiye). During the study, surveys in 9 districts (Ardeşen, Çayeli, Derepazarı, Fındıklı, Güneysu, İyidere, Kalkandere, Centre and Pazar) were carried out between May and September with the random sampling method, corresponding to the tea plant's 1st, 2nd and 3rd shoot period. Studies were conducted in 73 tea gardens in 9 districts in the first shoot period, 107 tea gardens in the 2nd shoot period and in 97 tea gardens in the 3rd shoot period and plant samples were taken. At the end of the study, *Polyphagotarsonemus* latus (Banks) (Acari: Tarsonemidae) was the most common and highest number detected species with 49.7%. Other mites identified are *Calacarus carinatus* (Green) (Prostigmata: Eriophyidae) with 24.3%, *Brevipalpus phoenicis* (Geijskes) (Prostigmata: Tenuipalpidae) with 19.9%, *Tydeus californicus* (Banks) (Prostigmata: Tydeidae) with 17.1%, *Neoseiulus californicus* (Oudemans) (Mesostigmata: Phytoseiidae) with 5.9%, *Czenspinskia transversostriata* (Oudemans) (Acari: Winterschmidtiidae) with 2.9%, *Tuckerella* sp. (Prostigmata: Tuckerellidae) with 1.8%, and Oribatida species, respectively. The mites found in this study have been completed in a way that integrates with previous studies.

INTRODUCTION

Camellia sinensis (L.) Kuntze is a plant from the family Theaceae, grown in humid climates, and its leaves and buds are used as a beverage. The history of this plant is much older than expected, dating back to the BC Chinese Empire. It dates back to 2737 years. Tea, which was started to be used for medical reasons at first, has turned into a beverage that cannot be given up over time. Although its gene center is in China, India, Vietnam, Laos, Cambodia, and Myanmar, it is also grown in tropical and subtropical regions around the World (Üstün and Demirci 2013). While tea cultivation iscarried out for 12 months in tropical and equatorial regions, tea production is carried out only 6 months of the year in countries with high latitudes such as Türkiye and Iran (Anonymous 2022). According to FAO statistics, China has 41%, India 21%, Kenya 8%, Sri Lanka 5%, Vietnam 4%, Türkiye 4%, Indonesia 2%, Iran 2%, and other producing countries 13% of world tea production in 2018 (Anonymous 2022). Tea which has been known for 400 years in Türkiye and has been produced economically for the last 70 years is a type of beverage that is widely consumed and adopted by all segments of society. It is also an important market in which the public and private sectors are active. Türkiye is among the world's leading countries in tea production and consumption. Tea farming is carried out in the Eastern Black Sea Region, from the Georgian border to the Fatsa district of Ordu province (Anonymous 2013). 66.4% of the tea areas are in Rize with 554.4 thousand decares, 20.3% in Trabzon

with 169.6 thousand decares, 10.8% in Artvin with 90.2% and 2.4 of them are located in Giresun with 20.3 thousand decares. All tea-producing areas in Türkiye are located in these four provinces. In the 2020 production period, tea agriculture increased by 6.21% compared to the previous production period and was carried out on 834 thousand hectares. Many pests have been identified in tea plant around the world. Among these, there are very important mite families that damage plants such as Tetranychidae, Tarsonomidae, Tenuipalpidae, Eriophyidae, and Acaridae.

In our country, 14 mite species, 5 of which are harmful, 3 are predators, and 6 are saprophytes and mycophagous, have been found in tea gardens in the Black Sea Region. (Ozman-Sullivan et al. 2006). Also, Ozman-Sullivan et al. (2006, 2007) studied the distribution and population densities of mites found in tea gardens. However, in these studies, the prevalence and density of mites were determined very little and were not found to be at a level that required struggle. Over the years, it has been decided to study this subject after complaints from tea producers. For this purpose, this study was carried out between 2017-2019 to determine the mite species found in the tea gardens in the districts of Rize province (Türkiye). In our study, the densities of the mites were found to be high, especially the yellow tea mite density was at a level that required serious struggle.

MATERIALS AND METHODS

Material

The main material of the study consisted of clone tea gardens infested with mites.

Field studies

Field studies were carried out in the tea gardens of Rize Province Centre, Ardeşen, Çayeli, Derepazarı, Fındıklı, Güneysu, Iyidere, Kalkandere, and Pazar districts between May and September 2017, coinciding with the 1st, 2nd and 3rd shooting periods (Figure1 a, b and c). Accordingly, surveys were conducted in 73 tea gardens in the first shoot period, 107 tea gardens in the second shoot, and 97 tea gardens in the third shoot. Field studies were carried out according to the random sampling method in cooperation with the Atatürk Tea and Horticultural Research Institute. For this purpose, surveys were carried out to represent these areas, provided that the sampling error is not less than 1% of the tea cultivation area in Rize province and the sampling error is not greater than 0.05 (Yazıcıoğlu and Erdoğan 2014). GPS was used to determine the horizontal and vertical position of the sample area. An average of 25 leaf samples were taken from different edges and shoot tips of the tea plant in each garden, placed in paper bags and then in polyethylene bags, and brought to the laboratory. While the samples were collected from the orchards determined in the study area, the collection date was recorded together with the locality name, altitude and geographical coordinate. The samples brought to the laboratory were examined under a stereomicroscope.

