



Molecular Characterization of Natural Fungal Flora in Black Olives: From Field to Table

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ABSTRACT

In this study, molecular markers were used to determine fungal flora in black olive fruits from field surveys to the table, following the fermentation process. Field samples were collected from different locations of Çanakkale province, including Gokceada (Imbros), where organic farming is employed. Some of the fruits from field samples were used for black table olive production and then fungal flora was tracked during the fermentation process. Fungal isolation was also conducted on some commercial samples. Fifty seven isolates from field samples, 56 isolates from the fermentation process and 17 isolates from commercial products were obtained. Among these isolates, 41 *Alternaria*, 43 *Penicillium*, 19 *Aspergillus*, 8 *Monascus* and 19 other genera were determined using amplified sizes of the *Beta-tubulin* gene region. Species level identification was carried out based on sequences of *Beta-tubulin* amplicons, which provided accurate identification, especially where the genera were morphologically highly similar. The occurrence and prevalence of fungal species changed in fungal collections from the field to the fermentation process. While *Alternaria alternata* was common in field samples, they were absent during fermentation. Many of these identified species, such as *Penicillium expansum*, *Aspergillus niger* and *Monascus pilosus*, which are known as potential toxin producers such as aflatoxin, ochratoxin A and citrinin, were found both in natural and fermented samples, even at the end of the fermentation process. These results showed that some fungal species which survive on olives from the field to the table are potential toxin producers and can be successfully characterized by amplification and sequencing of *Beta-tubulin* gene.

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Introduction

Olive is the common name for the greenish fruits of the *Oleaceae* family. Olive is an important part of the Mediterranean diet, and defined as a functional food because of their antimicrobial, antimutagenic, anticarcinogenic and antioxidant properties (Sahin et al., 2010; Marsilio, 2001). They also differ from other fruits due to their high oil content and bitter taste which is mainly caused by oleuropein, a phenolic compound. Anthocyanins, flavanols, phenolic acid and phenolic alcohols are important phenolic compounds in olives (Baskou, 2015). These phenolic compounds play an important role in general antioxidant activity.

Olive is one of the most important agricultural products in Turkey with 1.73 million tons of olive production, and where 430,000 tons of these olives are used in the production of table olive (TUIK, 2016). Çanakkale provides about 4.2% of total olive production in Turkey (TUIK, 2013). Table olive processing is carried out by small family companies in Çanakkale (Ozkaya et al., 2010).

In Turkey, the most common process for black table olive is spontaneous fermentation. In this method, olive fruits are harvested when they have a black outer shell and a violet interior, then they are fermented in brine with a concentration of 6 to 10% salt (Aran, 2012). Fungal flora that form as a biofilm on the olive brine pool (called 'Kefeke' in Turkey), can occur in olives that are packaged or being prepared for packing. While *Penicillium* is the most common species, *Aspergillus*, *Cladosporium*, *Ulocladium*, *Rhizopus*, *Alternaria* and *Paecilomyces* are the other fungal species found in olives (Sahin and Korukoglu, 2000). The fungal flora may come from the field before the fermentation process, alive and reproduce during fermentation or appear due contamination during the process, and some of the fungal species may produce mycotoxins, which are harmful for human consumption.

Molecular techniques provide faster and more accurate diagnosis of fungal species than traditional approaches. For example, yeast in table olives have been identified by molecular methods (Arroyo-López et al.,

2006). Molecular methods are used for the identification and diversity of fungal flora in many foods such as coffee beans, maize grains, grapes (Magnani et al., 2005; Lumi et al., 2015; Wang et al., 2015). The ribosomal DNA region, and some protein-coding regions such as calmodulin, β -tubulin and elongation factors are commonly used in fungal systematics (Carbone and Kohn, 1999; Russell and Peterson, 2006; Gonvances et al., 2012). Primer pair developed for β -tubulin region could widely be used to detect different fungal species (Glass and Donaldson, 1995). In this study, fungal flora was characterized in black table olives from the field to the table using a molecular approach to observe diversity and changes in fungal species.

