

Fungal Agents Causing Fruit Rot in Sweet Cherry Orchards and Storages in Isparta Province

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Article History

Received 30 December 2024

Accepted 15 April 2025

First Online 25 April 2025

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Keywords

Disease

Fruit decay

Fungal rot

Latent infections

Virulent

Abstract

Fruit rot, causing significant yield losses not only in the orchards, but also in storage conditions, is among the most common diseases of sweet cherries. Isparta Province, an important region for fruit production in Türkiye, comes fourth in sweet cherry production. In this study, it was aimed to determine the fungal agents causing fruit rot on sweet cherries in the orchards and in the cold storages in Isparta province. Healthy and diseased fruit samples were collected before harvest, from the randomly selected 76 orchards, in the districts of Isparta province where sweet cherry production was made. Fruit samples were also taken after harvest from three cold storages in Eğirdir District, and from one sweet cherry processing center in Uluborlu District. As a result of the isolations, the most common agents on the rotten fruit samples were; *Alternaria alternata*, *Monilinia laxa* and *Botrytis cinerea*, respectively. *A. alternata*, *Aspergillus* spp., *B. cinerea*, *Cladosporium cladosporioides*, *Fusarium oxysporum* and *M. laxa* were isolated from the symptomless fruits indicating latent infections. The most common fungi isolated from the rotten fruits in the cold storages were; *A. alternata*, *Trichoderma* spp., *Penicillium* spp. and *B. cinerea*, while *A. alternata* was also isolated from the healthy looking fruits. *Penicillium* spp., *B. cinerea* and *Rhizopus stolonifer* caused severe rot in the pathogenicity experiment, where *Stemphylium botryosum* and *Fusarium lateritium* were found as the less virulent species. *Fusarium lateritium* and *Trichothecium roseum* were reported as fruit rot agents on sweet cherries for the first time in this study, while *Aspergillus* spp., *F. oxysporum*, *Geotrichum candidum*, *Sclerotinia sclerotiorum*, *Stemphylium botryosum* and *Trichoderma* spp. were first reports for Türkiye.

1. Introduction

Sweet cherry (*Prunus avium* L.), belonging to Rosaceae family, has known to spread throughout the world by birds, other animals and immigrants from its original area between Caspian sea, South Caucasia and North East Anatolia. Cherries are among the important fruits in human nutrition with their high mineral contents (Koç, 2023). Sweet cherry is a product that makes significant contributions to the Turkish economy, as it requires intensive labor in both production and marketing stages, creates extensive work opportunities, and provides significant foreign exchange income in

exports (Kaplan et al., 2022). Türkiye ranks first in the world's sweet cherry production with 656 041 tons, while it ranks third in exports (FAOSTAT, 2022). Isparta province has an important place in terms of fruit production in Türkiye, and comes fourth in sweet cherry production with 46 565 tons (TÜİK, 2023).

Pre and post harvest fruit rots cause significant losses in sweet cherry production. Various researchers from different countries mentioned *Penicillium* species such as *P. expansum*, *P. crustosum* and *P. chrysogenum* as the main rot agents (Ceponis, 1987; Spotts et al., 1998; Sanzani et al., 2013; Lopez et al., 2016). Other fungi

commonly causing fruit rot on sweet cherries are; *Alternaria alternata*, *Aspergillus niger*, *Botrytis cinerea*, *Cladosporium* spp., *Colletotrichum* spp. and *Monilinia* spp. (Thomidis and Exadaktylou, 2012; Tarbath et al., 2014; Borve and Stensvand, 2015; Barry et al., 2015). *Mucor piriformis* was also reported to cause cherry fruit decay by some researchers (Michailides and Spotts, 1990; Borve et al., 2000; Lopez et al., 2016).

Number of studies on the determination of fruit rot agents of sweet cherries is quite limited in Türkiye. In a study on the determination of the effects of modified atmosphere packages on storage and shelf life of sweet cherries, it was found that the main fungal agents causing storage rot were *B. cinerea*, *Rhizopus stolonifer*, *Monilinia* spp. and *A. alternata*, while *Penicillium expansum* and *Cladosporium* species were isolated in lower rates (Şen et al., 2016). In another study performed in the Egean Region during 2015-2017, it was determined that *Monilinia* species (*M. laxa* and *M. fructicola*) caused fruit rot in sweet cherry orchards at rates varying between 5.3-10.8%, with increasing rates after harvest (Morca et al., 2022).

