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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Investigation on phytoplasma diseases, their potential insect vectors and other hosts in pepper (*Capsicum annuum* L.) growing areas of Hatay-Turkey

Hatay ili biber (*Capsicum annuum* L.) üretim alanlarında fitoplazma hastalıklarının, potansiyel vektörlerinin ve diğer konukçularının araştırılması

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Ö Z E T / A B S T R A C T

Aims: This study was conducted to determine the phytoplasma diseases of pepper plants in Hatay province between 2016 and 2019.
Methods and Results: Samples of pepper (*Capsicum annuum*), sesame (*Sesamum indicum*), basil (*Ocimum basilicum*), tomato (*Solanum lycopersicum*) and bindweed (*Convolvulus arvensis*) which had typical phytoplasma symptoms and insect (Cicadellidae spp.) samples were collected from the pepper fields. *Empoasca* sp., *Cicadulina bipunctata*, *Psammotettix* sp., *Balchutha hebe*, *Euscelidius* sp., *Anaceratagallia laevis* and *Exitianus capicola* were found as potential insect vectors of the phytoplasmas as descending order of population densities. T-budding grafting technique was successful for phytoplasma transmission from infected sesame and basil plants to healthy periwinkle (*Catharanthus roseus*) plants in controlled conditions. Phytoplasmas were detected in pepper, tomato, basil, sesame and bindweed and from some insect samples. Total nucleic acid isolation was accomplished by the CTAB method. Direct and Nested PCR were used employing R16F1/R16R0 and R16F2n/R16R2 primer pairs respectively.
Conclusions: DNA of positive samples were sequenced, and uploaded to Genbank, and were identified as *Ca*. Phytoplasma trifolii on pepper (MT993358), sesame (MT994434), tomato (MT992754), basil (MT994432), *Empoasca* sp. (MT994430), *Exitianus capicola* (MT994433), *Euscelidius* sp. (MT994431); and as *Ca*. Phytoplasma solani on binweed (MT993422) and tomato (MT992796).
Significance and Impact of the Study: According to our knowledge, this study is the first to identify and upload to Genbank of *Ca*. Phytoplasma trifolii on basil and *Empoasca* sp., *Exitianus capicola* and *Euscelidius* sp. as insect vectors of the diseases in Turkey.

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INTRODUCTION

Pepper (*Capsicum annuum* L.) is an important field and greenhouse plant belonging to the nightshade (Solanaceae) family, native to South and Central America. It is known that pepper was used as a food in the Americas 9000 years ago and has been cultivated since 6000 years ago (Perry et al., 2007). It has been reported in historical documents that pepper has spread to the world after the discovery of America in

1492, and it has been commercially produced since the 1600s (Anonymous, 2020). It is thought that it entered to Turkey from Istanbul in the 16th century due to the trade with Europe during the Ottoman Empire (Andrews, 1999).

In the world, 36 million tons of fresh pepper is produced on an area of approximately 2 million hectares and 4,625,833 tons of dry pepper is produced from an area of 1 million 856 thousand hectares, and Turkey ranks 3rd in the world after China and Mexico with a production of 1.9 million tons (FAO, 2020).

There are 5 species among 30 pepper species in the genus Capsicum (*Capsicum annuum* L., *C. baccatum* L., *C. chinense* Jacq., *C. frutescens* L., and *C. pubescens* Ruiz & Pav.) cultivated in the world (Bosland, 1994). In Turkey, almost all of the pepper production belongs to the *C. annuum* species (Eken and Mavi, 2016), ranging from green peppers, bell peppers, capia, pointed to ornamental peppers as well as peppers for pepper paste are cultivated. In Turkey, Antalya province of Turkey is ranked at first with a production of approximately 500 thousand tons, while Hatay province ranks 12th with a production of 60 thousand tons (TUIK, 2020).

Many phytoplasma diseases that infect pepper have been reported in Turkey (Sertkaya et al., 2003; Sertkaya et al., 2007; Sertkaya, 2008; Sertkaya, 2012; Özdağ and Sertkaya 2017; Oksal et al., 2017; Yılmaz et al., 2019). In Hatay province, a phytoplasma disease belonging to 16SrXII-A group was determined in sesame, periwinkle, pepper, eggplant and tomato as well as in insect vector Orosius orientalis (Sertkaya et al., 2007; Sertkaya et al., 2013).

Ca. Phytoplasma trifolii was first reported to be the organism that caused the formation of clover proliferation (CP) in flowers of the alsike clover (*Trifolium hybridum*) plants in Canada in the early 1960s (Chiykowski, 1965); today it has been isolated from many vegetables, fruit trees and weeds (Usta et al., 2018; Zamharir, 2018; Oksal, 2020; Usta et al., 2021). In Turkey, *Ca*. Phytoplasma trifolii was first detected in pepper plants in Malatya (Oksal et al., 2017).