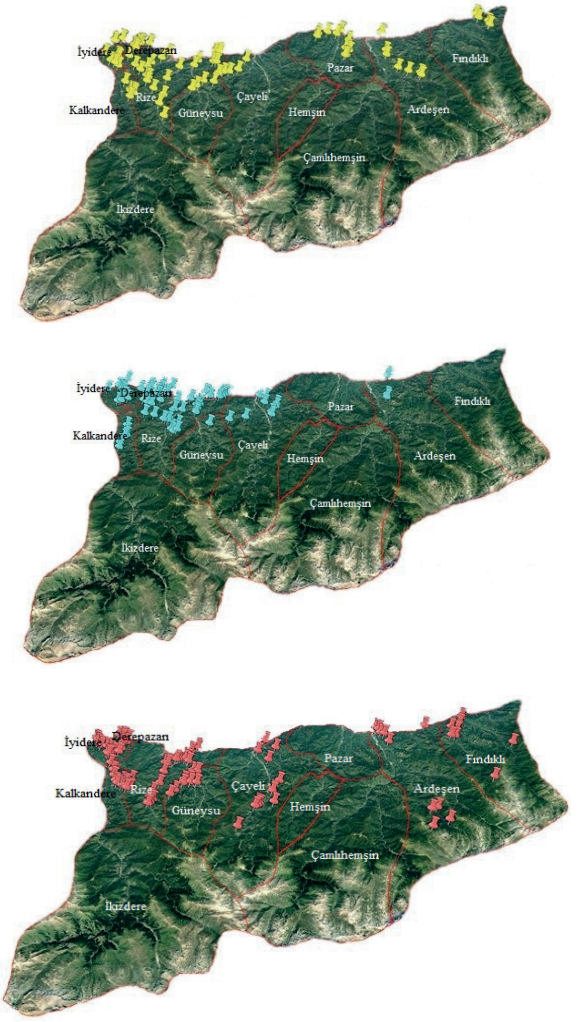

Figure 1. a Survey areas of the first exile period, b- Survey areas of the second exile period, c- Survey areas of the third exile period.

Laboratory studies

The upper and lower surfaces of the leaves were checked under a microscope and the mites found were taken into 70% ethyl alcohol. In addition, the same tea samples were extracted using the Berlese funnel method, and different types of mites were obtained (Düzgüneş 1980).

Mite preparation

The method suggested by Ecevit (1976) and Gutierrez (1985) was used in the preparation of mites. All of the mites were mounted on slides by using Hoyer's medium.

A Leica DM LB 2 microscope with phase contrast was used for identification purposes. All measurements are evaluated in micrometers.

Identification of mites detected in tea gardens

For species identification, Nucifora (1963), Dhooria and Bindra (1977), Costilla (1980), Fletchman and Rosa (1980), Aubert et al. (1981), Laffi (1982), Smith et al. (1997), Pritchard and Baker (1951, 1958), Baker (1965), Tuttle and Baker (1968), Muma and Denmark (1970), Jeppson et al. (1975), Krantz (1978), Gutierrez et al. (1979), Alaoğlu (1984), Chant and Yoshida-Shaul (1986), Çobanoğlu (1993 a,b,c,d, 1995, 1996, 2000), Geijskes (1939)'s definitions and keys were used. In addition, confirmation of the species descriptions was provided by Prof. Dr. Eddie A. Uckermann (*Neoseiulus californicus* and *Tydeus californicus*), Prof. Dr. Gerald W. Krantz (*Czenspinskia transversostriata*), Dr. Owen D. Seeman (*Tuckerella* sp.).

Identified species are preserved in the collection of the Directorate of Plant Protection Central Research Institute, Department of Agricultural Fauna and Microflora.

RESULTS AND DISCUSSION

As a result of this study, a total of 7 different mite species from 7 families were determined in the tea fields of 9 districts in Rize between the years 2017-2019. Of these, one species was identified at the genus level and the other at the family level (Table 1).

Table 1. Mite species determined in tea gardens in Rize province

Family	Species	Overall total	(%) Percentage
Tarsonemidae	*Polyphagotarsonemus latus*	305	49.7
Eriophyidae	*Calacarus carinatus*	149	24.3
Tenuipalpidae	*Brevipalpus phoenicis*	122	19.9
Tydeidae	*Tydeus californicus*	105	17.1
Phytoseiidae	*Neoseiulus californicus*	36	5.9
Winterschmidtiidae	*Czenspinskia transversostriata*	18	2.9
Tuckerellidae	*Tuckerella* sp.	11	1.8

Family: Tarsonemidae

Species: *Polyphagotarsonemus latus* Banks (1904)

Synonymous: *Tarsonemus latus* Banks (Lindquist, 1986)

Material examined: (316 ♀, 61 ♂)

