YYÜ TAR BİL DERG
(YYU J AGR SCI)
2017, 27(4): 543-549

Geliş Tarihi (Received): 20.09.2017
Kabul Tarihi (Accepted): 20.11.2017
doi: 10.29133/yyutbd.339081

Araştırma Makalesi/*Research Article (Original Paper)*

Screening Fungicide Resistance of *Alternaria* Pathogens Causing Alternaria Blight of Pistachio in Turkey

Hilal OZKILINC[1*], Sener KURT[2]

[1]Çanakkale Onsekiz Mart University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, Çanakkale, Turkey
[2]Mustafa Kemal University, Faculty of Agriculture, Department of Plant Protection, Hatay, Turkey
e-mail: hilalozkilinc@comu.edu.tr

Abstract: Alternaria blight is one of the important diseases of pistachio and wild relatives, and its management in pistachio orchards mainly relies on fungicide applications. But, it is observed the disease cannot be declined or controlled by fungicide treatment that might be due to development of fungicide resistance in *Alternaria* pathogens. On the other hand, wild *Pistacia* species and some pistachio trees which cultural practices are not applied are not exposed to fungicide treatments in nature. Isolates from orchards and wild systems may show difference in their sensitivities to fungicides. In this study, twenty-two isolates of *Alternaria* species from different hosts of *Pistacia* species were investigated *in vitro* for their sensitivity to different fungicides having different mode of action. Fungicide sensitivity were evaluated based on mycelial growth and spore germination of the isolates by using effective concentrations. The least effective fungicide was azoxystrobin and the most effective one was boscalid + pyraclostrobin among the fungicides tested in this study. Partial gene sequencing of succinate dehydrogenase genes presented boscalid sensitivity of the isolates according to the specific mutations related with boscalid resistance. This study is the first initiation to observe sensitivity of *Alternaria* pathogens from pistachio and wild relatives against to the most commonly used commercial fungicides in Turkey.

Keywords: *Alternaria*, pistachio, fungicide

Türkiye'de Antepfıstığı'nda Alternaria Yanıklık Etmeni *Alternaria* Patojenlerinin Fungisit Direncinin İncelenmesi

Özet: Alternaria yanıklığı, Antepfıstığı ve yabani akrabalarını etkileyen önemli hastalıklardan biridir ve Antepfıstığı bahçelerinde hastalık kontrolü fungisit uygulamalarına dayalı olarak yürütülmektedir. Fakat, fungisit kullanımı ile hastalığın azalmadığı veya kontrol edilemediği görülmüştür ki bu durum *Alternaria* patojenlerinde fungisit direnci gelişmiş olmasından dolayı olabilir. Diger taraftan, yabani *Pistacia* türleri ve kültürel pratiklerin uygulanmadığı bazı Antepfıstığı ağaçları doğada fungisit uygulamalarına maruz kalmamaktadır. Kültür bahcelerindeki ve yabani ekosistemdeki izolatlar, fungisitlere karşı duyarlılıkta farklılık gösterebilir. Bu çalışmada, *Pistacia* türlerinin farklı konukçularından örneklenmiş yirmi iki izolatın, farklı etki mekanizmasına sahip fungisitlere karşı *in vitro* duyarlılıkları araştırılmıştır. Fungisit duyarlılıkları izolatların miselyal gelişim ve spor çimlenmesi üzerinden etkili konsantrasyon verileri ile değerlendirilmiştir. Test edilen fungisitler arasında izolatlar üzerinde etkisi en düşük olan fungisit azoxystrobin, en etkili olan ise boscalid+pyraclostrobin olarak bulunmuştur. Boscalid direnci ile ilişkilendirilen polimorfizmlere göre suksinat dehidrogenaz genlerinin kısmi baz dizilim verileri izolatların duyarlı olduğunu göstermistir. Bu çalışma, Türkiye'de, Antepfıstığı ve yabani akrabalarından örneklenmis *Alternaria* izolatlarının yaygın ticari kullanımı olan fungisitlere karşı duyarlılıklarını belirlemek üzere yapılan ilk başlangıç çalısmasıdır.

