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Characterization of *Fusarium sambucinum* isolates associated with potato dry rot and evaluation of cultivar susceptibility and fungicides

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Abstract: Potato dry rot disease (*Fusarium* spp.) is one of the most important fungal storage rots on potato tubers after harvesting. The aims of this study were to identify the *Fusarium* species that cause dry rot in potato storages, evaluate the most virulent Fusarium species in vitro and in vivo for fungicide sensitivity and determine the susceptibility of the cultivars. In this context, as a result of surveys carried out in potato storages of Ödemiş and Torbalı counties in İzmir province in 2015–2016; *Fusarium* species obtained from diseased potato tubers were identified as *Fusarium sambucinum*, *F. oxysporum*, *F. avenaceum* and *F. equiseti*, using morphological and molecular methods. The most virulent species was determined according to the pathogenicity tests. To determine the susceptibility to disease, 13 potato cultivars, commonly stored in the region throughout the working period, were tested against the most virulent *Fusarium* species. The pathogenicity tests showed variation between the rank order of susceptibility of the cultivars to the most virulent pathogen. Fungicide sensitivity tests have been conducted with the most susceptible among these cultivars. There are not any plant protection products licensed against this disease in Turkey. On fungicide sensitivity tests, fludioxonil, flutolanil, thiophanate methyl, imazalil, fludioxonil + sedaxane, fluxapyroxad and tolclophos–methyl + thiram efficacy were investigated on the mycelial growth of *F. sambucinum*. In vitro assays; fludioxonil, fludioxonil + sedaxane and imazalil were the most effective fungicides preventing mycelial growth of the pathogen. In addition to chemicals that are effective in vitro tests, efficacy of *Bacillus subtilis* QST 713 strain 1.34%, *Pseudomonas fluorescens* strain Pf1 1.5% and 42% carboxylic acid were also evaluated. In vivo tests were conducted with Russet Burbank, Lady Olympia, Granola cultivars and fungicides were applied in two different methods including before and after inoculation. Results of this study indicated that the treatments with fludioxonil + sedaxane had the highest efficacy and treatments before inoculation was more effective.

Key words: Dry rot, fludioxonil, *Fusarium* spp., *Fusarium sambucinum*, potato

1. Introduction

Potato (*Solanum tuberosum* L.) is one of the major crops in the world and it has an important role in human nutrition. Being a highly nutritious food, potato is considered as one of the most promising crops to reduce hunger, malnutrition, and poverty in the world due to its high yield potential (Demirel et al., 2017). The potato tubers could be easily invaded by pests and pathogens and these directly affect the amount and quality of the product. Significant losses are observed due to many factors during both productions of potato planting and storage. Among diseases caused by fungal pathogens, storage diseases have an important role for potato production considering the losses they cause.

Fusarium dry rot is one of the most important fungal storage disease of potatoes worldwide (Boyd, 1997; Secor and Salas, 2001). The disease occurs in warehouses.[1] Dry rot disease affects both tubers in the warehouse and seed tuber parts in the field economically (Wharton et al., 2007). The disease begins primarily on the tubers in the form of dark and sunken superficial spots. In tuber flesh, necrotic areas are observed in colors ranging from light brown to black (Figure 1). When the rot progresses, gaps form in the tuber and tuber surface wrinkles.

The disease reduces yield, seed quality and marketable product. It causes product losses directly or indirectly. Most tuber infections consist of wounds that appear during planting, mechanical harvesting or classification by size before storage. It is estimated that the crop losses due to dry rot range from 6 to 25%, and during long-term storage, 60% of qualitative tubers can be affected by dry

[1] Tarım ve Orman Bakanlığı, Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü (2020). TAGEM [online]. Website https://www.tarimorman.gov.tr/TAGEM/Belgeler/BitkiSagligi/Bitki%20Hastal%C4%B1klar%C4%B1%20ve%20Yabanc%C4%B1%20Ot%20Zirai%20M%C3%BCcadele%20Teknik%20Talimatlar%C4%B1.pdf [accessed 07 June 2020].

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Figure 1. Symptoms of potato dry rot in the tuber.

rot (Secor and Salas, 2001; Stevenson et al., 2001; Estrada et al., 2010).

Thirteen different *Fusarium* species are considered as causal agents of *Fusarium* dry rot in potatoes worldwide (Cullen et al., 2005). When tubers injured during harvesting, the disease progresses in the storage and the presence of rots on tubers can potentially exclude the whole stock. *Fusarium* dry rot of seed tubers can reduce crop establishment by killing developing potato sprouts.

There have been some studies on potato dry rot in Turkey. In the previous studies, a number of pathogenic fungus species were isolated from potatoes in the storages of Turkey (Türkensteen and Eraslan, 1985). In a study on the presence of *Fusarium* spp., 31 *Fusarium* species were identified in 55 plant species (Özer and Soran, 1991). In a study in which 20 isolates including *Bacillus*, *Burkholderia* and *Pseudomonas* species were tested against *Fusarium sambucinum*, which causes dry rot in potatoes, in vitro and in vivo, only the *Burkholderia* OSU–7 species was found to be 95% effective in preventing the disease (Kotan et al., 1999). In a study on the biological control of potato dry rot caused by *Fusarium sambucinum* with *Trichoderma* isolates, the interaction of pathogens and antagonists was measured in the PDA medium and the activity of antagonists was investigated on potato tubers (Aydın, 2019).