Phytoplasmas are disease agents in the Mollicutes class of bacteria. The agent, which has spread throughout the world, causes yellows, stunting and witches' broom, phyllody, and virescence symptoms in plants.

Since the agent could not be cultured in artificial media, transmitted by vectors and its symptoms were similar to those of virus symptoms, it was thought that the cause of the disease was a virus until 1967, but it was identified as Mycoplasma-Like Organism after imaging under the electron microscope (Doi et al., 1967). At the 10th congress of The International Organization for Mycoplasmology (IRPCM) in 1994, the Phytoplasma Study Team adopted the name "Phytoplasma". It was suggested by IRPCM (The International Organization for Mycoplasmology) to be included in the new genus as '*Candidatus* (*Ca*.) Phytoplasma' in 2004 (IRPCM, 2004). Phytoplasmas were categorized into 33 ribosomal groups, each containing several subgroups, by 2018 (Bertaccini and Lee, 2018).

Phytoplasmas cause devastating losses in cultivated plants and natural ecosystems worldwide. The agent causes disease in forest trees, vineyards, orchards, ornamental plants, weeds and many plants including vegetables such as potatoes, tomatoes, peppers, eggplants. Vector insects such as leafhoppers, planthoppers and pysillids are known to transmit the diseases. Other ways of transmission are identified as grafting, natural root fusion and parasitic plants such as dodder (*Cuscuta* sp.) (Çıtır, 1985; McCoy et al., 1989; Şaş-Sertkaya, 1999; Lee et al., 2000; Singh and Singh 2000; Seemüller et al., 2002; Pracros. et al., 2006; Weintraub and Beanland, 2006; Bertaccini, 2007; Hogenhout and Music, 2010).

Phytoplasma belonging to 16 different ribosomal groups affecting vegetables has been reported worldwide and the aster yellows phytoplasma group (16SrI), which causes the highest number of diseases in different vegetables, is followed by the peanut witches' broom (16SrII) group (Kumari et al., 2019). Peanut witches' broom phytoplasma, which infects species belonging to Cucurbitacea (cucumber, zucchini), Solanaceae (pepper) and Cruciferae (radish) families, is transmitted by *Orosius albicinctus*, *Macrosteles laevis* and *Orosius argentatus* (Tran-Nguyen et al., 2003; Salehi et al., 2015), aster yellows phytoplasma is transmitted by *Macrosteles quadrilineatus* (Zheng-Nan et al., 2013). *Empoasca devastans*, *Hishimonus phycitis* and *Circulifer haematoceps* transmit clover proliferation (16SrVI) phytoplasma (Thomas and Krishnaswami, 1939; Salehi et al., 2007), which causes severe crop losses in eggplant and cabbage, while *Macrosteles laevis*, *Hyalesthes obsoletus* and *Circulifer tenellus* have been reported as vectors of stolbur phytoplasma (16SrXII-A) in vegetables. Especially, *C. tenellus* has been identified as a potential vector of potato purple top disease. A single insect species can transmit one or more phytoplasma diseases, or a phytoplasma can be transmitted by different insect species (Lee et al., 1998). The presence of these vector insects, which are known to transmit phytoplasma diseases, have been reported in cultivated plants belonging to the Solanaceae family grown in Hatay province (Kılıç and

Sertkaya, 2019).

In this study, phytoplasma diseases in pepper and weed species in and around pepper fields and other cultivated plants grown in the same fields in Hatay province and potential vector insect species that play a role in the spread of these diseases were investigated. Thus, basic data on the general situation of phytoplasma diseases in pepper fields, to control of the disease and especially the prevention of its spread was tried to be obtained.

MATERIALS and METHODS

Plant samples

The study was carried out in Antakya, Reyhanlı, Kırıkhan, Samandağ, Arsuz and Altınözü districts of Hatay province in Turkey between May and October in 2016 to 2019. Pepper plants and basil, sesame, tomato plants and bindweed in pepper fields were used as research material. In the areas visited in the study, symptoms specific to phytoplasmas such as general yellows, stunting, sterility, upward orientation of flower structures, virescence, phyllody, big bud, bushy plants, little leaves, upward curling of leaves and shoot samples were collected from the plants. During the study, a total of 450 peppers (*Capsicum annuum* L.), 35 sesame (*Sesamum indicum* L.), 5 tomatoes (*Solanum lycopersicum* L.), 7 basil (*Ocimum basilicum* L.) and 20 bindweed (*Convolvulus arvensis* L.) plant samples were collected.

Insect samples

Insects from pepper fields were collected at the end of the growing season (September-October) with the D-VAC. Insects were collected randomly with the help of D-VAC and in the form of zigzag patterns in the field. Among the collected insect species, samples belonging to the Cicadellidae (Hemiptera) family were separated and identified by Prof. Dr. Hüseyin BAŞPINAR (Adnan Menderes University).

