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Fungal Biodiversity of Strawberry Fields in Aydin, TURKEY

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Abstract: Strawberry is a kind of delicious and aromatic fruit, which can be consumed as fresh and also is suitable for industry. However, strawberry is exposed to many fungal diseases. The aim of this study is to determine the fungi that present in the field whether or not pathogenic. Samples were collected from different strawberry fields in Aydın in April 2015. Morphological identification was made according to the shape and color of the colonies, mycelium and spore structures. For molecular identification, ITS rDNA gene region was used. According to morphological and molecular methods, eleven different fungal genera were found on strawberries.

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1. Introduction

Biodiversity is the foundation living systems to which human success is actually associated [1]. It is one of the basic parts of nature and it ensures the survival of earth. Strawberries are known as plants belonging to the genus *Fragaria*. Taxonomically, the genus *Fragaria* is included in the family Rosaceae. Strawberry is a perennial herbaceous, short day plant. Strawberries are a consumable plant part, which also used in industry. Be that as it may, natural products are easily spoilt and as a rule have dynamic digestion amid the capacity organize [2]. The significance of organic products in human nourishment can't be overestimated as it gives basic development factors, for example, vitamins and minerals important for continuation of human life [3]. The high concentration of various sugars, minerals, vitamins, amino acids, and low pH also enhances the successful growth and survival of various forms of fungi [4]. Annual reports have shown that 20% of fruits and vegetables produced are lost to spoilage [5].

According to Food and Agriculture Organization (FAO) (2012), Turkey was in the 3rd place in strawberry production. But about 15% of the products were lost in the field before harvest due to the diseases according to farmers.

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The organic action is a fundamental factor in the physical and substance advancement of soils [6]. There are 110.000 defined fungi species were present in the World but it is estimated that 1.5 million fungi species exists [7]. The ITS region is considered to be a good candidate for accurate detection and can largely separate from all other species by this application.

It is essential to decide the decent variety of organisms, which cause infections on strawberries and their natural and hereditary impacts. In this study, fungi on strawberry fruits will be detected by morphological and molecular methods.

2. Material and Methods

2.1. Sample Collection

Samples were collected aseptically from the strawberry fields in Aydın (Yenipazar, Atça, Sultanhisar, Köşk and Umurlu) in April 2015 during harvest time. Rotten strawberry fruits were collected in sterile plastic bags and kept in the portable refrigerator until brought to the laboratory.

2.2. Isolation of Fungal Species

One gram of strawberry fruits was weighted and homogenized in 9 ml of 0.85% physiologic Saline Water (PSW). 100 µL of these homogenized samples were inoculated on Rose Bengal Choloramphenicol Agar and Potato Dextrose Agar. Samples were incubated at 27°C for 5 days. After the incubation, the differentiated fungi samples were selected and isolated from the mixed colony under the same incubation conditions.

2.3. Morphological Identification

Morphological identification of the samples was realized according to Samson [8]. Mycelium and spore structures smeared on a slide, dyed with lactophenol cotton blue and visualized under the microscope. Colonial shapes were determined and used to identify species microscopically.

2.4. Molecular Identification

Fungi samples were put in 1.5 ml eppendorf tubes using a sterile toothpick. After, samples have reduced the powder using liquid nitrogen. DNA isolation of the samples was realized with 2X CTAB isolation protocol according to Doyle and Doyle [9]. Concentration and purity of the samples were measured with a Nanodrop Spectrophotometer (Thermo). ITS rDNA gene region was used to identify the species (ITS1: 5'TCCGTAGGTGAACCTGCGG'3, ITS4: 5'TCCTCCGCTTATTGATATGC'3) [10]. PCR reaction conditions were: initial denaturation 94 ºC 5 min, denaturation 94 ºC 30 sec, annealing 60 ºC 30 sec, extension 72 ºC 60 sec with 35 cycles and a final extension at 72ºC 10 min. Reagents concentrations were: 10X Taq Buffer, 0.5M dNTP mix, 10 pM from each primer, 7.5 mM $MgCl_2$ and 1U Taq polymerase (ABM) with the final volume of 25 µl. Agarose gel electrophoresis of the samples was observed on 1.4% agarose concentration at 90 V 40 min. 100 bp DNA ladder was used for size comparison of the products. PCR products were sent to DNA sequencing (Macrogen, Holland).

