Turkish Journal of Agriculture - Food Science and Technology, 11(s1): 2525-2529, 2023
DOI: https://doi.org/10.24925/turjaf.v11is1.2525-2529.6420

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Identification of *Alternaria, Aspergillus, Fusarium* and *Penicillium* spp. on Onion Plant (*Allium cepa* L.) Growing in Hatay, Amasya and Tokat Provinces Using MALDI-TOF Mass Spectrometry

Merve Kara[1,a,*], Emine Mine Soylu[1,b]

[1]*Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Plant Protection, 31034 Antakya, Hatay, Türkiye*
[*]*Corresponding author*

ARTICLE INFO

Research Article

Received : 02.10.2023
Accepted : 21.11.2023

Keywords:
Onion
Disease pathogen
MALDI-TOF MS
Diagnosis
Proteomic

ABSTRACT

Plant fungal disease pathogens cause significant yield and quality losses in onion growing areas. In addition to yield losses, they cause negative effects that reduce the quality and export potential of the product, resulting in significant economic losses during harvest, post-harvest, processing and marketing stages. In recent years, Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) has emerged as a rapid, cost-effective, reproducible, and powerful technique for identifying microorganisms, and its impact on microbiological diagnosis has transformed workflow in equipped laboratories. In this study, proteomic analyzes were performed on *Alternaria, Aspergillus, Fusarium* and *Penicillium* species isolated from onion growing areas in Hatay, Amasya, and Tokat provinces. After extraction of mycelium from single spore cultures of the isolates with ethanol-formic acid, the spectra of the individual fungal isolates were determined using the Flex control software program. These spectra were compared with Maldi Biotyper Real-Time Classification (RTC) and identification was performed. Of 519 different fungal isolates, 435 representative fungal isolates (83.8%) were identified by MALDI TOF MS. Eighty-four fungal isolates could not be identified because they were not in a satisfactory range of purity and identification. Of the 435 isolates, 269 (61.8%) were identified as *Fusarium* spp., 80 isolates (18.4%) were identified as *Alternaria* spp., 60 isolates (13.8%) as *Aspergillus* spp., and 26 isolates (6.0%) as *Penicillium* spp. Among the fungal isolates, 72.5% of the *Fusarium* isolates, 78.8% of the *Alternaria* isolates, 90.0% of the *Aspergillus* isolates and 84.6% of the *Penicillium* isolates were identified as "highly probable" species with score values between 2.000-3.000 (green color). These species are *Alternaria alternata, Alternaria infectoria, Aspergillus flavus, Aspergillus niger, Fusarium culmorum, Fusarium moniliforme, Fusarium oxysporum, Fusarium proliferatum, Fusarium solani, Fusarium verticillioides, Penicillium commune* and *Penicillium glabrum*. The results clearly demonstrate that MALDI TOF MS biotyping may be used as a highly reliable and economical diagnostic method for routine diagnosis of diseases caused by *Alternaria, Aspergillus, Fusarium* and *Penicillium* species.

[a] *mervekara@mku.edu.tr* *https://orcid.org/0000-0001-7320-3376* | [b] *msoylu@mku.edu.tr* *https://orcid.org/0000-0001-5961-0848*

Introduction

Onion (*Allium cepa* L.) is one of the vegetables with a great variety when examined considering its different characteristics. Many factors prevent production in the areas where onion cultivation is carried out. Among them, fungal, bacterial and viral disease pathogens cause significant yield and quality loss in different development periods of the onion plant (Schwartz and Mohan, 2008). These diseases and pests create negative effects that reduce the quality and export potential of the product, which cause significant economic losses during harvest, post-harvest, processing and marketing stages, as well as a decrease in yield. Among the fungal and fungal-like disease pathogens, *Alternaria porri*, *Alternaria alternata*, *Alternaria tenuissima*, *Alternaria palandui*, *Alternaria brassicola*, *Botrytis cinerea*, *Botrytis aclada*, *Botrytis allii*, *Botrytis byssoidea*, *Botyrtis squamosa*, *Botrytis porri*, *Cladosporium* sp., *Stemphylium* sp., *Urocystis cepulae* and *Peronospora destructor* cause significant product losses by causing disease in the green parts of the plant. Fungal disease pathogens such as *Aspergillus niger, Aspergillus oryzae, Penicillium georgiense, Penicillium polonicum, Penicillium glabrum* and *Penicillium expansum* cause bulb rot in onions before and after harvest. Soil-borne fungal disease pathogens such as *Pythium* spp., *Fusarium* spp.,

Rhizoctonia solani, *Sclerotium rolfsii* and *Sclerotium cepivorum* cause diseases such as wilt, root and root rot and damping-off in onions (Smith, 1988; Haq et al., 2003; Chilvers et al., 2007; Schwartz and Mohan, 2008; Dumbre et al., 2011; Bayraktar et al., 2014; Oh et al., 2015; Duduk et al., 2017; Chethana et al., 2018).

