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Corresponding address:

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Çukurova Üniversitesi Kampüsü PTT Şubesi
P.O. Box: 33 - 01330, Adana - Turkey

E-mail: bimude@cu.edu.tr
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Tel: 0322 338 60 69
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Özgün makale (Original article)

Domates bakteriyel solgunluk ve kanser hastalığının (*Clavibacter michiganensis* subsp. *michiganensis* (Smith) Davis *et al.*) bakteriyel antagonistler kullanılarak mücadele imkânlarının araştırılması*

Evaluation of biological control possibilities for tomato bacterial wilt and canker (*Clavibacter michiganensis* subsp. *michiganensis* (Smith) Davis *et al.*) by using bacterial biocontrol agents

Parisa MOHAMMEDİ[1], Recep KOTAN[1]**

Abstract: Tomato bacterial wilt and canker disease (*Clavibacter michiganensis* subsp. *michiganensis* (Smith) cause significant losses in tomato cultivation in both Turkiye and worldwide. Cultural measures and chemical control methods have limited success against this pathogen. In this study, 1150 potential biocontrol agent bacterial strains were tested for activity against this pathogen in Petri dish experiments. Biochemicals, metabolites and enzymes from a total of 48 strains were effective against the pathogen. The disease prevention rates of these potential biocontrol agents, which were propagated in liquid media, were examined in pot trials. The tested strains reduced the development of the diseases by from 6.5% to 93.4%. *Bacillus megaterium* TV-49A (93.4%) and *B. cereus* TV-85D (86.9%) showed the highest effects in terms of disease prevention. These isolates produce ACC, indole acetic acid and salicylic acid, and high phosphate solubility. Furthermore, they were positive for lipase, amylase, protease, lysis decarboxylase, phytase and chitinase enzyme production.

Keywords: Bacterial wilt and canker, biological control, *Clavibacter michiganensis* subsp. *michiganensis*, tomato

Öz: Domates bakteriyel solgunluk ve kanser hastalığı (*Clavibacter michiganensis* subsp. *michiganensis* (Smith) Davis et al.) Dünyada olduğu gibi Türkiye'de de domates yetiştiriciliğinde önemli kayıplara neden olmaktadır. Bu patojene karşı; yapılan kültürel önlemler ve kimyasal mücadele yöntemlerinin yetersiz olduğu bilinmektedir. Bu çalışmada, toplam 1150 potansiyel antagonist bakteri izolatı patojene karşı antibakteriyel aktivitesi için Petri denemelerinde test edilmiştir. Etkili bulunan toplam 48 izolatın bazı biyokimyasal özellikleri, ürettikleri metabolitleri, enzim aktiviteleri belirlenmiş; sıvı besi ortamında geliştirilen bu izolatların saksı denemelerinde hastalığı engelleme oranları tespit edilmiştir. Test edilen tüm izolatların hastalığın gelişimini % 6.5 ile 93.4 oranında engellediği tespit edilmiştir. Hastalığı engelleme oranına göre en yüksek etkiyi *Bacillus megaterium* TV-49A (%93.4) ve *B. cereus* TV-85D (%86.9) bakteri izolatları göstermiştir. Bu iki izolatın ACC,

* Bu çalışma birinci yazarın doktora tez çalışmasından üretilmiştir.

[1] Atatürk Üniversitesi, Ziraat Fakültesi, Aydın, Bitki Koruma Bölümü, Erzurum, Türkiye
**Sorumlu yazar (Corresponding author) e-mail: rkotan@atauni.edu.tr
ORCID ID (Yazar sırasıyla): 0000-0002-1459-3287; 0000-0001-6493-8936

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indol asetik asit, salisilik asit üretimi ve fosfatı çözebilme özelliğinin yüksek olduğu; lipaz, amilaz, proteaz, liziz dekarboksilaz, fitaz ve kitinaz enzim üretimi sonuçlarının pozitif olduğu belirlenmiştir.

Anahtar Kelimeler: Bakteriyel solgunluk ve kanser, biyolojik mücadele, *Clavibacter michiganensis* subsp. *michiganensis*, domates

Giriş

Domates (*Lycopersicon esculentum* Mill.); dünya çapında yetiştiriciliği yapılan Solanacea familyasının Lycopersicon cinsine ait, ılıman iklimlerde tek yıllık, tropikal iklimlerde ise çok yıllık yetişen bir meyvedir (Seniz 1992). Dünyanın en önemli olan tarım ürünlerinin başında domates gelir. Günümüzde insan beslenmesindeki vazgeçilmez ürünlerden olması ve gıda sanayinde çok farklı kullanım alanlarına sahip olması nedeniyle Türkiye için de büyük önem arz etmektedir (Keskin & Gül 2004; Günay 2005).

Domateste pek çok bakteriyel, fungal ve viral hastalık etmenleri çeşitli faktörlere bağlı olarak zaman zaman önemli ürün kayıplarına neden olmaktadır. *Clavibacter michiganensis* subsp. *michiganensis* domates bakteriyel kanser ve solgunluk hastalığın etmenidir (Davis et al. 1984). Hastalığın ilk defa 1909 yılında Amerika Birleşik Devletleri'nin Michigan Eyaleti'nde domates üretim alanlarında görüldüğü ve hızlı bir şekilde diğer eyaletlere yayıldığı belirtilmiştir (Bryan 1930). Türkiye'de ilk kez Bremer et al. (1952) tarafından Ankara ilinde domates üretim alanlarında saptanmıştır. Daha sonra Güney Doğu Anadolu, Marmara ve Ege Bölgesi'nde (Tokgönül 1998), Doğu Akdeniz (Çınar 1980), Batı Akdeniz (Basim et al. 2004) ve Doğu Anadolu (Şahin et al. 2002) Bölgelerinde tespit edilmiştir. Domatesin en yıkıcı ve önemli patojenlerinden birisi olup dünya çapında ciddi ekonomik kayıplara neden olmaktadır (Gleason et al. 1993; Gartemann et al. 2003). Son yıllarda ülkemizde de zaman zaman ciddi verim kayıplarına sebep olmaktadır.

Patojenin yaralar, doğal açıklıklar, stomalar ve hidatodlar yoluyla bitkiye giriş yapar (Carlton et al. 1998). İlk enfeksiyonlar tohum veya hatalıklı bitki artıklarının bulunduğu toprak vasıtasıyla olup; bitkiden bitkiye aşı, böcek aktiviteleri ve budama gibi kültürel uygulamalarla yayılır. Genç fideler patojen tarafından enfekte edildiği zaman hızla solar ve çöker. Enfekteli gövdelerde iletim demetleri kahverengi bir renk alır. Patojen ksilemden yanındaki parankima ve floem hücrelerine yayıldığı için gövdede açık kahverengi sarı çizgiler şeklinde belirti gösterir. Çizgiler gittikçe koyulaşır ve bazen çatlaklar açılır. Eğer patojen açılan yaralardan veya doğal açıklıklardan bitkiye giriş yaparsa ilk olarak yaprak lekeleri ile kendini gösterir. Zamanla bu lekeler nekrozlara dönüşür. Nekrotik alanlar gittikçe genişler, yaprağın ve zamanla bütün gövdenin büzülmesine neden olur. Meyvelerdeki belirtiler ise kuşgözü lekesi olarak adlandırılan merkezi siyah bu siyah lekenin etrafı küçük beyaz hale ile çevrilidir (Çetinkaya & Yıldız 2007).

C. m. subsp. *michiganensis* mücadelesi en çok zor olan bitki patojenlerinden birisidir. Solgunluk hastalığına karşı kullanılan üç bakırlı bileşiğin (bakır hidroksit, bakır oksiklorür ve bakır sulfat), iki antibiotiğin (streptomycin ve kasugamycin) ve bitki aktivatorunün (ASM) önemli derecede patojenin populasyonunu ve gelişimini

etkilediği belirtilmektedir (Milijašević et al. 2009). Ancak antibiyotiklerin pek çok ülkede yasaklanmış olması, kimyasal ilaçların insan sağlığı ve çevre üzerindeki olumsuz etkileri, zamanla kimyasallara karşı patojenlerde oluşan dayanıklılık sorunundan dolayı sürekli bir alternatif ve daha çevreci yöntemlere ihtiyaç duyulmaktadır (O'Brien et al. 2009). Son yıllarda yapılan çalışmalarda bu patojene karşı bakteriyel antagonistlerle biyolojik mücadelenin alternatif bir yöntem olabileceği belirtilmektedir (Umesha 2001; Boudyach et al. 2001).

Bu çalışmada; toplam 1150 bakteri izolatının patojene karşı Petri denemelerinde antibakteriyel etkinlikleri test edilmiştir. Petri denemelerinde etkili bulunan tüm izolatlar viyollerde, viyollerde %70'in üzerinde hastalığın gelişimini engelleyen izolatlar ise saksı denemelerinde domates bakteriyel solgunluk ve kanser hastalığının biyolojik mücadelesinde kullanılabilirliği araştırılmıştır. Ayrıca viyol denemelerinde etkili bulunan antagonist bakteri izolatlarının bazı biyokimyasal özellikleri, ürettikleri metabolitleri ve enzim aktiviteleri belirlenmiştir.

Materyal ve Yöntem

Çalışmada kullanılan patojen ve potansiyel antagonist bakteri izolatları ve bitki çeşidi

Çalışmada patojen bakteri straini olarak daha önce yürütülen bir araştırmada Doğu Anadolu Bölgesi'nde Oltu, Ispir ve Yusufeli ilçelerinde tipik bakteriyel kanser simptomu sergileyen domates bitkilerinin yaprak, gövde ve meyvelerinden izole edilerek Koch postulatlarina göre virulanslıkları test edilen ve virulanslığının yüksek olduğu belirlenen Cmm RK-1248 izolatı kullanılmıştır (Şahin et al. 2002). Potansiyel antagonist bakteri izolatı olarak ise daha önce yapılmış olan farklı araştırmalarda yabani ve kültür bitkilerinin toprak altı veya toprak üstü aksamlarından izole edilen toplam 1150 bakteri izolatı kullanılmıştır (Çizelge 1). Bu izolatların tanısı yağ asidi metil esterlerine göre Microbial Identification Systemde (MIS) yapılmış ve Atatürk Üniversitesi. Ziraat Fakültesi, Bitki Koruma Bölümü Mikroorganizma Kültür Koleksiyonunda muhafaza edilmektedir (Kotan 1998; Kotan 2002; Karagöz & Kotan 2010). Patojen ve potansiyel antagonist bakteri izolatları daha sonraki çalışmalarda kullanılmak üzere %30 gliserol ve Lauryl Broth (LB) içeren stok besiyerinde -86 °C'de muhafaza edilmiştir. Viyol ve saksı denemelerinde Anamas Tohum firmasına ait Early Urban ticari isimli domates çeşidi kullanılmıştır.

Potansiyel antagonist bakterilerin patojene karşı Petride test edilmesi

Stok kültürleri yapılan patojen ve 1150 potansiyel antagonist bakteriler dondurucudan çıkarılarak çözülmesi beklenmiştir. Patojen bakteri Nutrient-Broth Yeast Extract Agar (NBYA), potansiyel antagonist bakteri kültürleri ise Nutrient Broth (NB) besi ortamı içeren Petrilere ekilmiş, 27°C'de inkübasyona bırakılarak 24 saatlik taze kültürleri elde edilmiştir. Gelişen taze bakteri kültürleri steril swap ile alınarak sdH_2O ile süspanse edilmiş ve hücre konsantrasyonu $1x10^8$ hücre/ml'ye ayarlanmıştır. Potansiyel antagonistler NBYA besi ortamı içeren Petrinin (çap 9 cm) tam ortasındaki disk üzerine 10 µL damlatılmıştır ve 27°C'de 24 saat süreyle

inkübasyona bırakılmıştır. $1x10^8$ hücre/mL'ye ayarlanan patojen bakteri süspansiyonu Petri yüzeyine sprey edilmiş, Petriler parafilm ile sarılarak 27°C'de 72 saat süreyle inkübasyona bırakılmıştır (Jung et al. 2014). Bu sürenin sonunda inhibisyon zonu veya hiperparazitik etki oluşturan izolatlar potansiyel antagonist olarak değerlendirilmiştir. Bu işlem her antagonist bakteri için 3 kez tekrar edilmiştir.

Potansiyel antagonist bakterilerin biyokimyasal, metabolit ve enzim testleri

Petri denemelerinde patojene karşı etkili olan toplam 47 bakteri izolatının; Potasyum Hidroksit (Sands 1990), Katalaz ve Oksidaz (Klement et al. 1990), Levan (Lelliot and Stead 1987), Aminosiklopropan Karboksilat Deaminaze Aktivitesi (Jacobson et al. 1994; Shahzad, et al., 2010), Azot Fiksasyonu (Mulder 1950, Brown et al. 1962), Fosfatı Çözebilme (Cisneros et al. 2017) özellikleri bakımından biyokimyasal testleri yapılarak sonuçları kayıt edilmiştir. Metabolit testlerden Hidrojen Siyanür (Nandhini et al. 2012), İndol Asetik Asit (Bent et al. 2001), Salisilik Asit (Meyer et al. 1992), Siderofor (Alexander ve Zuberer 1991) üretimi testi; Enzim aktivitesi testlerinden ise Kitinaz (Senol et al. 2014), Fitaz (Kim et al. 1998), Amilaz (Sung et al. 1993), Proteaz (Atlas 1997), Lipaz (Omidvari 2008) enzimi aktiviteleri belirlenerek sonuçları kaydedilmiştir. Biyokimyasal, metabolit ve enzim testleri ile ilgili geniş bilgiler Mohammedi (2018)'de verilmiştir.