Ardeşen: Akdere, 523 m, 20.09.2017, 4 ♀♀, 3 ♂♂, Akyazı, 454 m, 20.09.2017, ♀, Düz, 58 m, 20.09.2019, 2 ♀♀, Işıklı, 7 m, 20.09.2017, 3 ♀♀, ♂, Köprübaşı, 25 m, 20.09.2017, 2 ♀♀, Pinçuk, 125 m, 13.07.2017, 3 ♀♀, Pirinçlik, 165 m, 20.09.2017, ♀, Seslikaya, 223 m,13.07.2017, 5 ♀♀, ♂, Yeniyol (Oce), 2 m, 20.09.2017, ♀, ♂, Yeşiltepe, 360 m, 20.09.2017, 7 ♀♀; Çayeli: Altıntaş, 372 m, 18.07.2017, 2 ♀♀, 2 ♂♂, Aşıklar stream, 70 m, 12.07.2017, 4 ♀♀, Buzlupınar, 283 m, 26.09.2017, 2 ♀♀, ♂, 290 m, 26.09.2017, 3 ♀♀, Büyükköy, 460 m, 26.09.2017, 4 ♀♀, ♂, Çataldere, 719 m, 26.09.2017, 2 ♀♀, Çaykent, 55 m, 06.06.2017, ♀, 170 m, ♀, Gülpaşa, 90 m, 18.07.2017, 4 ♀♀, İncesırt, 20 m, 12.07.2017, 4 ♀♀, Kaptanpaşa, 414 m, 26.09.2017, ♀, 2 ♂♂, Madenli, 123 m, 12.07.2017, 3 ♀♀, ♂, Musadağı, 12 m, 12.07.2017, 4 ♀♀, 2 ♂♂, Sabuncular, 25 m, 26.09.2017, 5 ♀♀, Sarısu, 225 m, 12.07.2017, 2 ♀♀, ♂, Selimiye, 187 m, 26.09.2017, 3 ♀♀, Seslidere, 358 m, 26.09.2017, 3 ♀♀, Yamaç, 135 m, 12.07.2017, ♀, 2 ♂♂, Yeşiltepe, 716 m, 26.09.2017, ♀, 3 ♂♂; Derepazarı: Aşağı Subaşı, 190 m, 07.08.2017, 2 ♀♀, ♂, Bahattinpaşa, 239 m, 14.07.2017, 3 ♀♀, Bürücek, 112 m, 14.07.2017, 3 ♀♀, Centre, 26 m, 14.07. 2017, 2 ♀♀, Çakmakçılar, 87 m, 14.07.2017, 2 ♀♀, Çukurlu, 111 m, 07.08.2017, 2 ♀♀, Kaf Mountain, 235 m, 07.08.2017, 4 ♀♀, 241 m, 07.08.2017, 4 ♀♀, Kirazdağı, 405 m, 05.06.2017, 2 ♀♀, 425 m, 07.08.2017, ♀, Sandıktaş, 142 m, 07.08.2017, ♀, Tersane 50 m, 07.08.2017, ♀, Uzunkaya, 150 m, 14.07.2017, ♀, Yanıktaş, 285 m, 07.08.2017, ♀, Yukarı Fıçıcılar, 266 m, 07.08.2017, ♀; Fındıklı: Dağınıksu, 110 m, 18.07.2017, 3 ♀♀, Derbent, 310 m, 20.09.2019, ♂, Gündoğdu, Yenimahalle, 180 m, 18.07.2017, ♂, Hara, 268 m, 20.09.2019, 4 ♀♀, 3 ♂♂, Hatıra, 94 m, 18.07.2017, 4 ♀♀, Karaali, 317 m, 20.09.2019, 4 ♀♀, Kopuzlar, 358 m, 18.07.2017, 2 ♀♀, Kozmağa, 241 m, 20.09.2019, 2 ♀♀, Meyvalı, 193 m, 20.09.2019, 2 ♀♀, 2 ♂♂, Paçva, 53 m, 20.09.2019, ♀, ♂, Sahil, 14 m, 20.09.2019, ♀, Şentürktepe, 460 m, 20.09.2019, 2 ♀♀, Yukarı Dağınıksu, 128 m, 18.07.2017, 2 ♀♀, Yukarı Manastır, 52 m, 20.09.2019, 2 ♀♀; Güneysu: Adacami, 80 m, 19.09.2017, 3 ♀♀, Aşağı Kiremit, 185 m, 19.09.2017, 2 ♀♀, ♂, Ayane, 342 m, 19.09.2017, 3 ♀♀, Çankana, 140 m, 19.09.2017, 2 ♀♀, 3 ♂♂, Dumankaya, 422 m, 19.09.2017, 4 ♀♀, Kiremit, 343 m, 11.07.2017, ♂, Pazarköy, 102 m, 19.09.2017, ♀, Selamet, 200 m, 11.07.2017, 3 ♀♀, Tepebaşı, 405 m,19.09.2017, 3 ♀♀, Yeşilköy, 437 m, 19.09.2017, 4 ♀♀, Yüksekköy, 441 m, 19.09.2017, 2 ♀♀, Zincirliköprü, 49 m, 19.09.2017, 3 ♀♀; İyidere: Büyükçiftlik, 235 m, 22.09.2017, 3 ♀♀, 269 m, 07.08.2017, 3 ♀♀, Denizgören, 63 m, 12.07.2017, 2 ♀♀, Fethiye, 135 m, 07.08.2017, 4 ♀♀, Hazar, 117 m, 22.09.2017, 2 ♀♀, Kalecik, 128 m, 22.09.2017, 4 ♀♀, 3 ♂♂, 160 m, 22.09.2017, 4 ♀♀, ♂, Köşklü, 167 m, 22.09.2017, 2 ♀♀, Küçükçiftlik, 260 m, 22.09.2017, 3 ♀♀, Sarayköy, 15 m, 07.08.2017, 2 ♀♀, 25 m, 05.06.2017, ♂, 39 m, 22.09.2017, 4