In the provinces where onion cultivation is intense in Turkey, the definitive diagnosis of fungal pathogens seen in onion plants using the latest technological diagnostic devices is of great importance for the development of correct control methods.

In recent years, Matrix-assisted lasser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) has emerged as a powerful technique for the identification of microorganisms and its impact in microbiological diagnostics has changed the workflow in well-established laboratories. Compared to traditional diagnostic methods that rely on biochemical testing and require long incubation procedures, MALDI-TOF MS has the advantage of identifying bacteria and fungi directly from colonies grown in culture media in a few minutes and with simple procedures (Kurt et al., 2020; Uysal et al., 2022). Many studies available in different systems have proven the reliability and accuracy of the method (Carolis et al., 2014, Uysal et al., 2019; Soylu et al., 2021; Kara et al., 2022). In this respect, it offers a powerful alternative to microscopic and molecular biology methods. Today, commercial MALDI systems are available for diagnostic applications in clinical medicine, biotechnology, and industrial as well as biological research studies. Although it is mostly used in bacterial biotyping, many experimental strategies have been developed for the analysis of fungi. Members of many fungal genera such as *Aspergillus*, *Fusarium*, *Penicillium* or *Trichoderma*, as well as various yeasts from clinical specimens (e.g. *Candida albicans*) have been successfully identified with MALDI-TOF MS (Chalupová et al., 2014).

In this study, *Alternaria*, *Aspergillus*, *Fusarium* and *Penicillium* species isolated from onion growing areas in Hatay, Amasya and Tokat provinces were identified by MALDI-TOF MS system.

Materials and Methods

Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) Analysis

All fungal isolates were preliminary diagnosed morphologically using dichotomic keys. Colony color, reverse colony color, shape and size of conidia and conidiophores, shape and size of macro and/or microconidia, presence of sexual structures, sclerotia production and formation of chlamidospores on selective and general nutrient media such as CDA (Czapek Dox Agar), CMA (Corn Meal Agar), MEA (Malt Extract Agar), YEA (Yeast Extract Agar), CLA (Carnation Leaf Agar) and PDA (Potato Dextrose Agar) media were used as morphological fungal parameters as suggested for each fungal genera (Nelson et al., 1983; Ellis, 1993, Barnett and Hunter, 2003; Frisvad and Samson, 2004; Chethana et al., 2018).

Fungal isolates, previously obtained from onion growing areas in Hatay, Amasya and Tokat provinces of Turkey, were grown in PDA medium for 5-7 days. Up to 3-5 mycelial discs (0.5 cm) were transferred to plastic tubes containing an average of 8 ml potato dextrose broth (PDB) broth. The cultures were allowed to grow on the rotator (13.000 rpm) in plastic tubes for 2-3 days at room temperature. Formic acid ethanol extraction processes were used for MALDI-TOF MS analyses. First of all, each isolates were taken into a 1.5 ml Eppendorf tube. It was centrifuged at 13000 rpm for 2 minutes. The liquid part was removed with the help of a Pasteur pipette so that the pellet remained at the bottom. Then, centrifugation was repeated by adding 1 ml of distilled water at HPLC value. The liquid part was removed so that the pellet remained at the bottom again. Then 300 µl of HPLC distilled water was added to the pellet and vortexed. Then, 900 µl of ethanol was added, vortexed again and centrifuged at 13000 rpm for 2 minutes. After centrifugation, the ethanol was completely removed and left to dry for 5-10 min at 37°C. Depending on the size of the pellet, between 10 and 80 µl of 70% formic acid was added to the dried pellet and vortexed. Acetonitrile was added as much as the amount of formic acid added and vortexed again. Finally, it was centrifuged at 13000 rpm for 2 minutes. After centrifugation, 1 µl of the upper liquid part was loaded into the wells of the MALDI-TOF MS target and left to dry. After drying, it was covered with HCCA (α-Cyano-4-hydroxycinnamic acid) matrix liquid. After the wells were completely dry, they were loaded onto the target device. Spectra were taken with the Flex control software program. Then, these spectra were compared with the MALDI Biotyper V9.0 software program and the species identification was completed (Biotyper 3.0; Microflex LT; Bruker Daltonics GmbH, Bremen, Germany).