Fruit rot is the main problem both in the sweet cherry orchards and in the storages in Isparta province. Since it is well known that the latent pathogens on sweet cherry fruits in the orchards may cause infections after harvest, it is thought that the research on the determination of the fruit rot

agents should better be comprised the fruit samples both from the orchards and storages. Thus, determination of the fungal agents causing fruit rot in the sweet cherry orchards and storages in Isparta province was aimed in the present study.

2. Materials and Methods

2.1. Collection of the samples

Surveys were performed in the period of June 19 and July 7, 2023, just before harvest and fruit samples were collected from 76 sweet cherry orchards randomly selected in 13 districts of Isparta province, according to the numbers of sweet cherry trees of the districts (TÜİK, 2021). Number of surveyed orchards increased with the increasing tree numbers, where in the districts with less than 25 000 trees only 2 orchards were investigated, while 10 orchards were selected in the districts with tree numbers higher than 200 000 (Table 1). In the orchards, at least 100 representing trees were examined during the period near harvest and fruits with rot symptoms were taken (Figure 1). In addition, to determine the latent infections, healthy looking fruits from each orchard were also collected. Fruit samples were also taken from 3 cold storages in Eğirdir District and one sweet cherry processing center in Uluborlu District, after harvest. Similarly

Table 1. Sweet cherry tree numbers of the districts of Isparta province (TÜİK, 2021) and number of surveyed orchards.

Districts	Total tree numbers	Number of surveyed orchards
Aksu	2 370	2
Atabey	504 060	10
Eğirdir	41 570	4
Gelendost	10 000	2
Gönen	66 090	6
Keçiborlu	60 110	6
Merkez	336 800	10
Senirkent	419 000	10
Sütçüler	21 030	2
Uluborlu	552 849	10
Yalvaç	49 280	4
Yenişarbademli	6 790	2
Şarkikaraağaç	101 500	8
Total	2 171 449	76

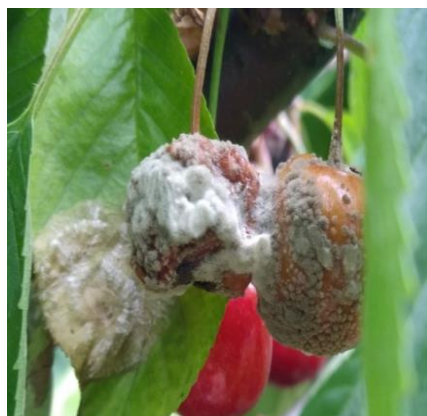


Figure 1. Some rotten fruit samples in the sweet cherry orchards in Isparta province.

rotten and healthy fruit samples were taken from the randomly selected boxes in the storage. Samples were separately packed with necessary labeling and brought to the laboratory in an ice container. They were kept at 4°C and isolations were performed as soon as possible.

2.2. Isolation and identification of the pathogens

Sections of about 3 mm², taken from the fruit samples including both healthy and decayed tissue were surface disinfected in 70% ethyl alcohol for 30 seconds. They were washed three times in sterile distilled water, blotted dry and transferred to Potato dextrose agar (PDA: Merck) or Malt extract agar (MEA: Merck) media. Fungal colonies were purified and single spore isolates were kept in two parallel agar slants at 4°C and in 20% glycerol at -80°C for later use in microscopic examinations and pathogenicity test (Kim et al., 2007; Lopez et al., 2016). Identifications of the fungi were done according to their cultural and microscopic features using related literature (Booth, 1971; Ellis, 1971; Samson et al., 1995; Kubicek and Harman, 2002; Pitt and Hocking, 2009; Houbraken et al., 2011; 2016; Morca et al., 2022).

2.3. Pathogenicity tests

To determine the pathogenicity of the fungal isolates, healthy fruits (genotype 219) picked before harvest from the sweet cherry orchard of Fruit Research Institute in Eğirdir District were used. For the pathogenicity test, randomly selected fungal isolates from each identified genus or species were used. Fruits were sterilized in 2% NaOCl solution for 30 seconds, washed two times in sterile distilled water and blotted dry under aseptic conditions. Fungi in stock cultures were activated on PDA and spore suspensions with 10⁶ conidia mL⁻¹ concentrations were prepared. Three wounds were made with a sterile needle in a small area (3×3 mm) on the dried fruits and inoculated with 10 µl spore suspension of the selected isolate. Same amount of sterile distilled water applied fruits served as

controls. Inoculated fruits were kept in sterilized plastic boxes with sterile blotter papers humidified with sterile distilled water. Five fruits were used for each box and 3 replicate boxes were used for each isolate. Boxes were kept at 22°C for seven days and virulence of the isolates were determined by measuring the lesion diameters on the inoculated fruits. Reisolations were made to confirm the similarity with the original isolate (Lopez et al., 2016; Peng et al., 2022). Data obtained by the pathogenicity test were subjected to analyses of variance and lesion diameter means were compared by Tukey's multiple range test ($p \leq 0.05$).