Materials and Methods

Plant Material

Olive fruits were collected from different locations of Canakkale province, including Gokceada, Ezine, Intepe, the city center of Canakkale and Assos in November of 2015 (Table 1). Healthy, physically injured excluding insect infested ones, diseased and moldy fruits were collected from both trees and ground. Sampling were done from 27 trees of olive cultivars Edremit and Ladolyes, and 7 wild olive trees (Table 1). Wild trees were not exposed to any cultivation practices except being used as rootstock. Fruit samples were categorized according to their collection source (state of health, from a tree or ground, from wild or cultivated trees, olive cultivars) to associate fungal isolates.

Black Table Olive Production

Fully mature black olives from Edremit cultivar were harvested from trees planted in the Ezine county of Canakkale and used for natural black table olive production. Lab-scale production (n=4) was carried out in plastic containers (10 L), filled with 7 kg of olive fruits and 3 L of brine (10% NaCl w/w). The containers were incubated for natural fermentation at room temperature. The brine was not replaced with a fresh solution during the fermentation process. Sampling was carried out during

the fermentation process: on the first day to see what initially occurs, on the day 120, which is when the biofilm begins to form and on the day 240, which is the end of the process. In addition, fermented fruit samples were obtained from five olive-producing commercial companies that produce table olive in Ezine/Canakkale.

Fungal Isolations and DNA Extraction

Surface sterilization of the fruit samples were carried out with 2.5% (w/w) sodium hypochlorite (NaOCl) for 3 min, and then rinsed three times with sterile distilled water and left to dry. After surface sterilization, healthy fruits were directly placed on Potato Dextrose Agar (PDA) (Merck, Germany). Fruits with disease, wounds or having naturally fungal hyphae before sterilization, were cut into small pieces and those parts were transferred to PDA Petri dishes and incubated under 12 h light and 12 h dark at room temperature for three to five days. Additionally, fungal isolations were performed from biofilm of fermented olive bottles. Sampling from biofilm was done at the end of the fourth and eighth months. Besides, biofilm sampling was taken from commercial olive-producing companies at the end of fermentation. Samples taken from the biofilm were transferred to a PDA medium and incubated at the same conditions stated above. All growing fungal samples from both fruit and biofilm sources were transferred to new PDA media, and single-spore isolations were done from pure cultures. Fungal cultures obtained from single spores were used in all analyses. Cultures from single-spore colonies were kept in sterile whatman no 1 paper at -20°C for long-term storage. Based on colony and spore morphologies, each isolate was evaluated morphologically, but molecular data was used for identification.

DNA was isolated from each fungal isolate obtained. Mycelia were grown in a Potato Dextrose broth (Merck, Germany) medium for five days in an orbital shaker at 150 rpm at room temperature. Mycelia were harvested and lyophilized to use in DNA isolation. Total genomic DNA extractions were carried out using an i-genomic Plant DNA Extraction Kit (Intron Biotechnology, Korea) according to the manufacturer's instructions.

Table 1 Plant materials obtained from Canakkale in 2014.

Region	Coordinates		Altitude (m)	Olive trees sampled		Number of fruits collected	Number of fungal isolates obtained
	Latitude	Longitude		Cultivar type	Number of trees sampled		
Gokceada	40°07'	25°44'	36	Edremit	4	20	15
				Ladolyes	9		
				Wild	2		
Ezine	39°46'	26°11'	12	Wild	2	28	23
				Edremit	10		
Assos	39°29'	26°19'	3-118	Wild	2	16	12
				Edremit	4		
Canakkale (around city center area)	40°07'	25°44'	86	Wild	1	6	7

Fungal Molecular Identification

The partial region of *Beta-tubulin* (β -*tubulin*) coding gene was used for molecular identifications. β -*tubulin* was amplified with primer pairs Bt2a and Bt2b (Glass and Donaldson, 1995), respectively. A 25 μ L volume of PCR containing 1x PCR buffer (Invitrogen), 3 mM of MgCl₂ (Applied Biological Materials Inc., Canada), 2 mM of each dNTP, 0.4 μ M of each primer, 1 U of Taq polymerase (Promega, Madison, WI) and 10 to 15 ng of DNA. The PCR conditions for the β -*tubulin* region were 95°C for 5 min, 45 cycles at 95°C for 20 s, 55 to 59°C for 30 s, and 72°C for 30 s, followed by a final step at 72°C for 5 min. All PCR reactions were carried out in a thermal cycler Bio-Rad T100 (Bio-Rad, USA).