As a result of the analysis; the scores between 2.300-3.000 (green color) probable species identification, 2.000-2.299 (green color) reliable genus level diagnosis and probable species level diagnosis, 1.700-1.999 (yellow color) probable genus level diagnosis, 0.000- a score of 1.699 (red color) was considered as an unreliable diagnosis (Uysal et al., 2019; Soylu et al., 2021; Kara et al., 2022; Uysal et al., 2022).

Results and Discussion

A total of 1691 fungal isolates were obtained from different fields and provinces in Tokat, Amasya and Hatay provinces where important onion cultivation was made. Pre-diagnosis of each isolates were made according to their morphological properties. Single spore cultures of 519 isolates pre-diagnosed as *Alternaria*, *Aspergillus*, *Fusarium* and *Penicillium* spp. were obtained for MALDI-TOF analysis. The spectra of the device were taken with the Flex control software program following the extraction of mycelium from single spore cultures of the isolates with ethanol-formic acid. These spectra were compared with the Maldi Biotyper Real-Time Classification (RTC) and diagnosis process was performed (Figure 1).

Among 519 different fungal isolates, 435 isolates were diagnosed (83.8%) using the MALDI TOF MS analysis. Eighty four fungal isolates could not be diagnosed because they were not in the satisfactory purity and identification spectrum. Of the 435 isolates identified, 269 (61.8%) were identified as *Fusarium* spp., 80 (18.4%) isolates were *Alternaria* spp., 60 isolates (13.8%) were *Aspergillus* spp., and 26 (6.0%) isolates were *Penicillium* spp.