F. sambucinum and *F. solani* are common pathogens that cause dry rot in potato tubers stored in temperate regions. It is stated in a study that some strain of *F. sambucinum* investigated were aggressive than the others and caused more severe tuber rots compared to *F. solani*. Many potato cultivars used in the same study were susceptible hosts to the pathogen *F. sambucinum* and *F. sambucinum* isolates were detected as more aggressive than *F. solani* in all cultivars (Aydın and İnal, 2018).

It has been observed that the studies conducted in the world on dry rot disease are mostly aimed at the detection, diagnosis and controlling of the disease. There have been many reports of work on tuber susceptibility, the effect of storage conditions and disease control (Boyd, 1972). One of the pathogens that cause dry rot, *F. sambucinum* has been reported as the main pathogen in many potato growing regions, for example; in the north of America, England and the Ivory coast (Hide and Cayley, 1985; Leach and Webb, 1981). It has also stated that *Fusarium oxysporum* is one of the most important species which causes rottings in the potato tubers in the world (Nelson et al., 1986). This species is one of a major pathogen of dry rot in seed potatoes in most potato–producing regions, such as India, South Africa and Europe (Theron and Holz, 1989). In Great Britain and in the Nordic countries, the most common species isolated from potato has been *F. coeruleum* (Peters et al., 2008).

Measures for controlling dry rot in storage are limited. Previous studies indicated that fungicide applications during the postharvest period can cause resistance after a while and thus they may not be efficient enough (Leach and Webb, 1981; Greyerbiehl and Hammerschmidt, 1998).

In recent years, the number of biological control studies has been increasing, as well as chemical control studies against stored product diseases. Biological control can be one of the low-cost and ecologically sustainable methods for managing plant diseases caused by soilborne pathogens like *Fusarium*, *Rhizoctonia*, and *Pythium* (Khan et al., 2014). It is also reported that different biological products such as fungi, bacteria and yeast are effective in the control of postharvest diseases such as dry and soft rot.

With this study carried out for the first time in potato warehouses in İzmir, which is a major potato region both in the Aegean region and Turkey, the objectives were to identify the species that cause dry rot of potatoes, determine the cultivar susceptibility and fungicide sensitivity. Disease management studies have been conducted with different fungicides in cold storage.

In this study, it was aimed to determine the *Fusarium* species causing dry rot disease in potato tubers stored in Ödemiş and Torbalı counties in İzmir, using morphological and molecular methods. It has also been

aimed to determine the susceptibility of some commonly stored potato cultivars to the most virulent *Fusarium* spp. determined by pathogenicity tests. *Fusarium sambucinum* was the most commonly isolated species. There is not registered fungicide for dry rot in Turkey. *F. sambucinum* tested for sensitivity to fludioxonil, flutolanil, thiophanate methyl, imazalil, fludioxonil + sedaxane, fluxapyroxad and tolclophos–methyl + thiram with in vitro experiments. In the experiments carried out in the storage, in addition to fungicides that are effective against the disease in vitro tests, the efficacy of *Bacillus subtilis* QST 713 strain 1.34%, *Pseudomonas fluorescens* strain Pf1 1.5% and carboxylic acid were also tested on Russet Burbank, Lady Olympia and Granola cultivars.

2. Material and methods

2.1. Materials

Fusarium spp. isolates were obtained from the symptomatic potato tubers collected from warehouses in Ödemiş and Torbalı. Potato dextrose agar [(PDA; 1 L; 40 g potato dextrose agar, 14 g agar and 1 L distilled water)] and water agar [(WA); 1 L of pure water, 10 g of agar] was used for isolation and diagnostic studies. In the susceptibility tests, Melody, Marabel, Granola, Madeleine, Van Gogh, Laura, Desiree, Lady Anna, Lady Amarilla, Alegria, Triplo, Lady Olympia and Russet Burbank cultivars, which are widely stored during the study, were used. The characteristics of fungicides tested for *Fusarium sambucinum* in vitro are listed in Table 1 and characteristics of the biological products tested in vivo are listed in Table 2. The potatoes were stored at 10 °C in storage after cultivar susceptibility tests and in vivo tests.

2.2. Sample collection

Sample collection studies carried out at the end of the spring and autumn production periods in Ödemiş and Torbalı in 2015–2016. Firstly, tubers were washed to remove excess soil, then the pieces with healthy and diseased tissue were cut and disinfected in 1% sodium hypochlorite (NaOCl) solution for 3 min and rinsed with sterile distilled water and dried. All the sterilized pieces were placed onto PDA and WA, the plates were incubated at 22 ± 2 °C for approximately 7 days (Ciesniewska et al., 2015). After the incubation period, the resulting *Fusarium* colonies were transferred to fresh PDA plates and maintained at 4 °C for further studies.