Biological indexing

Tissue grafting was done to healthy test plants with tissue pieces taken from pepper, tomato, basil and sesame plants showing phytoplasma symptoms in field conditions. Plant parts containing phloem tissue of 10-15 mm in length from infected plants were grafted onto healthy test plants. T budding was used in grafting and studies were carried out to transmit the disease. Grafting was attempted in the same species (pepper to pepper) and between species (sesame and basil to pepper and periwinkle) in the studies. Tissue fragments were taken from the plants and inoculated to at least 5 plants from the test plants, both intraspecific (pepper to pepper) and interspecies (pepper to sesame, sesame to periwinkle).

Nucleic acid isolation and polymerase chain reaction studies

Total nucleic acid isolation in plant samples was performed according to the CTAB method (Doyle and Doyle, 1990). Leaf midrib and flower parts were used for DNA isolation from plant samples. After identifying the insects collected from pepper fields, DNA isolation was performed with the MN (Macherey-Nagel) isolation kit. For the abundant insect species (i.e. *Empoasca* sp., *Cicadulina bipunctata*, *Psammotettix* sp., *Balclutha hebe* and *Euscelidius* sp.) groups of 5 individuals, in rare species (i.e. *Anaceratagallia laevis*, *Exitianus capicola*) each individual's DNA was isolated. PCR studies were carried out in two stages. Universal primer pairs R16F1/R16R0, F1 5'-AAGACGAGGATAACAGTTGG-3' -R0 5'-GGATACCTTGTTACGACTTAACCCC-3' (Lee et al., 1994) yielding 1.8 kbp were used in Direct-PCR, R16F2n/R16R2, F2n 5'-CGACTGCTAAGACTGG-3'-R2 5'-TGACGGGCGGTGTGTACAAACCCCG-3' (Gundersen and Lee, 1996) yielding 1,250 bp were used for Nested-PCR. Total nucleic acid and Direct PCR products used in Direct and Nested PCR studies were diluted to 1/30 ratio.

Genome sequencing

The sequencing for both forward and reverse reads was performed by a commercial firm (BM Yazılım Danış. ve Lab. Sis. Ltd. Şti.) using the Sanger method on 33 isolates selected from plant and insect samples with positive Nested PCR results. Only one of the isolates with the same DNA sequence in the sequencing results was selected and uploaded tothe NCBI Genbank.

In silico RFLP (Restriction Fragment Length Polymorphism) analysis

The samples for which DNA sequence analysis was performed were uploaded to Genbank, their codes were taken, and cut with 6 different restriction enzymes (*Alu*I, *Hae*III, *Hha*I, *Taq*I, *Mse*I and *Rsa*I) using the *i*PhyClassifier (Zhao et al, 2009) program.

Phylogenetic analyzes

Phylogenetic analysis was performed using MEGA-X (version 10.2.4 https://www.megasoftware.net) to determine the relationships of the (Hosseini et al., 2016) current isolates and their closely related (99%) previously registered isolates in Genbank. Thus, the differentiation and similarity of the obtained sequences with each other were determined and their phylogenetic relatedness levels were revealed. The Neighbor-Joining method was used to create a phylogenetic tree and the Maximum Likelihood method was used as a statistical method.

RESULTS and DISCUSSION

Phytoplasma detection

According to the evaluations made under field conditions, the symptoms that may be related to phytoplasma infections in pepper plants were observed in 2016 (0,5-3%).

While symptoms were rarely observed in pepper plants in the same regions in 2017 and 2018, a high rate of (3/10) phytoplasma symptoms was observed in sesame and basil plants grown in pepper fields in all of the three years (Figure 1). The most common symptoms thought to be related to phytoplasmas, along with abnormalities in flower structures and phyllody observed in pepper, tomato, basil, sesame, bindweed and periwinkle plants were shown in Table 1.