Potansiyel antagonistlerin viyol denemeleri

Petri denemelerinde patojene karşı etkili olduğu tespit edilen toplam 47 bakteri izolatı viyol denemelerinde kullanılmıştır. Domates tohumları %2.5 Sodyum Hipoklorit solüsyonunda 3 dakika bekletilmiş ardından steril saf su ile 3 kez durulanarak dezenfekte edilmiştir (Mew & Rosales 1986). King B besi yerinde 25±1°C'de 24-48 saat geliştirilmiş antagonist bakteri izolatlarının konsantrasyonu steril su ile spektrofotometrede 600 nm dalga boyunda 0.3 absorbans değerine ayarlanmıştır. Hazırlanan süspansiyonun içerisine domates tohumları 20 dakika daldırılarak vakumla izolatların tohum yüzeyine kolonize olmaları sağlanmıştır. Uygulamadan bir hafta sonra patojenin 24-48 saatlik taze kültürlerinden 10^8 hücre/mL konsantrasyonunda hazırlanan süspansiyonu ile tohumlar inokule edilmiştir. Sadece patojen ile enfekte edilen tohumlar pozitif kontrol, sadece suyla muamele görmüş tohumlar negatif kontrol olarak kullanılmıştır. Uygulama görmüş tohumlar torf içeren viyollere ekilmiştir (Krishnamurthy & Gnanamanickam 1998). Gerçek yapraklar çıkıncaya kadar viyoller nem çemberi içerisinde tutulmuş ve patojen uygulanan bitkilerde hastalık simptomları gözleninceye kadar bitkiler günlük olarak incelenmiştir. 0-5 skalasına göre (0: Bitkilerde hiçbir solgunluk belirtisi yok; 1: Bitkilerde yaprakların %1-10'de solgunluk mevcut; 2: Bitkilerde yaprakların %11-25'de solgunluk mevcut; 3: Bitkilerde yaprakların %26-49'de solgunluk ve kloroz mevcut; 4: Bitkilerde yaprakların %50-74'de solgunluk mevcut; 5: Bitkiler tamamen solmuş) hastalık şiddeti ve uygulamaların hastalık gelişimi üzerine etkinliği araştırılmıştır (Soylu et al. 2003). Deneme üç tekerrürlü olacak şekilde yapılmıştır.

$$\text{Uygulamaların etkinliği (\%)} = \frac{\text{Kontrol - Uygulama}}{\text{Kontrol}} \times 100$$

Potansiyel antagonistlerin serada saksı denemeleri

Viyol çalışmalarında hastalığın gelişiminde %70'in üzerinde etkili olan toplam 7 antagonist bakteri izolatı, daha uzun bir vejetasyon periyodunda hastalığın gelişimi üzerine etkisinin tespit edilmesi için saksı çalışmalarında test edilmiştir. Tohumlar viyol denemelerinde olduğu gibi dezenfekte edilmiştir. Tohumlar 12 saat boyunca hava ile kurutulduktan sonra kum ve torf (1:2 hacim) içeren viyollere ekilmiştir. Viyoller bitki büyütme kabinine alınarak aydınlatma süresi 12 saat; sıcaklık: 10°C gece ve 28°C gündüz, nem %80 ve her 2 günde bir sulanmıştır.

Patojen inokulum süspansiyonu 25 ml Tryptic Soy Broth (TSB) içeren steril tüplerde 27°C'de 200 rpm'de çalkalayıcılı etüvde 24 saat inkübasiyondan sonra elde edilmiştir. Tüpler 3500 rpm de 5 dakika santrifüj yapılmıştır, steril distile su ile iki kez yıkanmıştır. Konsantrasyon steril su ile 10^8 hücre/mL'ye (OD_{640}=0.12) ayarlanmıştır (Soylu et al. 2003). Hazırlanan bu süspansiyon steril olmayan kum ve torf (1:2 hacim) karışımına ilave edilmiştir. Patojen inokule edilen bu karışım 4 litrelik plastik saksılara dağıtılmış ve üzeri 5 cm kalınlığında patojen inokule edilmemiş kum ve torf (1:2 hacim) karışımı ile kaplanmıştır.

Viyollere geliştirilen üç yapraklı dönemdeki domates fideleri yavaşça çıkartılmıştır. Fidelerin kökleri viyol denemelerinde olduğu gibi 10^8 hücre/mL'ye konsantrasyonda hazırlanan ve yapıştırıcı olarak % 0.5 ksantan kullanılan bioajan süspansiyonuna 3 dakika boyunca daldırılmıştır (Boudyach et al. 2001). Sadece patojen ile enfekte edilen fideler pozitif kontrol, sadece suyla muamele görmüş fideler negatif kontrol olarak kullanılmıştır. Deneme 10 tekerrürlü ve 4 kez tekrar edilmiştir. Serada, çevre etkilerini en aza indirmek için saksılar tamamen tesadüfi olarak dağıtılmıştır. Bitkiler her 2 günde bir sulanmıştır. Hastalığın gözlenmesı 12 hafta süre ile onar gün aralıklarla yapılmış ve ortalaması alınmıştır. Hastalık şiddeti ve uygulamaların hastalık gelişimi üzerine etkinliği viyol denemelerinde kullanılan 0-5 skalasına göre yapılmıştır.

Potansiyel antagonistlerin bitki gelişimi üzerine etkileri

Bitkilerin kök boğazından en üstteki dalın ucuna kadar olan yükseklik bitki boyu (cm), kök boğazından 5 cm yukarısındaki kalınlık gövde çapı (cm), her bir bitkiye ait çiçekler sayılarak çiçek sayısı (adet/bitki), bitkilerin çiçek tomurcuğu oluşturduğu dönemde sağlam yapraklardan taşınabilir klorofil metre (SPAD-502, Konica Minolta Sensing. Inc., Japon) yardımıyla klorofil içeriği indeksi, her bir bitkiye ait dal sayılarak dal sayısı (adet/bitki), ilk-orta ve son dönemde her bir bitkiye ait meyveler sayılarak meyve sayısı (adet/bitki), meyveler yetişme mevsiminde tartılarak meyve ağırlığı (gr/meyve), yetişme mevsiminin sonunda bitkiler kök boğazından kesilerek yaş ağırlıkları (gr/bitki), kağıt torbalar içinde etüvde 65-70°C'de kurutularak kuru ağırlığı (gr/bitki), uygulamaların yapıldığı günden fideler ilk çiçek açmasına kadar geçen sure çiçeklenme süresi (gün) olarak değerlendirilmiştir. Bütün veriler ortalama olarak değerlendirilmiştir.

Sonuçların analizi

Çalışmada elde edilen sonuçlar SPSS (Statistical Package for Social Sciences, Version 9.0) istatistik programında analiz edilerek, aritmetik ortalamaları ve standart sapmaları hesaplanmıştır. Uygulamalar arasındaki farklılığın önem derecesini belirlemek için Duncan ($p \leq 0,01$) testi yapılmıştır.

Bulgular ve Tartışma

Petri denemeleri test sonuçları

Çalışmada kullanılan toplam 1150 potansiyel antagonist bakteri cinsleri, toplam izolat sayıları ve Petride patojene karşı bakteriyostatik veya bakteriyosidal etki gösteren toplam izolat sayıları Çizelge 1'de verilmiştir. Petri denemelerinde test edilen toplam 1150 bakteri izolatı MIS tanı sonucuna göre 78 farklı cinsten oluşmuştur. Test edilen bu bakteri izolatlarının 301 (%26,17) adeti Bacillus, 126 (%10,95) adeti Pseudomonas *v*e 54 (%4,69) adeti Panteoa cinsine ait izolatlardan oluşmuştur. Toplam 1150 izolatın 48 adeti bakteriyostatik veya bakteriyosidal etki göstermiştir. Antibakteriyel etki gösteren cinsler arasında 301 adet Bacillus cinsi olmuş ve etkili izolat sayısı 27 adet olarak belirlenmiştir. Test edilen Bacillus izolatlarının % 8.97'si antibakteriyel etki göstermiştir.

Potansiyel antagonist bakterilerin biyokimyasal, metabolit ve enzim testleri

Petri çalışmalarında antibakteriyel etki gösteren bakteri izolatlarının bazı biyokimyasal test ve metabolit üretimi sonuçları Çizelge 2'de verilmiştir. Bu sonuçlara göre izolatların tamamı katalaz, 10'u KOH, 7'si siderofor, 4'ü oksidaz ve birer izolatında levan ve HCN pozitif olduğu belirlenmiştir. Yine toplam 45 izolatın 44'ünün azot fikse ettiği, 41'inin farklı düzeylerde fosfatı çözebildiği, 7'sinin ise yüksek düzeyde ACC aktivitesi gösterdiği görülmüştür. İzolatların indol asetik asit üretimi değerlerinin 0.092 ile 0.959 µg/ml, salisilik asit üretimi değerlerinin ise 0.026 ile 0.181 µg/ml arasında değiştiği görülmüştür. En yüksek indol asetik asit üretimi *B. ceraus* TV-85D (0.959 µg/ml), *B. megaterium* TV-49A (0.833 µg/ml) ve *B. atrophaeus* TV-15B (0.622 µg/ml) izolatından elde edilmiştir. En yüksek salisilik asit üretimi değerleri ise *P. chlororaphis* PM-18 **(**0,189 µg/ml**),** *B. ceraus* TV-85D (0.181 µg/ml) ve *B. megaterium* TV-49A (0.177 µg/ml) izolatlarından elde edilmiştir.

Potansiyel antagonist bakterilerin viyol ve saksı deneme sonuçları

Potansiyel antagonist bakterilerin enzim aktivitesi test sonuçları, viyol ve saksı denemelerinde domates bakteriyel solgunluk hastalığı skala değerleri ve engelleme oranları ise Çizelge 3'de verilmiştir. Toplam 14 izolatın lizi dekarboksilaz, 13 izolatın lipaz, 7 izolatın proteaz ve 6 izolatın ise amilaz enzim üretim değerleri kuvvetli pozitif olarak belirlenmiştir. İzolatların fitaz enzim üretimi değerleri 0.090 ile 0.339 ve kitinaz enzim üretimi değerleri ise 0.060 ve 0.342 µg/ml arasında

değişmiştir. En yüksek fitaz ve kitinaz enzim üretimi sırası ile tanılanamayan RK-306 ile *P. alvei* DKG izolatlarından elde edilmiştir.

Viyol denemelerinde test edilen bakteri izolatlarının tümü hastalığı az ya da çok engellemiştir. Engelleme oranının %6.5 ile %93.4 arasında değiştiği görülmüştür. Hastalık gelişimini %70'in üzerinde engelleyen izolatlar ve engelleme oranları sırası ile *K. rosea* TV-14C %71.7, *B.lentimorbus* RK-340 %71.7, *B. atrophaeus* TV-15B %78.2, *B. megaterium* TV-13C %78.2, *P. chlororaphis* PM-18 %78.2, *B. cereus* TV-85D %86.9 ve *B.megaterium* TV-49A %93.4 olmuştur. Bu izolatlar saksı denemelerinde kullanılmıştır. Saksı denemelerinde de tüm izolatların hastalık gelişimini %68.0 ile %95.7 arasında engellediği tespit edilmiştir. Antagonist bakterilerin hastalığ engelleme oranları sırası ile *K. rosea* TV-14C % 68.0, *B.lentimorbus* RK-340 % 74.4, *B. megaterium* TV-13C % 78.7, *P. chlororaphis* PM-18 % 78.7, *B. atrophaeus* TV-15B % 85.1, *B. cereus* TV-85D % 89.3 ve *B.megaterium* TV-49A % 95.7 olmuştur.

Domates solgunluk ve kanser hastalığı, tohum ve toprak kökenli önemli bitki bakteri hastalıklarından birisidir. Bitki bakteri hastalıkları ile mücadelede bronopol, thiram, sodyum hipoklorit, hidroklorik asit ve bakır asetat gibi çeşitli kimyasallar, dayanıklılığın teşvik edilmesi, bitkisel esaslı ekstrakt ve uçucu yağlar, çeşitli antibiyotikler ve sıcak su gibi fiziksel uygulamalar tohumdaki inokulumu yok etmek veya azaltmak için kullanılan çok farklı yöntemlerdir (Fatmi et al. 1991; Özaktan, 1991; Soylu et al. 2003; Kotan et al. 2013; Horuz et al. 2019). Yine tohum kökenli inokulumu yok edebilecek veya azaltabilecek sodyum hipoklorit, üzüm sirkesi, elma sirkesi, sıcak su ve laktik asit gibi uygulamaların tohumdaki bakteri yoğunluğunu ve bulaşık tohum sayısını azaltığı belirtilmiştir (Horuz et al. 2019). Ancak PGPR (Planth Growth Promoting Rhizobacteria) olarak adlandırılan bitki büyümesini teşvik eden kökbakterileri hem bitkilerde hastalık oluşturan pek çok bakteriyel, fungal ve viral etmene karşı bitkide bulunan doğal dayanıklılığı teşvik etmekte, hem de bitki büyümesini artırıcı özelliklerinden dolayı son yıllarda çok tercih edilmektedir (Weller 1988; Wei et al. 1996).

Yapılan bir çalışmada; toplan 499 adet aday PGPR bakteri izolatı arasından en fazla fosforu çözme, azotu bağlama özelliklerine sahip 30 izolat seçilmiş, bu izolatlar arasında en etkili olan sekiz izolatın bakteriyel solgunluk hastalığını baskılayabilme potansiyelleri *in vivo* saksı çalışmaları ile araştırılmıştır. Saksı çalışmalarında iki bakteri izolatının domates bitkilerinde hastalık şiddetini ortalama % 38 ve % 54 oranında azalttığı, tarla çalışmalarında ise bu iki izolatın birlikte kullanıldığı uygulamada hastalık şiddetinde ortalama % 43 azalma gözlenmiştir. Bakteri uygulamalarının bazı bitki büyüme parametrelerinde de önemli artışlara sebep olduğu görülmüştür (Çetinkaya Yıldız & Aysan 2014). Bu çalışmada da kullanılan bakterilerin hem hastalığı engelleme hem de bitki gelişim parametreleri üzerine etkinliği açısından test edilen bakterilerden özellikle *B. cereus* TV-85D ve *B.megaterium* TV-49A izolatlarının hastalığı engellemede çok başarılı olduğu ve bitki gelişim parametrelerinde de önemli artışlara sebep olduğu görülmüştür.

Hastalığın baskılanmasında indol asetik asit ve salisilik asit üretiminin önemli olduğu düşünülmektedir. Çünkü etkili bulunan *B. ceraus* TV-85D izolatı 0.959 µg/ml ile en yüksek *B. megaterium* TV-49A izolatı ise 0.833 µg/mL ile ikinci en yüksek indol asetik asit üretimine sahiptir. *B. ceraus* TV-85D ve *B. megaterium*

TV-49A izolatlarının salisilik asit üretimi değerleri sırası ile 0.181 µg/mL ve 0.177 µg/mL ile en yüksek ikinci ve üçüncü izolat olarak görülmektedir.