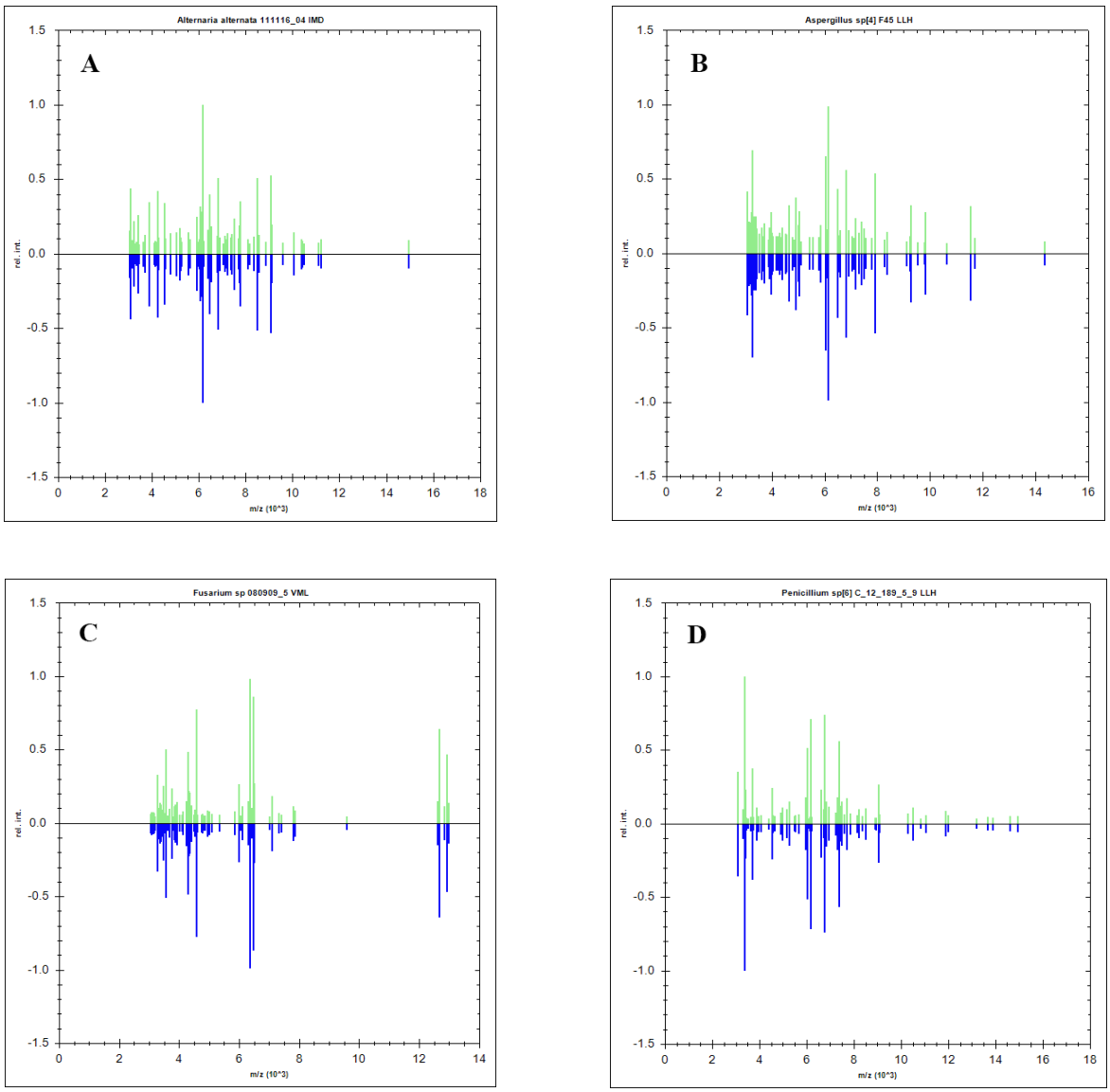

Figure 1. MALDI-TOF MS spectra of representative isolates belonging to *Alternaria* (A), *Aspergillus* (B), *Fusarium* (C) and *Penicillium* (D) genera identified using MALDI Biotyper 3.0 program

Amongst identified isolates, 72.5% of the isolates of the *Fusarium* genus, 78.8% of the *Alternaria* genus, 90.0% of the *Aspergillus* genus and 84.6% of the *Penicillium* isolates with score values between 2,000-3,000 (green color) were identified as "highly probable" species. These species are *Alternaria alternata, Alternaria infectoria, Aspergillus flavus, Aspergillus niger, Fusarium culmorum, Fusarium moniliforme, Fusarium oxysporum, Fusarium proliferatum, Fusarium solani, Fusarium verticillioides, Penicillium commune* and *Penicillium glabrum*.

The distribution of 435 isolates obtained from different provinces and identified with MALDI TOF MS according to the plant parts from which they were isolated on the basis of provinces and their % ratios are given in Tables 1 and 2. In this way, a preliminary diagnosis process was carried out according to protein analysis of species belonging to *Alternaria, Aspergillus, Fusarium* and *Penicillium*. This study is the first in Turkey to use the MALDI TOF MS technique for the identification of *Alternaria, Aspergillus, Fusarium* and *Penicillium* species in onions. When the MALDI TOF MS method is compared with molecular methods considering the economic cost aspect, it has clearly shown that MALDI TOF MS biotyping can be used as a highly reliable and economical diagnostic method for the routine diagnosis of diseases caused by *Alternaria, Aspergillus, Fusarium* and *Penicillium* species.

It has also been reported in many taxonomic identification studies that the MALDI-TOF MS technique is an effective device for the diagnosis of Ascomycetes phytopathogenic fungi belonging to the *Alternaria* genus, such as *Alternaria dauci* in carrots, *A. porri* in onions, *Alternaria solani* in potatoes, and *Alternaria tomatophila* in tomatoes (Brun et al., 2013; Chalupová et al., 2014). Hettick et al. (2008a) described 12 *A. flavus* species and 5 strains using the MALDI TOF MS technique. In another study, 12 *Penicillium* species were analyzed by MALDI TOF MS technique (Hettick et al., 2008b). The MALDI-TOF MS technique was also used to differentiate *P. expansum* and *Penicillium pinophilum* in apples and *P. citrinum, P. italicum* and *P. digitatum* in citrus fruits (Chen and Chen, 2005).