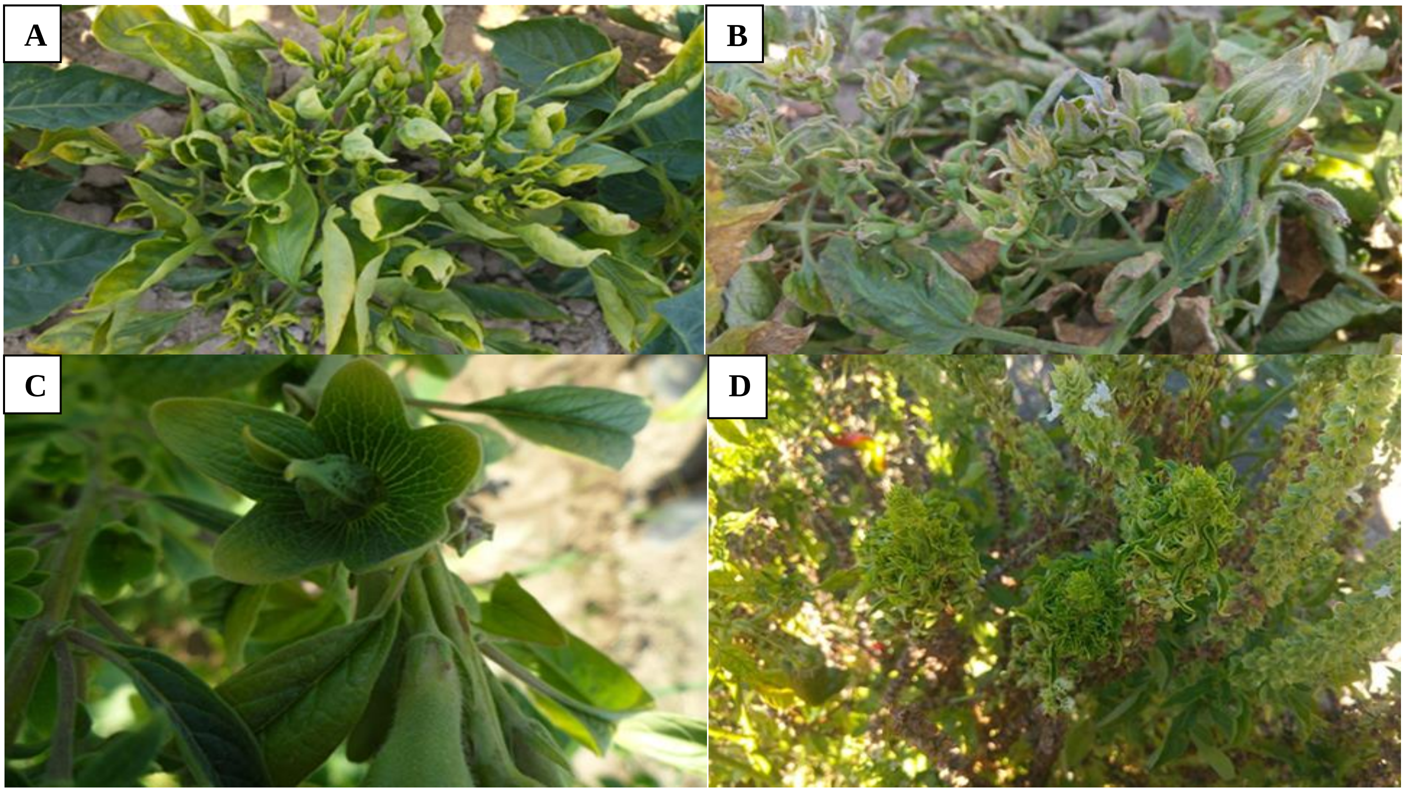

Figure 1. Phytoplasma symptoms; uprolling of the leaves on pepper (A), big buds on tomato (B), phyllody on sesame (C) and phyllody and witches' broom on basil (D) plants

According to studies of transmission of the disease through tissue grafting inoculation, the transmission rate of the disease was found to be approximately 50% (6/11), although the tissue pieces dried in about 1 week in the grafts made from infected sesame and basil to Periwinkle. However, inoculations made with tissue pieces taken from infected peppers, the disease could not be transmitted either to its own species or to other species. Therefore, it has been observed that the most successful plant in transferring the disease in grafting, even from different species, is periwinkle. As a result of laboratory studies and analyzes, *Ca*. Phytoplasma trifolii belonging to 16SrVI group was detected in pepper, tomato, basil and sesame samples, while *Ca*. Phytoplasma solani belonging to 16SrXII group was detected in tomato and bindweed plants. One sample from the tomato plants exhibiting big bud symptom on the margins of the pepper fields was found to be infected by *Ca*. Phytoplasma solani belonging to the 16SrXII and the other one was infected by *Ca*. Phytoplasma trifolii belonging to the 16SrVI group (Table 1), thus more than one phytoplasma species/group could infect the same plant species. As a matter of fact, in some studies conducted in different geographies, it has been reported that phytoplasmas belonging to different groups cause big bud disease in tomatoes. As a causative agent of tomato big bud disease phytoplasmas have been reported all around the world; namely aster yellows (16SrI) in Iran, paenut witches' broom (16SrII) in China and India, elm yellows (16SrV) in Mauritius, clover proliferation (16SrVI) in the U.S. and stolbur (16SrXII) in Russia (Gungoosingh-Bunwaree et al., 2007; Ember et al., 2011; Xu et al., 2013; Sichani et al., 2014; Kumari et al., 2018). Stolbur phytoplasma group (16SrXII-A) of symptoms associated with sesame and periwinkle with phyllody symptoms, pepper with stolbur symptoms, eggplant with yellow-little leaf and tomatoes with big bud, as well as *Orosius orientalis* vector insects were reported by Sertkaya et al. (2007) in Hatay province. Çağlar et al. (2010) detected "*Ca*. Phytoplasma solani" (16SrXII-A) on potato plants in Kayseri and Sivas provinces, on tomato plants and in *Cicadula inornata* (Cicadellidae) in Kahramanmaraş and Adana provinces.