2.3. Pathogenicity tests

The healthy potato tubers were used in this experiment. Five potato tubers, appearing healthy and uniform in size 100–120 g, were selected for each isolate and the trial was conducted with 3 replications. The tubers were first washed to remove excess soil, and then they sterilized with 0.5% sodium hypochlorite solution for 10 min, rinsed

Table 1. Characteristics of fungicides tested against *Fusarium sambucinum* in vitro experiments.

FRAC[1] code	Active ingredient (a.i)	Trade name	Formulation	In vitro dose*
E2:12	Fludioxonil 100 g/L	Celest Max 100 FS – Syngenta	FS	20 mL
C2:7	Flutolanil 464 g/L	Moncut 40 – AMC-TR	SC	17.5 mL
E2:12–C2:7	Fludioxonil 50 g/L + Sedaxane 40 g/L	Vibrance Premium – Syngenta	EC	50 mL
C2:7	Fluxapyroxad 300 g/L	Sercadis – BASF	SC	20 mL
F3:14–M:03	Tolclophos–methyl 20% + Thiram 30%	Rizolex-T 50 WP – Sumiagro	WP	40 g
B1:1	Thiophanate methyl 70%	Sumitop WP – SumiAgro	WP	60 g/100 L water
G1:3	Imazalil 500 g/L	Bestnate 50 EC – Agrobest	EC	30 mL/100 L water

*100 kg/seed.

[1] Fungicide Resistance Action Committee (2020). FRAC [online]. Website https://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list-2020-final.pdf?sfvrsn=8301499a_2 [accessed 07 June 2020].

Table 2. Characteristics of the preparations used in vivo trials.

FRAC[3] code	Active ingredient (a.i)	Trade name	Dose of application*
BM 02	*Bacillus subtilis* QST 713 1.34% (min.1 × 10^9 cfu/mL)	Serenade – Bayer	300 mL
BM 02	*Pseudomonas fluorescens* strain Pf1 1.5%, 1 × 10^8 kob/mL min	Cedriks – Agrobest	500 mL
-	42% Carboxylic acid	Supa Link – Hektaş	200 mL/100 L water

*100 kg/seed

Table 3. Potato dry rot disease scale.

Scale value	Definition
0	No enfection (in natural and artificially infected tubers)
1	1%–20% infected tissue
2	21%–40% infected tissue
3	41%–60% infected tissue
4	61%–80% infected tissue
5	Up to 81% orall tuber is infected

with sterile distilled water, and air dried. Dried tubers were wounded with a diameter of 4 mm and depth of 4 mm with a cork borer, then agar blocks, containing fresh Fusarium mycelium developed in PDA, were inoculated. Wounded parts were wrapped with parafilm (Choiseul et al., 2007; Peters et al., 2008; Chehri et al; 2011) and tubers were incubated in black polyethylene bags for 3 weeks at 20 °C in the dark (Manici and Cerato, 1994). After the incubation period, tubers were cut longitudinally through sites of inoculation, and disease severity was calculated using the index formula, according to the potato dry rot disease scale (Table 3) (Karman, 1971; TAGEM[2]).

$$\text{Disease severity (index)} = \frac{\Sigma\,(\text{scale value} \times \text{number of tuber included in scale value})}{\text{Total number of tubers}}$$

The most virulent *Fusarium* isolate as a result of pathogenicity tests was used as fungal material for studies of cultivar susceptibility tests.

2.4. Molecular identification

The DNA isolation was performed as in the study of Mishra et al. (2003) with the Thermo Scientific GeneJet Plant Genomic DNA Purification MiniKit protocol. The resulting template DNAs were subjected to PCR. PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA) (2×) protocol was used for each PCR application and the mixture was prepared in a total volume of 25 µL. Each PCR contained 12.5 µL PCR Master Mix, the concentration of 2 µL of forward and reverse primers (Table 4), 9.5 µL nuclease-free water, and 1 µL target DNA. DNA amplification was performed in Eppendorf Mastercycler Gradient PCR thermal cycler using the following conditions: initial denaturation for 1 min at 94 °C followed by 25 cycles of denaturation (94 °C for 60 s), 58 °C for 30 s and extension (72 °C for 60 s). A final extension step (72 °C for 7 min) was performed after the completion of the cycles. PCR products, along with a 100-bp GeneRuler DNA ladder plus, were loaded into 1.5% agarose gel containing SYBR® Safe DNA gel stain (Invitrogen) and submitted to electrophoresis in TAE buffer for 60 min at 100 V. The amplified DNA fragments were visualized under ultraviolet light.