Azot fiksasyonu yapan bakteriler ile yapılan çalışmalar, bu bakterilerin patojenlere karşı dayanıklılık sağladığını, büyüme süresinin kısalttığını, bitki gelişimini teşvik ettiğini, çiçeklenme, meyve iriliğini, verim ve/veya kaliteyi ve meyve tutumunu arttırdığını göstermiştir (Bashan & de-Bashan 2002; Shridhar 2012). Son yıllarda yapılan çalışmalarda bitkilerde kullanılan bazı biyolojik mücadele etmenlerinin içsel hormon konsantrasyonunu arttırarak bitki gelişimi teşvik ettikleri ortaya konmuştur (Patten & Glick 1996). IAA hormonu bitkilerde en önemli oksinlerden birisi olarak bilinir (Ali et al. 2009). Bu maddenin bitkilerde çeliklerin kök oluşumunu teşvik ettiği, meyve tutumunu artırdığı, adventif kök oluşumunu teşvik ettiği, meyve ve yaprak dökümünü engellediği, tomurcukların daha erken çiçek açmasını sağladığı bildirilmektedir (Çetin 2002; Greene 2006; Baktır 2010).

Bu çalışmada TV-49A ve TV- 85D salisilik asidi (SA) en yüksek oranda üretmişler. Salisilik asidin çiçek tomurcuğu oluşumunu ve çiçeklenmeyi teşvik ettiği (Lee & Skoog 1965; Eberhart et al. 1989), bitkilerde sistemik dayanıklılık mekanizmasında önemli rol oynadığı (Lawton 1994; Maurhofer et al. 1994; Van Loon et al. 1998; Ramamorthy et al. 2001). Günümüzde ise artık pratikte, bitkilerde SA büyüme düzenleyicisi olarak kullanılmaya başlanmıştır (Özeker 2005). Özellikle Bacillus ve Pseudomonas cinsi bakterilerin sistemik dayanıklılık mekanizmalarında önemli rol oynadıkları bildirilmektedir (Brain et al. 2004; Harman 2004; Haas & Defago 2005; Shanmugam & Narayanasamy 2008). Çalışmalar daha çok *B. cereus*, *B. amyloliquefaciens*, *B. mycoides*, *B. pumilus*, *B. subtilis* and *B. sphaericus* türlerinde yoğunlaşmıştır (Kloepper et al. 2004).

Sonuç olarak Petri, viyol ve saksı denemelerinde domates bakteriyel solgunluk ve kanser hastalığını engellemede başarılı bulunan *B. cereus* TV-85D ve *B.megaterium* TV-49A izolatlarının aynı zamanda bitki gelişim parametrelerinde de önemli artışlara sebep olduğu görülmüştür. Bunun üzerine bu bakteri izolatları ile ilgili çalışmalara devam edilmiştir. Bu antagonist izolatlar için sıvı taşıyıcı geliştirilmiş, sera ve tarla şartlarında hastalığın gelişimi üzerine etkisi araştırılmış, izolatların gen dizilimleri çıkarılarak gen bankasına kaydedilmiştir. Çalışmanın kapsamlı oluşundan sonuçlar tek bir makalede verilememiş ve daha sonra bir başka çalışmada paylaşılacaktır.

Şekil 1. 1: Petride *in vitro* testi (1a: antagonist ve patojen bakteri, 1b: Patojen bakteri); 2: Viyol denemeleri (2a: TV-85D+Patojen, 2b: TV-49A+Patojen, 2c: Patojen); Saksı denemeleri (2a: TV-85D+Patojen, 2b: TV-49A+Patojen, 2c: Patojen)

Figure 1. *In vitro* test in Petri dish (1a: Bioagent and pathogenic bacteria, 1b: Pathogenic bacteria); 2: Viol trials (2a: TV-85D+Pathogen, 2b: TV-49A+Pathogen, 2c: Pathogen); Pot trials (2a: TV-85D+Pathogen, 2b: TV-49A+Pathogen, 2c: Pathogen)

Çizelge 1. Çalışmada kullanılan potansiyel antagonist bakteri cinsleri, toplam izolat sayıları (TİS) ve toplam etkili izolat sayıları (EİS)

Table 1. Tested potential bacterial genera used in the study, the number of tested isolates (TIS) and the number of effective isolates (EIS)

Sıra	Bakteri cinsleri	TİS	EİS	Sıra	Bakteri cinsleri	TİS	EİS	Sıra	Bakteri cinsleri	TİS	EİS
1	*Bacillus* sp.	301	27	**18**	*Leclercia* sp.	13	0	**35**	*Chromobacterium sp.*	4	0
2	*Pseudomonas* sp.	126	3	**19**	*Vibrio* sp.	13	0	**36**	*Flavobacterium sp.*	4	0
3	*Stenetrophomonas* sp.	71	0	**20**	*Yersinia* sp.	15	1	**37**	*Actinomadura sp.*	3	0
4	*Panteoa* sp.	54	2	**21**	*Agrobacterium* sp.	10	0	**38**	*Cellulomonas sp.*	3	0
5	*Arthrobacter* sp.	34	0	**22**	*Kelpsiella* sp.	10	0	**39**	*Citrobacter sp.*	3	0
6	*Enterobacter* sp.	34	0	**23**	*Staphilococcus* sp.	15	0	**40**	*Hafnia sp.*	3	0
7	*Micrococcus* sp.	34	0	**24**	*Pseudoalteromonas* sp.	10	0	**41**	*Janthinobacterium sp.*	3	1
8	*Serratia* sp.	30	0	**25**	*Sphingobacterium* sp.	11	0	**42**	*Neisseria sp.*	3	0
9	*Paenibacillus* sp.	39	1	**26**	*Brevibacterium* sp.	8	2	**43**	*Photobacterium sp.*	3	0
10	*Alcaligenes* sp.	25	0	**27**	*Micobacterium* sp.	7	1	**44**	*Weeksella sp.*	3	0
11	*Salmonella* sp.	27	0	**28**	*Photorhabdus* sp.	7	0	**45**	*Curtobacterium sp.*	2	0
12	*Brevibacillus* sp.	27	1	**29**	*Achromobacter* sp.	8	0	**46**	*Deinococcus sp.*	2	0
13	*Chryseobacterium* sp.	28	1	**30**	*Myroides sp.*	7	0	**47**	*Kluyvera sp.*	2	0
14	*Kocuria* sp.	23	2	**31**	*Aeromonas sp.*	11	2	**48**	*Lysobacter sp.*	2	0
15	*Burkholderia* sp.	19	1	**32**	*Kurthia sp.*	7	0	**49**	*Proteus sp.*	2	0
16	*Acinetobacter* sp.	24	0	**33**	*Bergeyella sp.*	4	0	**50**	*Psychrobacter sp.*	2	0
17	*Erwinia* sp.	18	0	**34**	*Brevundimonas sp.*	5	0	**51**	*Ralstonia sp.*	2	0

Çizelge 1'in devamı

Table 1 continued

Sıra	Bakteri cinsleri	TİS	EİS	Sıra	Bakteri cinsleri	TİS	EİS	Sıra	Bakteri cinsleri	TİS	EİS
52	*Shewanella* sp.	2	0	**62**	*Dunganella sp.*	1	0	**72**	*Rahnella sp.*	1	0
53	*Streptoverticillium* sp.	2	0	**63**	*Edwardsiella sp.*	1	0	**73**	*Rhodococcus sp.*	1	0
54	*Acetobacter* sp.	1	0	**64**	*Escherichia sp.*	1	0	**74**	*Rothia sp.*	1	0
55	*Acidovorax* sp.	1	0	**65**	*Geobacillus sp.*	1	0	**75**	*Shigella sp.*	1	0
56	*Aerococcus* sp.	1	0	**66**	*Hydrogenophaga sp.*	1	0	**76**	*Sphingomonas sp.*	1	0
57	*Bordetella sp.*	1	0	**67**	*Kytococcus sp.*	1	0	**77**	*Variovorax sp.*	1	0
58	*Cellulosimicrobium* sp.	1	0	**68**	*Methylobacterium sp.*	1	0	**78**	*Zobellia sp.*	1	0
59	*Colwellia* sp.	1	0	**69**	*Pediococcus sp.*	1	0	**79**	*Tanılanamayan izolatlar*	3	3
60	*Delftia* sp.	1	0	**70**	*Providencia sp.*	1	0				
61	*Dermabacter* sp.	1	0	**71**	*Pseudoxanthomonas sp.*	1	0				

Çizelge 2. Antagonist bakterilerin biyokimyasal ve metabolit aktivite test sonuçları

Table 2. Biochemical and metabolic activity test results for bioagent bacteria

Strain	**Bakteri türleri**	**KOH[1]**	**KAT[1]**	**OKS[1]**	**LEV[1]**	**ACC[1]**	**AZO[1]**	**FOS[3]**	**HCN[1]**	**IAA**	**SİD[1]**	**SA[4]**
TV-99E	*Bacillus megaterium*	-	+	-	-	H	+	4	-	0,132	-	0,065
TV-96D	*Bacillus cereus*	-	+	-	-	L	+	2	-	0,152	-	0,032
TV-92C	*Aeromonas salmonicida*	+	+	-	-	L	+	0	-	0,138	-	0,046
TV-91D	*Bacillus megaterium*	-	+	-	-	L	+	4	-	0,118	-	0,067
TV-8C	*Bacillus pumilus*	-	+	-	-	L	+	1	-	0,139	-	0,066
TV-87D	*Bacillus laevolacticus*	-	+	-	-	L	+	1	-	0,257	+	0,047
TV-85D	*Bacillus cereus*	-	+	-	-	H	+	4	-	0,959	+	0,181
TV-6G	*Micobacterium flavescens*	-	+	-	-	L	+	1	-	0,318	-	0,065
TV-67C	*Bacillus pumilus*	-	+	-	-	L	+	1	-	0,279	-	0,028
TV-49A	*Bacillus megaterium*	-	+	-	-	H	+	4	-	0,833	+	0,177
TV-47B	*Bacillus atrophaeus*	-	+	-	-	L	+	1	-	0,239	-	0,031
TV-45E	*Bacillus atrophaeus*	-	+	-	-	L	+	1	-	0,374	-	0,101
TV-3C	*Bacillus pumilus*	-	+	-	-	L	+	3	-	0,134	-	0,061
TV-36D	*Aeromonas salmonicida*	+	+	-	-	M	+	0	-	0,146	-	0,034
TV-1D	*Bacillus megaterium*	-	+	-	-	L	+	1	-	0,175	+	0,059
TV-17C	*Bacillus subtilis*	-	+	-	-	L	+	1	-	0,357	-	0,073
TV-15B	*Bacillus atrophaeus*	-	+	-	-	H	+	2	-	0,622	-	0,170
TV-14C	*Kocuria rosea*	-	+	-	-	M	+	3	-	0,520	-	0,161
TV-13C	*Bacillus megaterium*	-	+	-	-	M	+	2	-	0,527	-	0,159
TV-131D	*Pantoea agglomerans*	+	+	+	-	L	+	4	-	0,093	-	0,063
TV-130B	*Bacillus subtilis*	-	+	-	-	H	+	2	-	0,108	-	0,096
TV-124C	*Chryseobacterium balustinum*	+	+	-	-	M	+	4	-	0,201	-	0,065
TV-10F	*Bacillus laevolacticus*	-	+	-	-	L	+	1	-	0,417	+	0,041
RK-447	*Bacillus subtilis*	-	+	+	-	L	+	2	-	0,169	-	0,038
RK-435	*Bacillus megaterium*	-	+	-	-	M	+	6	-	0,092	-	0,051

Çizelge 2'nin devamı

Table 2 continued

Strain	Bakteri türleri	KOH[1]	KAT[1]	OKS[1]	LEV[1]	ACC[1]	AZO[1]	FOS[3]	HCN[1]	IAA	SİD[1]	SA[4]
RK-413	*Pseudomonas putida*	+	+	-	-	M	+	4	-	0,110	-	0,078
RK-387	*Pseudomonas putida*	+	+	-	-	M	+	1	-	0,112	-	0,056
RK-347	*Janithobacterium lividum*	+	+	-	-	L	+	1	-	0,297	-	0,033
RK-340	*Bacillus lentimorbus*	-	+	-	-	M	+	1	-	0,302	-	0,154
RK-339	*Bacillus subtilis*	-	+	-	-	L	+	1	-	0,353	-	0,044
RK-324	*Brevibacillus brevis*	-	+	+	-	M	+	0	-	0,242	-	0,026
RK-32	*Pantoea agglomerans*	+	+	-	-	M	+	2	-	0,506	-	0,067
RK-308	*Burkholderia pyrrocinia*	+	+	-	-	L	+	3	-	0,172	-	0,052
RK-1253	*Bacillus atrophaeus*	-	+	+	-	L	+	1	-	0,321	-	0,079
PM-34	*Yersinia aldovae*	+	+	-	-	L	-	0	-	0,121	-	0,051
PM-25	*Bacillus cereus*	-	+	-	-	L	+	2	-	0,092	-	0,064
PM-24	*Bacillus cereus*	-	+	-	-	H	+	2	-	0,139	-	0,087
PM-23	*Bacillus megaterium*	-	+	-	-	L	+	2	-	0,100	-	0,103
PM-20	*Brevibacterium epidermis*	-	+	-	-	L	+	3	-	0,296	-	0,062
PM-19	*Kocuria rosea*	-	+	-	-	M	+	2	-	0,216	-	0,029
PM-18	*Pseudomonas chlororaphis*	+	+	-	+	H	+	5	+	0,726	+	0,189
PM-17	*Brevibacterium epidermis*	-	+	-	-	M	+	1	-	0,321	-	0,039
PM-16	*Bacillus subtilis*	-	+	-	-	L	+	2	-	0,275	-	0,088
PM-15	*Bacillus subtilis*	-	+	-	-	L	+	1	-	0,210	+	0,055
DKG	*Paenibacillus alvei*	-	+	-	-	L	+	1	-	0,297	-	0,101
RK-394	Tanılanamadı	-	+	-	-	M	+	1	-	0,138	-	0,055
RK-307	Tanılanamadı	-	+	-	-	L	+	2	-	0,124	-	0,039
RK-306	Tanılanamadı	-	+	-	-	L	+	1	-	0,168	-	0,075

KOH: Potasyum hidroksil, **KAT:** Katalaz, **OKS:** Oksidaz, **LEV:** Levan, **ACC:** 1-aminosiklopropan-1-karboksilik asit, **N:** Azot fiksasyonu aktivitesi, **P:** Fosfatı çözebilme, **HCN:** Hidrojen siyanür üretebilme, **IAA:** İndol asetik asit ((µg/ml)), **SİD:** Siderofor, **SA:** Salisilik asit, **LİP:** Lipaz, **PRO:** Proteaz, **AMİ:** Amilaz, **LİZ:** Lizi dekarboksilaz, **FİT:** Fitaz, **KİT:** Kitinaz, **HS:** Hastalık skala değeri, **YE:** Yüzde etki, **[1]:** +: Pozitif, -: Negatif; **[2]:** OD>0.15 Grup-H, OD 0.15-0.10 Grup-M ve OD <0.10) Grup-L; **[3]:** 0: Negatif , 1: Zayıf pozitif, 2: Pozitif , 3: Kuvvetli pozitif; +: Pozitif, -: Negatif **[4]:** OD527, +: Pozitif, -: Negatif