Table 1. The isolate numbers of fungal species obtained from Hatay, Amasya and Tokat provinces and identified by MALDI TOF MS analysis according to the samples from which they were isolated (piece)

Species Name	Hatay					Amasya					Tokat					T
	R	B	L	F	S	R	B	L	F	S	R	B	L	F	S	
Alternaria alternata	-	-	17	2	-	1	2	11	4	-	-	-	14	28	-	79
Alternaria infectoria	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
Aspergillus flavus	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1
Aspergillus niger	3	21	-	-	-	2	3	3	1	1	2	7	8	8	1	59
Fusarium culmorum	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1
Fusarium moniliforme	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1
Fusarium oxysporum	11	9	5	-	5	51	27	19	-	-	16	11	4	6	3	167
Fusarium proliferatum	2	6	8	-	-	8	4	15	1	1	3	4	7	1	-	59
Fusarium solani	2	1	-	-	-	4	-	1	-	-	1	-	-	-	-	9
Fusarium verticillioides	2	1	2	-	-	10	5	6	-	-	2	-	3	1	-	32
Penicillium commune	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1
Penicillium glabrum	5	4	-	-	-	3	5	1	-	-	2	3	-	2	-	25
Total	25	42	32	2	5	82	45	56	6	2	26	25	37	46	4	435

R: Root; B: Bulb; L: Leaf; F: Flower; S: Soil; T: Total

Table 2. Distribution of fungal species obtained from Hatay, Amasya and Tokat provinces of Turkey and identified according to MALDI TOF MS analysis according to the plant parts they isolated (%)

Species Name	Hatay					Amasya					Tokat				
	R	B	L	F	S	R	B	L	F	S	R	B	L	F	S
Alternaria alternata	-	-	89.5	10.5	-	5.6	11.1	61.1	22.2	-	-	-	33.3	66.7	-
Alternaria infectoria	-	-	-	-	-	-	-	-	-	-	-	-	100.0	-	-
Aspergillus flavus	-	-	-	-	-	100.0	-	-	-	-	-	-	-	-	-
Aspergillus niger	12.5	87.5	-	-	-	20.0	30.0	30.0	10.0	10.0	7.7	26.9	30.8	30.8	3.8
Fusarium culmorum	-	-	-	-	-	-	100.0	-	-	-	-	-	-	-	-
Fusarium moniliforme	-	-	-	-	-	100.0	-	-	-	-	-	-	-	-	-
Fusarium oxysporum	36.7	30.0	16.7	-	16.7	52.6	27.8	19.6	-	-	40.0	27.5	10.0	15.0	7.5
Fusarium proliferatum	12.5	37.5	50.0	-	-	27.5	13.7	51.7	3.4	3.4	20.0	26.7	46.7	6.7	-
Fusarium solani	66.7	33.3	-	-	-	80.0	-	20.0	-	-	100.0	-	-	-	-
Fusarium verticillioides	40.0	20.0	40.0	-	-	47.6	23.8	28.6	-	-	33.3	-	50.0	16.7	-
Penicillium commune	-	-	-	-	-	100.0	-	-	-	-	-	-	-	-	-
Penicillium glabrum	55.6	44.4	-	-	-	33.3	55.6	11.1	-	-	28.6	42.9	-	28.6	-

R: Root; B: Bulb; L: Leaf; F: Flower; S: Soil

Recently, two *F. solani* isolates (CFs4 and CFs8) that cause dry rot in citrus roots in the Eastern Mediterranean Region were identified with MALDI-TOF MS technique (Kurt et al., 2020). Similar to our study, Al-Hatmi et al. (2016) was successfully identified *Fusarium ficicrenscens* as a differential species in the *Fusarium fujikuroi* species complex by using the formic acid-ethanol extraction method. In our study, all obtained spectra were evaluated on the Bruker Mikroflex platform using MALDI Biotyper V2.0 software. As a result, the device made a reliable diagnosis of 3 isolates of *Fusarium* with 2.193, 2.200 and 2.226 score values. De Carolis et al. (2012) created their own library for Mucorales, *Fusarium* and *Aspergillus* species with the Biotyper system and identified 97% of 94 isolates at the species level. Santos et al. (2015) tested the MALDI-TOF MS technique to identify *Fusarium guttiforme* on pineapple side shoots in situ. The identification of a plant pathogen (*F. guttiforme*) and its antagonist (*Trichoderma asperellum*) using MALDI-TOF MS have been demonstrated. On the other hand, Masih et al. (2016) identified 95% of *Aspergillus* species using the Bruker system and the database they developed.