2.5. Cultivar susceptibility tests

The tests were carried out with the most virulent *Fusarium* spp., determined as a result of pathogenicity tests, with three replicates. Firstly; conidia of selected *Fusarium* isolate, grown on PDA, were washed from petri plates and transferred to a flask containing pure water and stirred for 30 min in a shaker. The remaining pieces were passed through cheesecloth to remove and it was adjusted to give a suspension of 5×10^4 spores/mL.[2] Twenty microliter of a conidial suspension was injected into the wounds opened with a cork borer at a depth of 4 mm (Gachango et al., 2012). After the incubation period of 3–4 weeks, tubers were cut longitudinally through sites of inoculation, and parameters of dry rot [maximal width (w) and depth (d)] were recorded. According to Lapwood et al. (1984), the pathogen penetration into tubers was calculated. Potato cultivars were considered as less or moderately susceptible, if the mean penetration value is ≤ 10 mm, susceptible if the mean penetration value is between 10 mm and 15 mm, and highly susceptible if the mean penetration is ≥ 15 mm (Remadi et al., 2006).

$$\text{Penetration (mm)} = [w / 2 + (d - 6)] / 2$$

The data obtained from the experiments as a result of the cultivar susceptibility tests were subjected to variance analysis using the statistics program of SPSS Statistics 19 (IBM, Armonk, NY, USA).

2.6. Disease management

2.6.1. In vitro fungicide sensitivity assays

These tests carried out in laboratory conditions. PDA medium was used to determine the efficacy and susceptibility levels of fungicides to *Fusarium sambucinum* isolate. Plates of PDA were prepared with each of the

[2] Tarım ve Orman Bakanlığı, Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü (2020). TAGEM [online]. Website https://www.tarimorman.gov.tr/TAGEM/Belgeler/Sitandard/Sebze%20Hastal%C4%B1klar%C4%B1%20Standart%20%C4%B0la%C3%A7%20Deneme%20Metotlar%C4%B1.pdf [accessed 07 June 2020].

Table 4. Primer, sequence (5',3') and product size (bp) of *Fusarium* species used in PCR studies.

Fusarium spp.	Primer	Sequence (5', 3')	Product size (bp)	Reference
TEF–1α	EF1–F EF2–R	5'-ATG GGT AAG GAR GAC AAG AC-3' 5'-GGA RGT ACC AGT SAT CAT GTT-3'	650–700	Du et al., 2012
F. sambucinum	FSF1 FSR1	5'-ACATACCTTTATGTTGCCTCG-3' 5'-GGAGTGTCAGACGACAGCT-3'	315	Mishra et al., 2003
F. oxysporum	FOF1 FOR1	5'-ACATACCACTTGTTGCCTCG-3' 5'-CGCCAATCAATTTGAGGAACG-3'	340	
F. equiseti	FEF1 FER1	5'-CATACCTATACGTTGCCTCG-3' 5'-TTACCAGTAACGAGGTGTATG-3'	389	
F. avenaceum	FAF1 FAR	5'-AACATACCTTAATGTTGCCTCGG-3' 5'-ATCCCCAACACCAAACCCGAG-3'	314	

fungicides at concentrations of 0 (control), 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30 ppm. The stock concentration of 10,000, 1000 and 100 ppm was prepared for each fungicide. The media were poured into sterile petri dishes and 3 agar discs, obtained from *F. sambucinum* isolates which were grown for 4 days and 4 mm diameter, were placed on the center. The experiment was conducted with three replicates for each fungicide. The plates were incubated at 22 ± 2 °C for 4 days. After the incubation period, growth of the fungal isolate on each replicate was measured. The efficacy of fungicides was determined according to the EC_{50} (effective fungicide concentration that caused 50% inhibition of mycelia growth) and MIC (minimum inhibition concentration) values for each fungicide were calculated.

2.6.2. In vivo tests

Bacillus subtilis QST 713 strain 1.34%, *Pseudomonas fluorescens* strain Pf1 1.5% and carboxylic acid were also investigated for efficacies on treated tubers to compare with fungicides. Russet Burbank, Lady Olympia and Granola; the susceptible cultivars determined as a result of cultivar susceptibility tests in this study, were used in these experiments. The experiment was carried out with five replicates. The selected potato tubers of Russet Burbank, Lady Olympia and Granola were washed with water, dipped in sodium hypochlorite (1%) for 5 min, rinsed abundantly with sterile distilled water (10 min), and air-dried properly. Then 4 mm depth and 4 mm diameter wounds were opened with cork borer in the tubers. Twenty microliter of spore suspension, contains 5×10^4 spore/mL, according to the method in cultivar sensitivity tests, was given to each wound. Tuber treatment was realized by dipping tubers for 10 min in fungicidal suspension prior (first dipping method) and after (second dipping method) inoculation and then dried completely. Tubers were incubated in black polyethylene bags for 4 weeks at 20 °C in the dark (Vatankhah et al., 2019). After the incubation period, tubers were cut longitudinally through sites of inoculation, and disease assessment was evaluated according to the dry rot disease scale. The efficacy of fungicides was calculated with the Abbott's formula.

Another different spraying program was conducted with Lady Olympia cultivar in vivo trials. In this program; a mixture of carboxylic acid with flutolanil, fluxapyroxad, and fludioxonil + sedaxane has been applied separately. The tubers were stored at 10 °C in the dark for at least 4 weeks. Disease assessment was performed according to the dry rot disease scale. The efficacy of fungicides was calculated with the Abbott's formula.

