

## EVALUATION OF SOME *TRICHODERMA* SPECIES IN BIOLOGICAL CONTROL OF POTATO DRY ROT CAUSED BY *FUSARIUM SAMBUCINUM* FUECKEL ISOLATES

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**Abstract.** *Fusarium* dry rot of potato is a major disease caused by several *Fusarium* species, and *Fusarium sambucinum* Fuckel is considered to be the most aggressive species in worldwide, including Turkey. Biological control based on the use of microorganisms to suppress tuber diseases offers an attractive alternative that has gained great attention due to the significant potential. Several fungal biocontrol agents have been used in plant disease control, and *Trichoderma* group has been reported as effective against tuber pathogens such as *F. sambucinum*. This study was carried out under *in vitro* and *in vivo* conditions. During *in vitro* conditions, the effects of fifteen *Trichoderma* isolates (*T. asperellum* ÖT1; *T. viride* VG18; *T. viride* VG19; *T. harzianum* TZ16, *T. harzianum* LO52; *T. gamsii* VG47; *T. gamsii* VG48; *T. virens* KB31; *T. strigosum* LO43, *T. strigosum* LO8; *T. neokoningii* A15; *T. atroviride* VG3; *T. tomentosum* VG2; *T. Inhamatum* KEB12; *T. hamatum* ÖT16) were studied against three isolates of *F. sambucinum* (Fs2, Fs3 and Fs4) in PDA medium by using duel culture technique in incubation at 22 ± 24 °C. The most effective isolates were *T. virens* KB31, *T. gamsii* VG47, *T. hamatum* ÖT16, *T. asperellum* ÖT1, *T. harzianum* LO52, *T. atroviride* VG3, respectively. During *in vivo* conditions, potato tubers, CV. Desire were wounded and inoculated with 1 ml of *Trichoderma* isolates suspensions (10<sup>7</sup> spores mL<sup>-1</sup>), 24 h prior inoculation by *F. Sambucinum*. Reduction rate of dry rot in tubers was recorded during 5-6 weeks of incubation at 20-24 °C to compare with control treatments. Tuber dry rot was reduced by the antagonistic fungal isolates with different rates. The most effective isolates were *T. viride* VG18, *T. asperellum* ÖT1, *T. harzianum* TZ16, *T. virens* KB31 and *T. inhamatum* KEB12, respectively. Potato tubers were also treated with commercial seed fungicides named Celest-Max<sup>®</sup> (Fludioxonil, SC 100 g/l) and Quadris<sup>®</sup> (Azoxystrobin, SC 250 g/l). The results revealed that Fludioxonil treatments were more effective compared to Azoxystrobin treatments and the biological control agents.

**Keywords:** potato dry rot, *Fusarium sambucinum*, virulence, *Trichoderma* sp., biocontrol

### Introduction

Potato dry rot disease is one of the major diseases of potato tubers (*Solanum tuberosum* L.) both in storage and planting. The rate of tubers infected in storage reaches up to 60%. Yield losses attributed to dry rot in field is almost 25% (Hanson et al., 1996; Stevenson et al., 2001). Primary causes of potato dry root disease are several species of *Fusarium* such as *F. sambucinum* Fuckel, *F. solani* (Mart.) Sacc, *F. avenaceum* (Fr.) Sacc, *F. culmorum* (W.G. Sm.) Sacc, and *F. oxysporum* Schltdl (Boyd., 1972; Nelson et al., 1981, 1983; Hanson et al., 1996; Eken et al., 2000; Borca and Carmen, 2013; Stefańczyk et al., 2016; Aydın et al., 2016). Chemical control strategy sometimes may not be quite effective and economical against dry rot in potato. Therefore, alternative methods are needed to control the plant diseases. *Trichoderma* is known as the most widely used antagonists in biological control.

Current study was carried out with a total of 15 isolates and ten *Trichoderma* species. The efficacy of *Trichoderma* species against 3 isolates of *F. sambucinum* (Fs2, Fs3, Fs4) was investigated by duel culture in nutrient media and tuber application methods. Thus, some effective antagonists have been identified. According to our information,

this is the first study on biological control using a large number of *Trichoderma* species against dry rot disease of potato.

## Review of literature

*Fusarium sambucinum* Fuckel - teleomorph *Giberella pulicaris* most (Fr.) Sacc. is one of the most common and aggressive species found in potato tubers throughout the world (Boyd, 1972; Secor and Salas, 2001; Choiseul et al., 2001; Cullen et al., 2005; Sun et al., 2008; Eken et al., 2000; Aydın et al., 2016; Peters et al., 2008). This pathogen also produces trichothecene toxins which are secondary metabolites and cause various problems on humans and animals (Senter et al., 1991).