Çizelge 3. Antagonist bakterilerin enzim aktivitesi test sonuçları, viyol ve saksı çalışmasında domates bakteriyel solgunluk hastalık şiddeti ve yüzde engelleme oranları

Table 3. Enzyme activity test results for bioagent bacteria, tomato bacterial wilt disease disease severity and percent prevention rate in viol and pot studies

Strain	Bakteri türleri	LİP[1]	AMİ[2]	PRO[1]	LİZ[2]	FİT[3]	KİT[4]	VHS*	VYE*	SHS*	SYE*
PM-20	*Brevibacterium epidermis*	2	1	3	4	0,156	0,080	4.3 ab	6.5	-	-
TV-91D	*Bacillus megaterium*	3	4	0	5	0,097	0,122	4.0 a-c	13.0	-	-
TV-6G	*Micobacterium flavescens*	2	1	2	6	0,159	0,219	4.0 a-c	13.0	-	-
RK-435	*Bacillus megaterium*	1	6	1	2	0,230	0,181	4.0 a-c	13.0	-	-
RK-32	*Pantoea agglomerans*	0	1	0	4	0,186	0,184	4.0 a-c	13.0	-	-
RK-308	*Burkholderia pyrrocinia*	0	1	2	1	0,108	0,139	4.0 a-c	13.0	-	-
PM-19	*Kocuria rosea*	1	1	3	3	0,190	0,142	4.0 a-c	13.0	-	-
TV-96D	*Bacillus cereus*	3	2	1	0	0,142	0,159	3.6 a-c	21.7	-	-
TV-45E	*Bacillus atrophaeus*	2	3	1	3	0,187	0,164	3.6 a-c	21.7	-	-
TV-1D	*Bacillus megaterium*	0	0	2	1	0,188	0,268	3.6 a-c	21.7	-	-
RK-387	*Pseudomonas putida*	1	3	1	1	0,103	0,174	3.6 a-c	21.7	-	-
RK-347	*Janithobacterium lividum*	2	6	1	3	0,205	0,159	3.6 a-c	21.7	-	-
RK-324	*Brevibacillus brevis*	1	2	2	0	0,349	0,195	3.6 a-c	21.7	-	-
PM-25	*Bacillus cereus*	3	6	2	1	0,196	0,133	3.6 a-c	21.7	-	-
PM-17	*Brevibacterium epidermis*	1	0	3	4	0,172	0,196	3.6 a-c	21.7	-	-
PM-15	*Bacillus subtilis*	3	1	3	1	0,148	0,125	3.6 a-c	21.7	-	-
RK-306	Tanılanamadı	0	0	2	2	0,339	0,182	3.6 a-c	21.7	-	-
TV-67C	*Bacillus pumilus*	1	0	1	4	0,131	0,113	3.3 a-c	28.2	-	-
TV-36D	*Aeromonas salmonicida*	3	1	0	6	0,185	0,146	3.3 a-c	28.2	-	-
TV-17C	*Bacillus subtilis*	1	3	3	6	0,134	0,116	3.3 a-c	28.2	-	-
PM-34	*Yersinia aldovae*	3	2	0	6	0,142	0,189	3.3 a-c	28.2	-	-
RK-394	Tanılanamadı	1	2	2	3	0,179	0,144	3.3 a-c	28.2	-	-
TV-87D	*Bacillus laevolacticus*	2	2	1	4	0,167	0,151	3.0 a-d	34.7	-	-
TV-47B	*Bacillus atrophaeus*	2	3	3	4	0,169	0,133	3.0 a-d	34.7	-	-
TV-124C	*Chryseobacterium balustinum*	2	6	3	5	0,179	0,074	3.0 a-d	34.7	-	-
TV-10F	*Bacillus laevolacticus*	2	4	2	6	0,148	0,180	3.0 a-d	34.7	-	-

Çizelge 3'ün devamı

Table 3 continued

Strain	Bakteri türleri	LİP[1]	AMİ[2]	PRO[1]	LİZ[2]	FİT[3]	KİT[4]	VHS*	VYE*	SHS*	SYE*
RK-413	*Pseudomonas putida*	2	4	0	5	0,132	0,163	3.0 a-d	34.7	-	-
PM-24	*Bacillus cereus*	3	0	1	1	0,185	0,146	3.0 a-d	34.7	-	-
TV-99E	*Bacillus megaterium*	3	1	2	2	0,119	0,133	2.6 b-e	43.3	-	-
TV-92C	*Aeromonas salmonicida*	3	1	0	5	0,157	0,167	2.6 b-e	43.3	-	-
TV-130B	*Bacillus subtilis*	1	2	2	2	0,284	0,200	2.6 b-e	43.3	-	-
RK-447	*Bacillus subtilis*	3	3	2	2	0,159	0,127	2.6 b-e	43.3	-	-
RK-1253	*Bacillus atrophaeus*	1	0	1	6	0,158	0,178	2.6 b-e	43.3	-	-
TV-131D	*Pantoea agglomerans*	2	2	0	3	0,126	0,171	2.6 b-e	43.4	-	-
PM-23	*Bacillus megaterium*	3	1	0	3	0,090	0,170	2.6 b-e	43.4	-	-
PM-16	*Bacillus subtilis*	3	1	2	3	0,156	0,142	2.6 b-e	43.4	-	-
TV-8C	*Bacillus pumilus*	1	4	0	4	0,152	0,165	2.3 c-f	50.0	-	-
TV-3C	*Bacillus pumilus*	1	1	2	2	0,202	0,186	2.3 c-f	50.0	-	-
RK-339	*Bacillus subtilis*	2	1	1	5	0,274	0,060	2.3 c-f	50.0	-	-
DKG	*Paenibacillus alvei*	2	0	1	5	0,164	0,342	2.3 c-f	50.0	-	-
RK-307	Tanılanamadı	0	0	1	3	0,173	0,194	2.3 c-f	50.0	-	-
TV-14C	*Kocuria rosea*	1	5	2	5	0,186	0,198	1.3 d-g	71.7	1.5 b	68.0
RK-340	*Bacillus lentimorbus*	2	1	1	0	0,248	0,173	1.3 d-g	71.7	1.2 bc	74.4
TV-15B	*Bacillus atrophaeus*	2	1	1	5	0,160	0,175	1.0 e-g	78.2	0.7 bc	85.1
TV-13C	*Bacillus megaterium*	2	4	2	4	0,125	0,144	1.0 e-g	78.2	1.0 bc	78.7
PM-18	*Pseudomonas chlororaphis*	3	6	3	4	0,189	0,186	1.0 e-g	78.2	1.0 bc	78.7
TV-85D	*Bacillus cereus*	3	1	1	4	0,163	0,120	0.6 fg	86.9	0.5 b-d	89.3
TV-49A	*Bacillus megaterium*	1	2	2	2	0,080	0,317	0.3 g	93.4	0.2 bc	95.7
Pozitif Kontrol		-	-	-	-			4.6 a	0.0	4.7 a	0.0
Negatif Kontrol		-	-	-	-			0.0 g	100.0	0.0 c	100.0

[1]: Lip (lipaz) ve Pro (proteaz) skala değerleri 0: Negatif , 1: Zayıf pozitif, 2: Pozitif , 3: Kuvvetli pozitif ; **[2]:** Amilaz ve Lizi dekarboksilaz skala değerleri 0: Negatif , 1 ve 2: Zayıf pozitif, 3 ve 4: Pozitif, 5 ve 6: Kuvvetli pozitif;**[3]:** Fitaz OD_{700}; **[4]:** Kitinaz OD_{540}; -: Test edilmedi

*Aynı sütun içerisinde aynı harfler ile gösterilen ortalama değerler arasındaki farkın önemli olmadığını göstermektedir (Duncan çoklu karşılaştırma testi, $p \leq 0.05$)

Sera denemelerinde elde edilen bazı bitki gelişim parametreleri Çizelge 4'de verilmiştir. Bakteri uygulamalarında ilk çiçeklenme gün sayısı pozitif ve negatif kontrole göre azalırken, *B. lentimorbus* RK-340 uygulamasındaki dal sayısı hariç diğer tüm parametrelerde artışlar kaydedilmiştir. Bu artışların büyük bir çoğunluğu da istatistiki olarak önemli bulunmuştur. Bitki gelişim parametrelerindeki en büyük artışlar *B. megaterium* TV-49a uygulamasından elde edilmiştir. Bu bakteri izolatının hem hastalık gelişiminde önemli bir azalmaya sebep olması hem de bitki gelişiminde önemli artışlara sebep olması oldukça dikkat çekici bulunmuştur.

Çizelge 4. Sera denemelerinde elde edilen bazı bitki gelişim parametreleri

Table 4. Some plant development parameters determind in greenhouse trials

Parametreler	TV-85D	TV-49A	TV-13C	TV-15B	TV-14C	PM-18	RK-340	PK	NK
BB (cm/bitki)	65.8a	68.0a	54.4cd	59.8b	55.4c	65.5a	52.5cd	**42.8e**	**52.1d**
GÇ (cm/bitki)	4.6a	4.8a	4.0bc	4.2b	4.0bc	4.5a	4.0bc	**3.2d**	**3.9c**
ÇS (adet/bitki)	31.7a	31.7a	31.5a	27.0b	25.0b	30.2a	26.2b	**5.5d**	**19.7c**
YK (SPAD)	43.4b	46.4a	40.2c	41.2bc	39.8c	41.5bc	37.4d	**29.3e**	**35.6d**
DS (adet/bitki)	16.0a	16.7a	14.2bc	15.5ab	14.0c	16.5a	13.5c	**11.2d**	**13.7c**
MS (adet/bitki)	27.5a	27.7a	27.2a	22.2b	20.0b	26.0a	22.5b	**3.2d**	**15.2c**
MA (gr/meyve)	128.9a	133.7a	116.2ab	121.1ab	117.6ab	127.7a	88.0cd	**69.7d**	**98.7bc**
YBA (gr/bitki)	506.1a	500.6a	476.9bc	487.4b	476.9bc	498.3a	473.5c	**389.2e**	**462.2d**
KBA (gr/bitki)	208.2ab	211.4a	201.1cd	204.8c	201.5cd	206.3abc	198.7d	**161.0f**	**185.7e**
İÇS (gün)	52.2d	52.2d	53.7cd	53.0d	54.7cd	54.2cd	56.7c	**74.0a**	**65.7b**

BB: Bitki boyu, GÇ: Gövde çapı, ÇS: Çiçek sayısı, YK: Yaprakta klorofil, DS: Dal sayısı, MS: Meyve sayısı, MA: Meyve ağırlığı, YBA: Yaş bitki ağırlığı, KBA: Kuru bitki ağırlığı, İÇS: İlk çiçeklemöe süresi, PK: Pozitif kontrol, NK: Negatif kontrol

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Original article (Özgün makale)

Effectiveness of four Turkish entomopathogenic nematode isolates against *Bactrocera oleae* (Diptera: Tephritidae) pupae at different temperatures

Ayşenur YILMAZ [1], Taylan ÇAKMAK [2], Uğur GÖZEL [1]*

Dört yerel entomopatojen nematod türünün farklı sıcaklıklarda *Bactrocera oleae* (Diptera: Tephritidae) pupaları üzerindeki etkinliği

Öz: Zeytinin anavatanı olan Türkiye, dünyanın önde gelen zeytin üreticilerinden biridir. Türkiye'de en fazla zeytin üretimi yapan iller arasında İzmir, Aydın, Çanakkale, Balıkesir, Muğla ve Bursa yer almaktadır. Zeytinin ana zararlısı olan *Bactrocera oleae* (Diptera: Tephritidae), ülkemiz zeytin yetiştiriciliğinde önemli bir sorundur. Daha önceki çalışmalarla ülkemizde tespit edilmiş 4 yerel entomopatojen nematod türünün (*Steinernema feltiae*/12, *Steinernema carpocapsae*/1133, *Heterorhabditis bacteriophora*/70, *Heterorhabditis bacteriophora*/91), tek doz ve 5 farklı sıcaklıkta (10, 15, 20, 25 ve 30 ^{o}C) laboratuvar koşullarında *B. oleae* pupaları üzerinde meydana getirdikleri infeksiyon oranları belirlenmiştir. Elde edilen sonuçlara göre en yüksek infeksiyon oranı 25 °C'de *Heterorhabditis bacteriophora*/91 izolatında, en düşük infeksiyon oranı 10 °C'de *Steinernema feltiae*/12 izolatında gözlemlenmiştir.

Anahtar Kelimeler: *Bactrocera oleae*, *Steinernema feltiae*, *Steinernema carpocapsae*, *Heterorhabditis bacteriophora*, biyolojik mücadele

Abstract: Türkiye, is the country of origin of the olive tree and is among the world's leading olive producers. Most of the olive production in Türkiye is concentrated in Izmir, Aydın, Çanakkale, Balıkesir, Muğla and Bursa Provinces. *Bactrocera oleae* (Diptera: Tephritidae), being one of the main pests of olive trees, is an important problem in olive cultivation in Türkiye. The efficacy of four Turkish populations of entomopathogenic nematodes against *B. oleae* pupae was determined under laboratory conditions. They were *Steinernema feltiae/12*, *S. carpocapsae*/1133, *Heterorhabditis bacteriophora*/70, *H. bacteriophora*/91, which were collected in different regions of Türkiye during our earlier studies. They were tested against the pest by using a single dose at 5 different temperatures (10, 15, 20, 25 and 30 °C). The highest infection rate was observed for*H. bacteriophora*/91 isolate at 25 °C, and the lowest infection rate was observed for *S. feltiae*/12 isolate at 10 °C.

[1] Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Plant Protection, Çanakkale, Türkiye
[2] Düzce University, Faculty of Agriculture, Department of Agricultural Biotechnology, Düzce, Türkiye
* Corresponding author (Sorumlu yazar): ugozel@gmail.com
ORCID ID (Yazar sırasıyla): 0000-0002-3873-6537; 0000-0003-4151-5724; 0000-0003-1363-1189
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Introduction

Türkiye is an important olive producer and exporter worldwide. Olive trees are widely grown due to the suitable climate and soil characteristics. Olive production is mostly concentrated in the Aegean, Marmara and Mediterranean Regions. In Türkiye, olives are generally divided into two main categories: olives for oil and table olives. Türkiye's olive oil is well known worldwide for its quality and taste. Olive production fluctuates over the years depending on weather conditionsand farming techniques. Therefore, Türkiye promotes sustainable agricultural practices for the table olive and olive oil industries. Nevertheless, as with all cultivated plants, some diseases and pests negatively affect the yield and quality of the crop. Important pests of olives includes the olive fly (*Bactrocera oleae* Rossi (Diptera: Tephritidae), the olive moth (*Prays oleae* Bern (Lepidoptera: Yponomeutidae), and the olive leaf moth (*Palpita unionalis* Hübn (Lepidoptera: Pyralidae).