Table 1. Phytoplasmas detected the plant species, number of infected plant/total plant sample and main symptoms observed on these plants

Plant Species	**Phytoplasma(s) detected (Number of infected plants/ Number of tested plants)**	**Phytoplasma Group**	**Common symptoms observed**
Pepper (*Capsicum annuum*)	*Ca.* Phytoplasma trifolii (135/450)	16SrVI	Stunting, yellowing, upward rolling of leaves, big buds and upward shooting
Tomato (*Solanum lycopersicum*)	*Ca.* Phytoplasma solani (1/5) *Ca.* Phytoplasma trifolii (1/5)	16SrVI, 16SrXII	Bushy, big buds and upward shooting
Basil (*Ocimum basilicum*)	*Ca.* Phytoplasma trifolii (7/7)	16SrVI	Sterility, phyllody, longer pedicel, leaf-like structures emerging from the flower
Bindweed (*Convolvulus arvensis*)	*Ca.* Phytoplasma solani (3/20)	16SrXII	Witches' broom, yellowing, little leaf and upward rolling of leaves
Periwinkle (*Catharanthus roseus*)	*Ca.* Phytoplasma trifolii (17/35)	16SrVI	Witches' broom, phyllody

In this study, it was observed that towards the end of the production period (September-October) there was an increase in populations of the Cicadellidae family especially after ceasing of the insecticide applications. In the study, insect species of *Empoasca* sp. (90), *Cicadulina bipunctata* (Melichar) (85), *Psammotettix* sp. (65), *Balclutha hebe* (Kirkaldy) (35), *Euscelidius* sp. (25), *Anaceratagallia laevis* (Ribaut) (4), *Exitianus capicola* (Stal) (3) were collected. *Empoasca* sp was the most abundant insect species collected by D-VAC, followed by *Cicadulina bipunctata*, *Psammotettix confinis* and *Psammotettix* sp. Other identified species included *Exitianus capicola*, *Euscelidius* sp. and *Balclutha hebe*, the insect samples determined to be Delphacidae could not be identified at the species level and were recorded as Delphacidae (Table 2).

It has been reported by Kılıç and Sertkaya (2019) that *Empoasca decipiens*, *Asymmetrasca decedens* and *Psammotettix provincialis* species were abundant/densely populated in the areas where important Solanaceae crops (potato, tomato, pepper, eggplant) are grown in Hatay. *Exitianus capicola* is known to be the vector of *Ca*. Phytoplasma cynodontis (Salehi et al., 2009). It has been reported that *Ca*. Phytoplasma trifolii could be successfully transmitted by *Ceratagallia nitidula* and *Empoasca abrupta* (Hemiptera: Cicadellidae) species that feed on ornamental peppers infected with *Ca*. Phytoplasma trifolii for one week and these species were vectors (Salas-Muñoz et al., 2018). In this study, the phytoplasma determined species, the number of tested insects and the determined phytoplasmas in pepper fields are given in Table 2.

Table 2. Insect species in which phytoplasmas determined

Insect Species	Phytoplasma determined	Phytoplasma Group	Number of Positive Sample/ Number of Total Samples
Empoasca sp.	*Ca.* Phytoplasma trifolii	16SrVI	9/18 (90)
Exitianus capicola	*Ca.* Phytoplasma trifolii	16SrVI	1/3 (3)
Euscelidius sp.	*Ca.* Phytoplasma trifolii	16SrVI	4/5 (25)
Delphacidae (unidentified species)	*Ca.* Phytoplasma trifolii	16SrVI	2/2 (2)

Empoasca sp., *Euscelidius* sp. and *Exitianus capicola* were determined to be able to transmit *Ca*. Phytoplasma trifolii belonging to the 16SrVI group. *Ca*. Phytoplasma trifolii was also determined in phytoplasma positive insect samples belonging to Delphacidae family, which could not be identified at the species level. The presence of any phytoplasma agent could not be determined in *Cicadulina bipunctata*, *Psammotettix* sp., *Balclutha hebe* and *Anaceratagallia laevis* species.

It was determined that *Ca*. Phytoplasma trifolii is the common phytoplasma species especially in pepper plants along with *Ca*. Phytoplasma solani in tomato and bindweed samples in pepper fields in Hatay province, potential insect vectors include species belonging to different families, and the presence of hosts other than pepper in the same field. Potential insect vectors of phytoplasma diseases identified in this study were mostly species belonging to the family Cicadellidae, which are common in pepper fields. One sample from each species was selected and uploaded to the NCBI Genbank.

