Fungal Agents Causing Diseases on Pomegranates Grown in Antalya, Turkey

Tuncay Ilgın and Gürsel Karaca*

Süleyman Demirel University, Faculty of Agriculture, Department of Plant Protection,
32260 (Isparta, Turkey)

**Corresponding author's email: gurselkaraca [AT] sdu.edu.tr*

ABSTRACT—*In order to determine the fungal disease agents causing losses in pomegranate orchards and storages in Antalya province, surveys were performed in 61 orchards and 21 storages, in 2012. Totally 12 fungus species were obtained as a result of isolations made from the leaf and fruit samples taken from the orchards and storages. It was determined that the most common fungus was Alternaria alternata both in leaf and fruit samples obtained from the orchards. Cladosporium herbarum from the leaf samples, Aspergillus and Penicillium species from the fruit samples were the other common fungi. Colletotrichum gloeosporioides and Coniella granati, known as the important pathogens of pomegranate, was also isolated in lower rates. Botrytis cinerea was the species with highest isolation frequency from the fruit samples taken from the storages and followed by Penicillium sp., Aspergillus niger, Alternaria alternata and Coniella granati. In the pathogenicity trials performed under laboratory conditions; A. alternata, B. cinerea, C. granati and Fusicoccum aesculi caused severe browning on pomegranate leaves, while C. gloeosporioides, C. herbarum, Pleospora herbarum, A. niger and Penicillium sp. were the other pathogens causing necrosis on the leaves. As a result of fruit inoculations, C. granati and F. aesculi caused severe symptoms on the fruits, where C. gloeosporioides, B. cinerea, A. niger, Epicoccum nigrum, Fusarium semitectum and Penicillium sp. caused moderate or slight fruit rot.*

Keywords— *Punica granatum* L., leaf spot, fruit rot, post-harvest decay

1. INTRODUCTION

Besides its fresh consumption, pomegranate fruit have recently been used as a raw material in food industry. Drug, dye, ink, animal food, tannin and marmalade are some of the products where pomegranates are used [1]. Pomegranate fruit is also an important product for health sector. Pomegranate juice, rich in B and C vitamins, calcium, sodium, potassium, iron, copper, chromium and fibers, is good for human health and prevents many diseases. There are studies showing the decreasing effect of tannin in pomegranate fruit on cholesterol and heart attack risk. It is also known as preventive on some cancer types [2,3]. Pomegranate cultivation can be made almost all regions of Turkey, but mainly in the Mediterranean region. Because of its increasing consumption, pomegranate production is also increasing year by year. Turkey comes 4th after India, Iran and China regarding pomegranate production [1]. According to the recent statistics, 397 335 tones of pomegranates were produced in 304 548 da land in Turkey, while Antalya province provided 27% of the total production with 108 786 tones [4].

Various fungi were reported to cause diseases and resulting yield loss on pomegranate, in different countries or regions. Wilt caused by *Ceratocystis fimbriata* and antrachnose caused by *Colletotrichum gleosporioides* were reported as the major diseases of pomegranate in India, while minor diseases were Alternaria blight, leaf or fruit spots caused by *Cercospora*, *Sphaceloma*, *Fusarium*, *Phomopsis* and *Drechslera* species [5]. *Alternaria alternata* was determined to cause 40-50% yield loss by causing fruit rot in Greece [6]. The main pathogens causing post harvest rot on pomegranate fruits were reported as *Penicillium* spp., *Botrytis cinerea*, *Aspergillus niger* and *Coniella granati* [7,8]. *P. granati* was recently reported as one of the important pathogens causing rot from different countries like Greece [9], Spain [10], U.S.A. [11] and Iran [12]. With few studies on pomegranate diseases in Turkey, *Alternaria alternata*, *Botrytis cinerea*, *Colletotrichum gleosporioides*, *Coniella granati*, *Penicillium*, *Aspergillus*, *Phytophthora* and *Fusarium* species were reported as the pathogens causing diseases on pomegranate [13,14,15]. In a recent research performed in Antalya province, it was determined that *Fusarium* spp., *Phytophthora* spp. and *Rhizoctonia solani* were the major agents causing root and crown rot [16]. The aim of this study was the determination of the fungal pathogens causing diseases on pomegranate trees in the orchards and on the fruits in the storages where they were kept after harvest, in Antalya province.