In conclusion, MALDI-TOF MS technique for the early detection of filamentous fungi infecting agricultural products has not been extensively studied and data is lacking in the literature.

Acknowledgements

This study was supported by Hatay Mustafa Kemal University Scientific Research Projects Commission (HMKU BAP-18.D.001 project) and contains a part of the first author's doctoral thesis. This research was presented at the 3rd International Congress of the Turkish Journal of Agriculture - Food Science and Technology, Malatya, Turkiye, held on 13 and 16 September 2023 (as an oral presentation).

References

Al-Hatmi SMA, Mirabolfathy M, Hagen F, Normand A, Stielow BJ, Karami-Osbo R, Van Diepeningen DA, Meis FJ, De Hoog SG. 2016. DNA barcoding, MALDI-TOF, and AFLP data support *Fusarium ficicrescens* as a distinct species within the *Fusarium fujikuroi* species complex. Fungal Biology, 120: 265-278. doi: 10.1016/j.funbio.2015.08.001

Barnett HL, Hunter BB. 2003. Illustrated Genera of Imperfect Fungi, Fourth Edition. APS Press, St. Paul Minnesota. pp218.

Bayraktar H, Tekin K, Özer G. 2014. Soğan üretimi ile ilişkili farklı *Fusarium* türlerinin PCR-RFLP analizi. Anadolu Journal of Agricultural Sciences, 29 (3): 194-198. doi: 10.7161/anajas.2014.29.3.194-198

Brun S, Madrid H, Gerrits Van Den Ende B, Andersen B, Marinach-Patrice C, Mazier D, De Hoog S. 2013. Multilocus phylogeny and MALDI-TOF analysis of the plant pathogenic species *Alternaria dauci* and relatives. Fungal Biology, 117: 32-40. doi: 10.1016/j.funbio.2012.11.003

Chalupová J, Raus M, Sedlářová M, Šebela M. 2014. Identification of fungal microorganisms by MALDI-TOF mass spectrometry. Biotechnology Advances, 32: 230-241. doi: 10.1016/j.biotechadv.2013.11.002

Chalupová J, Raus M, Sedlářová M, Šebela M. 2014. Identification of fungal microorganisms by MALDI-TOF mass spectrometry. Biotechnology Advances, 32: 230-241. doi: 10.1016/j.biotechadv.2013.11.002

Chen HY, Chen YC. 2005. Characterization of intact *Penicillium* spores by matrix-assisted laser desorption/ionization mass spectrometry. Rapid Communications in Mass Spectrometry, 19: 3564-8. doi: 10.1002/rcm.2229

Chethana BS, Girija G, Rao AS, Bellishree K. 2018. Morphological and molecular characterization of *Alternaria* isolates causing purple blotch disease of onion. International Journal of Current Microbiology and Applied Sciences, 7: 3478-3493.

Chilvers MI, du Toit LJ, Akamatsu H, Peever TL. 2007. A real-time, quantitative PCR seed assay for *Botrytis* spp. that cause neck rot of onion. Plant Disease, 91: 599-608. doi: 10.1094/PDIS-91-5-0599

De Carolis E, Posteraro B, Lass-Florl C, Vella A, Florio AR, Torelli R, Girmenia C, Colozza C, Tortorano AM, Sanguinetti M, Fadda G. 2012. Species identification of *Aspergillus*, *Fusarium* and *Mucorales* with direct surface analysis by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clinical Microbiology and Infection, 18: 475-484. doi: 10.1111/j.1469-0691.2011.03599.x

De Carolis E, Vella A, Vaccaro L, Torelli R, Spanu T, Fiori B, Posteraro B, Sanguinetti M. 2014. Application of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. The Journal of Infection in Developing Countries, 8 (9): 1081-1088.

Duduk N, Lazarević M, Žebeljan A, Vasić M, Vico I. 2017. Blue mould decay of stored onion bulbs caused by *Penicillium polonicum*, *P. glabrum* and *P. expansum*. Journal of Phytopathology, 165: 662-669. doi: 10.1111/jph.12605

Dumbre S, Guldekar D, Potdukhe RS. 2011. Sörvey of seed borne fungi of onion (*Allium cepa* L.) from various locations of Maharashtra. Journal of Soils and Crops, 21: 221-224.

Ellis MB. 1993. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew.

Frisvad JC, Samson RA. 2004. Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. Studies in Mycology, 49: 1-174.