The main pest of olives is *B. oleae* which is aninsect that damages olive fruits and is especially common in the Mediterranean Region. *Bactrocera oleae* damages the olive fruit through females laying eggs and the larvae hatching and feeding inside the fruit. This causes deterioration in the internal structure of the olive, loss of quality and decrease in the commercial value. The larvae feed on the fleshy part of the fruit and drop to the soil close to pupation time and pupate in the soil. Various management methods have been used against *B. oleae*. These methods include biotechnical, biological, chemical and cultural methods. Due to the negative effects of chemical pesticides on the environment, biological control has recently gained importance for controlling pests and pathogens (Lacey 2001).

Many nematode species are associated with insects, and so far, 23 families of nematodes that have parasitic relationships with insects have been reported. Seven of which are insect parasites. Nematodes from Steinernematidae Travassos, 1927 and Heterorhabditidae Poinar, 1976 (Nematoda: Rhabditida) are used as microbial insecticides to infect insects with pathogenic microbes, and are commercially produced and marketed by various companies (Koppenhöfer 2007) for biological control studies and are used effectively against many insect pests species (Grewal et al. 2005). EPNs, which are obligate parasites of insects with a part of their life-cycle in the soil, can be effective biological control agents oft insects pests. The host specificity of EPNs is one of the main features that distinguish them from insecticides (Smart 1995). EPNs particularly provide a unique option for controlling a pest such as *B. oleae*; because when applied to plants they actively seek out their hosts, cause no harm to vertebrates, and effectively can kill insects even when applied at very low dose levels. EPNs can kill their hosts within approximately 48 hours by causing septicemia (blood poisoning) in the host through the activity of symbiotic bacteria associated with them (Ünlü & Özer 2003). In host infection, the third larval stage (IJ or dauer juvenile) is the most active larval stage found in the soil seeking its host. The third larval stage (J3) can

survive in the soil without a host for at least a year. TheJ3 of EPNs enters the host's hemocoel through the host's orifices (mouth, anus, stigma) or thinned parts of the cuticle (only in Heterorhabditidae, which have dorsal labial teeth in the mouth) (Bedding & Molyneux 1982; Wang & Gaugler 1998). Nematodes and their symbiotic bacteria which feed on damaged tissues of their hosts, develop and reproduce within the insect's cadaver (Poinar & Grewal 2012). Feeding of IJs continues for approximately 2-3 generations until the infected host is consumed. Nematodes at the J3 stage, which have consumed all the tissues of the insect host, leave the cadaver, move to the soil and begin to look for new hosts (Poinar 1979; Akhurst & Boemare 1990).

Entomopathogenic nematodes can be mass produced in vivo or in vitro in solid or liquid media (Grewal & Georgis 1998). EPNs can be applied against insects pests in the soil, animal manure, aquatic habitats and on leaves. However, the most common of these applications is soil application (Klein 1990). In biological control, EPNs that exist naturally in the soil have a significant advantage in their use on harmful insect species that spend at least one period of their life cycle in the soil. *Bactrocera oleae* is one of the insects that pupate in the soil where it is potentialy vulnerable to EPNs.

The effectiveness and virulence of EPN species have been investigated for many insect species in Türkiye (Ataş et al. 2020; Gözel et al. 2020; Özdemir & Evlice 2020; Şahin & Gözel 2021; Erdoğuş et al. 2023). This study aimed to investigate the effectiveness of four EPN species, which are capable of suppressing many important pests, against the pupal stage of *B. oleae* under laboratory conditions.

Materials and Methods

Obtaining *Bactrocera oleae*

The main material of the study was *B. oleae* pupae and EPN isolates obtained from Edirne, Çanakkale and Sakarya Provinces. Olives damaged by *B. oleae* were collected from Çanakkale center and Geyikli and brought to the Nematology Laboratory of Çanakkale Onsekiz Mart University, Faculty of Agriculture and stored at 9 ^{o}C. In the course of time, pupaes of *B. oleae* were obtained from damaged olives (Figure 1).

Figure 1. Obtaining pupae of *Bactrocera oleae* from damaged olives

Mass production of *Galleria mellonella*

Since the last instar larvae of *Galleria mellonella* L. (Lepidoptera: Pyralidae) are very vulnerable to EPNs, it is used to obtain EPNs from soil and in mass production (Bedding & Akhurst 1975). *Galleria mellonella* larvae were reared in an artificial nutrient medium (a mixture of 500 g coarse bran, 50 g honey, 65 ml glycerin and 25 ml pure water) in incubators in glass jars at 27±1 ^{o}C (Kaya & Stock 1997). Some of the grown larvae were returned to the glass jars to continue the culture, and some were used in the mass production of EPNs.

Mass production of entomopathogenic nematodes

EPN isolates obtained from soil in Türkiye were mass produced on the last instar of larvae of *G. mellonella* (Table 1) (Figure 2). Depending on the EPN species, emergence from each *G. mellonella* larva was observed within 2-4 days under optimal conditions (25 ^{o}C, 70% humidity).

Table 1. Entomopathogenic nematode species used against the olive fruit fly, *Bactrocera oleae*

No of the EPN isolate	Name of EPN species	Obtained place
12	*Steinernema feltiae* Filipjev, 1934	Edirne
1133	*Steinernema carpocapsae* Weiser, 1955	Sakarya
70	*Heterorhabditis bacteriophora* Poinar, 1976	Çanakkale
91	*Heterorhabditis bacteriophora* Poinar, 1976	Çanakkale

Figure 2. Production of EPNs obtained from soil in Turkiye on last instar of *Galleria mellonella* larvae

Determining of the effectiveness of entomopathogenic nematodes on *Bactrocera oleae* pupae

In the laboratory, Whatman filter papers were placed in Petri dishes with a diameter of 3 cm. All Petri dishes were labelled according to the treatment. For one

repetition, 20 Petri dishes were prepared and then one *B. oleae* pupa was placed in each Petri dish. In the experiment, 5 different temperatures (10, 15, 20, 25 and 30 ^{o}C) and four different EPN isolates were used. Two hundred IJ/pupae in 200 µl of pure water were placed in each Petri dish (Figure 3). The experiment was set up with 3 replications. After inoculation, the Petri dishes were placed in incubators at one of five different temperatures. The Petri dishes were moistened for 15 days. The pupae were then dissected under a binocular microscope and checked for EPN emergence. The Petri dishes in which emergence was observed were recorded asconfirmation that the deaths of the *B. oleae* pupae were caused by the EPNs.

Figure 3. Infecting pupae of *Bactrocera oleae* with EPNs in pure water

Statistical analysis

The data generated in this study were evaluated with one-way analysis of variance and the differences were grouped with the Tukey test.

Results and Discussion

There weredifferences in efficacy against the the olive fruit fly, *Bactrocera oleae* among four different Turkish EPN isolates and at different temperatures in a laboratory study (Table 2).

Table 2. Levels ofinfection of *Bactrocera oleae* pupae by four entomopathogenic nematode isolates collected in Turkiye

Place	Nematode Species /Isolate Code	Temperature (°C)				
		10	**15**	**20**	**25**	**30**
Edirne	*Steinernema feltiae*/12	10 ±5,77 *ABc	15 ± 2,89 ABbc	25 ± 5,77 Aab	25 ± 2,89 Bab	30± 2,89 Aa
Sakarya	*Steinernema carpocapsae*/1133	15 ± 2,89 ABb	25 ± 5,77 Aab	30 ± 5,77 Aa	35 ± 2,89 ABa	35 ± 0,00 Aa
Çanakkale	*Heterorhabditis bacteriophora*/70	15 ± 5,77 ABc	20 ± 2,89 Abc	30 ± 2,89 Aab	30 ± 0,00 ABab	35 ± 0,00 Aa
Çanakkale	*Heterorhabditis bacteriophora*/91	20 ± 2,89 Ac	25 ± 5,77 Abc	35 ± 2,89 Aab	40 ± 2,89 Aab	35 ± 0,00 Aa**

* Statistical differences between EPN groups at the same temperature are indicated with a capital letter (P<0,05).
** Statistical differences between temperatures in the same EPN group are indicated by lowercase letters (P<0,05).

The highest infection rate was observed for *Heterorhabditis bacteriophora*/91 isolate at 25 °C, and the lowest infection rate was observed for *Steinernema feltiae*/12 isolate at 10 °C. The efficacy rates of the EPNs against *B. oleae* pupae varied significantly, depending on the EPN strain and the temperature applied.

Similar results have been obtained in studies conducted with *Ceratitis capitata* Wied. (Diptera: Tephritidae), *Rhagoletis cerasi* L. (Diptera: Tephritidae), *Anastrepha suspensa* (Loew) (Diptera: Tephritidae) and *Bactrocera dorsalis* Handel (Diptera: Tephritidae), which are in the same family as *B. oleae*. It has been reported that insects in the order Diptera are vulnerable to infection by species in the genera, *Steinernema* and *Heterorhabditis* (Rohde et al. 2012; Kepenekci et al. 2015; Minas et al. 2016; Heve et al. 2017; Godjo et al. 2018).

In studies investigating the effectiveness of EPNss against *B. oleae* larvae and pupae, *S. fetiae* (Sirjani et al. 2009; Torrini et al. 2020) and *S. carpocapsae* (Torrini et al. 2017) were effective at high application rates.

In the present study, the infection rates of *B. oleae* pupae by EPNs varied, depending on the nematode species and strain? and the temperature applied, with the rate of infection increasing with increasing temperature. *Steinernema fetiae*/12 isolate hadthe lowest activity against *B. oleae* pupae. Separately,, *S. carpocapsae*/1133 and *H. bacteriophora*/70 isolates caused similar mortality of *B. oleae* pupae at all temperatures. Overall, *H. bacteriophora*/91 had the highest efficacy against *B. oleae* at almost all temperatures.

The use of biological control methods as an alternative to chemical control against insect pests is increasing. The EPNs used in biological control have a wide

host range, cause high rates of mortality of their hosts, are easy to apply, and are compatible withhuman and environmental health, which facilitates their use.

In this study, the four EPN isolates had similar efficacy rates to those reported in earlier studies of infection levels of*B. oleae* larvae and pupae. *Bactrocera oleae* larvae after feeding on the fleshy part of the olive fruit fall to the soil where they pupate and are vulnerable to EPNs. the isolate *Heterorhabditis bacteriophora*/91 infected *B. oleae* pupae more effectively than the other EPN isolates and it is a potential biological contol agent of this major pest.

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Original article (Özgün makale)

Morphological characteristics and density of *Bracon (Habrobracon) concolorans* Marshall,1900 (Hymenoptera: Braconidae), a native Turkish parasitoid of *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), on greenhouse-grown tomatoes in Antalya, Türkiye

Nurdan TOPAKCI [1]*, Ahmet BEYARSLAN [2]

Antalya/Türkiye'de Serada Yetiştirilen Domateslerde Bulunan *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae)'nın Yerli Parazitoidi *Bracon (Habrobracon) concolorans* Marshall, 1900 (Hymenoptera:Braconidae)'ın Morfolojik Özellikleri ve Yoğunluğu

Öz: *Tuta absoluta* **(**Meyrick, 1917) (Lepidoptera: Gelechiidae) domates bitkisinin en önemli zararlılarından biridir. Zararlı ile mücadelede kimyasal mücadeleye öncelik verilmekte, bu durum çevre ve insan sağlığı risklerini de beraberinde getirmektedir. Mücadelede zaman zaman ticari biyolojik ajanlar kullanılsa da zararlının mevcut parazitoid ve predatörlerinin yoğunluğunun bilinmesi entegre mücadelenin etkinliği açısından önemli olmaktadır. Bu çalışmada, Antalya'da *T. absoluta*'nın istila ettiği domates bitkilerinden elde edilen yerli parazitoid, *Bracon* (*Habrobracon*) Marshall, 1900 (Hymenoptera: Braconidae)'ın yoğunluğu araştırılmıştır. Antalya/Elmalı'da 2019 yılı Ekim-Kasım aylarında seradan haftalık olarak toplanarak kültüre alınan domates yaprak örnekleri günlük olarak takip edilmiş ve ergin döneme ulaşan parazitoidlerle birlikte zararlı sayısı da kayıt altına alınmıştır. Sonuçlara göre 221 bulaşık yaprak örneğinden toplam 2004 adet *T. absoluta* ve 478 adet *B.* (*H.*) *concolorans* ergini elde edilmiştir. Ergin döneme ulaşan parasitoid sayısı en az 1.7 ergin/yaprak, en fazla 2.6 ergin/yaprak olarak belirlenmiştir. Çalışmada parazitoidin Antalya populasyonunun morfolojik karakterlerine ilişkin değerlendirmelere de yer verilmiştir. Buna göre parazitoid türün vücut uzunluğu erkek bireylerde ortalama 2.13 mm dişi bireylerde 2.37 mm olarak belirlenmiştir. Erkek ve dişi bireylerde anten uzunluğu sırasıyla ortalama 2,04 mm ve 1,80 mm, anten segment sayısı 20-23 ve 18-21 olarak saptanmış, ovipozitör uzunluğu ise ortalama 0,59 mm olarak bulunmuştur.