Anahtar kelimeler: *Alternaria*, antepfıstığı, fungisit

Introduction

Alternaria blight is one of the most important disease of pistachio (Pryor and Michailides 2002; Michailides 2005; Ozkilinc et al. 2015). Morphologically three (*Alternaria alternata, A. tenuissima* and *A. arborescens*) and phylogenetically two species (*A. alternata/tenuissima* and *A. arborescens*) is responsible for the disease (Pryor and Michailides 2002; Ozkilinc and Sevinc 2016; Ozkilinc et al. 2017). Fungicide treatment is widely used

against pathogens. But, fungicide application cannot provide enough protection, besides, fungicide resistant pathogen population may arise (Maand Michailides 2005).

Fungicide efficiency and fungicide resistance in pathogens have been studied for *Alternaria* blight of pistachio in California/U.S.A. In the first studies, copper hydroxide and copper oxide were successful for protection against to the disease (Michailides and Morgan 1993). Moreover, it is reported that benomyl was reduced spots on the fruits (Michailides and Morgan 1993). In continuous studies, azoxystrobin was shown as another efficient fungicide against *Alternaria* blight (Michailides et al. 1999). But, later on, fungicide resistant *Alternaria* isolates were found (Ma et al. 2003; Ma and Michailides 2004a and b). Then, *Alternaria* isolates from pistachio were found resistant to boscalid (Avenot and Michailides 2007) and pyraclostrobin + boscalid (Avenot et al. 2008). Thus, it is shown that fungicides can loss their efficiency by the time and fungicide resistant isolates may appear in the populations. In these situations, control strategies should be revised and updated considering changes in the pathogen population structure.

It is known that there is no registered fungicide against to alternaria blight disease of pistachio. But, some fungicides are used against to *Alternaria* diseases on different hosts. For example, fungicide including azoxystrobin (trade name is Quadris SC) is registered against to *A. solani* from tomato and *A. cucumerina* from watermelon; fungicide including pyraclostrobin + boscalid (trade name is Bellis) is registered against to *A. solani* from potato and *A. cucumerina* from watermelon; fungicide including azoxystrobin + difenoconazole (trade name is Quadris Maxx) is registered against to *A. solani* from potato, *A. cucumerina* from melon and *A. alternata* from pomegranate; fungicide including trifloxystrobin (Flint 50 WG) is registered against to *A. alternata* from apple and *Citrus* spp., dodine is widely used against Septoria disease of pistachio in Turkey. Iprodione is also used against *Alternaria* diseases in Turkey and worldwide. Difenoconazole + propiconazole, dodine and copper oxychloride are widely used against to the disease even if these fungicides cannot completely prevent the disease. *Alternaria* blight disease has been observed both in pistachio and its wild relatives in Turkey and the disease agents were characterized based on morphology, pathology and molecular/phylogenetic aspects (Ozkilinc et al. 2016). The disease is common wherever pistachio trees are grown.

Considering common prevalence of the disease even though fungicide application, it is thought that the pathogen bear fungicide resistance traits in the populations. Determination fungicide resistance and which fungicide loss their efficiency against the pathogen should be determined for better control strategies. On the other hand, it has been known that some of the pistachio trees grown in wild or wild hosts (*Pistacia* species) are not exposed to the fungicide treatments. Thus, it is possible that fungicide application may favour *Alternaria* pathogens for different selection pressures in view of fungicide resistance trait in wild and agricultural ecosystems.

Thus, different fungicides were chosen considering wide range of application of fungicides for different alternaria diseases as well as different fungicide classes based on the mode of action of active ingredient to test on *Alternaria* pathogens from pistachio and wild relatives. *Alternaria* spp. isolates were chosen from the wild and cultivated pistachio hosts where has been exposed to the fungicide treatment or not. Thus, both resistance of isolates against different fungicide groups and effect of fungicide treatment history on the isolates were evaluated.

Materials and Methods

Fungal isolates

In vitro tests were conducted using 10 isolates from the hosts which has fungicide treatment history and 12 isolates from the hosts which has no fungicide treatment history (Table 1). A total of 22 isolates were used in the study (Table 1). Information about fungicide treatments were known from the growers. Hosts not treated with fungicides are mostly wild species which have not exposed to any cultural practices or some pistachio trees which grown out of an orchard. All these isolates were from single spore cultures, morphologically and phylogenetically identified and known as pathogenic from previous studies (Ozkilinc et al. 2017; Ozkilinc and Sevinc 2016; Ozkilinc H unpublished data).