2. MATERIALS AND METHODS

2.1 Sampling

Surveys were performed in 61 pomegranate orchards in 14 districts of Antalya province, using simple random sampling method, in 2012 [17]. Orchards were visited two times, after bloom (May-June), and before harvest (October-November) and samples with disease symptoms were taken. Number of orchards in each district for sampling was determined according to the numbers of pomegranate trees in the districts [18] and 2, 4, 6, 8 and 10 randomly selected orchards were investigated in the districts with tree numbers between 0-100 000, 100 000-200 000, 200 000-400 000, 400 000-800 000 and more than 800 000, respectively). During surveys, crown, stem, twigs, leaves and fruits of the trees were examined and diseased samples were taken to the laboratory in polyethylene bags. Storage surveys were performed in 21 cold storages found in 9 districts of the province. Storages where pomegranate fruits were kept after harvest were visited once 2-3 months after harvest and fruit samples with disease symptoms were taken to the laboratory. Storage temperatures were about 5-7°C during sampling.

2.2 Isolation and identification of the fungi

Plant samples with disease symptoms were macroscopically examined for fungal growth and structures under stereomicroscope. Samples without any fungal structure on them were incubated on sterile blotter papers in petri dishes for sporulation, in a climatic room with 22±2°C temperature, 12-12 hour of light and dark conditions and examined again. Isolations were also made by placing small pieces with diseased and healthy part of the plant tissue on PDA (Potato dextrose agar-Merck) and WA (Water agar-15 g agar, 1000 ml tap water) media, after surface disinfection with 1% NaOCl for 2-3 minutes. Three replicate plates were used for each sample. Growing hyphal tips were transferred to separate plates after 5-7 days incubation in the climatic room. Preparations were made from the single colonies and fungi were identified according to their cultural and microscopic features by using related literature [19,20,21,22]. Isolates were maintained on agar slants at 4°C temperature. Distribution, isolation frequency and isolation rates of fungi isolated from the leaf and fruit samples were calculated by the following formulae.

Distribution (%) = (NO/TNO) x 100, where NO is the number of orchards from which the pathogen species was isolated and TNO is the total number of orchards,

Isolation frequency (%) = (ND/TND) x 100, where ND is the number of plant tissue segments with disease symptoms used for isolation, and TND is the total number of segments examined,

Isolation rate (%) = NI/TNI) x 100, where NI is the number of isolates of a given species, and TNI is the total number of isolates.

2.3 Pathogenicity experiments

Pathogenicity tests were performed by using detached healthy leaves and newly harvested healthy fruits. Care was taken to use leaf and fruits from an orchard where no chemical was used. Leaf and fruits were firstly surface disinfected with 1% NaOCl solution for three minutes and dried. Then leaves were transferred onto sterile blotter papers in petri dishes, humidified with sterile distilled water. Fungi were activated on PDA and each leaf sample was inoculated with a 3 mm diameter agar piece with mycelia, cut by a cork borer from the growing edge of the culture. Lesion diameters were determined one week after incubation at 22±2°C and 12h light: 12h dark conditions. Pomegranate fruits were wounded at opposite sides by using a 3 mm diameter cork borer, similarly inoculated and incubated in polyethylene bags under same conditions for one week. Disease severity was evaluated by using 0-4 scale, where 0=healthy leaf or fruit, 1=lesion or rot on leaves or on fruits less than 15 mm diameter, 2=Lesion or rot with a diameter between 16-25 mm, 3=half of the leaf or fruit became brown or rotted, and 4=totally brownish or rotted leaf or fruit. Leaf and fruits inoculated with agar plugs without pathogen mycelia were used as controls. Disease severity (%) was determined by using Townsend and Heuberger [24] formula. Pathogenicity trials were conducted in completely randomized design with 3 replicates. Data were subjected to analyses of variance after $\sqrt{x+1}$ transformation for scale values and arcsin transformation for percent values, and means were compared by Tukey test. Reisolations were made from the leaf and fruit samples showing symptoms in order to confirm the inoculated fungi.

3. RESULTS AND DISCUSSION

3.1 Fungi isolated from pomegranate orchards and storages

During the surveys in May-June period, only leaf spots were scarcely observed in some orchards. *Alternaria alternata* and *Cladosporium herbarum* were the major fungi isolated from the leaves in this period. Severity of the leaf spots was again very low but they were seen in almost all of the orchards during the surveys before harvest and different fungi were isolated. As a result of isolations made from 58 leaf samples showing disease symptoms, 11 fungus species were determined (Table 1).