♀♀, 2 ♂♂, Yalıköy, 171 m, 07.08.2017, 3 ♀♀, Yapraklar, 160 m, 07.08.2017, 4 ♀♀; Kalkandere: Adalar, 223 m, 25.09.2017, ♀, 2 ♂♂, Akarsu, 265 m, 06.06.2017, 2 ♀♀, 2 ♂♂, Ambarlık, 337 m, 19.09.2017, 2 ♀, ♂, Ayane, 538 m, 18.07.2017, 3 ♀♀, ♂, Azaklıhoca, 96 m, 14.07.2017, 5 ♀♀, Beştepe, 322 m, 05.06.2017, 4 ♀♀, Çağlayan, 240 m, 25.09.2017, 2 ♀♀, ♂, 336 m, 25.09.2017, 3 ♀♀, Çaykent, 128 m, 19.09.2017, 2 ♀♀, Centre, 55 m, 06.06.2017, ♀, 11.07.2017, ♀, Çiftekavak, 20 m, 14.07.2017, 3 ♀♀, Çorapçılar, 187 m, 17.07.2017, 3 ♀♀, 157 m, 19.09.2017, ♀, Dörtyol, 75 m, 06.06.2017, 2 ♀♀, Düzköy, 200 m, 17.07.2017, 2 ♀♀, Düzler, 526 m, 17.07.2017, 2 ♀♀, Geçitli, 50 m, 25.09.2017, 3 ♀♀, Gölgeli, 190 m, 14.07.2017, 5 ♀♀, 2 ♂♂, Hamzabey, 149 m, 18.07.2017, 4 ♀♀, ♂, 167 m, 19.09.2017, 2 ♀♀, Hayrat, 193 m, 14.07.2017, 3 ♀♀, Kambursırt, 229 m, 14.07.2017, 4 ♀♀, 2 ♂♂, Karayemiş, 246 m, 19.09.2017, 4 ♀♀, Kömürcüler, 193 m, 19.09.2017, 4 ♀♀, Kuruköy, 118 m, 25.09.2017, ♀, Medrese, 137 m, 25.09.2017, 4 ♀♀, 2 ♂♂, Melek, 456 m, 17.07.2017, 3 ♀♀, 2 ♂♂, Muradiye, 327 m, 17.07.2017, 2 ♀♀, ♂, Pekmezli, 130 m, 06.06.2017, ♀, Portakallık, 20 m, 14.07.2017, 3 ♀♀, ♂, 48 m, 19.09.2017, 3 ♀♀, Salarha (Çaykent), 155 m, 17.07.2017, 4 ♀♀, Seyrantepe, 437 m, 25.09.2017, 3 ♀♀, ♂, Topkaya, 99 m, 14.07.2017, 5 ♀♀, Ünalan, 560 m, 25.09.2017, ♀, Yeşildere, 470 m, 19.09.2017, 2 ♀♀, Yolveren, 151 m, 17.07.2017, 2 ♀♀, 125 m, 19.09.2017, 2 ♀♀, Yumurtatepe, 102 m, 25.09.2017, 2 ♀♀, ♂; Pazar: Boğazlı, 56 m, 29.05.2017, ♀, Selimiye, Ayane, 598 m, 06.06.2017, ♀.

Host: The Yellow tea mite has a wide host range in tropical areas. It attacks greenhouse plants in temperate and subtropical regions. Crops listed; apple, avocado, cantaloupe, castor, chilli, citrus, coffee, cotton, eggplant, grapes, guava, jute, mango, papaya, passion fruit, pear, potato, sesame, string or pole beans, tea, tomato, African violet, ageratum, azalea, begonia, chrysanthemum, cyclamen, dahlia, gerbera, gloxinia, ivy, jasmine, impatiens, lantana, marigold, peperomia, pittosporum, snapdragon, verbena, and zinnia (Baker 1997, Peña and Campbell 2005).

Distribution in Türkiye: Antalya, Adana, İçel, Hatay, Rize (Çobanoğlu 1995, Tunç and Göçmen 1995, Uygun et al. 1995, Yabaş and Ulubilir 1995, Bulut and Göçmen 2000, Vatansever and Ulusoy 2002, Ozman-Sullivan et al. 2006, 2007, Akyazı et al. 2019, Aşık-Çuhadar et al. 2019).

Distribution in the World: Found in tropical regions around the World (Zhang 2003). There are no detailed studies on detecting mite species in tea gardens in our country. Yellow tea mite was first detected in our country in 1992 in Antalya. Especially on young plants newly transferred to the greenhouse in autumn (Tunç and Göçmen 1995). It was determined that P. latus was common and a significant pest on pepper plants in the greenhouses of İçel province (Yabaş and Ulubilir 1995). In the world, the Yellow tea mite was first detected in the tea plant in Colombo (Sri Lanka) in 1890. It was later reported on fig and mango plants in New York in 1904 (Lin and Zhang 2002).

Family: Eriophyidae

Species: *Calacarus carinatus* (Green, 1890)

Synonymous: *Typhlodromus carinatus* Green; *Eriophyes carinatus* Nalepa; *Calacarus carinatus* Keifer; *Epitrimerus adornatus* Keifer

Material examined: (144 ♀, 5 ♂)

Ardeşen; Işıklı, 7 m, 20.09.2017, 6 ♀♀, Seslikaya, Ortamahalle, 302 m, 13.07.2017, 5 ♀♀, ♂; Çayeli: Centre, 230 m, 05.06.2017, 3 ♀♀, Kaptanpaşa, 433 m, 26.09.2017, 5 ♀♀, ♂, Sabuncular, 25 m, 26.09.2017, 5 ♀♀, Sarısu, 225 m, 12.07.2017, 5 ♀♀, Yeşilköy, 110 m, 09.06.2017, 3 ♀♀; Derepazarı: Fıçıcılar, 124 m, 05.06.2017, 4 ♀♀, Tersane, Cumhuriyet, 50 m, 22.09.2017, 4 ♀♀, Yukarı Fıçıcılar, 266 m, 22.09.2017, ♀; Fındıklı: Meyvalı, Ortamahalle, 193 m, 20.09.2017, 4 ♀♀, Sümer, 0 m, 30.05.2017, 2 ♀♀; Güneysu: Aşağı Kiremit, 185 m, 19.09.2017, 2 ♀♀, Pazarköy, 100 m, 11.07.2017, 8 ♀♀, ♂, Tepebaşı, 405 m, 19.09.2017, 5 ♀♀; İyidere: Hazar, 137 m, 22.09.2017, 4 ♀♀, Kalecik, 55 m, 22.09.2017, 6 ♀♀, Köprü, 0 m, 05.06.2017, 4 ♀♀, Sarayköy, 14 m, 12.07.2017, 5 ♀♀; Kalkandere: Akarsu, Valanda, 265 m, 11.07.2017, 8 ♀♀, Azaklıhoca, Çaycılar, 86 m, 14.07.2017, 3 ♀♀, Çağlayan, Karamanlar, 317 m, 25.09.2017, 7 ♀♀, ♂, Çorapçılar, Boyama, 150 m, 19.09.2017, 5 ♀♀, Kuruköy, 104 m, 05.06.2017, 4 ♀♀, Pekmezli, 60 m, 11.07.2017, 5 ♀♀, Portakallık, 0 m, 11.07.2017, 4 ♀♀, ♂, Yemişlik, 600 m, 06.06.2017, 7 ♀♀, Yumurtatepe, 102 m, 25.09.2017, 4 ♀♀; Pazar: Hunarsu, 6 m, 29.05.2017, 6 ♀♀, Sivritepe, 355 m, 06.09.2017, 10 ♀♀.