Table 1. *Alternaria* spp. isolates used in this study

Isolates which were exposed to fungicide			Isolates which were not exposed to fungicide	
Isolate code*	Host	Fungicide**	Isolate code	Host
27-34-G[1]	*P. vera*	Armure	46-02-G/1[3]	*P. khinjuk*
27-42-G/1[1]	*P. vera*	Bellis	02-49/2-G[1]	*P. vera*
27-50[1]	*P. vera*	Armure	47-64/G[1]	*P. vera*
02/28[2]	*P. vera*	Bellis	47-61-GY[1]	*P. vera*
27-39-1/G[1]	*P. vera*	Dodine	47-73-GY[1]	*P. vera*
47-71-G[1]	*P. vera*	Armure	35-22-1[1]	*P. lentiscus*
47-46-GY[1]	*P. vera*	Armure	35-45[1]	*P. lentiscus*
47-43/1-GY[1]	*P. vera*	Armure	46-02-1	*P. vera*
63-02-2[1]	*P. vera*	Armure	45-42[1]	*P. khinkuk*
63-02-4[1]	*P. vera*	Armure	27-02/1-G[1]	*P. terebinthus*
			35/08[1]	*P. atlantica*
			33-67-GY-1[1]	*P. mutica*

*Isolates morphologically were defined as [1] *A.alternata*, [2] *A. arborescens*, [3] *A. tenuissima*
**Growers' information about fungicide application

Fungicides

Six different fungicides were used in the experiments. Fungicides used in *in vitro* experiments were formulated. Active ingredients, trade names and some other technical properties were presented in Table 2. Different concentrations for each fungicide were applied in *in vitro* tests. Concentrations were decided based on some preliminary studies. Aliquots of fungicide solutions were added into potato dextrose agar medium at 50 ºC.

Table 2. Fungicides used in this study

Active ingredient	Chemical Group	Fungicide trade name	Company	Form
Iprodione / %50	Dicarboxamide	Rovral 50 WP	Bayer	WP
Azoxystrobin / 250 g L^{-1}	Strobilurin- QoI	Quadris SC	Syngenta	SC
Tebuconazole / 250 g L^{-1}	Triazole-DMI	Folicur EC 250	Bayer	EC
Dodine / %65	Guanidine	Dodene 65 WP	Tarkim	WP
Difenoconazole + propiconazole/ 150 g+150 g L^{-1}	Triazole-DMI	Armure 300 EC	Syngenta	EC
Pyraclostrobin + boscalid/ % 12.8 + 25.2	Strobilurin- QoI + carboxamide	Bellis WG	BASF	WG

Assay on mycelium

In order to determine mycelial sensitivity of 22 isolates of *Alternaria* spp. to the six fungicides were used following concentrations: 0.01, 0.1, 0.5, 1, 10, 50 µg mL^{-1} for Iprodione; 0.0025, 0.01, 0.04, 0.16, 0.625, 2.5, 10, 50, 100 µg mL^{-1} for azoxystrobin; 0.1, 0.5, 1.0, 2.0, 5.0, 10 µg mL^{-1} for tebuconazole; 0.01, 0.1, 1.0, 10, 50, 100, 200 µg mL^{-1} for dodine; 0.01, 0.1, 0.5, 1.0 µg mL^{-1} for propiconazole + difenoconazole; and, 0.1, 0.5, 1.0, 3.0 µg mL^{-1} for boscalid + pyraclostrobin. Mycelial discs of pathogens (5 mm in diameter) taken from the margins of 7 days old cultures were transferred to PDA media amended with the fungicide at tested concentration. Three replicates were used per treatment. Control plates were included PDA without fungicide. Cultures were incubated at 20 ºC in the dark for 7 days. Mycelial colony growth was measured daily and average of colony diameter was used. Results were expressed as effective concentration (EC_{50}) which is the concentration reducing mycelial growth by 50 %, determined by regression the inhibition of radial growth values (% control) against the log10 values of the fungicide concentration. All experiments were repeated twice. EC_{50} values from the experiments were evaluated and the range of EC_{50}, mean and SE values were determined for mycelial growth.