Fungi is mostly transported by tubers. However, *Fusarium* species such as *F. sambucinum* are common in most potato grown soils and can survive as resistant spores free in soil for a long time (Adams and Lapwood, 1983; Secor and Salas, 2001; Carnegie et al., 1998).

The disease can temporarily be suppressed by some postharvest applications to the tubers. However, when tubers exposed to the pathogen in soil, no measures can be taken. Previous studies indicated that fungicide applications during postharvest period cause resistance to *F. sambucinum* after a while and thus they may not be efficient enough (Hide et al., 1992; Desjardins, 1995; Peters et al., 2001; Gonzalez et al., 2002; Daami-Remadi et al., 2006; Gachango et al., 2012). A large number of studies have been conducted to identify cultivars resistant to dry rot disease of potato caused by *F. sambucinum*, but commercial potato cultivars are often susceptible to this disease (Schisler et al., 1997; Jellis, 1975; Langerfeld, 1979; Hooker, 1981; Jellis and Starling, 1983; Wastie et al., 1989; Ayed et al., 2006b; Aydın and İnal, 2018). Therefore, effective tuber treatments along with other applications prior to planting or during storage may reduce the severity of dry rot disease.

Several studies have been carried out on biological control of plant pathogens. The studies showed that *Trichoderma* species can be used as biological control agents against pathogens in soil microflora, especially in plant roots and tubers (Boosalis, 1964; Wilhelm, 1973; Lockwood, 1977; Cook and Baker, 1983; Whipps et al., 1993; Elad, 2000; Harman et al., 2004; Chaube et al., 2002; Aydın and Turhan, 2009). The role of *Trichoderma* species in biological control can be explained by interactions of biological control mechanisms such as antibiosis, hyperparasitism and competition (Kredics et al., 2003). When *Trichoderma* species are applied to tuberous plants, they colonize on newly formed organs of plants (tubers, roots and stolons) during the production season and maintain their activities (Harman, 2000; Howell, 2003; Aydın and Turhan, 2013). Some studies have reported that *Trichoderma* species can successfully control important tuber and soilborne phytopathogenic fungi such as *F. sambucinum* and *Rhizoctonia solani* Kuhn (Chet and Baker, 1981; Elad et al., 1980; Bell et al., 1982; Manczinger et al., 2002; Aydın and Turhan, 2013). A large number of studies have been conducted to investigate the effects of *Trichoderma* species on dry rot disease of potato caused by *F. sambucinum*. Some of these studies are; *T. viride* Pers. (Sadfi et al., 2001; Ayed et al., 2006a); *T. harzianum* Rifai. (Cherif et al., 2001; Ru and Di., 2012. Wharton and Kirk, 2014) *T. harzianum*, *T. viride* (Daami-Remadi et al., 2006); *Trichoderma* spp. (Schisler et al., 1998); *T. longibrachiatum* Rifai, *T. atroviride* Bissett., *T. virens* (*G. virens*) J.H., Mill., Giddens & A.A Foster (Ru and Di., 2012). The studies generally reported suppressing the dry rot disease of potato at certain rates.

## Material and methods

### *Microorganisms*

#### *Pathogen*

Three isolates of *F. Sambicunum* (Fs2, Fs3, Fs4) used in the study were obtained from the isolate collection of Phytopathology laboratory in the Department of Plant Protection, Faculty of Agriculture at Siirt University, Turkey. The isolates were isolated from potatoes with signs of dry rot disease. Identifications were performed by classical and DNA based technics, and pathogenicity of the isolates have been previously determined (Aydın et al., 2016; Aydın and İnal, 2018). Before being used in studies, clean tubers were contaminated with the isolates and isolates were re-isolated. Thus, the virulence of the isolates was protected. Isolates purified with single spore culture and stored at +4 °C were used in the study after culturing for 15 days in the dark at 15 °C in Potato Dextrose Agar (PDA, 38 g and 1 L completed with sterile water).

#### *Antagonists*

*Trichoderma* species (Table 1) were selected from isolate collection of Phytopathology laboratory in the Faculty of Agriculture at Siirt University. These isolates were previously isolated from soils in different regions of Turkey and identified both based on colony and conodial morphology (Aydın and Turhan, 2009). Isolates were stored at + 4 °C in oblique agar tubes containing PDA and used in the study after culturing in Potato Dextrose Agar (PDA) medium for 15 days at 24 °C (12 h dark, 12 h light).

#### *Potato cultivars*

The potato variety, cv. DESIRE® known to be sensitive to *F. Sambucinum* was used in the study (Aydın and İnal, 2018). The certified tubers weighing 80-100 g and 50-60 mm in diameter, which were not contaminated with any disease, were obtained from a field harvested in the same year.