Table 1: Number of orchards from which the fungi were isolated and number of isolates of each fungi obtained from pomegranate leaf and fruit samples taken from the orchards and storages in Antalya province

Fungi	Leaf isolates		Fruit isolates of orchards		Fruit isolates of storages	
	No of orchards	No of isolates	No of orchards	No of isolates	No of storages	No of isolates
Alternaria alternata	57	221	45	196	8	24
Aspergillus niger	9	15	41	130	14	64
Botrytis cinerea	1	5	1	2	20	150
Cladosporium herbarum	37	145	34	136	2	2
Colletotrichum gloeosporioides	6	12	13	71	2	15
Coniella granati	2	5	3	9	7	52
Epicoccum nigrum	4	4	8	8	0	0
Fusarium semitectum	5	6	6	6	1	2
Fusicoccum aesculi	0	0	2	8	0	0
Penicillium sp.	2	3	23	55	15	73
Pleospora herbarum	7	16	4	6	0	0
Trichothecium roseum	1	1	1	1	1	1
Total numbers of orchards, storages and isolates	**61**	**433**	**61**	**628**	**21**	**383**

Among 433 leaf isolates, *A. alternata,* isolated from leaf samples obtained from 57 orchards, related with the small dark brown lesions on the leaves, was the most common fungi. It was previously reported as a leaf and fruit pathogen of pomegranate [25,26]. *C. herbarum,* isolated from the smaller, blistered brown leaf spots was the other fungi with high isolation rate (Figure 1).

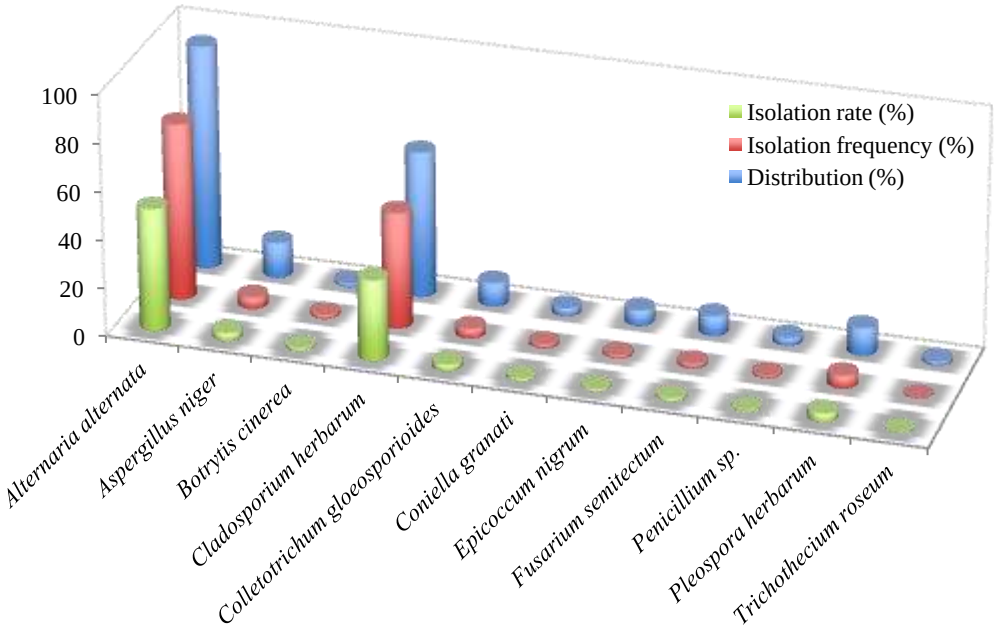

Figure 1: Distribution, isolation frequency and isolation rates of fungi isolated from the leaf samples taken from pomegranate orchards in Antalya province, Turkey

Fruit infections were more common and observed in all the orchards surveyed in Antalya province. Isolations made from 61 fruit samples taken from the orchards yielded 12 fungus species with a total of 628 isolates. *A. alternata* was again the most frequently isolated fungi, followed by *A. niger* and *C. herbarum* (Figure 2). *A. alternata* was isolated from dark brown lesions starting from calix region and on some fruits it was observed that the pathogen totally covered the

inside of the fruit as a black rot (Figure 3). *A. niger* was previously reported to cause fruit rot and cracking on pomegranates [13,27]. It was isolated from the similar dark brown lesions with black mold originating from calix. *Penicillium* sp., isolated from 24 fruit samples, was another common pathogen. Anthracnose disease agent *C. gloeosporioides* was found on 14 samples with an isolation frequency of 17.5 %. It was isolated from the small, sunken brown lesions with light brown center. *C. granati*, which is known as one of the important pathogens of pomegranate, was isolated from three fruit samples and *F. aesculi*, which could not be found on the leaf samples was isolated from two fruit samples. *B. cinerea* [13] and *T. roseum* [28], known to cause storage rot on pomegranate fruits, were isolated each from only one fruit sample.