3. Results and discussion

3.1. Sample collection

As a result of surveys, a total of 468 potato tubers with dry rot symptoms were collected. These potato tubers were sourced from 42 storages, located in Ödemiş and Torbalı counties in İzmir and represented an important potato storage areas in these counties. In this study, as a result of isolations made from diseased tubers, 79 *Fusarium* spp. isolate was obtained. According to the pathogenicity tests, 41 isolates (51.89%) were determined as pathogens. Disease rates of 41 isolates range from 4.00% to 82.66% (Table 5). Considering morphological characteristics such as colony development and colony color; microscopic structures such as macroconida form, microconida, phialide types, and presence of chlamydospore, 41 isolates were divided into 4 groups. *Fusarium sambucinum* was the most commonly isolated species (39.02%), followed by *F. oxysporum* (26.82%) and *F. avenaceum* (19.51%). *F. equiseti* was the fourth most prevalent (14.63%). The highest disease incidence belongs to *F. sambucinum*

Table 5. Potato cultivar, isolate number and disease incidence (%).

Cultivar	Isolate number	Disease incidence (%)	Cultivar	Isolate number	Disease incidence (%)
Granola	21	16.00	Innovator	13	13.20
	23	20.00		14	5.20
	1.4.5	37.33		1.2.8	52.00
	4.5.3	44.00		1.14.1	57.33
	3.5.3	61.33	Safari	22	13.00
	4.12.4	46.66		1.7.5	65.20
	4.2.5	60.00	Agria	3.4.4	40.00
	4.9.5	50.66		4.12.7	74.66
	1.12.7	45.33	Triplo	2.6.2	33.33
	1.13.3	49.20		1.13.4	54.66
	4.1.4	53.33	Karera	2.7.3	44.00
	1.5.1	37.33	Russet Burbank	2.8.3	30.66
	1.10	50.60		1.2.7	34.66
	1.1.3	52.00		3.7.4	48.00
	1.5.2	70.66		2.10.3	38.60
Marabel	3.6.4	28.00		3.25	82.66
	4.2.4	4.00		4.8.2	28.00
	1.1.1	32.00		1.5.7	70.66
	1.6.2	34.66		3.5.4	64.00
	1.4.2	48.00			
	3.9.4	61.33			
	1.5.4	49.33			

isolate, isolated from Russet Burbank potatoes with 82.66%. *Fusarium sambucinum* (Figure 2) was used as a fungal material for cultivar susceptibility tests.

3.2. Molecular identification

Molecular tests were conducted to confirm the morphological identification. *Fusarium* species are intended to be diagnosed by TEF–1α gene analysis using primers EF–1 (ATG GGT AAG GAR GAC AAG AC) and EF–2 (GGA RGT ACC AGT SAT CAT GTT) (O'Donnell et al., 1998) (Figure 3). According to molecular studies; 315 bp band was obtained using primers of FSF1 (ACA TAC CTT TAT GTT GCC TCG) and FSR1 (GGA GTG TCA GAC GAC AGC T) belonging to *Fusarium sambucinum* (Figure 4).

3.3. Cultivar susceptibility tests

According to the mean pathogen penetration value of potato cultivars used in cultivar susceptibility tests; 8 cultivars (Melody, Marabel, Lady Anna, Laura, Madeleine, Lady Amarilla, Triplo, Desiree) with mean penetration value ≤ 10 mm are less or moderately susceptible compared to other cultivars. Alegria, Granola and Van Gogh were susceptible with mean penetration value between 10 mm and 15 mm. Russet Burbank and Lady Olympia were highly susceptible with mean penetration value of 18.19 mm and 15.39 mm, respectively (Table 6).

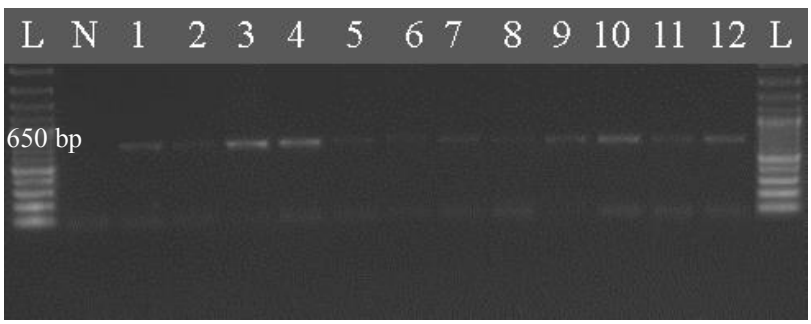
Russet Burbank was included in the first group (a) with an average penetration value of 18.19 mm and was determined to be the most susceptible cultivar. Lady Olympia and Alegria followed this with mean penetration value of 15.39 and 14.58 mm, respectively, and were in the second group (b). Granola and Van Gogh are in the third (c) group, while Melody and Marabel cultivars are in the fourth (cd) and fifth (de) group, respectively. Desiree was less susceptible showing mean penetration of 3.52 mm.