3. Results and Discussion

3.1. Fungi isolated from the sweet cherry orchards in Isparta province

As a result of the isolations made from the rotten sweet cherry fruits taken from 76 orchards in Isparta province, 9 fungi were identified at the species level. In addition, nine fungi, two of them belonging to *Aspergillus*, 5 of them to *Penicillium* and two to *Trichoderma* genera, were also identified according to the related literature (Samson et al., 1995; Kubicek and Harman, 2002). *A. alternata* was the fungus with the highest prevalence and isolation rates, followed by *M. laxa* and *B. cinerea* (Table 2). Likewise these fungi were found to be the pathogens causing highest losses in Bulgaria during 1999-2003 (Borovinova, 2004). In addition to these fungi, *R. stolonifer* and *Cladosporium* spp. were isolated from the rotten fruits in Italy (Romanazzi et al., 2008). Similar with our findings, *C. cladosporioides*, *Fusarium oxysporum*, *Geotrichum candidum* (Serradilla et al., 2021), *Sclerotinia sclerotiorum* (Förster and Adaskaveg, 2000; Ruan et al., 2023), *Stemphylium botryosum* (Dugan and Roberts, 1994), *Penicillium* (Romanazzi et al., 2008; Borge and Stensvand, 2015), *Trichoderma* (Serradilla et al., 2021) and *Aspergillus* species (Thomidis and Exadaktylou, 2012; Ali et al., 2024) were isolated from the sweet

Table 2. The prevalence and isolation rates of the fungi isolated from the rotten and healthy fruits in the sweet cherry orchards in Isparta province.

Fungi	Isolations from the rotten fruits		Isolations from the healthy fruits	
	Prevalence rate (%)	Isolation rate (%)	Prevalence rate (%)	Isolation rate (%)
<i>Alternaria alternata</i>	86.84	45.71	18.42	38.77
<i>Aspergillus</i> spp.	5.26	1.94	1.32	2.04
<i>Botrytis cinerea</i>	50.00	16.90	15.79	28.57
<i>Cladosporium cladosporioides</i>	2.63	0.83	1.32	2.04
<i>Fusarium lateritium</i>	1.32	0.28	-	-
<i>Fusarium oxysporum</i>	2.63	0.55	2.63	4.08
<i>Geotrichum candidum</i>	1.32	0.28	-	-
<i>Monilinia laxa</i>	60.53	23.82	13.16	24.48
<i>Penicillium</i> spp.	27.63	7.48	-	-
<i>Sclerotinia sclerotiorum</i>	2.63	0.55	-	-
<i>Stemphylium botryosum</i>	1.32	0.28	-	-
<i>Trichoderma</i> spp.	2.63	1.11	-	-
<i>Trichothecium roseum</i>	1.32	0.28	-	-

Table 3. The prevalence and isolation rates of the fungi isolated from the rotten and healthy sweet cherry fruits in the cold storages in Isparta province.

Fungi	Isolations from the rotten fruits		Isolations from the healthy fruits	
	Prevalence rate (%)	Isolation rate (%)	Prevalence rate (%)	Isolation rate (%)
<i>Alternaria alternata</i>	100	51.83	50	100
<i>Botrytis cinerea</i>	75	8.43	-	-
<i>Fusarium oxysporum</i>	25	2.41	-	-
<i>Monilinia laxa</i>	50	3.62	-	-
<i>Penicillium</i> spp.	100	18.07	-	-
<i>Rhizopus stolonifer</i>	25	2.41	-	-
<i>Stemphylium botryosum</i>	25	1.21	-	-
<i>Trichoderma</i> spp.	75	12.05	-	-

cherry fruits in different countries. However, *Fusarium lateritium* and *Trichothecium roseum* were mentioned for the first time as sweet cherry fruit rot pathogens in this study, while *Aspergillus* spp., *F. oxysporum*, *G. candidum*, *S. sclerotiorum*, *S. botryosum* and *Trichoderma* spp. were first reports for Türkiye.