PCR products were visualized on 1.5% agarose gels dyed with 5 μ L/100 mL of nucleic acid dye (SafeView, Applied Biological Materials Inc., Canada) and under UV light of a gel documentation system – Vilber Lourmat Quantum ST4 1100 (Vilber Lourmat, France). Some of the PCR products were sequenced in an ABI 3500xL Genetic Analyser (Applied Biosystems, MedSanTek, Istanbul, Turkey). Bioedit v7.0.53 for Windows software (Hall, 1999) was utilized to check and edit DNA sequences and, then, sequences were aligned using clustalW implemented in BioEdit software. Each sequence data was searched for similarities using BLASTn from the Basic Local Alignment Search Tool (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) (Altschul et al., 1990).

Results and Discussion

Molecular Identifications

A total of 130 fungal isolates were obtained in this study: 57 from field samples, 22 from the first day of table olive production, 17 isolates from the biofilm formation stage (day 120) during fermentation and 17 isolates from the final fermented product (day 240) before packaging (Table 2). Seventeen fungal isolates were obtained from the commercial product sampling at the end of fermentation (Table 2). β -*tubulin* amplification was successful in distinguishing different fungal genus even with size amplification on agarose gel: 400 bp for *Alternaria*, 500 bp for *Penicillium* and 600 bp for *Aspergillus* (Figure 1). So, this region is suggested for rapid identification at genus level. *Monascus* produced 600 bp product for *Beta-tubulin*, which is the same size produced from the *Aspergillus* genus. But, these two genus are easy to distinguish morphologically. Moreover, sequences of the β -*tubulin* region were resolved for species-level identification (Table 3). Sequencing was performed for 77 isolates within a total collection of 130 isolates. The sequences were compared with sequences recorded in the National Center for Biotechnology Information (NCBI) gene bank and the most closely related sequences were presented in Table 3. Among the species detected, *P. expansum*, *A. niger*, *A. tubingensis* and *M. pilosus* are known to be potential mycotoxin producers such as aflatoxin, ochratoxin A and citrinin. Thus, rapid and accurate identification of these fungi is

important so that control strategies could be applied against to the fungal contaminations.

The fungal flora and frequency of the species differed between the field collection and the fermentation process (Table 2). β -*tubulin* sequence data was not able to resolve species-level identification for *Alternaria*. Morphologically, it belongs to small-spored *Alternaria* section and it is known that species-level identification for this group is highly challenging. We identified it as *A. alternata*, based on morphology and *Endopolygalacturanase* data from another study (Ozkilinc H., unpublished data).

Fungal Flora from Field Survey

Forty-two isolates from cultured olive trees, and 15 isolates from wild olive trees were obtained (Table 2). Among these isolates, forty-seven were isolated from olive fruits and ten from olive leaves. Fifteen *Alternaria*, four *Penicillium* and two *Aspergillus* fungi were isolated from damaged parts of the olive fruits. *Alternaria* causes pathogenic symptoms (Ozkilinc H., unpublished data) and the pathogenicity of *Alternaria* in olives was shown in other studies (Piccolo et al., 2014). *Penicillium* and *Aspergillus* are not known as olive pathogens, but these fungi may colonize easily, especially in damaged tissues. By contrast, only four isolates of *Penicillium* and two isolates of *Aspergillus* were isolated from healthy olives, which indicates that some fungi may present without any sign. Even though *Penicillium* and *Aspergillus* do not cause disease on the fruit, they may potentially be dangerous due to being toxin-producers. In addition to these major fungal groups, some other species such as *Fusarium*, *Cladosporium*, *Epicoccum*, *Phoma* and *Clonostachys* were detected at a low frequency. Similar diversity of fungal species in olives in a natural environment has been reported in previous studies. Chliyeh et al. (2014) studied all the fungal species associated with olive trees in Morocco and reported that *A. alternata* was identified in ranges from 13 to 84.5% for all studied regions. In another study, fungi were isolated from sub-cortical brown streaking and from the cankers of wilted olive plants (Carlucci et al., 2013). The total isolation frequency of *A. alternata*, *Aspergillus* spp. and *Penicillium* spp. was reported as 3.3, 8.9 and 5.4%, respectively (Carlucci et al., 2013). For example, 42% of the total isolates from olive trees were *Alternaria*, *Penicillium* and *Fusarium* were identified from olive trees in Portugal and the 42% of the total isolates was reported as *Alternaria* (Oliveira et al., 2012). Baffi et al. (2012) reported that *Aspergillus* and *Penicillium* species were major fungal genera in olive ecosystems such as olive fruits, olive pomace and olive paste. Al-Ameri et al., (2015) has isolated *A. niger* (13% sample frequency) from olive fruit rot. Roussos et al. (2006) reported that *Aspergillus* and *Penicillium* were the dominant mold flora in olive oil production efforts in Morocco and that *A. niger* was the most widely distributed. Thus, the dominant species in this study are consistent with those isolated in previous studies.