3.4. Disease management

3.4.1. In vitro fungicide sensitivity assays

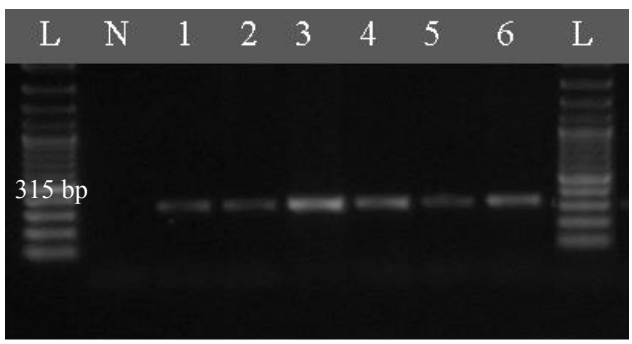
The efficacy of fungicides to the mycelial growth of *F. sambucinum* was determined. The efficacy of fungicides has been evaluated by using diameter measurement values. EC_{50} and MIC (μg/mL) values of the fungicides arelisted in Table 7. According to the EC_{50} of fungicides, it was determined that *F. sambucinum* isolate shows different

Figure 2. Colony development in PDA (a) and macroconidia (b, c) of *F. sambucinum*.



L: 100 bp ladder, N: water kontrol, 1-12: (1.5.4, 1.6.2, 2.7.3, 1.13.3, 4.5.3, 1.5.1, 1.1.3, 3.5.3, 14, 1.7.5, 4.12.7, 3.9.4)

Figure 3. PCR image conducted using EF–1 and EF–2 primers of *Fusarium* isolates.



L: 100 bp ladder, N: water control, 1-6: (2.10.3, 1.2.8, 3.25, 3.6.4, 1.13.4, 13)

Figure 4. PCR image obtained using FSF–1 and FSR–1 primers of *F. sambucinum* isolates.

effects on mycelial development. The lowest EC_{50} value was determined as fludioxonil with 0.029 µg/mL. This was followed by fludioxonil + sedaxane with 0.052 µg/mL and imazalil with 0.22 µg/mL, respectively. According to these results; fludioxonil, fludioxonil + sedaxane and imazalil have been the most effective fungicides in preventing the mycelial growth of the pathogen. Fludioxonil has been the most effective fungicide on spore germination from 0.1 µg/mL doses.

3.4.2. In vivo tests

In these tests, fungicides were applied in two different methods as before (first dipping method) and after (second dipping method) inoculation on Granola, Lady Olympia and Russet Burbank cultivars. The efficacy of fungicides obtained from dipping was calculated with the Abbott's formula, disease rates and efficacy of fungicides are listed in Tables 8 and 9. Applications conducted in first dipping method, fludioxonil + sedaxane (77.18%) showed the

Table 6. Potato cultivars used in cultivar susceptibility tests, their susceptibility and mean pathogen penetration (mm).

Potato cultivar	Susceptibility	Mean pathogen penetration (mm)
Russet Burbank	Highly susceptible	18.19 ± 1.29 a*
Lady Olympia	Highly susceptible	15.39 ± 2.68 b
Alegria	Susceptible	14.58 ± 2.54 b
Granola	Susceptible	11.69 ± 0.58 c
Van Gogh	Susceptible	11.07 ± 1.37 c
Melody	Less or moderately susceptible	9.39 ± 0.81 cd
Marabel	Less or moderately susceptible	8.48 ± 0.53 de
Lady Anna	Less or moderately susceptible	7.90 ± 1.76 def
Laura	Less or moderately susceptible	7.31 ± 1.02 def
Madeleine	Less or moderately susceptible	7.21 ± 0.69 def
Lady Amarilla	Less or moderately susceptible	6.05 ± 0.79 ef
Triplo	Less or moderately susceptible	5.51 ± 0.46 fg
Desiree	Less or moderately susceptible	3.52 ± 0.12 g

* Values followed by the same letter are not significantly different.

Table 7. EC_{50} and MIC values (µg/mL) of fungicides on *F. sambucinum*.

Fungicide	**EC_{50}**	**MIC (µg/mL)**
50 g/L Fludioxonil + 40 g/L Sedaxane	0.052	1
100 g/L Fludioxonil	0.029	0.1
464 g/L Flutolanil	> 30	> 30
300 g/L Fluxapyroxad	> 30	> 30
70% Thiophanate methyl	> 30	> 30
500 g/L Imazalil	0.22	1
20% Tolclophos–methyl + 30% Thiram	19	> 30

highest effect for Granola cultivar. This was followed by fludioxonil (51.66%) and imazalil (50.23%), respectively. Fludioxonil + sedaxane (74.49%) showed the highest effect for the Lady Olympia, followed by imazalil (66.44%) and fludioxonil (49.27%), respectively. In Russet Burbank, fludioxonil + sedaxane showed the highest efficacy with 70.36% in dipping before inoculation. Imazalil efficacy was 62.73% where fludioxonil efficacy was 45.39%. Generally, in the applications before inoculation; fludioxonil + sedaxane, fludioxonil and imazalil were the most effective on all cultivars.