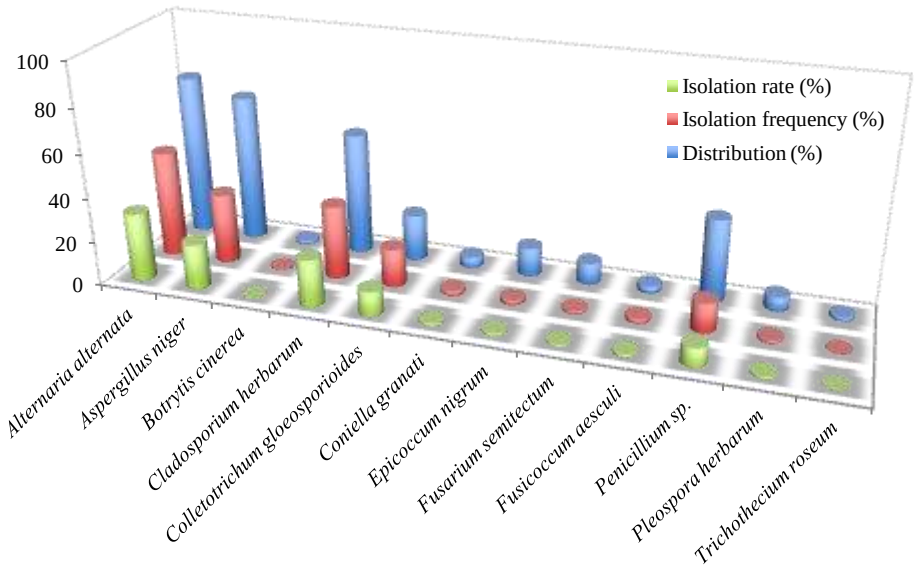

Figure 2: Distribution, isolation frequency and isolation rates of fungi isolated from the fruit samples taken from pomegranate orchards in Antalya province, Turkey

Figure 3: Fruit samples from which *Alternaria alternata* was isolated

Isolations made from the fruit samples taken from 21 storages in Antalya province yielded 9 fungus species (Table 1). *B. cinerea*, which was found on almost almost all the storages, was the most common pathogen with the highest isolation frequency and isolation rate (Figure 4) and generally caused stem end rot (Figure 5). *Penicillium* sp., *A. niger*, *A. alternata* and *C. granati* were the other common fungi causing fruit rot, but their isolation rates were lower. *C. gloeosporioides*, *T. roseum*, *C. herbarum* and *F. semitectum*, isolated from the fruit samples from one or two storages, had very low isolation frequencies and rates.