2.5. Data Analysis

Sequence results were aligned with the ones in GenBank using BLASTn software to find out the species of the samples. MEGA6 was used to infer phylogenetic tree.

3. Results

3.1. Morphological Identification

Morphological methods showed eleven different fungal species (Table 1). Colony shape, mycelium and spore structures were observed to this purpose.

Table 1. Morphological identification of the species

No	Name	Location
1	*Rhizopus* sp.	Yenipazar, Atça
2	*Lichtheimia* sp.	Yenipazar
3	*Alternaria* sp.	Yenipazar, Atça, Sultanhisar
4	*Fusarium* sp.	Yenipazar, Atça, Sultanhisar, Köşk, Umurlu
5	*Syncephalastrum* sp.	Yenipazar, Atça
6	*Aspergillus* sp.	Yenipazar, Atça, Sultanhisar
7	*Cladosporium* sp.	Sultanhisar
8	*Trichoderma* sp.	Yenipazar, Atça
9	*Talaromyces* sp.	Atça
10	*Botrytis* sp.	Yenipazar, Atça, Sultanhisar, Köşk, Umurlu
11	*Syncephalastrum monosporum*	Yenipazar, Atça

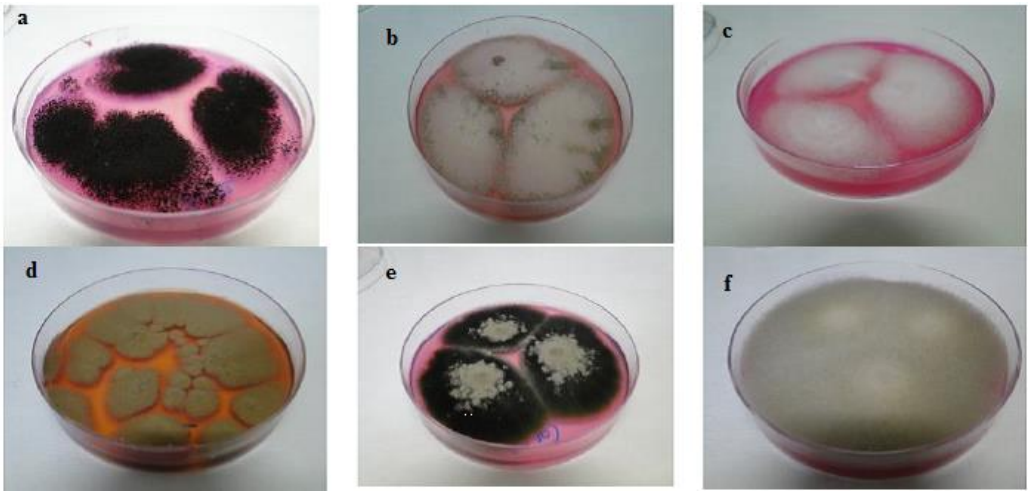

Figure 1. Colonial shapes of some fungi isolated. a: *Aspergillus niger*, b: *Rhizopus oryzae*, c: *Lichtheimia corymbifera*, d: *Cladosporium cladosporioides*, e: *Trichoderma atroviride*, f: *Botrytis cinera*

3.2. Molecular Identification

ITS rDNA gene region was used to identify fungal samples at the species level. PCR products were sent to sequencing to Macrogen (Holland). Molecular identification was made by comparing sequences with GenBank using BLASTn. Nine fungal species were found in contrast with morphological results (Table 2).