According to the applications performed in the second dipping method; fludioxonil + sedaxane had the highest efficacy of 63.53% for Granola. This was followed by fludioxonil 35.41% and imazalil 32.02%, respectively. Fludioxonil + sedaxane had the highest efficacy in Lady Olympia with 60.56% and followed by imazalil with 30.26%. While the fludioxonil + sedaxane in Russet Burbank had the highest efficacy with 58.36%, it was followed by imazalil with 31.40%. Fludioxonil efficacy was 22.83%. Generally, on applications after inoculation; fludioxonil + sedaxane was the most effective application in all cultivars, followed by fludioxonil and imazalil, depends on cultivars.

Flutolanil and fluxapyroxad, which are not effective in vitro tests, were used as a mixture with carboxylic acid, and its efficacy was also evaluated on Lady Olympia cultivar. Fludioxonil + sedaxane was mixed with carboxylic acid and applied with two spraying methods for comparison in this program. The results obtained from sprayings are listed in Table 10. According to the results; in both spraying methods, applications of fludioxonil + sedaxane

Table 8. Efficacies of fungicides in vivo on *Fusarium sambucinum* before inoculation.

Active ingredient (a.i)	Cultivar					
	Granola		Lady Olympia		Russet Burbank	
	Incidence (%)	Efficacy (%)	Incidence (%)	Efficacy (%)	Incidence (%)	Efficacy (%)
B.subtilis	38.83 ± 5.24 bc*	43.41	26.83 ± 5.61 b	45.92	37.15 ± 5.60 b	40.18
Fludioxonil	33.16 ± 2.07 b	51.66	25.16 ± 6.78 b	49.27	33.91 ± 4.14 b	45.39
Carb. acid	78.36 ± 3.23 e	14.21	55.61 ± 2.69 c	12.09	56.36 ± 5.49 c	9.26
Flud + Sed	15.65 ± 3.89 a	77.18	12.65 ± 2.24 a	74.49	18.40 ± 8.08 a	70.36
P. fluorescens	41.20 ± 3.42 c	39.95	31.20 ± 3.42 b	37.11	39.95 ± 7.64 b	35.68
Imazalil	34.14 ± 7.49 b	50.23	16.64 ± 2.47 a	66.44	23.14 ± 4.11 a	62.73
Control	68.61 ± 1.78 d	–	49.61 ± 6.44 c	–	62.11 ± 6.42 c	–

B. subtilis: *Bacillus subtilis* QST 713 strain 1.34%, Fludioxonil: Fludioxonil 100 g/L, Carb. acid: 42% Carboxylic acid, Flud + Sed: 50 g/L fludioxonil + 40 g/L sedaxane, *P. fluorescens*: *Pseudomonas fluorescens* strain Pf1, Imazalil: Imazalil 500 g/L.
* Values followed by the same letter are not significantly different at $P < 0.05$.

Table 9. Efficacies of fungicides in vivo on *Fusarium sambucinum* after inoculation.

Active ingredient (a.i)	Cultivar					
	Granola		Lady Olympia		Russet Burbank	
	Incidence (%)	Efficacy (%)	Incidence (%)	Efficacy (%)	Incidence (%)	Efficacy (%)
B. subtilis	34.33 ± 11.54 bcd*	21.28	41.83 ± 3.96 cd	18.16	46.08 ± 5.28 cd	17.14
Fludioxonil	28.16 ± 2.61 b	35.41	38.66 ± 2.45 bc	24.35	42.91 ± 1.68 bc	22.83
Carb. acid	39.13 ± 4.79 cd	10.27	44.88 ± 1.92 d	12.18	49.88 ± 4.71 de	10.30
Flud + Sed	15.90 ± 2.91 a	63.53	20.15 ± 0.92 a	60.56	23.15 ± 4.52 a	58.36
P. fluorescens	34.95 ± 3.37 bcd	19.86	42.20 ± 2.73 cd	17.43	47.20 ± 3.88 cd	15.12
Imazalil	29.64 ± 7.13 bc	32.02	35.64 ± 3.77 b	30.26	38.14 ± 6.21 b	31.40
Control	43.61 ± 5.43 d	–	51.11 ± 4.50 e	–	55.61 ± 2.20 e	–

B. subtilis: *Bacillus subtilis* QST 713 strain 1.34%, Fludioxonil: Fludioxonil 100 g/L, Carb. acid: 42% Carboxylic acid, Flud + Sed: 50 g/L fludioxonil + 40 g/L sedaxane, *P. fluorescens*: *Pseudomonas fluorescens* strain Pf1, Imazalil: Imazalil 500 g/L.
* Values followed by the same letter are not significantly different at $P < 0.05$.

Table 10. Efficacies of fungicides on Lady Olympia before and after inoculation.