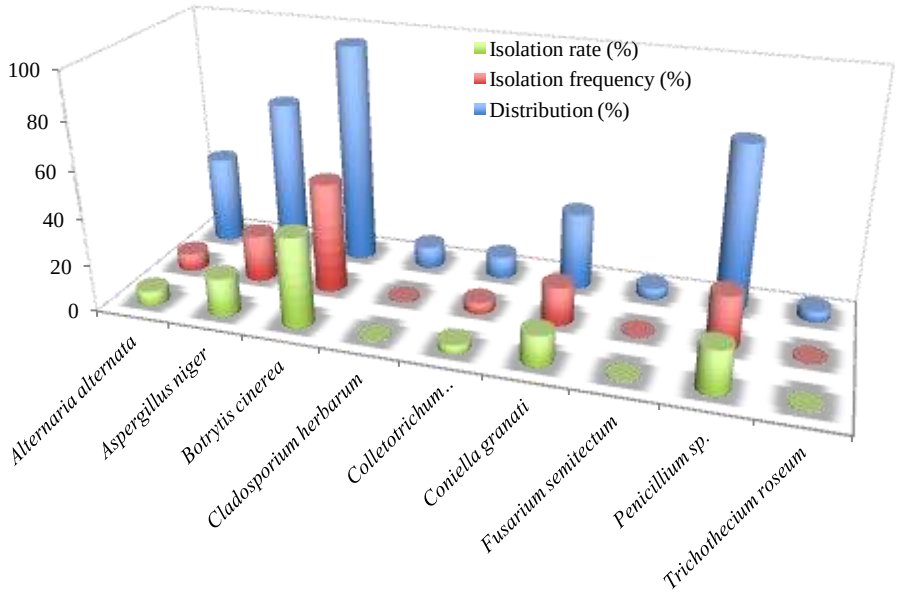

Figure 4: Distribution, isolation frequency and isolation rates of fungi isolated from the pomegranate fruit samples taken from storages in Antalya province, Turkey

Figure 5: Fruit sample from which *Botrytis cinerea* was isolated

Nine of the 12 fungus species determined on the fruit samples taken from the orchards were also isolated from the fruit samples taken from the storages. *E. nigrum*, *P. herbarum* and *F. aesculi* which were isolated from the fruits taken from the orchards in low frequencies were not determined on the fruit samples from the storages. Isolation frequency of *A. alternata* was high on fruit samples from the orchards, while the pathogen was isolated in lower rates from the storage samples. On the contrary, *B. cinerea* was isolated from 95% of the fruit samples from the storages, but its frequency was very low on the fruits from the orchards. This shows that this pathogen infected fruits later in the orchards near harvest, or transferred to storages with harvested fruits and infection occurred in storages. There is a report supporting our results, that it caused stem end decay of pomegranate fruits developing in cold storage [29]. However, in another study performed in the Çukurova region of Turkey, this pathogen was commonly isolated from the fruit samples taken from the orchards [15]. Similarly preharvest fruit rot caused by the pathogen was reported from central Greece with 10% loss in 2008 [30].

3.2 Pathogenicity of the fungi isolated from pomegranate leaf and fruits

As a result of pathogenicity trials performed with detached healthy leaves and fruits, it was determined that the symptoms and severity of the diseases caused by them were different from each other. Investigation on the pathogenicity of the 12 fungus species obtained from the leaf samples showed that *A. alternata* caused the highest disease severity with 94% (Table 2). This pathogen was previously known to cause leaf spots from different regions of the world [26,31]. It was reported that it caused big brown spots on the leaves and infections starting from the young shoots could cause the death of young leaves also could cause dieback symptom [32]. The pathogen was isolated from the similar symptoms in the present study, and all the leaves inoculated by the pathogen became brown in the pathogenicity test (Figure 6).

Table 2: Mean disease severity index and disease severity rates (%) of fungi isolated from pomegranate leaf and fruit samples taken from the orchards and storages in Antalya province

Fungi	On leaves		On fruits	
	Mean disease index	Severity rate (%)	Mean disease index	Severity rate (%)
Alternaria alternata	3.78 a*	94.43 a	0.89 c	22.23 d
Aspergillus niger	2.33 abc	58.33 ab	1.67 bc	41.67 cd
Botrytis cinerea	3.67 a	91.67 ab	1.56 bc	38.90 cd
Cladosporium herbarum	1.33 abc	33.33 ab	0.00 d	0.00 e
Colletotrichum gloeosporioides	2.89 abc	72.23 ab	1.89 b	47.20 c
Coniella granati	3.11 ab	77.77 ab	4.00 a	100.00 a
Epicoccum nigrum	0.33 bc	8.33 ab	1.56 bc	38.90 cd
Fusarium semitectum	0.11 c	2.78 b	1.00 bc	25.00 cd
Fusicoccum aesculi	3.33 ab	83.33 ab	3.11 a	77.77 b
Penicillium sp.	2.45 abc	61.13 ab	0.89 c	22.23 d
Pleospora herbarum	1.33 abc	33.33 ab	0.00 d	0.00 e
Trichothecium roseum	0.44 bc	11.11 ab	0.00 d	0.00 e

*Means in the same column shown by the same letter were not statistically different from each other according to Tukey's test ($P<0.05$)

Figure 6: Leaf samples from which *Alternaria alternata* was isolated (left) and leaves becoming brown after inoculation with the pathogen