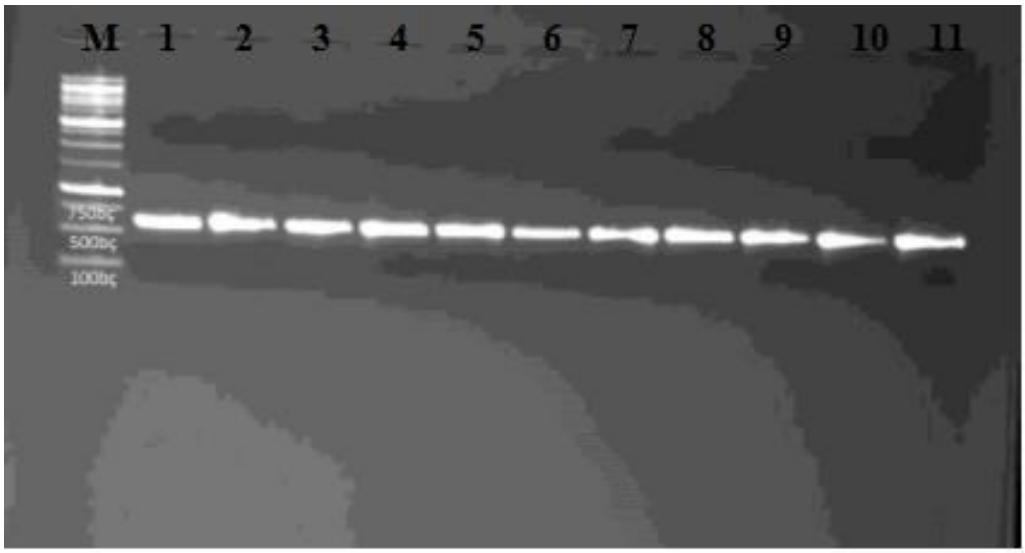

Figure 2. ITS PCR results of samples. (M: 100bp marker (ABM), 1-11: Samples)

Table 2. Molecular Identification of species

No	Name	Accession No	Location
1	*Rhizopus oryzae*	KJ417550.1, AY213685.1	Yenipazar, Atça
2	*Lichtheimia corymbifera*	LN812956.1	Yenipazar
3	*Alternaria alternata*	KP131535.1, KX463014.1, KP131533.1	Yenipazar, Atça, Sultanhisar
4	*Fusarium proliferatum*	GU074010.1, GQ856689.1, EU151490.1	Yenipazar, Atça, Sultanhisar, Umurlu, Köşk
5	*Syncephalastrum monosporum*	JQ954886.1	Yenipazar, Atça
6	*Aspergillus niger*	AF108474.1	Yenipazar, Atça, Sultanhisar, Umurlu, Köşk
7	*Cladosporium cladosporioides*	EF405864.1	Sultanhisar
8	*Trichoderma atroviride*	AF456920.1, KX538952.1	Yenipazar, Atça
9	*Bortytis cinerea*	KX766413.1, KX387891.1, KP234034.1	Yenipazar, Atça, Sultanhisar, Umurlu, Köşk

MEGA6 was used to construct the phyloenetic tree. Maximum likelihood method based on the Jukes-Cantor model was used (Figure 3). MP tree was obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value.