Assay on conidia

Concentrations of 0.01, 0.1, 0.5, 1, 10, 50 ve 100 µg mL^{-1} for Iprodione, concentrations of 2.5, 10 for azoxystrobin, concentrations of 50, 100 µg mL^{-1} for azoxystrobin, concentrations of 10, 25, 50, 100 and 200 µg mL^{-1} for tebuconazole, concentrations of 5, 10, 20, 25, 50 µg mL^{-1} for dodine, concentrations of 10, 20, 50, 80 µg mL^{-1} for propiconazole + difenoconazole, concentrations of 0.01, 0.1, 0.5, 1.0 µg mL^{-1} for boscalid + pyraclostrobin were tested against to 22 isolates of *Alternaria* spp. for their effects on spore germination. Spore

suspension ($1x10^6$ conidia per mL) was prepared in distilled water from 7 days old cultures. Then, 100 µL aliquots of the spore suspensions were spread onto the surface of PDA plates supplemented with fungicide at specific concentration. Each treatment was replicated three times. After incubation of 14-18 h at 20 ºC in the dark, the percentage of spore germination (100 spores for each treatment) and the length of germ tube (the germ tube length was about at least three times of the conidium length) were estimated under a microscope. Results were expressed as effective concentration (EC_{50}) which is the concentration causing 50 % reduction in the length of germ tubes. All experiments were repeated twice. EC_{50} values from the experiments were evaluated and, the range of EC_{50}, mean and SE values were determined for conidia germination.

Partial sequencing of SDH regions

Succinate dehydrogenase B, C, and D genes of *Alternaria* species were investigated for any related mutation against fungicides. Primers were designed based on data available in GenBank (EU178851.1 for sdhB, FJ437067.1 for sdhC, FJ 437068.1 for sdhD). Primer3 software was used to design primers (Rozen and Skaletsky 2000). Primers were sdhB-F: CGTATAGCGCACAGTATCACAG and sdhB-R: CTAGCGCAGGGTTCAGTCC for sdhB; sdhC-F: TTCTCAGCGGGTATTTCAGC and sdhC-R: CTGAATGCGACGGTCAAG for sdhC; sdhD-F: GTCATGCGTCCCGGTCTC and sdhD-R: CTATGCGTGCCACAACCTC for sdhD. A 25 µL PCR reaction contained 15-20 ng of template DNA, 1X PCR buffer (Applied Biological Materials Inc., Canada), 1.5 mM $MgCl_2$ (Applied Biological Materials Inc., Canada) 200 µM dNTPs, and 0.4 µM of each primer. The PCR conditions were 95 °C for 5 min, 35 cycles at 94 °C for 20 s, 55 °C (sdhB and sdhC) or 58 °C (sdhD) for 30 s, and 72 °C for 30 s followed by a final step at 72 °C for 10 min. Reactions were carried out with a Bio-Rad T100 thermalcycler (Bio-Rad, USA). PCR products were detected on 1.5 % agarose gels, stained with 5 μL/100 mL of SafeView (Applied Biological Materials Inc., Canada) dye and visualized under UV light on a gel documentation system Vilber Lourmat Quantum ST4 1100 (Vilber Lourmat, France). After successful amplifications, PCR products were sequenced in ABI 3500xL Genetic Analyzer (Applied Biosystems, MedSantek Lab., İstanbul, Turkey). DNA sequences were controlled and edited with Bioedit v7.0.53 for Windows software (Hall 1999) and aligned using clustalW implemented in BioEdit software.

Results and Discussion

Effects of fungicides on mycelium growth and spore germination

Twenty-two isolates of *Alternaria* spp. were tested towards fungicide effect on mycelial growth and spore germination. Range of EC_{50} values for mycelial growth was shown in Table 3. $EC_{50} \geq 0.1$ µg/mL value is considered to determine sensitivity of the pathogens.