Host: *Camellia caudata* Wallich, *Camellia sinensis* (L.) Kuntze, *Camellia kissi* Wallich, *Camellia japonica* L., *Camellia sasanqua* Thunb., *Capsicum annuum* L. (Solanaceae), *Viburnum opulus* L. (Adoxaceae) (Anonymous 2022).

Distribution in Türkiye: Giresun (Düzgüneş 1963); Central and Eastern Black Sea Region (Ozman-Sullivan et al. 2007).

Distribution in the World: Korea (JeollaNam-do, Jeju-do); Cambodia, China, Japan, India, Indonesia, Laos, Malaysia, Sri Lanka, Taiwan, Vietnam, Italy, Portugal, Spain, The Soviet Union, America, Australia, New Zealand (Manson 1984,, Channabasavanna 1996, Hong and Zhang 1996).

The *Calacarus carinatus* detected in this study belongs to the Eriophyoidea superfamily and it was first found in Türkiye by Ozman et al. (2006), and first in Indian 1890 in the World

(Green 1890, Watt 1898). It was later determined in China, India, Japan and America, respectively (Li et al. 2014). It was found in the Camellia (Theaceae) plant in Korea (Lee et al. 2014). Ozman-Sullivan et al. (2007) conducted surveys between 2005 and 2006 in the Black Sea Region in order to determine the distribution of mites in tea gardens. As a result of study, *C. carinatus* (80.00%) was determined as the most harmful species and *Brevipalpus* spp. (*Brevipalpus obovatus* Donn. and *B. phoenicis* (34.78%) species followed. *P. latus* was detected at a very low rate (0.87%) in the Georgian border.

Family: Tenuipalpidae

Species: *Brevipalpus phoenicis* (Geijskes, 1939)

Synonymous: *Tenuipalpus phoenicis* Geijskes; *Brevipalpus phoenicis* Sayed; *Brevipalpus yothersi* Baker; *Brevipalpus mcbridei* Baker; *Brevipalpus papayensis* Baker; *Brevipalpus pseudocuneatus* Baker

Material examined: (120 ♀)

Ardeşen: Akyazı, 454 m, 20.09.2017, ♀, Işıklı, 7 m, 20.09.2017, 3 ♀♀, Köprübaşı, 250 m, 20.09.2017, 4 ♀♀, Tunca, 455 m, 09.06.2017, 2 ♀♀; Çayeli: Büyükköy, Esentepe, 460 m, 18.07.2017, 5 ♀♀; Derepazarı: Kirazdağı, 252 m, 22.09.2017, 6 ♀♀; Güneysu: Dörtyol, 75 m, 11.07.2017, 2 ♀♀, Dumankaya, 422 m, 19.09.2017, 2 ♀♀, Gündoğdu, Taşlık, 105 m, 12.07.2017, 6 ♀♀, Selamet, 200 m, 11.07.2017, 3 ♀♀, Tepebaşı, 405 m, 19.09.2017, 5 ♀♀, Yeşilköy, 437 m, 19.09.2017, 4 ♀♀, Zincirliköprü, 49 m, 19.09.2017, 3 ♀♀; Fındıklı: Derbent, 310 m, 30.05.2017, 10 ♀♀, Hürriyet, 55 m, 21.07.2017, 8 ♀♀, Karaali, 317 m, 20.09.2017, 5 ♀♀, Meyvalı, Ortamahalle, 193 m, 20.09.2017, 8 ♀♀; İyidere: Ambarlık, 337 m, 19.09.2017, 5 ♀♀, Boyama, 150 m, 19.09.2017, 10 ♀♀, Hamzabey, 167 m, 19.09.2017, 6 ♀♀, Sarayköy, 39 m, 22.09.2017, 4 ♀♀, Yeşildere, 527 m, 19.09.2017, 10 ♀♀; Pazar: Boğazlı, 43 m, 29.05.2017, 5 ♀♀, Centre, 0 m, 06.09.2017, 8 ♀♀.