Alternaria alternata, *M. laxa* and *B. cinerea* had also highest prevalence and isolation rates from the healthy fruit samples taken from the orchards. *Aspergillus* sp., *C. cladosporioides* and *F. oxysporum* were the other fungi causing latent infections (Table 2). Similarly, *A. alternata* and *C. cladosporioides* were among the fungi isolated from symptomless sweet cherry fruits (cv. Bing) and found pathogenic (Dugan and Roberts, 1994). In another study made to determine the latent infections on the raw and mature sweet cherry fruits using specific primers, *M. laxa* and *B. cinerea* were determined (Förster and Adaskaveg, 2000). Similar research made in Spain showed that *A. alternata* and *C. cladosporioides* were among the fungi isolated from the fruit surfaces after harvest (Venturini, 2002). Additionally, Tarbath et al. (2014) stated that *B. cinerea* was found on 50% of the healthy fruits, while it was isolated from 94% of the rotten fruits in Tasmania.

3.2. Fungi isolated from the sweet cherry fruits taken from the cold storages in Isparta province

With the isolations made from the rotten fruit samples taken from the three cold storages in Eğirdir District and one sweet cherry processing center in Uluborlu District, 83 fungal isolates were obtained. *A. alternata* had the highest isolation rate and was determined in all four samples taken from different storages (Table 3). *B. cinerea*, *F. oxysporum*, *M. laxa*, *Penicillium* spp., *R. stolonifer*, *S. botryosum* and *Trichoderma* spp. were the other fungi isolated in lower rates, from the rotten sweet cherry fruits from the cold storages. It was determined that only *A. alternata* caused latent infections on the fruits from the two cold storages in Eğirdir District, while no latent infection was found on the healthy fruit samples taken from the sweet cherry processing center in Uluborlu District. Among these agents, *B. cinerea*, *M. laxa*, *Penicillium* spp. and *R. stolonifer* were previously isolated from the

fruits in cold storages in Türkiye and other countries (Romanazzi et al., 2008; Akbudak et al., 2008; Børve ve Stensvand, 2015; Şen et al., 2016). *S. botryosum*, *F. oxysporum* and *Trichoderma* spp. were reported among the fungi colonizing the sweet cherry fruits during the period between petal fall and harvest in eastern Washington, and thought to be the possible agents of storage rot of sweet cherries (Dugan and Roberts, 1994).

3.3. Virulence of the fungal isolates

In the pathogenicity test performed to determine the virulence of the fungi isolated from the rotten and healthy sweet cherry fruits from the orchards and cold storages in Isparta province, statistically significant differences were found among the selected isolates. *Penicillium* sp., *R. stolonifer* and *B. cinerea* caused the largest lesions on the fruits, while the lowest level of virulence was obtained by *F. lateritium* (Table 4). *Penicillium* sp. caused browning starting from the inoculation area, then formed white mycelia and green spores on the lesion and expanded throughout the fruit. *R. stolonifer* grewed all over the fruit forming aerial dark grey mycelia and black sporangia, while *B. cinerea* formed grey mycelia on sunken lesions (Figure 2). *F. lateritium* was isolated from cold-stored Chinese cherry fruits and infected healthy fruits in the pathogenicity test (Wang et al., 2021). It was also among the *Fusarium* species causing leaf spots on sweet cherries in China (Zhou et al., 2022).

4. Conclusion

Sweet cherry is among the fruit species grown and exported in Isparta province, where fruit production is intensive and cold storage facilities are common. Pre and post harvest fruit rot is an important disease causing losses in sweet cherry production. Since sweet cherry fruit has a short storage life depending on its rapid physiological aging and is sensitive to injuries and bruising, it is easier for rot pathogens to develop. In this study, it was aimed to determine the fungal agents causing rot on sweet cherry fruits, to form a basis for studies related to the control of the disease. As a result of the isolations made from the rotten and

Table 4. Mean lesion diameters on the sweet cherry fruits caused by the fungi isolated from the rotten and healthy sweet cherry fruits in the orchards and cold storages in Isparta province.