B. cinerea, with 91% disease severity, was the second most virulent pathogen after *A. alternata.* Although there wasn't any report regarding its pathogenicity on pomegranate leaves, it caused brown lesions covering almost all of the leaf area, in the pathogenicity test. *F. aesculi*, *C. granati*, *C. gloeosporioides*, *Penicillium* sp. and *A. niger* were the other pathogens with mean disease severities over 50%. Mean disease severity caused by *C. herbarum* and *P. herbarum* isolates were 33%, while they were statistically in the same group with the above mentioned fungi. Among those pathogens, *C. granati* was known as one of the most important pathogens of pomegranate all over the world. While it was generally reported as a fruit rot agent [10], it was found that it caused twig blight and dieback on young trees in Iran [12]. In the present study, it was isolated both from the leaf and fruit samples in lower rates and caused reddish brown spots on the leaves and most severe rot symptoms on the fruits, in the pathogenicity test.

C. gloeosporioides was known to cause antrachnose disease on plants like mango, citrus, kaju and also on pomegranate [33]. It was reported that it started as small purplish black spots, later with borders becoming darker and center light brown, and severely infected leaves dropped [32]. It was isolated from the similar spots and caused dark brown lesions on the leaves in the pathogenicity test. *F. aesculi*, *A. niger* and *Penicillium* sp. which caused brown lesions on some leaves, were also known as fruit rot agents [34,35,10,27]. *F. aesculi* was also reported to cause scab lesions on pomegranate twigs and stem [36]. In this study, *E. nigrum*, *T. roseum* and *F. semitectum* which didn't cause any symptoms on pomegranate leaves, were the agents with lowest virulence.

In the pathogenicity test performed with healthy pomegranate fruits and 12 fungus species isolated from the leaf and fruit samples, severity of the symptoms were again different (Table 2). *C. granati*, which was previously reported as fruit rot agent [11,37], was the most virulent pathogen with 100% disease severity, that all inoculated fruits totally rotted (Figure 7). *F. aesculi* having high virulence on pomegranate leaves, caused severe disease also on the fruits. It was reported that it caused rot on the fruits in the storage in China [34]. Antrachnose agent *C. gloeosporioides*, which was reported to cause post harvest fruit rot [38], caused moderate rot on the fruits. *A. niger*, *B. cinerea*, *A. alternata* and *Penicillium* sp., known as the pre and post harvest fruit rot agents, caused moderate or slight rot symptoms on the fruits in the pathogenicity test. This may because of the lower virulence of the isolates randomly selected for the pathogenicity trial. *C. herbarum*, *E. nigrum*, *F. semitectum*, *P. herbarum* and *T. roseum*, having lower isolation frequencies, caused no or very small lesions around the inoculation area. That's why they were thought as secondary parasites or saprobes. As a result of the reisolations made after the evaluation of pathogenicity trial, inoculated pathogens were obtained.

Figure 7: Pomegranate fruits inoculated with *Coniella granati*

Among the fungi isolated from pomegranate leaves and fruits in this study, *A. alternata*, *B. cinerea*, *C. granati*, *C. gloeosporioides*, *A. niger*, *Fusarium* spp. and *Penicillium* sp. were previously reported as pomegranate pathogens, but this is the first report of pathogenicity of *F. aesculi*, *C. herbarum*, *P. herbarum*, *T. roseum* and *E. nigrum* on this plant from Turkey. *F. aesculi* which was isolated both from leaves and fruits, and *C. granati*, which had low isolation frequency, were found as pathogens with higher virulence. However, *A. alternata*, *A. niger* and *Penicillium* sp., which were more common and had higher isolation frequency, didn't cause considerable disease symptoms in the pathogenicity trial. On the other hand, it doesn't mean that these are pathogens of no significance. Since they were common on fruit samples taken both from the orchards and storages, it can be thought that they infected the fruits before harvest, transferred to the storages and caused post harvest losses.

In order to manage and control pomegranate diseases effectively, there should be sufficient information on disease agents. In the present study, disease agents which can cause yield and quality losses on pomegranate were determined. According to the data obtained by the study, cultural measures which may decrease the losses caused by the pathogens should be taken and research should be done on the control methods which can prevent losses both in the orchards and in the storages.

4. ACKNOWLEDGEMENT

This study was performed as a M.Sc. thesis by the first author and accepted by the Graduate School of Natural and Applied Sciences, Suleyman Demirel University, Isparta, Turkey. Part of the study was presented as a poster at the 5th Plant Protection Congress of Turkey (2014). We would like to thank to the Scientific Research Projects Management Service of the Suleyman Demirel University for their financial support with the project number "3393-YL1-12".