Host: 146 plant species belonging to families Acanthaceae, Aizoaceae, Altingiaceae, Amaranthaceae, Anacardiaceae, Annonaceae, Apocynaceae, Aquifoliaceae, Araceae, Araliaceae, Apocynaceae, Asparagaceae, Balsaminaceae, Begoniaceae, Bignoniaceae, Bixaceae, Boraginaceae, Cannabaceae, Caricaceae, Clethraceae, Combretaceae, Compositae, Convolvulaceae, Cucurbitaceae, Dioscoreaceae, Euphorbiaceae, Garryaceae, Geraniaceae, Hydrangeaceae, Juglandaceae, Lamiaceae, Phyllanthaceae, Poaceae, Rubiaceae and Salicaceae are its hosts (McGregor 1916, Pritchard and Baker 1951, 1958, Morishita 1954, Michelbacher 1956, Dosse 1957, Carmona 1960, 1962, Milne et al. 1962, DeLeon 1965 a, b, Cranham 1966, Manson 1967, Ehara 1969, Haramoto 1969, CABI 1970, 1975, 1988, Livshitz et al. 1972, Chandra and ChannaBasavanna 1974, Chaudhri 1974, Roa 1974, Wahab et al. 1974, Baker et al. 1975, Gonzalez 1975, Jeppson et al. 1975, Murray 1976, Sadana and Joshi 1976, Lal and Mukharji 1977, 1979, Lal 1978;, Smith Meyer 1979, Moralde et al. 1982, Sadana and Gupta 1982, Sadana et al. 1982, 1983, Jagadish et al. 1983, Ghai and Shenhmar 1984, Goyal et al. 1984, 1985, Ochoa 1985, Sadana 1985, Hatzinikolis 1986, Heugens 1986, Baker and Tuttle 1987, Kumari and Sadana 1990, Evans et al. 1993, Trinidade and Chiavegato 1994, Ochoa et al. 1994, O'Dowd 1994, Smiley and Gerson 1995, Yano et al. 1995, Morse et al. 1996, Randeep et al. 1999, Walter 1999, Kitajima et al. 2003).

Distribution in Türkiye: Ankara (Sağlam and Çobanoğlu 2010).

Distribution in the World: America, Germany, Argentina, Hawaii Island, India, Holland, Spain, Cuba, Ceylon, Egypt, Trinidat, Tanjanika, Kongo, Kenya, Malaya, Sinaloa, Vera Cruz, Oaxaca, Mexican, Greece, Portugal, Sicily, Italy, Ethiopia, Taiwan, Brazil, Venezuela, Philippines and Australia (Düzgüneş 1965, Baker and Tuttle 1987, Jeppson et al. 1975, Baker et al. 1975).

Brevipalpus phoenicis, which belongs to the Tenuipalpidae family, was first identified in Türkiye by Düzgünes (1963). It was first found in the coffee plant in Brazil (Geijskes 1939). This mite causes brownish coloration in its hosts as a result of feeding. It is also the vector of *Citrus leprosis virus* (CiLV) (USDA 2004).

Family: Tuckerellidae

Species: *Tuckerella* sp.

Material examined: (11 ♀)

Çayeli: Kaptanpaşa, 433 m, 26.09.2017, 5 ♀♀; Derepazarı: Kirazdağı, 252 m, 22.09.2017, 6 ♀♀.

Family: Tydeidae

Species: *Tydeus californicus* (Banks)

Synonymous: *Tetranychoides californicus* Banks 1904; *Tydeus spathulatus* (Oudemans)

Material examined: (105 ♀).

Ardeşen: Düz, 58 m, 13.07.2017, 6 ♀♀; Çayeli: İnce Sırt, 20 m, 12.07.2017, 7 ♀♀; Derepazarı: Çakmakçılar, 87 m, 14.07.2017, 6 ♀♀, Kafdağı, 323 m, 22.09.2017, 8 ♀♀; Fındıklı: Şentürktepe, 460 m, 20.09.2017, 7 ♀♀; Güneysu: Çankana, 140 m, 19.09.2017, 6 ♀♀, Zincirliköprü, 49 m, 19.09.2017, 3 ♀♀; Kalkandere: Azaklıhoca, Çaycılar, 86 m, 14.07.2017, 5 ♀♀, Çorapçılar, 187 m, 17.07.2017, 10 ♀♀, Çağlayan, Eminoğulları, 272 m, 05.06.2017, 4 ♀♀, Çaykent, 128 m, 19.09.2017, 8 ♀♀, Hamzabey, 204 m, 19.09.2017,

9 ♀♀, Ortapazar, 386 m, 06.06.2017, 2 ♀♀, Pekmezli, 314 m, 11.07.2017, 4 ♀♀; Pazar: 0 m, 06.09.2017, 8 ♀♀, Kesikköprü, 180 m, 21.07.2017, 6 ♀♀, Sivritepe, 355 m, 06.09.2017, 6 ♀♀.

Host: Common worldwide in fruit, citrus and ornamental plants (Tempfli et al. 2015). Obtained from plum, apricot, cherry, peach, cherry, mahaleb and different plants in Türkiye (Çobanoğlu 1991, 1992, Çobanoğlu and Kazmierski 1999, Ozman and Çobanoğlu 2001, İncekulak and Ecevit 2002, Akyazı and Ecevit 2003, Yanar and Ecevit 2005, Kasap and Çobanoğlu 2007, Kumral and Kovancı 2004, Özşişli and Çobanoğlu 2011, Yeşilayer and Çobanoğlu 2011, Kasap et al. 2013, Kasap 2014, Akyazı et al. 2017, Soysal and Akyazı 2018).

Distribution in Türkiye: Bursa, Çanakkale, Denizli, İstanbul, İzmir, Manisa, Ordu, Tokat, Samsun (Akyazı and Ecevit 2003, Kumral and Kovancı 2004, Göven et al. 2009, Yeşilayer and Çobanoğlu 2011, Kasap et al. 2014, Erdoğan and Yanar 2015, Yeşilayer and Çobanoğlu 2016, Akyazı et al. 2018, Altunç and Akyazı 2019).

Distribution in the World: Türkiye, Hungary, Azerbaijan, Lebanon, Syria, Egypt, Jordan, Israel, Iranian, Iraq and North Africa (Anonymous 2008).