Active ingredient (a.i)	Before inoculation		After inoculation	
	Incidence (%)	Efficacy (%)	Incidence (%)	Efficacy (%)
Flud + sed + carb	5.20 ± 4.11 a*	87.42	5.95 ± 3.05 a	83.85
Flux + carb	29.16 ± 1.15 b	29.48	28.16 ± 2.84 b	23.59
Flut + carb	32.15 ± 2.08 c	22.25	26.68 ± 2.11 b	27.62
Control	41.36 ± 3.20 d	–	36.86 ± 4.23 c	–

Flud + sed + carb: 50 g/L fludioxonil + 40 g/L sedaxane + 42% Carboxylic acid, Flux + carb: fluxapyroxad 300 g/L + 42% Carboxylic acid, Flut + carb: flutolanil 464 g/L + 42% Carboxylic acid.
* Values followed by the same letter are not significantly different at $P < 0.05$.

+ carboxylic acid mixture had the highest efficacy. When fludioxonil + sedaxane was applied as a mixture with carboxylic acid, its effect was 87.42% before inoculation, while its effect was 83.85% in applications after inoculation. According to the results with other applications; the efficiency of carboxylic acid and fludioxonil + sedaxane was 12.09% and 74.49%, respectively, when they used separately before inoculation. When carboxylic acid used as a mixture with fludioxonil + sedaxane, its efficacy was increased to 87.42%. Carboxylic acid had 12.18%, fludioxonil + sedaxane had 60.56% efficacy when used separately after inoculation and as a mixture, its efficacy has increased to 83.85%.

4. Conclusion

This study carried out in the potato storages in Ödemiş and Torbalı counties of İzmir, which has an important place in terms of potato cultivation both in the Aegean regionand in Turkey. Surveys were carried out in potato storages on postharvest periods and it was firstly aimed to identify *Fusarium* species that cause potato dry rot disease. The sensitivity of potato cultivars, which are widely stored in the region during the study, to the most virulent *Fusarium* isolate was determined. In this study, it was determined that the pathogens causing dry rot in potato storages in İzmir belong to four *Fusarium* species. It is estimated that there are about 13 *Fusarium* species causing potato dry rot in the world (Cullen et al., 2005). According to the diagnostic studies using morphological and molecular methods, it was determined that these species were *Fusarium sambucinum*, *F. oxysporum*, *F. avenaceum,* and *F. equiseti*. The most isolated and most virulent isolate among these species belongs to *Fusarium sambucinum*. *F. sambucinum* has been reported as the main pathogen of dry rot disease in many potato cultivated regions such as North of America, Great Britain, Europe (Leach and Webb, 1981; Hide and Cayley, 1985), and some studies have revealed that it is a dominant species (Esfahani, 2005). Hanson et al. (1996) reported the most prevalent species in the northeastern US were *F. sambucinum*, *F. oxysporum,* and *F. solani*. Lacy and Hammerschmidt (1993) reported that *F. sambucinum* was the predominant species affecting potato tubers in storage and caused seed piece decay after planting and it was the most aggressive.

In molecular studies, the predicted size of PCR products for *Fusarium sambucinum* is 315 bp, for *F.oxysporum* 340 bp, for *F. avenaceum* 314 bp, and for *F. equiseti* 389 bp were obtained. The identification of *Fusarium* species using molecular methods, 650 bp band was obtained with the primers EF–1 and EF–2. Du et al., (2012); in their study to identify the *Fusarium* species obtained from potato tubers infected with dry rot; used 2 primers of the specific TEF–1α gene and obtained ~700 bp band. Diagnostic studies, which obtained 315 bp band by using specific primers of *F. sambucinum*, were found to be compatible with the diagnostic studies of Ciesniewska et al. (2015) and Mishra et al. (2003) using species-specific primers of *F. sambucinum*.

Dry rot is a storage disease and its main host is potato. More than one *Fusarium* species can cause this disease. It is important that the damaged tubers are not stored in the warehouses after harvest or used as seeds so that the disease does not spread to the warehouses and infect other tubers.

The present study showed that Russet Burbank is the most susceptible cultivar according to the cultivar susceptibility tests. This potato cultivar is one of the most widely used in the industry and has a long storage life. Due to the fact that its encrustation binds easily, the risk of injury in machine disassembly can be high. Desiree was found to be the least susceptible to dry rot disease among the 13 cultivars tested. Storing susceptible cultivars can directly affect the development of dry rot in the warehouse. In cases where the storage period is long and the stored cultivar does not have the advantages of long storage; improper storage conditions will result also in increased losses. Tuber infections may increase depending on the temperature and humidity of the storages as well as directly contact with the pathogen. Providing proper air circulation in the warehouses is very important in terms of controlling the disease. Temperatures above 10 °C and taking injured tubers into the warehouse are conditions that promote disease development.

In this study; it has been observed that protective applications before inoculation were more successful and among the fungicides used in the trials, fludioxonil + sedaxane was one of the most effective. This was followed by fludioxonil and imazalil. There are not any registered fungicides for potato dry rot in Turkey. Fludioxonil is a registered fungicide for potato seed treatment against *Fusarium* dry rot in the US (Wharton et al., 2007). It is a protective fungicide and can be used alone or in combination with other active ingredients to control potato dry rot. Fludioxonil can reduce seed piece decay as well as the incidence of diseased sprouts that develop into unhealthy plants (Wharton et al., 2007).

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