Ca. Phytoplasma trifolii (16SrVI) isolates from pepper (MT993358), tomato (MT992754), sesame (MT994434), basil (MT994432), *Empoasca* sp. (MT994430), *Exitianus capicola* (MT994433) and *Euscelidius* sp. (MT994431) were uploaded in Genbank. In norder to determine the sub-group of the Phytoplasma, virtual RFLP analysis were accomplished by selecting 16SrVI group with *Alu*I, *Hae*III, *Hha*I, *Taq*I, *Mse*I and *Rsa*I digests in the *i*PhyClassifier (Zhao et al, 2009) program (Figure 2).

Samples of *Ca*. Phytoplasma trifolii uploaded to Genbank matched with 98.33% similarity rate to Van (Turkey) isolate MG732925 (Usta et al., 2018), with 99.76% similarity rate to Mexican isolate MK996152 (unpublished data), and 99.68% similarity rate to both Mexico MF092789 (Swisher et al., 2018), Iran MK379605 (Babaei et al. 2020) and MG788318 (unpublished data), 99.60% similarity rate to Iran isolate JF508509 (Jamshidi et al. 2014). The isolate MT992796 identified as *Ca*. Phytoplasma solani was similar to MN047263 (Jakovljević et al., 2020) and to Bulgaria isolate JN561702 (unpublished data) with a rate of 99.76%, and the isolate coded MT993422 was similar to KF614623 (Adamovic et al., 2014) with a rate of 99.60% (Figure 3). With this study, *Ca*. Phytoplasma trifolii was detected in *Empoasca* sp., *Exitianus capicola* and *Euscelidius* sp. in Turkey for the first time.