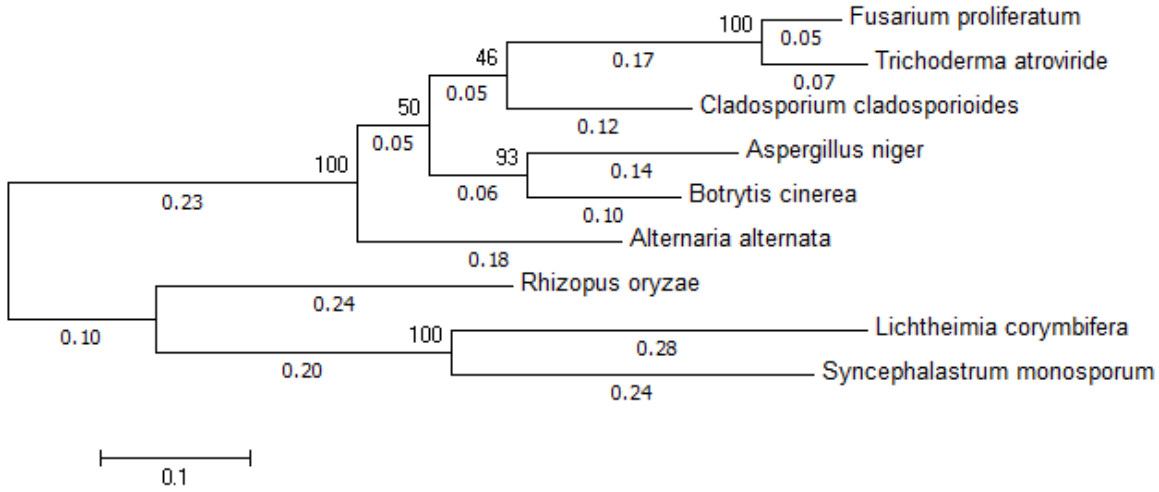

Figure 3. The evolutionary history was inferred using the Maximum Likelihood method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA6. Percenteces of the branches were shown on the nods.

4. Discussion

Eleven kinds of fungi differentiated from each other in morphological are identified from these strains. For molecular identification, rDNA ITS gene part of 78 examples is multiplied and as a result of the compare with GENEBANK data, 20 different kinds that belong to 9 species were determined. Literature shows that procedures, such as gathering and transporting, natural products may experience physical damage that builds post-reap misfortune and the likelihood of contagious pollution [11, 12].

Kasiamdari et al. (2002), isolated *R. solani* CFM1 isolate from cabbage, designed two primers from the ITS gene region and suggested that molecular methods would provide more accurate results than classical methods [13].

Staats et al. (2004) used the DNA sequence of 3 nuclear protein-coding genes (RPB2, G3PDH and HSP60) to classify *Botrytis* spp. They also compared them to conventional methods. The results of phylogenetic analyses were showed that *Botrytis* spp. were separated from *Sclerotiniaceae* species [14].

Khairnar et al. (2011) investigated soil-borne fungal biodiversity of some fruit crops in India and found 21 different fungal species and suggested that all twenty one fungal species can be controlled with 500 ppm Moximate, a fungicide [15].

Mailafia et al. (2017) researched fungi associated with fruit species and identified six different fungi and one yeast species [5].

Botrytis cinera is the cause of gray mold disease [16]. *Lichtheimia corymbifera* is the principle pathogen causing human and animal infections. Though only one sample was found, it wasn't widespread in the sample location [17]. *Syncephalastrum monosporum* is the endophytic fungal community of cacao and can also be found in the eyes of healthy horses, nests of laboratory reared leaf cutter ants, poultry feed, and spices [18]. *Rhizopus oryzae* is commonly found on dead organic matter and cause of disease [19]. *Alternaria alternata* is a common plant pathogen [20].

5. Conclusion

This study was made to detect fungal biodiversity on strawberries in Aydın, Turkey. As a result, nine fungal species were identified both by morphological and by molecular methods. Despite the usage of fungicides fungal diseases, such as gray mold, leaf spot disease can still be seen frequently both pre- and post-harvest. These species only were found on fruits of the plant. Investigation of soil and other plant parts can be resulted in more fungal species to be found.

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6. References

[1] Reddy, C.S., Ghai, R., Rashmi, K., & Kalai, V.C. (2003). Polyhydroxyalkanoates: an overview. *Bioresource Technol*., 87(2), p.137-146.

[2] Singh, D., & Sharma, R.R. (2007). Postharvest diseases of fruit and vegetables and their management. In: Prasad, D., editor. Sustainable Pest Management. Daya Publishing House, New Delhi, India.

[3] Al-Hindi, R.R., Al-Najada, A.R., & Mohamed, S.A. (2011). Isolation and identification of some fruit spoilage fungi: Screening of plant cell wall degrading enzymes. *Afr. J. Microbiol. Res.*, 5(4), 443-448.

[4] Droby, S. (2006). Improving quality and safety of fresh fruits and vegetables after harvest by the use of biocontrol agents and natural materials. Acta Hortic., 709, 45-51.