[1] Akdeniz University, Vocational School of Technical Sciences, Environmental Protection and Control Program, Antalya, Türkiye
[2] Department of Biology, Faculty of Science, Trakya University, (retired) Edirne, Türkiye
* Corresponding author (Sorumlu yazar): ntopakci@akdeniz.edu.tr
ORCID ID (Yazar sırasıyla): 0000-0001-5577-5522; 0000-0002-6948-8257
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Anahtar Kelimeler: *Bracon* (*H.*) *concolorans, Tuta absoluta*, örtüaltı, yoğunluk, morfolojik özellikler

Abstract: *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) is one of the most significant pests of tomato. Chemical pesticides are prioritized in pest control, and this poses risks to both the environment and human health. Although commercial biological agents are occasionally used for control, understanding the density of existing parasitoids and predators of the pest is crucial for the effectiveness of integrated pest management. In this study, the density of the native parasitoid *Bracon* (*Habrobracon) concolorans* Marshall, 1900 (Hymenoptera:Braconidae) collected from *T. absoluta* infesting tomato plants Antalya was investigated. Tomato leaf samples were collected weekly from a greenhouse in Elmalı/Antalya in October-November 2019, and cultured. The samples were monitored daily to record the number of pests and parasitoids that reached the adult stage. According to the results, 2004 *T. absoluta* and 478 *B.* (*H.*) *concolorans* adults were obtained from 221 infected leaves. The number of parasitoids reaching the adult stage ranged from 1.7 adults to 2.6 adults per leaf. The study also included determinations of the morphological characteristics of the Antalya population of the parasitoid. Accordingly, the average body length of the parasitoid was 2.13 mm in males and 2.37 mm in females. The average antenna length in males and females was 2.04 mm and 1.80 mm, respectively,and the number of antenna segments was 20-23 and 18-21, respectively.The average ovipositor length was 0.59 mm.

Keywords: *Bracon* (*H.*) *concolorans, Tuta absoluta*, greenhouse, density, morphological characteristics

Introduction

The tomato is an important cultivated plant and is susceptible to many pests. *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is one of the most important and destructive pest. *T. absoluta*, which originates from South America, was first observed in Argentina in 1964 and quickly spread to many countries due to its high damage potential (EPPO 2005; Desneux et al. 2010, 2011). This pest, which was unknown in Türkiye until 2009, has rapidly spread to all regions where tomato production occurs (Kılıç 2010; Doğanlar & Yiğit 2011; Karut et al. 2011; Ünlü 2011; Mamay & Yanık 2012; Aksu & Çıkman 2014; Aksu Altun & Çıkman 2021a; Aksu Altun & Çıkman 2021b; Portakaldalı et al. 2013; Erdoğan et al. 2014; Tatlı & Göçmen 2019). *Tuta absoluta*, which causes significant economic losses in Solanaceae family plants, mainly tomatoes, can damage all parts of the tomato plant except the roots. If left uncontrolled, product losses of up to 100% may occur (Biondi et al. 2018; Öztemiz 2012).

The management of *T. absoluta* is quite challenging. Promising predators (Cabello et al. 2009a; Molla et al. 2009; Desneux et al. 2010; Calvo et al. 2012; Portakaldalı et al. 2014) and parasitoids (Luna et al. 2007; Molla et al. 2008; Cabello et al. 2009b; Zappala et al. 2012; Chailleux et al. 2013) against *T. absoluta* were revealed to be insect species. Among the parasitoid species, in addition to egg

parasitoids, larval parasitoid Braconid species are also important. Braconidae (Hymenoptera) is a large group of 1103 genera and approximately 21223 described species (Yu et al. 2016). Studies in Türkiye show that some braconid species are parasitoids of *T. absoluta*. Doğanlar & Yiğit (2011) conducted research on *T. absoluta* in the areas of Hatay province where intensive tomato cultivation is practiced and identified the species *Bracon* (*Habrobracon*) *didemie* Beyarslan and *Bracon* (*Habrobracon*) *hebetor* (Say) as effective parasitoids. Effective parasitoids of *T. absoluta* were investigated in the Southeastern Anatolia Region, and *B. (H.) hebetor* and *Apanteles* sp. were recorded (Bayram et al. 2014). Natural enemies of *T. absoluta* were investigated in the tomato growing areas of Şanlıurfa, and *Bracon* (*Habrobracon*) *concolorans* Marshall, *B. (H.) didemie*, *B. (H.) hebetor*, *Bracon* (s. str.) *intercessor* Nees, and *Apanteles* sp. (Hymenoptera: Braconidae), were found to be effective braconid parasitoids (Altun & Çıkman 2019). Topakci et al. (2022) recorded *B.* (*H.*) *concolorans*, and *Bracon (*s.str.*) variegato*r Spinola, as the natural enemies of *T. absoluta* in their research conducted in the Antalya tomato cultivation areas. In research conducted in tomato cultivation areas in Adana, *B. (H.) didemie* and *Apanteles (Dolichogenidea*) *appellator* Telenga, (Hymenoptera, Braconidae) species, which are natural enemies of *T. absoluta* and promising species for control, were recorded (Karut et al. 2023). Yüksekyayla et al. (2023) detected *B. didemie* as the sole larval parasitoid of *T. absoluta* in tomato greenhouses across various districts of Antalya province.

Bracon (Habrobracon) concolorans Marshall (syn. *Bracon Habrobracon nigricans* Szépligeti) is a species distributed in the Oriental and Palaearctic Zoogeographic regions. Distribution in Türkiye: Adıyaman, Edirne, İcel (Beyarslan 1999), Aegean region (Beyarslan et al. 2002a), Imbros (Beyarslan et al. 2002b), Kastamonu (Beyarslan et al. 2005), Amasya, Çorum, Tokat (Beyarslan et al. 2008), Türkiye (Papp 2008), Türkiye (Beyarslan et al. 2010), Bayburt, Gümüşhane (Beyarslan & Cetin Erdogan 2010), Amasya, Ankara, Bayburt, Cankırı, Elazığ, Eskisehir, Gumushane, Kayseri, Kırıkkale, Konya, Malatya, Niğde, Samsun, Sivas, Tokat, Yozgat (Beyarslan & Cetin Erdogan 2012), Diyarbakır, Mardin, Şanlıurfa (Beyarslan et al. 2014), Ardahan, Erzurum, Iğdır, Kars (Beyarslan 2016), Antalya (Topakcı et al. 2022).

Although there are studies on the presence, density, and parasitism status of the larval parasitoid *Bracon* species of *T. absoluta* in Türkiye, few studies have been conducted on *B.* (*H.*) *concolorans*. This study aimed to determine the density and morphological characteristics of *B.* (*H.*) *concolorans* detected in the tomato greenhouse in Elmalı, Antalya.

Materials and Methods

Sampling

In this study, leaf samples were taken from tomato plants (Do-pink variety) which were heavily damaged by *T. absoluta* in a plastic greenhouse covering

approximately 1300 m^2,in the Elmalı district of Antalya Province (36°48′13.0″N, 30°01′21.4″E) at the end of the 2019 production season. Pheromone traps (0.5 mg (EZZ-3,8,11) -Tetradecatrienyl Acetate (95%) + EZ-3,8-tetradecadienyl acetate (5%) / capsule + Delta Trap) were changed weekly (1 trap/greenhouse) for *T. absoluta* in the greenhouse, from the beginning of the season, and the use of traps ended on 23.10.2019. In addition, 35% chlorantraniliprole, 5% emamectin benzoate and 25 g/l deltamethrin were used during the production season. However, there was no pesticide application during the leaf sampling period toward the end of the production season. On each sampling date, a minimum of 45 composite leaf samples, each 15-20 cm long, that had been damaged by various larval stages of *T. absoluta*, were collected.. These samples were placed in a paper bag, then transferred to a polyethylene bag, and finally brought to the laboratory at Akdeniz University Vocational School of Technical Sciences. To obtain tomato moths and adult parasitoids, leaf samples were cultured separately at ambient temperature in glass containers covered with thin gauze, with a maximum of five composite leaf (with 4-5 leaflets) samples in each container (Figure 1). Emerged parasitoids were aspirated from the containers and placed in Eppendorf tubes containing 70% alcohol. The specimens were then sent to the Trakya University, Faculty of Science, Department of Biology (Entomology) for identification. Each tube was numbered, and relevant information was recorded.

The diagnosis of specimens

Adult braconids were identified based on their morphological characteristics by the second author. Relevant literature (Belokobylskij & Tobias 2000; Beyarslan & Fischer 1990; Papp 1990, 2000, 2008; Samartsev 2011, 2013; Samartsev & Belokobylskij 2013; Tobias 1986; van Achterberg, 1993) and comparison material in the second author's collection were used to identify the samples.

The study also included measurements of the morphological characteristics of the *B.* (*H.*) *concolorans* obtained from greenhouse production under plateau conditions. For this purpose, 25 male and female individuals were examined under a microscope, and the data were evaluated. Leica DM1000 and DMSZ7PZB microscopes were used to capture digital images and take measurements. The specimens were deposited at the Trakya University, Faculty of Science, Department of Biology (Entomology) in Edirne and Akdeniz University Vocational School of Technical Sciences in Antalya.

Numbers of *Bracon* (*Habrobracon*) *concolorans* and *Tuta absoluta*

To monitor the emergence of both *T. absoluta* and parasitoid adults, glass containers were checked twice daily, in the morning and evening. Both moth and parasitoid adults were collected from the containers by using a mouth aspirator. The mean number of individuals per leaf was determined by dividing both the total number of moths and adult parasitoids about the total number of leaves. The

monitoring of the samples continued until approximately one month after the emergence of adults began.

Figure 1. Cultured leaf samples infested with *Tuta absoluta*

Results and Discussion

Morphological studies

The only parasitoid to emerge from *Tuta absoluta* was *Bracon* (*Habrobracon*) *concolorans.* Figure 2 shows images of the dorsal, ventral and lateral views, as well as the thorax, abdomen, antenna, and wing structures of male and female individuals from the Antalya population of species *B.* (*H.*) *concolorans*.

Figure 2 continued

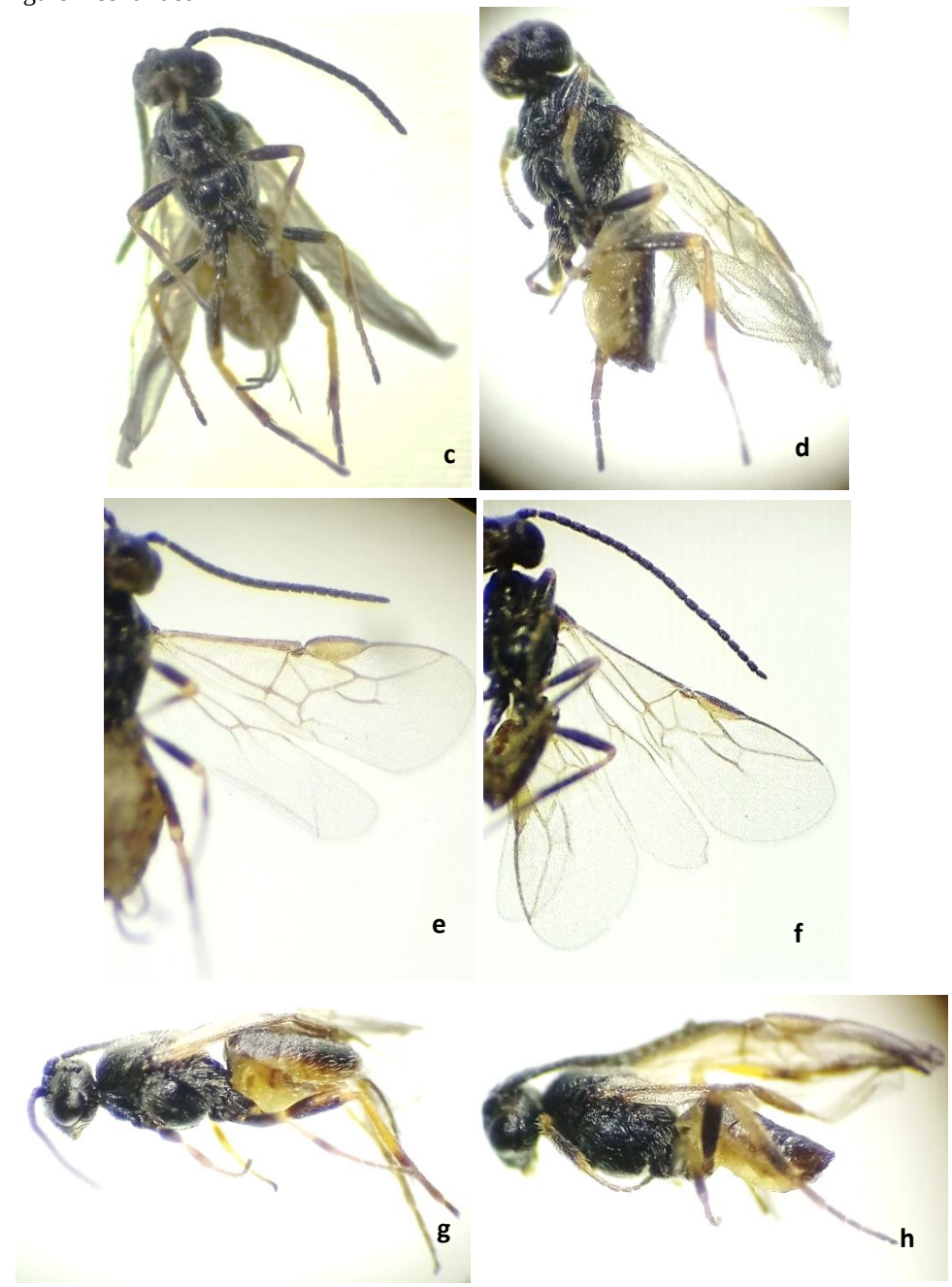

Figure 2 continued

Figure 2. *Bracon (Habrobracon) concolorans* a-b: dorsal (female-male), c-d: ventral (female-male), e-f: antenna and wing (female-male), g-h: lateral (female-male), k-l: thorax-abdomen (female), m-n: thorax-abdomen (male)

The measurements forbody length, head length, mesosoma length, metasoma length, ovipositor length and antenna length of both male and female *B.* (*H.*) *concolorans* are shown in Table 1. The average body length of *B.* (*H.*) *concolorans* males was 2.13 mm whereas the average body length of the females was 2.37 mm. The average antenna length in male and female individuals was 2.04 and 1.80 mm, respectively. In addition, the number of antenna segments was determined to be 20-23 in males and 18-21 in females.

The development and growth of parasitoids developing in hosts fed different diets also differ. For example, there were differences in adult sizes of *B. hebetor* adults reared on larvae of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) feeding on nine different host plants (Nivedita et al. 2021). In earlierstudies, the morphological evaluation of *B.* (*H.*) *concolorans* was conducted based on location information rather than the hosts from which it was obtained. Therefore, in this study, the parasitoid could not be compared morphologically with individuals obtained from different hosts. However, the morphological measurements obtained were compared with the similarities and differences revealed in earlier studies.

Similar to this study, in Iran, Ameri et al. (2014) determined that the body of *B. nigricans* is generally dark brown with yellowish brown spots, metasoma yellowish brown on the dorsal surface, the head width is approximately twice the head length, and the mesonotum is densely covered with uniform hairs. Unlike the data obtained in this study, they determined that the ovipositor had a length of 0.7-0.8 times the metasoma length, and that the antenna of females were 34-segmented. In our study, the ovipositor was approximately 0.6-0.7 times the length of the metasoma. In addition, the number of antenna segments was a maximum of 21 in females and 23 in males.