Table 2 Fungal species frequency from field to during table olive fermentation.

Source		ALT		PEN				ASP			MN	O*	T	
		SP1	SP2	SP3	SP4	SP5	SP6	SP7	SP8	SP9	SP10			SP11
Field Sampling	Olive Fruits	35	3	1	1	1	1	-	1	3	-	-	10	57
Fermentation process	Olive Fruits LSP	5	6	4	-	-	-	-	1	3	-	3	1	26
	Olive Fruits C	-	1	-	-	-	-	-	-	-	1	1	-	
	Brine LSP	1	7	2	2	-	-	2	1	2	-	1	-	24
	Brine C	-	-	-	1	-	-	-	-	-	3	-	3	
Biofilm (Kefeke)	LSP	-	6	3	-	-	-	-	3	-	-	2	2	23
	C	-	1	1	-	-	-	-	-	-	1	1	3	
Total		41			43					19		8	19	130

ALT: *Alternaria*, PEN: *Penicillium*, ASP: *Aspergillus*, MN: *Monascus*, O: Others, T: Total, LSP: Lab-scale production, C: Commercial, SP1: *A. alternata*, SP2: *Penicillium* spp., SP3: *P. crustosum*, SP4: *P. roquefortii*, SP5: *P. brevicompactum*, SP6: *P. glabrum*, SP7: *P. expansum*, SP8: *Aspergillus* spp., SP9: *A. niger*, SP10: *A. tubingensis*, SP11: *M. pilosus*, **Fusarium*, *Cladosporium Epicoccum*, *Phoma*, *Clonostachy*.

Table 3 Fungal species identified based on partial sequence data of *Beta-tubulin* region.

Isolates	Source	SL	Identity ^a	The best match reference ^b
<i>A. alternata</i>	Field sampling-olive fruit	280	99%	KU512287.1- <i>Alternaria alternata</i>
<i>P. crustosum</i>	Day 1 of olive fermentation-olive fruit	392	100%	FJ004401.1- <i>Penicillium crustosum</i>
<i>P. glabrum</i>	Field sampling-olive fruit	353	99%	EU128585.1- <i>Penicillium glabrum</i>
<i>P. roquefortii</i>	Day 1 of olive fermentation -brine	445	99%	AY674382.1- <i>Penicillium roquefortii</i>
<i>P. expansum</i>	Day 1 of olive fermentation -brine	362	99%	LN896431.1- <i>Penicillium expansum</i>
<i>P. brevicompactum</i>	Field sampling-olive fruit	264	99%	AY674436.1- <i>Penicillium brevicompactum</i>
<i>A. niger</i>	Field sampling-olive fruit	535	100%	KC175288.1- <i>Aspergillus niger</i>
<i>A. tubingensis</i>	Commercial sampling	447	99%	HQ632767.1- <i>Aspergillus tubingensis</i>
<i>M. pilosus</i>	Day 240 of olive fermentation/biofilm	484	99%	AB607170.1- <i>Monascus pilosus</i>

^aSequence similarity with one of the most related sequences from NCBI. ^bGenebank accession numbers with one of the most related sequences from NCBI-GeneBank, SL: Sequence length (bp)