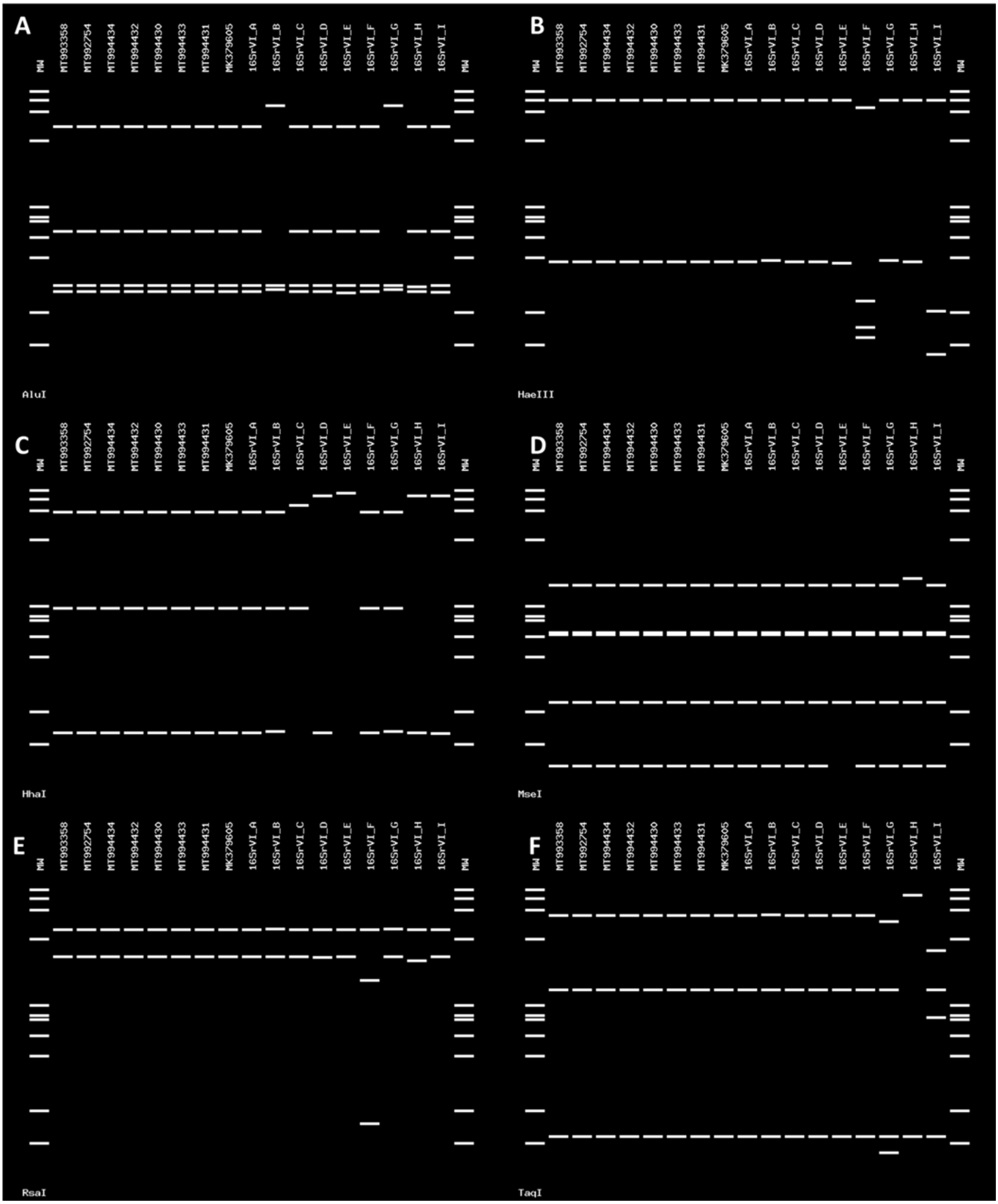

Figure 2. Digestions by AluI (A), HaeIII (B), HhaI (C), MseI (D), RsaI (E) ve TaqI (F) enzymes determined by iPhyclassifier (Zhao et al., 2009) (MW:1 kb DNA marker) Reference sample (MK379605) (Babaei et al., 2020) shown in the blue rectangulars

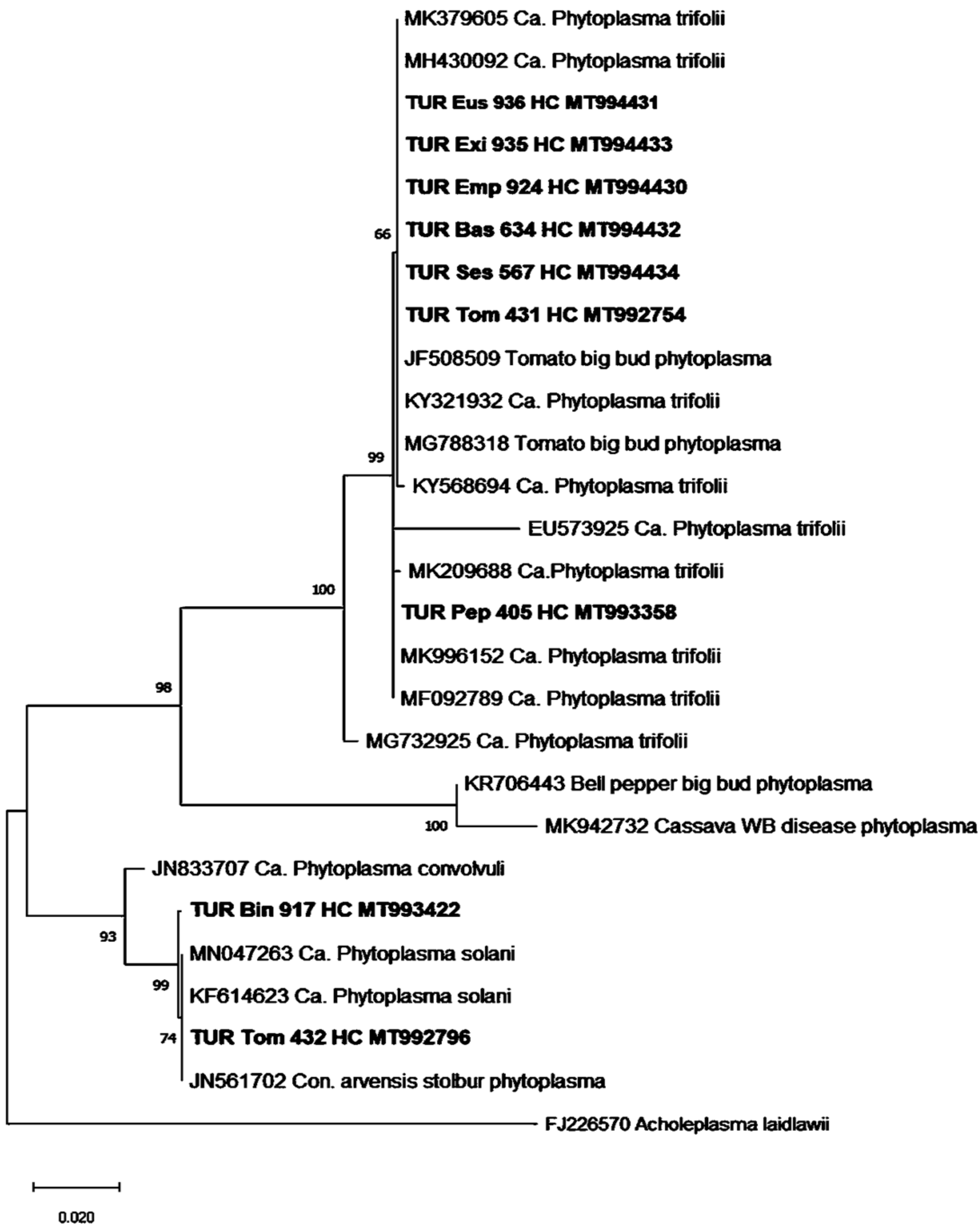

Figure 3. Phylogenetic tree created with the neighbour-joining method by MEGA-X, Bootstrap (1000 replicates) analysis was used. *Acholeplasma laidlawii* (FJ226570) was selected as root. Isolates written in bold characters are recorded in this study

In conclusion, in this study, the expected level of amplification was obtained as a result of PCR analysis in pepper, sesame, bindweed, tomato and basil plants. *Ca*. Phytoplasma trifolii belonging to group 16SrVI was detected in pepper, tomato, basil and sesame samples, while *Ca*. Phytoplasma solani belonging to group 16SrXII was detected in tomato and bindweed plants.