In South Korea, Smartsev & Ku (2021) determined that the antenna was 21-23 segmented in females and bodies of what species mainly brownish black. Yu et al. (2016) determined that the ovipositor length of *B.* (*H.*) *concolorans* was 0.7 -0.8 of the metasoma length. Females had 20-34 antenna segments and males had 22-28 antenna segments, and the body length was 2.0-3.2 mm in females and 2.0-3.1 mm in males, and the head width was 1.7-1.9 times head length. In the current study, the body length range was 1.8-2.4 mm in males and 2.1-2.7 mm in females, and head width was approximately 1.9 times head length.

Table 1. Morphological measurements of male and female *Bracon (Habrobracon) concolorans* collected in Antalya Province, Türkiye

Species characteristics	**Average measurements (mm) (male)**	**Measurement range (mm) (male)**	**Average measurements (mm) (Female)**	**Measurement range (mm) (Female)**
Body length	2.13±0.035	1.8-2.4	2.37±0.030	2.1-2.7
Mesosoma length	0.81±0.014	0.7-0.9	0.93±0.011	0.8-1.0
Metasoma length	0.97±0.018	0.8-1.1	1.07±0.023	0.8-1.3
Head length	0.32±0.008	0.3-0.4	0.37±0.009	0.3-0.4
Head width	0.59±0.009	0.5-0.7	0.65±0.011	0.5-0.7
Antenna length	2.04±0.023	1.9-2.3	1.80±0.026	1.6-2.1
Ovipositor length	-	-	0.59±0.008	0.5-0.7

In Türkiye, besides *Trichogramma* species which are egg parasitoids of *T. absoluta*, predominantly species of Eulophidae, and Braconidae were identified as larval parasitoids in open field and greenhouse tomato production areas in Hatay, Izmir, Antalya, Şanlıurfa, Diyarbakır, and Mardin Provinces (Doğanlar& Yiğit 2011; Öztemiz 2013; Bayram et al. 2014; Keçeci & Öztop 2017; Altun & Çıkman 2019; Topakcı et al. 2022; Çaylak & Başpınar 2022; Karut et al. 2023; Yüksekyayla et al. 2023).

Density of *Bracon (H.) concolorans*

The larval ectoparasitoid *B.* (*H.*) *concolorans* was athigh population densities across all three surveyed weeks from October to November. The 2004 *T. absoluta* adults and 478 *B.* (*H.*) *concolorans* were obtained from 221 plant samples (Table 3). The emergence of *T. absoluta* and *B.* (*H.*) *concolorans* was 825 and 248 individuals in the first sampling week, 733 and 113 individuals in the second week, and 446 and 117 individuals in the third week, respectively. (Table 3). After the leaf samples were cultured, parasitoid emergence started within 2-4 days (with an average of three days), and adult emergence was completed within 15-20 days. *Tuta absoluta* adult emergence started within 9-12 days (average 11 days) after the leaf samples were cultured and continued for 10-18 days (average 14 days).

Table 2. Number of *Bracon* (*H.*) *concolorans* and *Tuta absoluta* obtained from tomato leaves

Sampling Date	**Number of leaf samples**	**Number of *Tuta absoluta* reared**	***Tuta absoluta/ leaf***	**Number of *Bracon* (*H.*) *concolorans* reared**	***Bracon* (*H.*) *concolorans*/ leaf**
23.10.2019	108	825	7,6	248	2,3
30.10.2019	68	733	10,8	113	1,7
07.11.2019	45	446	9,9	117	2,6
Total	221	2004	9,1	478	2,2

Management of *T. absoluta* is generally carried out using chemical methods. The development of resistance to chemicals (Yalçın et al. 2015, Lietti et al. 2005), the presence of larvae in the tissues, and the ability to reproduce rapidly make it difficult to control the pest (Keçeci & Öztop 2017). Therefore, aligning with integrated pest management practices and leveraging natural enemies is crucial for successful pest control.

Many natural enemies of *T. absoluta* are egg parasitoids (Cabello et al. 2009a; Chailleux et al. 2013) In addition, larval parasitoids of *T. absoluta* across the world include *Diadegma ledicola* Horstmann, *Diadegma pulchripes* (Kokujev) (Hymenoptera: Ichneumonidae), *Bracon (Osculobracon) osculator* Nees, *Pseudapanteles dingus* (Muesebeck) (Hymenoptera: Braconidae), *Necremnus* sp.*;* near *tidius* (Walker), *Necremnus* sp. *near artynes* (Walker), *Neochrysocharis formosa* (Westwood), *Pnigalio soemius* s.l. (Walker), *Pnigalio cristatus* (Ratzeburg), *Pnigalio incompletus* (Boucek) (Hymenoptera: Eulophidae), *Halticoptera aenea* (Walker) (Hymenoptera: Pteromalidae) (Luna et al. 2007; Mollá et al. 2008; Lara et al. 2010; Riciputi 2011; Zappala et al. 2012).

Biondi et al. (2013b) stated that *B. nigricans* (syn. *B.concolorans*) should be considered as a potential biological control agent in newly invaded areas of the Palaearctic Region. In this study, the parasitoid species obtained from all leaf samples was *B. concolorans*. In the current study, the number of *T. absoluta* adults per leaf was 9.1, while the number of *B. concolorans* was 2.1. Sampling was carried out at the end of the tomato growing season. Therefore, since no pest management was conducted during this period, *T. absoluta* was found to be abundant on the leaves. It can be assumed that the population of parasitoids increased in response to the number of pests. In open-field tomato growing areas in Adana, *T. absoluta* was found at a maximum of 4.21 individuals per leaf. In comparison, the parasitoid *B. didemie* was found at a maximum of 28 individuals per 100 leaves (Karut et al. 2023).

Bracon (*H.*) *concolorans* is an idiobiont ectoparasitoid of mature larvae of *T. absoluta* (Biondi et al. 2013b). Parasitoids significantly reduce the population of *T. absoluta* through stinging and host feeding a(Biondi et al. 2013b). Under laboratory conditions, *B. nigricans* produced a higher number of offspring in fourth instar larvae of *T. absoluta* compared to third instar larvae, and deaths due to stinging behavior or host feeding were significantly higher in third instar larvae (Idriss et al. 2018). Zappala et al. (2012) determined that *B. nigricans*, along with *Necremnus* sp. and *Neochrysocarys formosa* Westwood, were the most dominant species in northern Italy. *Bracon (H.) concolorans* has been identified as one of the natural enemies of *Tuta absoluta* in Jordan (Al-Jboory et al. 2012).

In a study investigating the local natural enemies of *T. absoluta* in France, in addition to predatory and egg parasitoid species, two eulophid species and the braconid *B. nigricans* were identified as larval parasitoids. (Biondi et al. 2013a). *Bracon* (*H.*) *concolorans* is one of the larval parasitoids of *T. absoluta* in open-field tomato production in Iraq (Al-Gerrawy 2021). One of the two larval parasitoids of *T. absoluta* detected in Kenya's open field and greenhouse tomato production areas was identified as *B.(H.) nigricans* (Mama Sambo et al. 2022). In Türkiye, this species was determined to be a parasitoid of *T. absoluta* in Şanlıurfa (Altun & Çıkman 2019) and Antalya (Topakci et al. 2022). *Bracon* (*H.*) *concolorans* was obtained from *Etiella zinckenella* (Treitschke) (Lepidoptera: Pyralidae), *Pexicopia malvella* (Hübner) (Lepidoptera: Gelechiidae), *Cnephasia (Cnephasia) sedana* (Constant) (Lepidoptera: Tortricidae), (Beyarslan et al. 2005),

apple ermine moth, and *Yponomeuta malinellus* Zeller (Lepidoptera: Yponomeutidae) (Narmanlıoğlu & Çoruh 2017) but not *T. absoluta* in Türkiye.

In Hatay, it was determined that *C. clarus* had the highest rate of parasitism at 37.0%, and the parasitism rates of *B. hebetor* and *B. (H.) didemie* were 1.1% and 7.0%, on *T. absoluta*, respectively (Doğanlar & Yiğit 2011). A study conducted in greenhouse and open field tomato fields in Kenya revealed that *B. nigricans* parasitized *T. absoluta* at a rate of up to 21% (Mama Sambo et al. 2022). *Bracon* (*H.*) *concolorans*, whose parasitism rate on *T. absoluta* was 12%, 18%, and 23.5% in open field tomato production in 3 different regions in Iraq, has the highest parasitism rate among parasitoid species (Al-Gerrawy 2021). In Türkiye, the parasitism rate of *B. concolorans* on a different host, the apple ermine moth, *Y. malinellus* was similarly determined to be 19% (Narmanlıoğlu & Çoruh 2017). Luna et al. (2007) found the parasitism rate of the braconid *P.dignus*, which they reported as the most important natural enemy of *T. absoluta* in South America, to be 30% under laboratory conditions. Sanchez et al. (2009) revealed that *P. dignus* in Argentina had a 26.47% parasitism rate in organic fields and 45.95% under greenhouse conditions. Idriss et al. (2018) reported that the indigenous parasitoid species *B. (H.) nigricans and A.(D.) appellator*) in Sudan could be very promising, with parasitism reaching 55% of *T. absoluta* under laboratory conditions. It has been reported that parasitism rates vary between sanplingareas and regions (Mama Sabo et al. 2022). Although no calculation was made on the parasitism rate, numerical data of the parasitoid species were obtained from leaf samples. The ectoparasitoid *B. concolorans* female first paralyzes and kills larvae of *T. absoluta* and lays her eggs on or near the host.

AS adult individuals obtained from cultivated tomato leaves were evaluated in this study, the density of *B. concolorans* was determined from the number of adults. Accordingly, 117-248 parasitoid *B. concolorans* were obtained from at least 45, and at most, 108 tomato leaves, and no other species was identified. Yüksekyayla et al. (2023), in their study conducted in Antalya Province, determined the number of the parasitoid *B. didemie* obtained from 100 tomato leaves taken weekly as 24 at most and did not encounter any other species. Karut et al. (2023) identified *B. (H.) didemie* and *A.(D.) appellator* as parasitoids of *T. absoluta* in open-field tomato production in Adana and determined that *B. (H.) didemie* was much more common, constituting 93.2% of the total parasitoids.

Conclusion

Pesticides are used extensively against *T. absoluta*, which causes significant damage in tomato cultivation. The intensive use of pesticides has many negative effects on human health and the environment. Since chemical control alone is insufficient, integrated pest management, including biological control, should be applied for effective control of *T. absoluta.* Determining the natural enemies of *T. absoluta* and their activities contributes to sustainable, environmentally friendly control.

This study revealed the numbers of *Bracon* (*H.*) *concolorans* in a tomato greenhouse infested with *T. absoluta*. During the sampling period, which occurred towards the end of the production season, no control methods wereapplied against *T. absoluta*. However, chemical control was applied against the pest throughout the majority of the production season. Despite this, an average of 2.2 parasitoid individuals per leaf was recorded.

In future studies, it would be helpful to evaluate the mass rearing and releasing techniques of native, natural enemies and to conduct studies on the effects on this species of pesticides applied in tomato greenhouses. In addition, natural enemies may show some different morphological characteristics in different locations, and the extent to which they may affect their biological activity could also be evaluated. Determining the natural enemies of pests in their environment and evaluating the parasitism status of local natural enemies will support integrated control efforts.

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Original article (Özgün makale)

Morphological and Morphometric Identification of *Steinernema abbasi* (Nematoda: Steinernematidae) from Düzce, Türkiye

Taylan ÇAKMAK[1*]

Düzce İlinde Saptanan *Steinernema abbasi* (Nematoda: Steinernematidae)'nin Morfolojik ve Morfometrik Tanısı

Öz: Nematodların biyolojik mücadele etmeni olarak kullanılma potansiyeli son yıllarda artış göstermiştir. Entomopathogenik nematodlar (EPN) (*Steinernema* and *Heterorhabditis*), tarla ve bahçe tarımında zararlı sorununun çözümünde başarılı olmuşlardır. Biyolojik mücadele pragramlarında zararlıların ve doğal düşmanların doğru tanısı ile bu doğal düşmanların etkiliğinin belirlenmesi kritik öneme sahiptir. Bu çalışmada amaç, Düzce'den fındık bahçelerinden toplanan entomopatojenik nematodların tanısının yapılmasıdır. Morfolojik ve morfometrik karakterlere bağlı olarak, nematodların tanısı için metod sunulmuş ve bilinmeyen entomopatojenik nematod, ırk1, *Steinernema abbasi* olarak tanımlanmıştır.

Anahtar Kelimeler: Biyolojik Mücadele, entomopatojenik nematode, fındık, nematod tanısı, toprak faunası, Türkiye

Abstract: Interest in the use of nematodes as biological pest control agents has grown exponentially over the past two decades. Entomopathogenic nematodes (EPNs) (*Steinernema* and *Heterorhabditis*) have been particularly successful in managing pest problems in agriculture and horticulture. One of the most important requirements in biological control programmes is the accurate identification of pests and any beneficial organisms with biocontrol potential. The aim of this study was to identify an unknown entomopathogenic nematode that was collected from a hazelnut orchard in Düzce, Türkiye. Our study reports methods for the identification of *Steinernema abbasi*, based on morphological and morphometric characterization. The unknown entomopathogenic nematode, Strain 1, was identified as *S. abbasi.*

[1] Düzce University, Faculty of Agriculture, Department of Agricultural Biotechnology, Düzce, Türkiye
[*] Sorumlu yazar (Corresponding author): taylancakmak@duzce.edu.tr
ORCID ID (Yazar sırasıyla): 0000-0003-4151-5724
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Introduction

Insect pests are a major problem in agriculture, causing significant reductions in crop yield and quality. In horticulture, the negative impact of certain insects on the quality of fruit and ornamental plants is a major concern. As a result, various strategies have been employed to control these insects, both locally and systemically, such as the use of various agrochemicals. While most of these chemicals suppress the targeted insects, their use has been found to have a major impact on the environment (Nguyen & Hunt, 2007). There have also been reports of bioconcentration, bioaccumulation and biomagnification of these toxic materials. Therefore, the use of environmentally friendly biological control strategies is desirable.