Azoxystrobin which is from strobilurins (Qol inhibitors) were not found effective on *Alternaria* spp. isolates. The isolates were resistant even its highest concentration. The highest concentration of azoxystrobin (100 µg mL^{-1}) just reduced mycelial growth at 68%. EC_{50} values were 0.48-58.42 µg mL^{-1} for azoxystrobin showed that isolates are resistant to this fungicide. Even the highest concentration of azoxystrobin (100 µg mL^{-1}) provided just 60% reduction of spore germination. EC_{50} values were 12.82-168.13 µg mL^{-1} for azoxystrobin on spore germination (Table 3). It is known that azoxystrobin resistance is commonly seen in *A. alternata* populations from pistachio in California (Ma et al. 2003; Avenot et al. 2008a). Continuous application of this fungicide might be useless in near future even in the highest concentration of azoxystrobin due to resistance development.

The high EC_{50} values indicated that *Alternaria* spp. isolates were resistant against dodine. Even the highest concentration of dodine (200 µg mL^{-1}) just was able to reduced 46% of mycelial growth of the isolates. Dodine reduced 100 % of spore germination with the highest concentration. But, high EC_{50} values both for mycelial growth and spore germination indicated that isolates are resistant against dodine. Dodine is from aliphatic nitrogen fungicides having broad spectrum. It has not been found any report for dodine sensitivity of *Alternaria* from pistachio or from any other host.

EC_{50} values of iprodine were between 0.17-3.14 µg mL^{-1} on mycelial growth indicating reduced sensitivity against this fungicide. EC_{50} values were 0.01- 13.46 µg mL^{-1} for iprodione on spore germination. By increasing concentration for iprodione, spore germination of *Alternaria* spp. isolates were reduced gradually. Iprodione concentration differed to prevent mycelial growth and spore germination (Table 3). Ma and Michailides (2004a) showed that iprodione resistant *Alternaria* isolates from pistachio were sensitive to azoxystrobin and tebuconazole. But, it was not found any strict correlation for the cross-resistance among the fungicides tested in this study.

Isolates were tested against to demethylation inhibitors (DMI) such as tebuconazole, difenoconazole and propiconazole. By increasing concentration for tebuconazole, mycelial growth of *Alternaria* spp. isolates were reduced gradually. EC_{50} values were 0.53-5.62 µg mL^{-1} for tebuconazole which indicates resistance of the isolates against tebuconazole, as well. The highest concentration of tebuconazole (10 µg mL^{-1}) reduced mycelial growth of the isolates at 80%. Comparing effect on mycelial growth, higher concentration of tebuconazole was effective to inhibit spore germination. EC_{50} values were 7.72- 55.60 µg mL^{-1} on spore germination. Propiconazole + difenoconazole was effective on *Alternaria* spp. isolates even application of the lowest concentration. EC_{50} values of propiconazole + difenoconazole were 0.01-0.02 µg mL^{-1} on mycelial growth and 38.05-129.79 µg mL^{-1} on spore germination. EC_{50} values for propiconazole + difenoconazole differed for the effects on mycelial growth and spore germination (Table 3). The highest concentration of this fungicide mixture (80 µg mL^{-1}) inhibited 49 % of spore germination. Once *Alternaria* isolates from pistachio in California tested against to difenoconazole, propiconazole, and tebuconazole based on mycelial growth assay, the DMI-exposed population was found to be less sensitive compared with the populations collected in 1998-2003 and 2010 which is the time before fungicide registration (Avenot et al. 2016). Propiconazole + difenoconazole was effective in reducing mycelial growth, but, less effective on conidia germination in this study. Thus, application of this fungicide mixture when spore dispersion and primary inoculation of the pathogen, the initiation of the disease by spore germination will not be prevented.

Boscalid + pyraclostrobin was effective to inhibit mycelial growth of *Alternaria* spp. isolates with EC_{50} values of 0.04-1.72 µg mL^{-1}. Boscalid + pyraclostrobin was effective on spore germination even its the lowest concentration. Its highest concentration reduced spore germination at 100 %. EC_{50} values were 0.03-0.11 µg mL^{-1} for boscalid + pyraclostrobin on spore germination. Boscalid + pyraclostrobin was the most effective one both on mycelia and conidia of the fungus. Since isolates were not sensitive against to the strobilurin group considering azoxystrobin effects, boscalid seems responsible for the strong effect of this fungicide mixture. Boscalid works as succinate dehydrogenase inhibitor and cause inhibition of respiration. Recently, resistance against boscalid and boscalid + pyraclostrobin were detected among the isolates of *A. alternata* from pistachio in California (Avenot et al. 2008a and b).