Fungal Flora from the Table Olive Fermentation Process

Twenty-three isolates from olive fruits, seventeen isolates from brine and sixteen isolates from biofilm were obtained during the fermentation process (Table 2). While *Alternaria* was the most common fungus in field samples, it was not detected in fermented products. As an independent test, *Alternaria*, *Penicillium* and *Aspergillus* species (two isolates per genus) were inoculated into 100 mL of sterilized brine. While *Penicillium* and *Aspergillus* grew successfully, *Alternaria* did not grow. High salt concentration or antioxidants, especially oleuropein, may inhibit *Alternaria* growth. But, these conditions do not seem to restrict reproduction of *Aspergillus* and *Penicillium*. A previous study showed that KCl and NaCl together with cassia oil have an antifungal effect on *A. alternata* isolates (Feng and Zheng, 2006). Moreover, antifungal effect of phenolic compounds in olive is also known (Pereira et al., 2007). It is thought that the antifungal properties of phenolic components are much more effective on *Alternaria* than the other fungi in this study. Thus *Alternaria* is mainly a problem as a disease agent for olives in a natural environment, but *Aspergillus* and *Penicillium* can be present from field to table. Hence, if toxin production occurs, it should be determined at which stage or under what conditions toxin production is favored.

The dominant mold flora during fermentation was *Penicillium* (49%) and *Aspergillus* (20%). *P. crustosum* (14%) was the most common *Penicillium* species. Other species were *P. roquefortii* (4%) and *P. expansum* (2%), respectively. *P. brevicompactum* and *P. glabrum* were, however, not isolated during fermentation. Two species of *Aspergillus* (*A.niger* and *A.tubingensis*) was found at the same frequency (7%). Similarly, Sahin and Korukoglu

(2000) reported that *Penicillium* is the dominant species in the brine of pickled food. In addition, *P. roquefortii*, *P. brevicompactum* and *P. citrinum* were isolated from naturally fermented black olives in previous studies from Turkey (Heperkan et al., 2006; Heperkan et al., 2013). *P. expansum* and *A. niger* species detected in the study have potential to produce mycotoxins such as ochratoxin A and citrinin. If these species produce toxins, it will result in a toxin contaminated final product. Adlouni et al. (2006) stated that aflatoxin, ochratoxin A and citrinin was found in some of the black table olive samples from retailer and supermarket. Ghitakou et al., (2006) reported aflatoxin B1 and ochratoxin A production by natural microflora and *A. parasiticus* in black and green olives of Greek origin. Our research will continue to determine whether this threat exists.

Commercial sampling was taken from five olive companies in the Canakkale area. The major mold flora was *Penicillium*, *Aspergillus* and *Monascus*. Additionally, *P. crustosum*, *A. tubingensis* and *M. pilosus* were the most common species. *M. pilosus* was 10% of all isolates. Many isolated *M. pilosus* was from olive fruits and biofilm. Fermented products from laboratory experiments and commercial sampling presented similar fungal flora, but in different numbers: the number of *Monascus* isolates were higher in commercial products compared to the samples taken from laboratory scale production. Some of the *Monascus* species such as *M. purpureus* and some *M. pilosus* are known as Mon K toxin producers (Su et al., 2003; Hong et al., 2011). *P. crustosum* was reported as a major fungal species in commercial olive samples in Turkey and *P. expansum* and *P.roquefortii* were also reported from commercial olive products in previous similar studies (Tokusoglu et al., 2010).

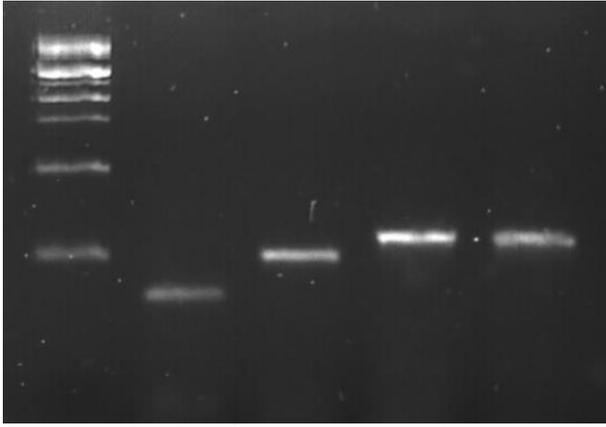


Fig. 1 Identification of *Alternaria*, *Penicillium*, *Aspergillus* and *Monascus* fungal genus by using amplification sizes of PCR with β -tubulin primers. The first line is DNA ladder (1 kb DNA ladder, Geneaid) and following lines for *Alternaria* (~400 bp), *Penicillium* (~500 bp), *Aspergillus* (~500 bp), *Monascus* (~600 bp), respectively.