One group of biological agents that can be used as biocontrol agents for insect pests are the entomopathogenic nematodes (EPNs) (Johnigk & Ehlers, 1999). The two most commonly used genera are *Steinernema* and *Heterorhabditis*. Steinernema species (Nematoda: Rhabditida) are produced in large-scale liquid culture for the biological control of insect pests (Ehlers 2001). These soil-dwelling nematodes have a specific third instar juvenile adapted to long-term survival, which is called the "dauer juvenile" (DJ). They carry cells of specific enteric bacterial symbionts of the genus *Xenorhabdus* in a pouch in the anterior part of the intestine (Ciche et al. 2006). DJs actively search for insects, enter the insect body through orifices or less sclerotic parts of the cuticle and release the bacteria. Approximately two days after invasion, the insects die of septicemia (Dowds & Peters 2002). The aim of this study was to identify an unknown EPN isolated from a hazelnut orchard in Düzce Province, Turkiye, using morphometrics and morphological analysis.

Materials and Methods

Collection and examination of nematodes

Samples were collected from a hazelnut orchard (40°56'40.1"N 31°15'04.8"E) in Düzce Province in the western Black Sea region of Turkiye. A 100 g soil sample from each sampling site was placed in a glass container with three last instar larvae of the wax moth, *Galleria mellonella* (L.), and covered with a lid. Samples were then stored at room temperature. After 10 days, dead larvae were collected and transferred to White traps to collect the emerging IJs. Five juveniles were collected from the test tube containing the unknown EPN, placed on a glass slide, and examined under a light microscope to identify the genus/genera to which the nematode(s) belonged, based on certain morphological characteristics, such as the type of cuticle (striated or smooth) and the presence or absence of horns. Twenty

G. mellonella larvae were placed in each of two different Petri dishes lined with slightly moistened double-layer filter paper. Infective juveniles (IJs) from the tube were used to inoculate the larvae at 100 IJs/larva. The Petri dishes were then covered and sealed with parafilm to prevent contamination. The Petri dishes was then stored at a temperature of around 15°C and left for five days. The *G. mellonella* larvae died, which is typical of infection by *Steinernema* spp. The larval carcasses were placed in a white trap to obtain the IJs (Kaya & Stock, 1997). The *G. mellonella* carcasses were dissected on day 5 to obtain first generation males. The males were transferred from the dissected cadaver to a staining block containing water and then immediately transferred to permanent fixation for further morphometric analysis.

Nematode preparation

The males were fixed and mounted which observations and all measurements were made on males using a calibrated light microscope with a drawing tube. Thirty (30) males were measured. The measurement of the infective IJs was done by making temporary slides followed by immediate measurement. Twenty-five (25) juveniles were measured. The following morphometrics were used to identify the nematode: length (L), maximum body diameter (MBD), excretory pore (EP), nerve ring (NR), esophagus (ES), tail length (T), hyaline (H), anal body diameter (ABD), spicule length (SL) and gubernaculum length.

Other calculated parameters were:

a= L/MBD c'= T/ABD H%= H/T*100
b= L/ES D%= EP/ES*100 GS%= GL/SL*100
c= L/T E%= EP/T*100 SW%= SL/ABD*100

Results and Discussion

Identification

Although some overlap in morphometric values was common for other species in the *Steinernema bicornutum* group (IJs with "horn like" structure in the head region) and *S. carpocapsae*, the infective juveniles and first generation males of the unknown EPN showed the greatest similarity to *Steinernema abbasi*, based on morphometric and morphological comparison only (Elawad et al. 1997).

Morphometrics and morphology of the infective juvenile

The average morphometric values of the unknown IJs showed some overlap with *Steinernema abbasi*, *S. bicornutum*, *S. ceratophorum* and *S. carpocapsae*, so that most of the values were interchangeable with the standard morphometric values used to describe the third instar juveniles of these four species. The calculated ratios a, b, c of the unknown nematode appear to have similar values to the

standard average ratios of *S. abbasi* and *S. carpocapsae* consistently (Table 1). However, there were inconsistencies with *S. bicornutum* and *S. ceratophorum*. Two horn-like structures were also observed on the IJs of the unknown strain. Nevertheless, the group comparison is based on the "bicornutum group". Other species of this group that were beyond the range of measurements were eliminated to facilitate comparison.

Comparison of the unknown nematode, based on the length of IJs, also showed that the unknown nematode (535 µm) was closest to *S. abbasi* (541 µm) and *S. carpocapsae* (558 µm). However, morphologically there is no horn-like structure in *S. carpocapsae*. Therefore, *S. carpocapsae* was discarded as a possibility. Furthermore, the measurements of the unknown IJ were almost outside the range of measurements of *S. bicornutum* and *S. ceratophorum*.

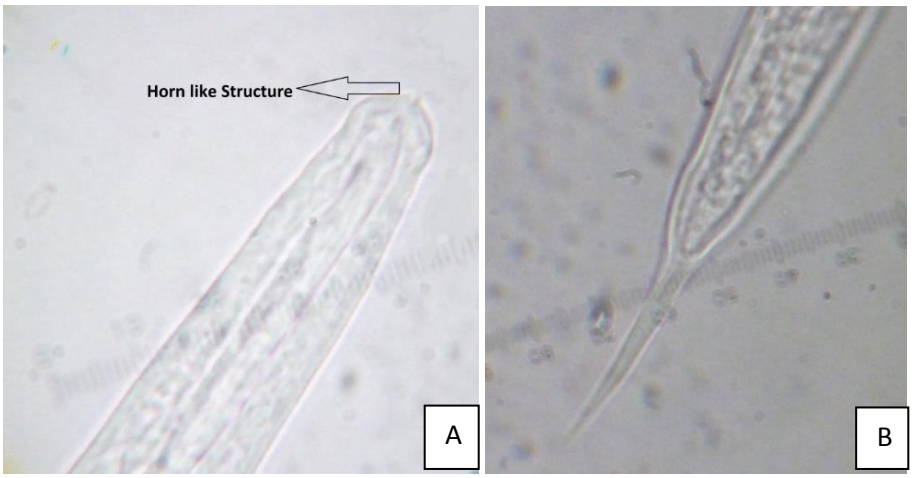

Figure 1. A. Anterior end of infective juvenile showing the horn-like structure. B. Posterior end of infective juvenile

Infective juveniles of *S. abbasi* are characterised by horn-like structures; thin, elongated body; sheath (J2 cuticle) present but sometimes lost; excretory pore always weak; near posterior end of metacorpus. Distance from anterior end to excretory pore always greater than body width at the same level. Oesophagus with cylindrical procorpus and slightly swollen metacorpus. Nerve ring just above metacorpus. Tail gradually tapering, dorsally curved at the tip, with a slight ventral depression that closely matches the unknown nematode.

This comparison of the unknown nematode with the above morphometric features of the infective juveniles shows that the nematode shares most of the morphometric characteristics of *S. abbasi*. Although the morphometric values within the "bicornutum group" don't show much overlap with other species, except *S. abbasi*, in order to make the identification more accurate, the characteristics of the first generation of males were diagnosed to give additional information (Fig. 1).

Table 1. Morphometric measurements of infective juveniles of Strain 1 and comparison with the closest species

Species/ Character	Strain 1	*S. abbasi*	*S. bicornutum*	*S. ceratophorum*	*S. carpocapsae*
n	25	15	20	45	55
L	535,2±21,7	541 ± 24	769,5±52,3	706 ± 62	558
	(480-565)	(496-579)	(648-873)	(591–800)	(438-650)
a	22,5±2,5	18 ± 0.91	26,5±1,5	25.9 ± 1.1	21
	(17-26)	(17-20)	(23-29)	(23.7–27.9)	(19-24)
b	5,9±0,3	6 ± 0.32	6±0,3		4,4
	(5,2-6.7)	(5.5-6.6)	(5,6-6,9)		(4.0-4.8)
c	11±0,9	9.8 ± 0.83	10,7±0,66	10.6 ± 0.9	10
	(9,93-14)	(8.1-10.8)	(9,7-12)	(8.8–12.9)	(9.1-11.2)
Body diam.	23,7±2,5	29 ± 1	29,5±1,6	27 ± 3	25
	(20-29)	(27-30)	(25-32,5)	(23–34)	(20-30)
EP	45±3,5	48 ± 1.5	60,6±3,3	55±5	38
	(39-52)	(46-51)	(53,5-65)	(47–70)	(30-60)
NR	70±2,5	68 ± 2.4	92±3,5	92 ±6	85
	(64-75)	(64-72)	(87,5-100)	(79 -103)	(76-99)
ES	91±4,8	89 ± 1.8	123,9±6	123 ± 7	120
	(80-105)	(85-92)	(112,5-135)	(108–144)	(103-190)
T	48,5±3,3	56 ± 3.2	72±4,97	66 ± 5	53
	(40-54)	(52-61)	(62,5-77,5)	(56–74)	(46-61)
ABD	12,9±0,9	29±1		15 ± 2	
	(12-15)	(27-30)		(9–18)	
D%	49,6±4,7	53 ± 0.02	50±3	44.9 ± 3.1	26
	(41-60)	(51-58)	(40-60)	(40.0–55.8)	(23-28)
E%	93±8,2	86 ± 0.05	80±6	84.2 ± 6.0	60
	(81-113)	(79-94)		(73.8–96.4)	(54-66)
Two "Horns" on Head	+	+	+	+	-
Reference		Elawad Ahmad & Reid (1997)	Tallosi & Ehlers (1995)	Jian et al. (1997)	Poinar (1967)

* Measurements are in µm and in the form: mean±SD (range).

Justification based on the morphometrics and morphology of first generation males

The length of the first generation males of the unknown nematode (1269 µm) showed closeness to *S. abbasi*, *S. bicornutum* and *S. ceratophorum*. In addition to the measurements, the absence of mucrons in first generation males was also used in the diagnosis (Table 2). Maximum body diameter, excretory pore (EP), nerve ring (NR), eusophagus length (ES), spicule length (SL), spicule width (SW), gubernaculum length (GL) and gubernaculum width (GW) of the unknown nematode had values similar to *S. abbasi*. In addition, the observation of caudal and precloacal papillae also helped supportour reasoning. We also observed one of the diagnostic features of *S. abbasi*, a single large midventral precloacal papilla. The tail was also short, conoid and relatively similar to the drawings of *S. abbasi*.

Furthermore, comparison of excretory pore (EP) and spicule length (SP) showed similarities with *S. carpocapsae*, *S. bicornutum* and *S. ceratophorum*. The presence of mucrons showed a clear difference between *S. carpocapsae* and the unknown nematode. In additionfurther comparisons made by looking at characteristic features of *S. abbasi* helped to eliminate other species, e.g., shape of spicule and gubernaculum, golden dark yellow coloured spicules and absence of terminal mucrons. Comparison using both morphological and morphometric measurements showed great similarities between the unknown species and *S. abbasi*. Therefore, it is possible to state that the unknown nematode most closely approximated *S. abbasi* in terms of 1st generation males (Fig. 2).

Figure 2. *Unknown nematode* A. General overview of 1st generation male, B. Excretory pore, C. Tail region - spicule and gubernaculum shape

Table 2. Morphometric measurements of 1st generation males of Strain 1 of an unknown nematode and a comparison with the closest species

Species/ Character	Strain 1	*S. abbasi*	*S. bicornutum*	*S. ceratophorum*	*S. carpocapsae*
n	20	15	20	35	25
L	1269±116,7	1252 ± 189	1352±149	1358±134	1450
	1080-1498	(999-1534)	(945-1539)	(1136-1694)	(1090-1710)
Body diam.	85,76±5,7	87 ± 6,7	108±11	146±21	102
	76-98	(82-98)	(80-127)	(104-185)	(77-131)
EP	74,32±5,2	80 ± 7,8	82±8	85±11	61
	(66-82)	(68-89)	(67,5-97,5)	(50-104)	(47-74)
NR	97,6±14,2	103.20 ± 6,48	123±8	123±14	110
	(68-124)	(99-123)	(107-137)	(90-147)	(93-124)
ES	134,56±20	133 ± 6	156±7	165±10	155
	(100-166)	(121-144)	(137-167)	(149-190)	(136-167)
Testis reflection	176,48±28	274 ± 33		393±94	563
	(110-220)	(234-319)		(163-574)	(400-808)
T	17,52±2,5	26 ± 3	31,5±2,5	30±4	30
	(14-22)	(20-31)	(25-35)	(23-38)	(23.4-39)
ABD	28,12±4	43 ± 4,90		52±5	42.6
	(22-38)	(37-55)		(45-70)	(32.5-54.6)
SL	67±4,8	65 ± 5,70	65±4	71±7	64.6
	(60-78)	(57-74)	(52,5-70)	(54-90)	(58.5-71.5)
Spicule Width	13,36±4,8	12 ± 1.30		11	11.Oca
	(10-18)	(10-14)		(9-16)	(9.1-13)
GL	47,72±6	45 ± 4.30	47,9±3,5	40±4	47
	(39-62)	(33-50)	(37,5-50)	(25-45)	(39-56)
Gubernaculum Width	6,56±0,89	7 ± 0.10		7±1	5,2
	(5-8)	(6-8,5)		(5-9)	(3.9-6.5)
D%	56±9	60 ± 5	50±3	51,4±7,2	
	(42,5-74)	(51-68)	(50-60)	(32.8-64.8)	
E%	424,2±77,3		260±24		
	(300-571)		(220-310)		

Table 2 continued

SW%	238,2±37,4	156±22		140±20	
	(173-327)	(107-187)		(10-20)	
GS%	71,2±10,5	70±0,07		60±10	
	(56-93)	(58-85)		(40-80)	
Mucron	Absent	Absent	Absent	Absent	Present
Reference		Elawad Ahmad & Reid (1997)	Tallosi & Ehlers (1995)	Jian et al. (1997)	Poinar, 1967.

* Measurements are in µm and in the form: mean±SD (range).

Conclusion

Previous work using morphological and morphometric measurements has shown the high intraspecific variation of *Steinernema* species (Tabassum & Shahina, 2004). Our study combined and compared different identification methods for *Steinernema* spp. based on morphological and morphometric characterization. However, as some identifications cannot be justified by the current methods, further studies need to be conducted with different methods.

Based on the morphometric values and morphological analysis of the unknown nematode in the current study, namely strain 1, *S. bicornutum*, *S. ceratophorum*, *S. carpocapsae* and *S. abbasi* had values that most often matched those of the unknown nematode. However, it had more values in common with *S. abbasi* which justified the unknown nematode being identified as *S. abbasi*.

Although identification based on morphometrics can be helpful in giving a good idea of the possible identification of the nematode species in question, errors due to inconsistencies in morphometric values are not uncommon due to obscuration of certain anatomorphological structures or poor fixation of the specimen. Therefore, molecular analysis is required in addition to morphological and morphometric characteristics for a more holistic and accurate and identification.

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