#### *Antagonism in vitro (duel culture)*

Three isolates of *F. sambicunum* (Fs2, Fs3, Fs4) and *Trichoderma* species were grown on PDA and discs with a diameter of 8 mm were taken from the margins using a cork borer. The discs were planted (duel culture techniques) on the opposite side of the plate at equal distance from the periphery. The planting process was performed at the same time to each petri dish containing 20 ml of PDA medium in dotted form. Experiments were performed in an incubator at 22-24 °C. The study was conducted in 6 replications.

The evaluation time was determined based on the time that the pathogen colony was first fully covered by an antagonist. The mycoparasitic activity considering the rate and intensity of *F. sambicunum* colonization by the antagonist was evaluated according to the scale suggested by Turhan (1990) (Table 1).

#### *Inoculation ad tuber treatment*

The tubers were thoroughly washed in tap water and then kept in 5% sodium hypochlorite (NaOCI) for 5 min prior to the treatments. They were washed twice in

sterile water and allowed to dry on the drying paper for one day. Colonies taken from 7 to 10-day cultures of *F. sambucinum* isolates were placed into the 8 mm deep and 8 mm diameter holes drilled by an appropriate knife in the mid-belly of tubers. Water was added to the antagonistic *Trichoderma* grown in the PDA for a week, then scraped with a spatula, transferred to a double layer cheesecloth, and the spores were separated from the medium. The density of spore was counted with a haemocytometer slide in a microscope and adjusted to  $1 \times 10^6$  spores  $\text{ml}^{-1}$ . Carboxy methyl cellulose in an amount of 0.05% was added to the spore suspensions to strengthen the adhesion, and three drops of Tween 20 were added to ensure uniform distribution of spores in the suspension. Finally, the suspensions were kept in a shaker for 15 min. to maintain the homogeneity. Twenty-four hours after placing the *F. sambucinum* colony into the tubers, 1 ml of the prepared suspensions was added to the drilled holes, and the holes were covered with the tissues taken from the potatoes. Only sterile water and PDA parts were used for the untreated tubers. For comparison purposes, fungicides such as Celestine-Max® (Fludioxonil, 100 g  $\text{L}^{-1}$ , Syngenta Crop Protection Inc.) registered against *Rhizoctonia solani* of potato in Turkey and Quadris® (Azoxystrobin, 250 g  $\text{L}^{-1}$ , Syngenta Crop Protection Inc.) usually licensed to the late blight disease caused by *Phytophthora infestans* in potatoes and vegetables were used. Fungicides were mixed with water at the indicated doses (20 ml  $\text{L}^{-1}$  for Fludioxonil and 75 ml 100  $\text{L}^{-1}$  for Azoxystrobin) and 1 ml was added into each hole drilled in tubers.

The treatments of experiment were 1) no treatment (Negative control), 2) treated only with *F. sambucinum* isolates (Fs2, Fs3, Fs4) (Positive control), 3) treated with *Trichoderma* species and pathogen isolates, and finally 4) treated with fungicides. All three tubers were considered as units and the experiment was set up with four replications in a randomized plot design. The tubers over a humidified cloth on a large plastic tray were placed in a climate cabinet set at 15-20 °C. The experiments were conducted in the laboratories of Plant Protection Department, Faculty of Agriculture, Siirt University, Turkey.

### ***Evaluation of tuber treatments***

The tubers were cut longitudinally after 5 weeks of incubation; width (w) and depth (d) were measured, the penetration value was calculated using *Equation 1* developed by Lapwood et al. (1984).

$$\text{Penetration (mm): } [w/2 + (d-6)]/2 \quad (\text{Eq.1})$$

The percent (%) effect of the treatments was evaluated according to the Abbott formula (*Eq. 2*), by comparing the values of positive control after computing the average values of each application.

$$\text{Effect of applications (\%): } \frac{X - Y}{X} \times 100 \quad (\text{Eq.2})$$

where:

x: Mean disease severity in positive control plots (%)

y: Mean disease severity in treated plots (%)

Statistical analysis of the experiment was carried out by “JMP 8” statistical software (SAS Institute Inc.) and the differences among treatments were grouped by the LSD (LS Means Differences Student’s t) test