Conclusion

Variety in fungal species on olives from field to table were shown in this study, and a fungal diagnosis was performed based on amplification and sequences of partial region of β -tubulin coding gene. The study showed that the amplification size of partial β -tubulin gene is very useful to discriminate between major fungal groups related to olive products at both a genus level and, with further sequencing information of β -tubulin, at a species level – except for *Alternaria*. While *Alternaria* poses a danger as a pathogen for fruit on trees, many species from *Aspergillus* and *Penicillium* are present in the product from field to table. Many of these species are known as mycotoxigenic species. Therefore, it is crucial to determine the conditions for toxin production by these species during this whole process.

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References

Adlouni CE, Tozlovanu M, Naman F, Faid M, Pfohl-Leszkowicz A. 2006. Preliminary data on the presence of mycotoxins (ochratoxin A, citrinin and aflatoxin B1) in black table olives "Greek style" of Moroccan origin. *Mol. Nutr. Food Res.* 50, 507-512. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J. Mol. Biol.*, 215: 403-410.

Aran N. 2012. Gıda Biyoteknolojisi, Geliştirilmiş 2. Baskı, Nobel Yayınevi. İstanbul Teknik Üniversitesi. pp. 49.

Arroyo-López FN, Dura'n-Quintana MC, Ruiz-Barba JL, Querol A, Garrido-Fernandez A. 2006. Use of molecular methods for the identification of yeast associated with table olives. *Food Microbiol.* 23:791–796.

Baffi MA, Romo-Sanchez S, Ubeda-Iranzo J, Briones-Perez AI. 2012. Fungi isolated from olive ecosystems and screening of their potential biotechnological use. *New Biotechnology.* 29: 451-456.

Baskou D. 2015. Olive and olive oil bioactive constituents. Olive oil chemistry and technology, 2nd edition, AOCS press.

Carbone I, Kohn LM. 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia*, 91: 553-556.

Carlucci A, Raimondo ML, Cibelli F, Phillips AJL, Lops F. 2013. *Pleurostomophora richardsiae*, *Neofusicoccum parvum* and *Phaeoacremonium aleophilum* associated with a decline of olives in Southern Italy. *Phytopathologia Mediterranea.* 52(3): 517–527.

Chliyah M, Rhimini Y, Selmaoui K, Touhami AO, Filali-Maltouf A, El Modafar C, Moukhli, A, Oukabli, A, Benkirane R, Douira A. 2014. Survey of the fungal species associated to olive-tree (*Olea europaea* L.) in Morocco. *Int. J. Rec. Biotech.* 2: 15-32.

Ghitakou S, Koutras K, Kanellou E, Markaki. 2006. Study of aflatoxin B1 and ochratoxin A production by natural microflora and *Aspergillus parasiticus* in black and green olives of Greek origin. *Food Microbiol* 23: 612-621.

Glass NL, Donaldson GC. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl. Environ. Microb. Apr.*, 1323–1330.

Gonvances JS, Ferracin LM, Vieira MLC, Iamanaka BT, Taniwaki MH, Pelegrinelli Fungaro MH. 2012. Molecular analysis of *Aspergillus* section Flavi isolated from Brazil nuts. *World J Microbiol Biotechnol.* 28: 1817–1825.

Feng W, Zheng X. 2006. Control of *Alternaria alternata* by cassia oil in combination with potassium chloride or sodium chloride. *Journal of Applied Microbiology.* 101: 1317–1322.

Hall TA. 1999. Bioedit: A user-friendly biological sequence alignment editor and analysis program for Windows95/98/NT. *Nucleic Acids Symposium Series.* 41: 95–98.

Heperkan D, Meric BE, Sismanoglu G, Dalkılıç G, Güler FK. 2006. Mycobiota, mycotoxigenic fungi and citrinin production in black olives. *Advances in Food Microbiology.* 571, 203-210.

Heperkan D. 2013. Microbiota of table olive fermentations and criteria of selection for their use as starters. *Frontiers in Microbiology.* 4: 1-11.

Hong SY, Oh JH, Lee I. 2011. Simultaneous enrichment of deglycosylated ginsenosides and monacolin K in red ginseng by fermentation with *Monascus pilosus*. *Bioscience, biotechnology, and biochemistry.* 75: 1490-1495.