Table 3. Responses of *Alternaria* spp. isolates to different fungicides based on EC_{50} values

	Mycelial growth			**Conidia germination**		
Fungicides	**Range of EC_{50}**	**Mean**	**±SE***	**Range of EC_{50}**	**Mean**	**±SE**
Iprodione	0.17-3.14	0.87	0.17	0.01-13.46	7.43	0.06
Azoxystrobin	0.48-58.42	5.89	0.06	12.82-168.13	54.50	0.10
Tebuconazole	0.53-5.62	2.27	0.16	7.72-55.60	30.41	0.13
Dodine	17.82-966781.12	73751.4	0.06	9.48-15.80	13.18	0.21
Propiconazole+ difenoconazole	0.01-0.02	0.01	0.18	38.05-129.79	90.06	0.19
Boscalid+pyraclostrobin	0.04-1.72	0.48	0.23	0.03-0.11	0.08	0.11

*± SE:Standard Error is the standard deviation of range of EC_{50}.

Partial sequencing results for succinate dehydrogenase gene regions

Boscalid inhibits respiration and boscalid resistance is related with mutations on succinate dehydrogenase (sdh) genes. Amplicons about 1000 bp, 600 bp and 630 bp were amplified for sdhB, sdhC and sdhD regions, respectively. Partial sequences of sdhB, sdhC and sdhD showed no variation among *A. alternata/A. tenuissima* isolates, but, showed polymorphisms between *A. alternata/tenuissima* and *A. arborescens* which indicates that these regions might be used for species level identifications, as well. Sequences of 519 bp and 552 bp for sdhC and sdhD, respectively, were blasted in NCBI nucleotide database. *A. alternata/tenuissima* isolates were matched with *A. alternata* isolates with accession numbers FJ437067.1 (between 68-586 nucleotide positions) and KJ426274.1 (between 72-623 nucleotide positions) for sdhC and sdhD, respectively. *A. arborescens* isolate matched with *A. arborescens* with accession numbers KR091579.1 (between 68-586 nucleotide positions) and KR091587.1 between 72-563 nucleotide positions) for sdhC and sdhD, respectively. 914 bp sequence length of sdhB for *A. alternata/tenussima* isolates matched with KJ426260.1 (between 104-1017 nucleotide positions). *A. arborescens* isolate matched with *A. arborescens* / KR091575.1 for sdhB (between 112-635 nucleotide positions). Mutations at positions 277 in sdhB, 134 in sdhC, and 133 in sdhD are mostly carried by sdh inhibitory fungicide-resistant isolates of *A. alternata* from pistachio in California (Avenot et al. 2008b; Avenot et al. 2009). Vega and Dewdney (2015) investigated boscalid sensitivity of 15 isolates of *A. alternata* from Citrus and looked-for polymorphism at sdhB, sdhC and sdhD regions. These isolates showed variability against to boscalid and all the three regions exhibited polymorphisms, but, no mutations were detected at specific position reported by Avenot et al. (2008b) and Avenot et al. (2009) (Vega and Dewdnet 2015). Since the isolates tested in this study

were sensitive against boscalid, we did not have any chance to compare sequences of sdh genes for resistant isolates. However, our isolates presented sensitive type sequences at codon 134 in sdhC and at codon 133 in sdhD. In view of sdhB mutation, *A. alternate/tenuissima* isolates used in this study were also found as sensitive in comparison with sequences boscalid sensitive (EU178851.1) and resistant (EU178852.2) *A. alternata* isolates (Avenot et al. 2008b).

Conclusion

This study is an initiation to monitor sensitivity of *Alternaria* isolates from pistachio and wild relatives against the most commonly used commercial fungicides. First findings indicate that isolates show different fungicide phenotypes to different fungicides groups and their sensitivity differ in their mycelial growth and conidial germination. This is quite important in view pathogen life cycle and disease development. Azoxystrobin was the least effective on both mycelia and conidia. Among the fungicides tested in this study, boscalid + pyraclostrobin was the most effective one on both mycelia and conidia of the fungus, even it's the lowest concentration. On the other hand, iprodione, tebuconazole and dodine were not effective enough against to the *Alternaria* pathogens. Thus, it seems that fungicide resistance may increase in the populations. Sensitivity of the isolates against to the fungicides did not show any differences according to the sampling source such as fungicide treatment history. This could be due the mainly clonal growth/expansion of the pathogen. There was one available *A. arborescens* isolates in the collection and it was also did not show any different fungicide phenotype comparing to the *A alternata/tenuissima* isolates. It is required to conduct tests on more fungal isolates. Besides, it would be beneficial to support *in vitro* experiment by testing *in vivo* experiments. Furthermore, effects of fungicides having site specific actions could be explored by mutations on related genes. If fungicide related mutation was detected, pathogen population could be screened easily for their fungicide sensitivity. Fungicide application is one of the strong directional selection factor on pathogens. Increased resistance could be so dangerous such a clonal pathogen due to maintain of beneficial mutations in the populations.