5. REFERENCES

[1] Kurt H, Şahin G, "Bir Ziraat Coğrafyası Çalışması: Türkiye'de Nar (*Punica granatum* L.) Tarımı", Marmara Coğrafya Dergisi, vol. 27, pp. 551-557, 2013.
[2] Tamer CE, "Nar: Bileşimi ve İnsan Sağlığı Üzerindeki Etkileri", Gıda ve Yem Bilimi Teknolojisi, vol. 9, pp. 48-54, 2006.
[3] Anoosh E, Mojtaba E, Fatemeh S, "Study the Effect of Juice of Two Variety of Pomegranate on Decreasing Plasma LDL cholesterol", Procedia Social and Behavioral Sciences, vol. 2, pp. 620–623, 2010.
[4] TUİK, Bitkisel Üretim İstatistikleri, http://tuik.gov.tr, 2015.
[5] Jamadar MM, Sataraddi AR, Patil PV, Jawadagi RS, Patil DR, "Status of Pomegranate Diseases of Northern Karnataka in India", Acta Horticulturae, vol. 890, pp. 501-508, 2011.
[6] Tziros GT, Lagopadi AL, Tzavella-Klonari K, "*Alternaria alternata* Fruit Rot of Pomegranate (*Punica granatum*) in Greece", http://www.bspp.org.uk/ndr/july2007/2007-20.asp, 2007.
[7] Palou L, Taberner V, Guardado A, Del Rio MA, Montesinos-Herrero C, "Incidence and Etiology of Postharvest Fungal Diseases of Pomegranate (*Punica granatum* cv. Mollar de Elche) in Spain", Phytopathologia Mediterranea, vol. 52, pp. 478-489, 2013.
[8] Kınay Teksur P, "Alternative Technologies to Control Postharvest Diseases of Pomegranate", Stewart Postharvest Review, vol. 4, pp. 1-7, 2015.
[9] Tziros GT, Tzavela-Klonari K, "Pomegranate Fruit Rot Caused by *Coniella granati* Confirmed in Greece", http://www.bspp.org.uk/ndr/jan 2007/2007-83.asp, 2007.
[10] Palou L, Guardado A, Montesinos-Herrero C, "First report of *Penicillium* spp. and *Pilidiella granati* Causing Postharvest Fruit Rot of Pomegranate in Spain", New Disease Reports, vol. 22, pp. 21, 2010.
[11] Michailides TJ, Puckett R, Reyes H, Morgan DP, "Fruit Decay Caused by *Pilidiella granati* in California", Phytopathology, vol. 100, S83, http://ucanr.edu/sites/ Pomegranates/files/122874.pdf, 2011.
[12] Mirabolfathy M, Groenewald JZ, Crous PW, "First Report of *Pilidiella granati* Causing Dieback and Fruit Rot of Pomegranate (*Punica granatum*) in Iran", Plant Disease, vol. 96, No. 3, pp. 461-461, 2012.
[13] Turan K, Başpınar N, Çetin V, "Bahçe ve Depo Koşullarında Nar Meyvelerinde Oluşan Fungal Hastalıklar Üzerinde Araştırmalar", In Proceedings of the 7th Phytopathology Congress of Turkey, pp. 118-121, 1995.
[14] Pala H, Canıhoş E, Öztürk N, Ulusoy R, Bayhan E, Yılmaz C, "Important Pests of Pomegranate Cultivation in the Eastern Mediterranean Region", In Proceedings of the First Plant Protection Congress of Turkey, pp. 207, 2004.
[15] Çetin H, "Determination of Phytopathological Problems on the Pomegranate Plantations of Çukurova and Investigation of the Effectivity of Some Fungicide Applications Against Postharvest Diseases", Master Thesis, University of Çukurova, Institute of Natural and Applied Sciences, Department of Plant Protection, Turkey, 2008.
[16] Basım E, Basım H, "Antalya İli Nar (*Punica granatum* L.) Bahçelerinde Görülen Kök ve Kök Boğazı Çürüklük Etmenlerinin Tespiti", Meyve Bilimi, vol. 1, No. 1, pp. 23-26, 2013.
[17] Bora T, Karaca İ, Kültür Bitkilerinde Hastalığın ve Zararın Ölçülmesi, Ege Üniv. Ziraat Fak. Yardımcı Ders Kitabı, Yayın No.167, Turkey, 1970.
[18] TUIK, "Bitkisel Üretim İstatistikleri", http://www.tuik.gov.tr, 2010.
[19] Barnett HL, Hunter BB, Illustrated Genera of Imperfect Fungi, Fourth Edition, APS Press, USA, 1998.
[20] Ellis MB, Dematiaceous Hyphomycetes, CAB International, UK, 1971.
[21] Ellis MB, More Dematiaceous Hyphomycetes, CAB International, UK, 1976.
[22] Sutton BC, The Coelomycetes, CMI, UK, 1980.
[23] Samson RA, Hoekstra ES, Frisvad JC, Filtenborg O, Introduction to Food-borne Fungi, Centraalbureau Voor Schimmelcultures, The Netherlands, 1995.
[24] Townsend GR, Heuberger JV, "Methods for Estimating Losses Caused by Diseases in Fungicide Experiments", Plant Disease Report, vol. 24, pp. 340-343, 1943.
[25] Pala H, Tatlı A, Yılmaz C, Özgüven AI, "Important Diseases of Pomegranate Fruit and Control Possibilies in Turkey", Acta Horticulturae, vol. 818, pp. 285-289, 2009.
[26] Gat T, Liarzi O, Skovorodnikova Y, Ezra D, "Characterization of *Alternaria alternata* Causing Black Spot Disease of Pomegranate in Israel Using a Molecular Marker", Plant Disease, vol. 96, pp. 1513-1518, 2012.
[27] Yehia HM, "Heart Rot Caused by *Aspergillus niger* Through Splitting in Leathery Skin of Pomegranate Fruit", African Journal of Microbiology Research, vol. 7, no. 9, pp. 834-837, 2013.