Fungi	Mean lesion diameter (mm)
<i>Alternaria alternata</i>	13.80 cf*
<i>Aspergillus</i> sp.	10.33 eg
<i>Botrytis cinerea</i>	22.87 ab
<i>Cladosporium cladosporioides</i>	10.80 dg
<i>Fusarium lateritium</i>	5.33 gh
<i>Fusarium oxysporum</i>	15.73 be
<i>Geotrichum candidum</i>	19.40 ac
<i>Monilinia laxa</i>	15.00 be
<i>Penicillium</i> sp.	27.33 a
<i>Rhizopus stolonifer</i>	26.87 a
<i>Sclerotinia sclerotiorum</i>	13.67 cf
<i>Stemphylium botryosum</i>	6.00 fh
<i>Trichoderma</i> sp.	14.13 ce
<i>Trichothecium roseum</i>	18.47 bd
Control	0.00 h

* Means in the column shown by the same letters are statistically not different from each other according to Tukey's multiple range test ($P \leq 0.05$).

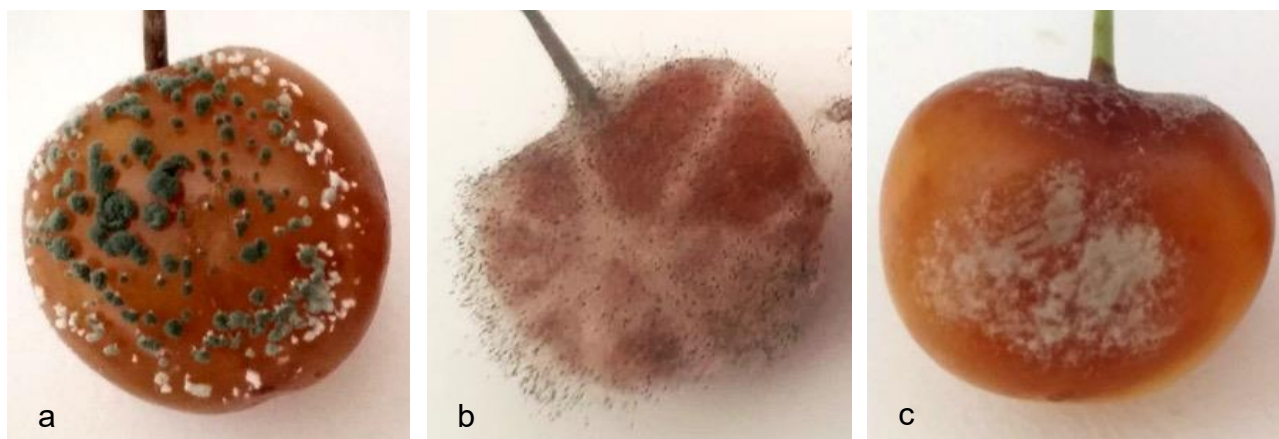


Figure 2. Lesions caused by *Penicillium* sp. (a), *Rhizopus stolonifer* (b) and *Botrytis cinerea* (c) on sweet cherry fruits in the pathogenicity test.

symptomless fruit samples taken from the orchards in the province, *A. alternata*, *M. laxa* and *B. cinerea* were the most common agents isolated both from the rotten and symptomless fruits. *A. alternata* was also isolated from the rotten and healthy fruits in the cold storages, while other fungi, mainly *Penicillium* species, were isolated only from the rotten fruits. *Penicillium* species, *R. stolonifer* and *B. cinerea* were found as the most virulent fungi in the pathogenicity test. Some of the pathogens found in the present study were previously reported to cause sweet cherry fruit rot in Türkiye, however *Aspergillus* spp. *F. oxysporum*, *F. lateritium*, *G. candidum*, *S. sclerotiorum*, *S. botryosum*, *Trichoderma* spp. and *T. roseum* were new findings. In addition no information could be found on the isolation and pathogenicity of *F. lateritium* and *T. roseum* on sweet cherry fruits. These findings have shown once again the importance of pre-harvest fruit rot disease in sweet cherry production. Besides, it was found that latent infections of fungi before harvest can also cause rotting in storage conditions resulting serious economic losses. Within the framework of sustainability from orchard to table, it is important to adopt and disseminate

alternative control methods instead of commonly used fungicides. Fungi isolated in this study will provide an important material for future studies on alternative control methods.

Acknowledgements

This study was financially supported by Isparta University of Applied Sciences, Scientific Research Projects Coordination Unit (Project number: 2023-YL1-0191). The authors would like to thank Fruit Research Institute (MAREM) for the use of facilities and researchers for their help during the research.

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