Acknowledgement

This research is supported by TUBITAK, TUBITAK-TOVAG-112O554 project. The authors would like to thank Pistachio Research Institute for technical supports.

References

Avenot H, Solorio C, Morgan DP, Michailides TJ (2016) Sensitivity and cross-resistance patterns to demethylation-inhibiting fungicides in California populations of *Alternaria alternata* pathogenic on pistachio. Crop Protection 88: 72-78.

Avenot H, Morgan DP, Michailides TJ (2008a) Resistance to pyraclostrobin, boscalid and multiple resistance to Pristine® (pyraclostrobin + boscalid) fungicide in *Alternaria alternata* causing Alternaria late blight of pistachios in California. Plant Pathology 57: 135-140.

Avenot H, Sellam, A, Karaoglanides G, Michailides TJ (2008b) Characterization of Mutations in the Iron-Sulphur Subunit of Succinate Dehydrogenase Correlating with Boscalid Resistance in *Alternaria alternata* from California Pistachio. Phytopathology 98: 736-42.

Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis. Nucleic Acids Symposium Series 41: 95-8.

Hu J, Chen C, Peever T, Dang H, Lawrence C, Mitchell T (2012) Genomic characterization of the conditionally dispensable chromosome in *Alternaria arborescens* provides evidence for horizontal gene transfer. BMC Genomics 6: 13-171.

MA Z, Michailides TJ (2005) Advances in understanding molecular mechanisms of fungicide resistance and molecular detection of resistant genotypes in phytopathogenic fungi. Crop Protection 4: 853-863.

MA Z, Michailides TJ (2004a) Characterization of Iprodione-resistant *Alternaria* isolates from pistachio in California. Pesticide Biochemistry and Physiology 80: 75-84.

MA Z, Michailides TJ (2004b) An allele-specific PCR assay for detecting azoxystrobin-resistant *Alternaria* isolates from Pistachio in California. Journal of Phytopathology 152: 118-121.

MA Z, Felts D, Michailides TJ (2003) Resistance to azoxystrobin in *Alternaria* isolates from pistachio in California. Pesticide Biochemistry and Physiology 77: 66-74.

Michailides T (2005) Above Ground Fungal Diseases. Pistachio Production Manual. Ed: In: Ferguson, L. CA, USA.

Pryor B M, Michailides TJ (2002) Morphological, pathogenic and molecular characterization of *Alternaria* isolates associated with Alternaria late blight of pistachio. Phytopathology 92: 406-16.

Ozkilinc H., Sarpkaya K., Kurt S., Can C., Polatbilek H., Yasar A., Sevinc U., Uysal A., Konukoglu, F. (2017) Pathogenicity, Morpho-Species and Mating Types of *Alternaria* spp. causing Alternaria blight in *Pistacia* spp. in Turkey, Phytoparasitica, https://doi.org/10.1007/s12600-017-0624-8.

Ozkilinc H, Sevinc U (2016) Molecular phylogenetic approaches to defining Alternaria species associated with Alternaria blight of pistachio and its wild relatives. Turkey 6th Plant Protection Congress with International Participation. Page: 487.

Ozkilinc H, Kurt S, Can C, Konukoğlu F, Aktan A, Uysal A, Polatbilek H, Yasar A, Sevinc U and Sarpkaya K (2015) Alternaria blight of Pistacia species in Turkey. XVI GREMPA Meeting. Page 64.

Rozen S, Skaletsky H (2000) Primer3 on the WWW for general users and for biologist programmers, Methods Mol Biol 132: 365–386.