Ca. Phytoplasma solani belonging to group 16SrXII was detected in one of 2 tomato plants showing big buds, and *Ca*. Phytoplasma trifolii belonging to group 16SrVI were detected in the other sample. According to field observations, it was determined that the populations of insect species belonging to the Cicadellidae family increased at the end of the production period in pepper fields. Among the insect species collected by D-VAC, the most abundant species was *Empoasca* sp, followed by *Cicadulina bipunctata* and *Psammotettix* sp. species followed.

Although there are different approaches for the control of phytoplasma diseases, there is no method that can be effective alone in the fight against the disease. Controlling vector insects will reduce the spread of phytoplasma diseases. However, although it is not possible to eradicate all vector insects in field conditions, over-spraying will not be an economical and healthy practice. Eradication of plants exhibiting symptoms of disease in field conditions can also significantly reduce the spread of phytoplasma diseases. Phytoplasma diseases can remain in perennial weeds for many years and these weeds play a role as an alternative host in field conditions.

In this study, it was determined that cultivars and weed species in pepper fields could also be hosts for phytoplasmas. In addition, the detection of phytoplasmas in vector insect species collected from pepper fields shows that phytoplasma diseases can be transmitted naturally to other crops.

In future studies, it is important to investigate other potential vector species belonging to Delphacidae family, together with the species belonging to Cicadellidae family, to determine the role of the transfer of these pathogens to uninfected areas and to other plant species.

ÖZET

***Amaç*:** Bu çalışma 2016-2019 yılları arasında Hatay ili biber alanlarındaki fitoplazma hastalıklarının belirlenmesi amacıyla yürütülmüştür.

***Yöntem ve Bulgular*:** Biber arazileri içerisindeki fitoplazmalara özgü belirtiler gösteren biber (*Capsicum annuum*), susam (*Sesamum indicum*), fesleğen (*Ocimum basilicum*), domates (*Solanum lycopersicum*), tarla sarmaşığı (*Convolvulus arvensis*) ve böcek (Cicadellidae spp.) örnekleri toplanmıştır. Ziyaret edilen alanlarda, popülasyon yoğunlukları sıralamasına göre, fitoplazmaların böcek vektörleri olarak *Empoasca* sp., *Cicadulina bipunctata*, *Psammotettix* sp., *Balchutha hebe*, *Euscelidius* sp., *Anaceratagallia laevis* ve *Exitianus capicola* bulunmuştur. Kontrollü koşullarda yapılan T göz aşısı ile, hastalıklı susam ve fesleğen örneklerinden Cezayir menekşesine (*Catharanthus roseus*) yapılan aşılamalarda hastalık bulaştırılmıştır. Toplam nükleik asit izolasyonu CTAB metoduna göre yapılmıştır. Direkt ve Nested PCR çalışmalarında sırasıyla R16F1/R16R0 ve R16F2n/R16R2 primer çiftleri kullanılmıştır.

***Genel Yorum*:** Fitoplazma bulunan bazı örneklerde DNA dizisi belirlenmiş ve Genbankası'na yüklenmiştir. Biber, domates, fesleğen, susam ve tarla sarmaşığında ve bazı böcek türlerinde fitoplazma tespit edilmiştir. Genbankası'na yüklenen biber (MT993358), susam (MT994434), domates (MT992754), fesleğen (MT994432), *Empoasca* sp. (MT994430), *Exitianus capicola* (MT994433), *Euscelidius* sp. (MT994431) örneklerinde Candidatus Phytoplasma trifolii; tarla sarmaşığı (MT993422) ve domates (MT992796) örneklerinde *Candidatus* Phytoplasma solani tespit edilmiştir.

***Çalışmanın Önemi ve Etkisi*:** Mevcut literatür bilgilere göre *Ca*. Phytoplasma trifolii, fesleğende ve vektör böcek olarak *Empoasca* sp., *Exitianus capicola* ve *Euscelidius* sp.'de ülkemizde ilk kez bu çalışma ile tespit edilmiştir.

***Anahtar Kelimeler*:** *Ca*. Phytoplasma trifolii, *Ca*. Phytoplasma solani, *Exitianus capicola*, *Euscelidius* sp., fesleğen (*Ocimum basilicum*).

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CONFLICT OF INTEREST

The authors declare no conflict of interest for this study.

AUTHOR'S CONTRIBUTIONS

The contribution of the authors is equal.

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