Tydeus californicus belongs to the family Tydeidae. Although there is no evidence of this mite species feeding on leaves, it has been observed that these species generally coexist with predatory mite species on the leaves. It is probably thought that they form the food of beneficial mites and serve as an additional food source for these species, especially when phytophagous mite species are not present in the environment. There are not many studies on the Tydeidae family in our country and the world. Generally, this family is considered a neutral species and feeds on lichens, fungi, plant and insect residues (Yanar and Erdoğan 2013). Yanar and Ecevit (2005) reported that *T. californicus* and *Tydeus* sp. species, and their findings support our results. Castagnoli (1989) determined that *T. californicus* is a common species and is found in grape, pear and peach plants in Italy. It has not been determined whether this species is harmful to plants in general or not.

Family: Phytoseiidae

Species: *Neoseiulus californicus* (McGregor)

Synonymous: *Typhlodromus californicus* McGregor, 1954; *Amblyseius californicus* Schuster and Pritchard, 1963; *Cydnodromus californicus* Athias-Henriot 1977; *Amblyseius (Amblyseius) californicus* Ueckerman

Material examined: (34 ♀, 2 ♂)

Ardeşen: Pirinçlik, 165 m, 20.09.2017, ♀, Seslikaya, 223 m, 13.07.2017, 2 ♀♀, 2 ♂♂; Çayeli: Madenli, Yenimahalle, 123 m, 12.07.2017, 2 ♀♀, Musadağı, 12 m, 09.06.2017, ♀, Yenipazar, 5 m, 26.09.2017, ♀; Derepazarı: 283 m, Yanıktaş, 22.09.2017, 2 ♀♀; Fındıklı: Derbent, 310 m, 30.05.2017, 2 ♀♀, Gündoğdu, Taşlık, 105 m, 12.07.2017, 2 ♀♀, Şentürktepe, 460 m, 20.09.2017, ♀; Güneysu: Selamet, 200 m, 11.07.2017, 2 ♀♀, Yüksekköy, 511 m, 19.09.2017, ♀; İyidere: Denizgören, 63 m, 05.06.2017, 2 ♀♀, Küçükçiftlik, 260 m, 22.09.2017, 2 ♀♀; Kalkandere: Çağlayan, Seymenler, 313 m, 25.09.2017, ♀, Çiftekavak, 20 m, 14.07.2017, 3 ♀♀, Dörtyol, 90 m, 06.06.2017, 2 ♀♀, Kendirli, Beştepe, 322 m, 05.06.2017, ♀, Pekmezli, 314 m, 11.07.2017, ♀, Salarha, Güneşli, 272 m, 17.07.2017, ♀, Selimiye, Centre, 380 m, 06.06.2017, ♀, Yeşildere, 470 m, 19.09.2017, ♀; Pazar: Kocaköprü, 52 m, 21.07.2017, ♀, Topluca, 120 m, 06.09.2017, ♀.

Host: *Neoseiulus californicus* usually feeds on tetranychid mites, but in the absence of its prey, it can survive by feeding on pollen and other harmful mites such as *P. latus* and *Tarsonemus pallidus* Banks (Castagnoli and Liguori 1994, McMurtry and Croft 1997).

Distribution in Türkiye: Ankara, Aydın, Balıkesir, Bursa, Çanakkale, Isparta, Ordu, Yalova (Çakmak and Çobanoğlu 2006, Yorulmaz and Ay 2012, Kasap et al. 2013, Çobanoğlu and Kumral 2014, Soysal and Akyazı 2018).

Distribution in the World: Algeria, Argentina, Azores, Brazil, Canada, Canary Islands, Chile, Colombia, Cuba, Cyprus, France, Greece, Guatemala, Italy, Japan, Mexico, Morocco, Peru, Portugal, Reunion Island, Senegal, Serbia, Slovenia, South Africa, South Korea, Spain, Syria, Taiwan, Tunisia, USA, Venezuela, Vietnam and Türkiye (Sahraoui et al. 2012, Demite et al. 2022).

Neoseiulus californicus, belonging to the Phytoseiidae family, was found for the first time in the world in 1954 by McGregor on lemon trees in California (Rhodes and Liburd 2005). It was found for the first time on strawberry, peach, bean, and pepper plants in Kuşadası, Aydın. It was found together with *Tetranychus urticae* Koch and *Panonychus ulmi* (Koch) (Acari: Tetranychidae) between 2001 and 2003 (Çakmak and Çobanoğlu 2006). This predator usually feeds on tetranychid mites. But in the absence of prey, they feed on harmful mites such as *Polyphagotarsonemus latus* and *Tarsonemus pallidus* Banks (Acari: Tarsonemidae) (Castagnoli and Liguori 1994, McMurtry and Croft 1997).

Family: Winterschmidtiidae

Species: *Czenspinskia transversostriata* Oudemans, 1931

Material examined: (15 ♀, 3 ♂)

Ardeşen: Kambursırt, 229 m, 14.07.2017, ♀, Pinçuk, 125 m, 09.06.2017, 2 ♀♀; Çayeli: Gülpaşa, 90 m, 12.07.2017, 3 ♀♀; Derepazarı: Fıçıcılar, 124 m, 05.06.2017, ♀; Fındıklı: Paçva, 53 m, 20.09.2017, ♀; Güneysu: Çaykent, 128 m, 19.09.2017, ♀, Düzler, 526m, 17.07.2017, ♀, Muradiye, Kayalık, 510 m, 17.07.2017, ♀, Yüksekköy, 511 m, 19.09.2017, 2 ♀♀; Pazar: Hunarsu, 6 m, 29.05.2017, ♀.

Distribution in Türkiye: Central and Eastern Black Sea Region (Ozman and Çobanoğlu 2001, Ozman-Sullivan et al. 2007).

Distribution in the World: Costa Rica, Brazil (Vega et al. 2008, Lofego et al. 2013).