Haq MA, Collin HA, Tomsett AB, Jones MG. 2003. Detection of *Sclerotium cepivorum* within onion plant using PCR primers. Physiological and Molecular Plant Pathology, 62: 185-189. doi: 10.1016/S0885-5765(03)00023-7

Hettick JM, Green BJ, Buskirk AD, Kashon ML, Slaven JE, Janotka E, Blachere FM, Schmechel D, Beezhold D. 2008a. Discrimination of *Penicillium* isolates by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry fingerprinting. Rapid Communications Mass Spectrometry, 22: 2555-60. doi: 10.1002/rcm.3649

Hettick JM, Green BJ, Buskirk AD, Kashon ML, Slaven JE, Janotka E, Blachere, FM, Schmechel D, Beezhold DH. 2008b. Discrimination of *Aspergillus* isolates at the species and strain level by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry fingerprinting. Analytical Biochemistry, 380: 276-81. doi: 10.1016/j.ab.2008.05.051

Kara M, Soylu S. 2022. Isolation of endophytic bacterial isolates from healthy banana trees and determination of their *in vitro* antagonistic activities against crown rot disease agent *Fusarium verticillioides*. (Mustafa Kemal University Journal of Agricultural Sciences, 27 (1): 36-46. doi: 10.37908/mkutbd.1021349

Kurt Ş,·Uysal A, Soylu EM,·Kara M, Soylu S. 2020. Characterization and pathogenicity of *Fusarium solani* associated with dry root rot of citrus in the eastern Mediterranean region of Turkey. Journal of General Plant Pathology, 86: 326-332. doi: 10.1007/s10327-020-00922-6

Masih A, Singh PK, Kathuria S, Agarwal K, Meis JF, Chowdhary A. 2016. Identification by molecular methods and matrix-assisted laser desorption ionization-time of flight mass spectrometry and antifungal susceptibility profiles of clinically significant rare *Aspergillus* species in a referral chest hospital in Delhi, India. Journal of Clinical Microbiology, 54: 2354-2364. doi: 10.1128/JCM.00962-16

Nelson PE, Toussoun TA, Marasas WFO. 1983. *Fusarium* Species: An Illustrated Manual For Identification. Pennsylvania State University, University Park.

Oh JY, Han GD, Jeong J, Sang MK, Chun S, Kim KD. 2015. First report of *Penicillium georgiense* as a fungal pathogen of onion (*Allium cepa* L.). Crop Protection, 72: 83-89. doi: 10.1016/j.cropro.2015.02.009

Santos C, Ventura JA, Costa H, Fernandes PMB, Lima N. 2015. MALDI-TOF MS to identify the pineapple pathogen *Fusarium guttiforme* and its antagonist *Trichoderma asperellum* on decayed pineapple. Tropical Plant Pathology, 40: 227-232. doi: 10.1007/s40858-015-0027-7

Schwartz HF, Mohan SK. 2008. Compendium of Onion and Garlic Diseases and Pests, Second Edition. APS Press.

Smith IM, Dunez J, Lellioth RA, Phillips PH, Archer SA. 1988. European Handbook of Plant Disease. Blackwell Scientific Publications, Oxford.

Soylu S, Kara M, Uysal A, Kurt Ş, Soylu E M. 2021. Determination of antagonistic potential of endophytic bacteria isolated from lettuce against lettuce white mould disease caused by *Sclerotinia sclerotiorum*. Zemdirbyste-Agriculture, 108 (4): 303-312. doi: 10.13080/z-a.2021.108.039

Uysal A, Kurt Ş, Soylu S, Kara M, Soylu EM. 2022. Hatay ilinde yer alan turunçgil paketleme tesislerinde meyve ve hava kökenli mikrobiyata içerisindeki fungal ve bakteriyel türler ile yoğunluklarının belirlenmesi. Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi, 27 (2): 340-351. doi: 10.37908/mkutbd.1095692

Uysal A, Kurt Ş, Soylu S, Soylu EM, Kara M. 2019. Yaprağı yenen sebzelerdeki mikroorganizma türlerinin MALDI-TOF MS (Matris Destekli Lazer Desorpsiyon/İyonizasyon Uçuş Süresi Kütle Spektrometresi) tekniği kullanılarak tanılanması. Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi, 29: 595-601. doi: 10.29133/yyutbd.627850