[28] Singh R, Basu M, "*Trichothecium roseum* Link on *Punica granatum* L. – A New Record From India", Indian Phytopath., vol. 59, no. 4, pp. 532, 2006.
[29] Michailides TJ, "Black heart and tree decline issues of pomegranate", http://ucanr.edu/sites/pomegranates/files/134012.pdf, 2011.
[30] Bardas GA, Tzelepis GD, Lotos L, Karaoglanidis GS, "First Report of *Botrytis cinerea* Causing Gray Mold of Pomegranate (*Punica granatum*) in Greece", Plant Disease, vol. 93, no. 12, 1,346.3, 2009.
[31] Archana BC, Jamadar MM, Management of Leaf Spot and Fruit Spot/rot of Pomegranate (*Punica granatum* L.) Caused by *Alternaria alternata* (Fr.) Keissler", Karnataka J. Agric. Sci., vol. 27, no. 2, pp. 247-249, 2014.
[32] Jamadar MM, Sataraddi AR, Patil PV, Jawadagi RS, Patil DR, "Status of Pomegranate Diseases of Northern Karnataka in India", Acta Horticulturae, vol. 890, pp. 501-508, 2011.
[33] Venkateswarlu D, Murthy KVMK, Reddy MLN, Lakshmi MSM, "Isolation and Identification of *Colletotrichum gloeosporioides* from Cashew Seedlings in Nurseries", Cashew, vol. 18, no. 1, pp. 36-40, 2004.
[34] Fu JN, Liu XH, Cai FD, Kou LP, "Identification of Pathogenic Fungus Causing a Decay of Stored Pomegranate Fruits Using Molecular Biology Technique", Acta Horticulturae Sinica, vol. 34, pp. 877-882, 2007.
[35] Spadaro D, Amatulli MT, Garibaldi A, Gullino ML, First Report of *Penicillium glabrum* Causing a Postharvest Fruit Rot of Pomegranate (Punica granatum) in the Piedmont Region of Italy", Plant Disease, vol. 94, 1,066.2, 2010.
[36] Liu HX, Li XD, Zhu XP, Liu AX, "First Report of Pomegranate Stem Scab Caused by *Botryosphaeria dothidea* in China", Plant Pathology, vol. 58, pp. 400, 2009.
[37] Thomidis T, "Pathogenicity and Characterization of *Pilidiella granati* Causing Pomegranate Diseases in Greece", Eur. J. Plant Pathol., vol. 141, pp. 45-50, 2015.
[38] Joshi MS, Sawant DM, "Variation in Virulence and Cross Infectivity Potential of *C. gloeosporioides* Isolates Infecting Tropical Fruits", The Journal of Agriculture and Natural Resources Sciences, vol. 1, no. 2, pp.127-131, 2014.