Czenspinskia transversostriata (Oudemans), belonging to the Winterschmidtiidae family, was detected for the first time in hazelnut orchards in Türkiye (Ozman and Çobanoğlu 2001). This species usually inhabits decaying matter, fungi, plant leaves, and stored food (Barbosa and Moraes 2021).

Species: Oribatidae Family

Oribatid mites were first successfully cultured by Michael (1884) (Shereef 1972). In our study, individuals belonging to the Oribatidae family were found. But species has not been determined.

With this study, many individuals belonging to harmful and beneficial mite fauna were obtained in the tea gardens and contributed to the mite fauna of the country. We believe that the obtained beneficial fauna, especially phytoseid and other predator mites, will be helpful for future studies on the use of biological control. In particular, it should be aimed to determine the distribution area of the yellow tea mite, to protect the beneficial ones at the right time of struggle, to explain the importance of this issue to the farmers and to provide training.

It is thought that the results of the study will be decisive in terms of preventing erroneous applications and will shed light on future studies.

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ÖZET

Bu çalışma, Rize ili çay bahçelerinde bulunan akar türlerini tespit etmek amacıyla 2017-2019 yılları arasında gerçekleştirilmiştir. Çalışma süresince, 9 ilçede (Ardeşen, Çayeli, Derepazarı, Fındıklı, Güneysu, İyidere, Kalkandere, Merkez ve Pazar) tesadüfi örnekleme yöntemi ile çay bitkisinin 1., 2. ve 3. sürgün dönemine denk gelecek şekilde mayıs-eylül ayları arasında sürvey çalışmaları yürütülmüştür. Birinci sürgün döneminde 9 ilçede toplam 73 çay bahçesinde, ikinci sürgün döneminde 107 çay bahçesinde ve üçüncü sürgün döneminde ise 97 çay bahçesinde arazi çalışmaları yapılmış ve çay örnekleri toplanmıştır. Çalışma sonunda en yaygın ve en yüksek sayıda tespit edilen tür %49.7 ile *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) olmuştur. Belirlenen diğer akarlar ise sırasıyla; %24.3 ile *Calacarus carinatus* (Green) (Prostigmata: Eriophyidae), %19.9 ile *Brevipalpus phoenicis* (Geijskes) (Prostigmata: Tenuipalpidae), %17.1 ile *Tydeus californicus* (Banks) (Prostigmata: Tydeidae), %5.9 ile *Neoseiulus californicus* (McGregor) (Mesostigmata: Phytoseiidae), %2.9 ile *Czenspinskia transversostriata* (Oudemans) (Acari: Winterschmidtiidae), %1.8 ile *Tuckerella* sp. (Prostigmata: Tuckerellidae) ve Oribatida türleridir. Bu çalışmada bulunan akarlar, daha önceki yıllarda yapılan çalışmalarla bütünleşecek şekilde tamamlanmıştır.

Anahtar kelimeler: çay, *Polyphagotarsonemus latus*, Acarina, fauna, Türkiye.

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BİTKİ KORUMA BÜLTENİ YAYIN İLKELERİ

1. Yayınlanan eserlere ait tüm sorumluluk yazarlarına aittir.

2. Bitki Koruma Bülteni bitkisel ürünlerde zarar oluşturan hastalık, zararlı ve yabancı ot konularında yapılan taksonomik, biyolojik, ekolojik, fizyolojik ve epidemiyolojik çalışmaların ve mücadele yöntemleri ile ilgili araştırmaların yanı sıra, zirai mücadele ilaçlarının kalıntı, toksikoloji ve formülasyonları ile zirai mücadele alet ve makinaları ilgili araştırmaları yayınlamaktadır.

3. Bitki Koruma Bülteni'nin yayın dili İngilizce ve Türkçe'dir. Gerekli hallerde Türkçe özet editör ofisi tarafından hazırlanır.

4. Bitki Koruma Bülteni'nde tek yıllık ve tek bir bahçe veya tarlada gerçekleştirilmiş biyolojik gözlemler, Türkiye için tek bir türe ait ilk kayıtları bildirilen kısa biyolojik notlar gibi eserler kabul edilmemektedir.

5. Bitki Koruma Bülteni'ne gönderilen makaleler, daha önce herhangi bir yayın organında yayınlanmamış veya aynı zamanda başka bir yayın organında değerlendirme aşamasında olmamalıdır.

6. Lisansüstü tezler veya TÜBİTAK, DPT, TAGEM, BAP gibi çeşitli kurumlarca desteklenen projelerin sonuçlarından kısımlar içeren eserler ilgililerinden gerekli izinler alındıktan sonra yayına hazırlanmalı, bu durum teşekkür kısmında mutlaka belirtilmelidir.

7. Bitki Koruma Bülteni'nde yayınlanması istenilen eserler için makale başvurusu DERGİPARK sistemi (http://dergipark.gov.tr/bitkorb) üzerinden yapılmalıdır.

8. Sisteme yüklenen makale "Yazarlar için" sekmesinde yer alan "Makale taslağı"na göre hazırlanmalı, sisteme "Makale giriş sayfası" ve tüm yazarlar tarafından doldurulup imzalanan "Bitki Koruma Bülteni Telif Hakkı Devir Formu" ve "Çıkar Çakışması ve Hakem Önerileri Formu" ile birlikte yüklenmelidir.

9. Bitki Koruma Bülteni'nde kör hakemlik değerlendirme süreci izlenmektedir.

10. Değerlendirme sürecine dahil edilen makaleler konu editörü ve belirlenen hakemler tarafından incelenip, onların önerileri doğrultusunda yazarları tarafından düzeltildikten sonra yayınlanır.

11. Bitki Koruma Bülteni'nde yayınlanan makaleler için baskı ücreti alınmamaktadır.