Lumi Abe, C. A., Bertechini Faria, C., Fernandes de Castro, F., et al. 2015. Fungi Isolated from Maize (*Zea mays* L.) Grains and Production of Associated Enzyme Activities. *International Journal of Molecular Sciences.* 16(7): 15328–15346.

Magnani M, Fernandes T, Prete CEC, Homechim M, Ono EYS, Vilas-Boas LA, Satori D, Furlaneto MC, Fungaro MHP. 2005. Molecular identification of *Aspergillus spp.* isolated from coffee beans. *Sci. Agric. (Piracicaba, Braz.)*. 62: 45-49.

Marsilio V, Campestre C, Lanza B, De Angelis M. 2001. "Sugar and polyol compositions of some European olive fruit varieties (*Olea europaea* L.) suitable for table olive purposes". *Food Chem.* 72: 485–490.

Oliveria I, Pereira JA, Lino-Neto T, Bento A, Baptista P. 2012. Fungal diversity associated to the olive moth, *Prays Oleae bernard*: A survey for potential entomopathogenic fungi. *Microb Ecol.* 63: 964-974.

Özkaya MT, Tunalioglu R, Eken Ş, Ulaş M, Tan M, Danacı A, İnan N, Tibet İ. 2010. "Türkiye zeytinliğinin sorunları ve çözüm önerileri". *Türkiye Ziraat Mühendisliği V. Teknik Kongresi* 11-15 Ocak 2010.

Pereira AP, Ferreira ICFR, Marcelino F, Valentao P, Andrade PB, Seabra R, Estevinho L, Bento A, Pereira JA. 2007. Phenolic compounds and antimicrobial activity of olive (*Olea europaea* L. cv. cobrançosa) leaves. *Molecules.* 12: 1153-1162.

Piccolo SL, Mondello V, Giambra S, Conigliaro G, Torta L, Burruano S. 2014. *Arthrinium phaeospermum*, *Phoma cladoniicola* and *Ulocladium consortiale*, New Olive Pathogens in Italy. *J Phytopathol* 162: 258–263.

- Roussos S, Zaouia N, Salih G, Tantaoui-Elaraki A, Lamrani K, Cheheb M, Hassouni H, Verhe F, Perraud-Gaime I, Augur C, Ismaili-Alaoui M. 2006. Characterization of filamentous fungi isolated from Moroccan olive and olive cake: Toxinogenic potential of *Aspergillus* strains. *Molecular Nutrition & Food Research*. 50: 500–506.
- Russel R, Peterson M. 2006. Identification and quantification of mycotoxigenic fungi by PCR. *Process Biochemistry*. 41: 1467–1474.
- Su YC, Wang JJ, Lin TT, Pan TM. 2003. Production of the secondary metabolites c-aminobutyric acid and monacolin K by *Monascus*. *J Ind Microbiol Biotechnol*. 30: 41–46.
- Şahin I, Korukoglu M. 2000. *Küf-Gıda-İnsan*, Uludağ Üniversitesi Güçlendirme Vakfı, Yayın No: 155, Bursa.
- Şahin OI, Aka A, Akpınar-Bayizit A, Baltaş-Minas E. 2010. Sofralık zeytin üretim tesislerinde gıda güvenliği yönetim sisteminin uygulanması. *U. Ü. Ziraat Fakültesi Dergisi*, 24(1): 11-24.
- Tokuşoğlu Ö, Alpas, H, Bozoğlu F. 2010. High hydrostatic pressure effects on mold flora, citrinin mycotoxin, hydroxytyrosol, oleuropein phenolics and antioxidant activity of black table olives. *Innovative Food Science and Emerging Technologies*. 11: 250–258.
- TUIK. 2016. http://www.tuik.gov.tr/PreTablo.do?alt_id=1001. Access date:28.06.2017.
- TUIK. 2013. <http://www.tuik.gov.tr/ilGostergeleri/iller/CANAKKALE.pdf>. Access date:28.06.2017.
- Wang C, García-Fernández D, Mas A, Esteve-Zarzoso B. 2015. Fungal diversity in grape must and wine fermentation assessed by massive sequencing, quantitative PCR and DGGE. *Frontiers in Microbiology*, 6, 1156.