[5] Mailafia S, Okoh GR, Olabode HOK, Osanupin R (2017) Isolation and identification of fungi associated with spoilt fruits vended in Gwagwalada market, Abuja, Nigeria, *Veterinary World*, 10(4), 393-397.

[6] Bardgett, R.D. (2005). *The biology of soil: a community and ecosystem approach*. Oxford University Press Inc, New York.

[7] Hawksworth, D. L. (2001). The magnitude of fungal diversity: the 1.5 million species estimate revisited** Paper presented at the Asian Mycological Congress 2000 (AMC 2000), incorporating the 2nd Asia-Pacific Mycological Congress on Biodiversity and Biotechnology, and held at the University of Hong Kong on 9-13 July 2000. *Mycological research*, *105*(12), 1422-1432.

[8] Samson, R.A., Hoekstra, E.S., & Frisvad, J.C. (2004). *Introduction to Food-and Airborne Fungi*, Laboratory Manual Series 2, Food and Indoor Fungi, 389pp.

[9] Doyle, J.J., Doyle, J.L., (1987). Isolation of Plant DNA From Fresh Tissue, *Focus*, 12, 13-15.

[10] Boysen, M., Skoube, P., Frisvad, J., Rossen, L. (1996). Reclassification of the *Penicillium roqueforti* group into three species on the basis of molecular genetic and biochemical profiles. *Microbiology*. 142, 541-519.

[11] Baiyewu, R.A., Amusa, N.A., Ayoola, O.A. and Babalola, O.O. (2007). Survey of the postharvest diseases and aflatoxin contamination of marketed Pawpaw fruit (*Carica papaya* L.) in South Western Nigeria. *Afr. J. Agric. Res*., 2(4), 178-181.

[12] Chukwuka, K.S., Okonko, I.O. and Adekunle, A.A. (2010). Microbial ecology of organisms causing pawpaw (*Carica papaya* L.) fruit decay in Oyo State, Nigeria. *Am. Eurasian J. Toxicol. Sci*., 2(1), 43-50.

[13] Staats, M., Baarlen, P.V., Kan J.A.V. (2004). Molecular Phylogeny of the Plant Pathogenic Genus Botrytis and the Evolution of Host Specificity. *Molecular Biology and Evolution*, 22(2), 333-346.

[14] Kasiamdari, R.S., Smith, E.S., Scott, E.S., Smith, F.A. (2002). Identification of binucleate Rhizoctonia as a contaminant in pot cultures of arbuscular mycorrhizal fungi and development of a PCR-based method of detection. *Mycol. Res.* 106 (12), 1417–1426.

[15] Khairnar. D. N., Kelhe. A. S., Khairnar. A. B. (2011). Soil-borne Fungal Biodiversity of Some Fruit Crops of Nashik District and Control Measures. *Nature Environment and Pollution Technology*, 10(1), 127-128.

[16] Adrian, M., Jeandet, P., Veneau, J., Weston, L. A., & Bessis, R. (1997). Biological activity of resveratrol, a stilbenic compound from grapevines, against *Botrytis cinerea*, the causal agent for gray mold. *Journal of Chemical Ecology*, *23*(7), 1689-1702.

[17] Bellanger, A. P., Reboux, G., Botterel, F., Candido, C., Roussel, S., Rognon, B., Millon, L. (2010). New evidence of the involvement of Lichtheimia corymbifera in farmer's lung disease. *Medical mycology*, *48*(7), 981-987.

[18] Gautam, R., Singh, S. K., & Sharma, V. (2016). Molecular diagnosis and intraspecific genetic variability of root pathogens of arid legumes in Western Rajasthan, India. *Revista de Biología Tropical*, *64*(4).

[19] Howell, C. R. (2003). Mechanisms employed by Trichoderma species in the biological control of plant diseases: the history and evolution of current concepts. *Plant disease*, *87*(1), 4-10.

[20] Lagopodi, A. L., & Thanassoulopoulos, C. C. (1998). Effect of a leaf spot disease caused by Alternaria alternata on yield of sunflower in Greece. *Plant disease*, *82*(1), 41-44.