## Results

### *Efficiency of antagonists in vitro*

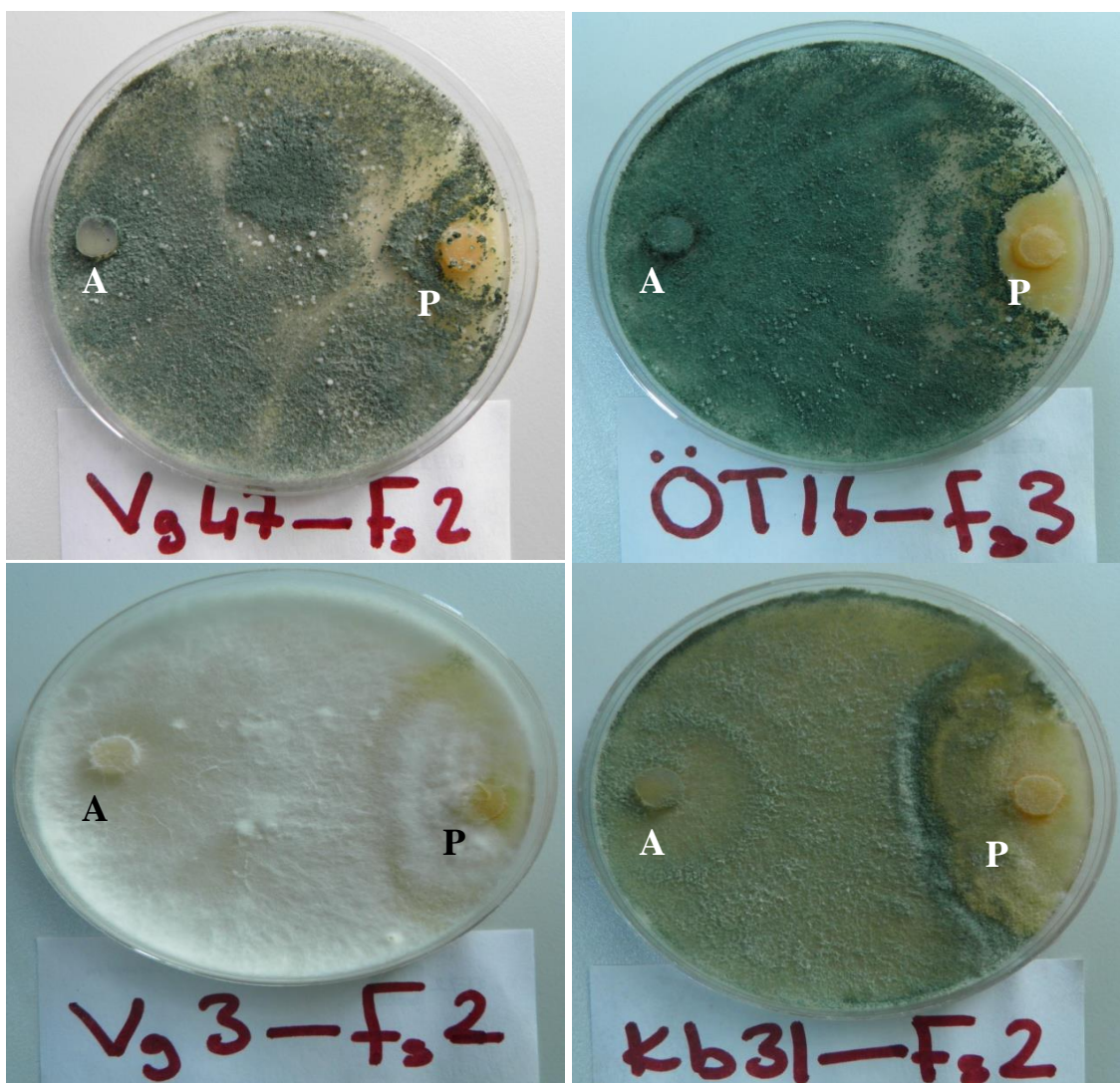
The effects of *Trichoderma* species on *F. sambucinum* isolates (Fs2, Fs3 and Fs4) were presented in *Table 1*. Antagonists and pathogen isolates were cross-planted in dotted form at the same time on the PDA medium. The assessment was based on appearance of the first antagonist that completely covered the pathogen colony in the petri dish. This antagonist was considered to be a very strong hyperparasitic and the others were assessed according to the scale given in *Table 1*. The most efficient antagonists for *F. sambucinum* Fs1 were *T. atroviride* VG3, *T. gamsii* VG47, *T. hamatum* ÖT16 and *T. viride* VG18, respectively. The antagonists for *F. sambucinum* Fs2 were *T. atroviride* VG3, *T. hamatum* ÖT16, *T. gamsii* VG47 and *T. viride* VG18, and those for *F. sambucinum* Fs3 were *T. atroviride* VG3, *T. gamsii* VG47, *T. strigosum* LO43, *T. viride* VG18 and *T. hamatum* ÖT16, respectively. Weakly developed antagonist isolates on three *F. sambucinum* isolates were *T. tomentosum* VG2, *T. neokoningii* A15 and *T. strigosum* LO8, respectively (*Table 1*).

**Table 1.** Effects of in-vitro antagonists on *F. sambucinum* isolates (Fs2, Fs3, Fs4)

Antagonists	<i>F. sambucinum</i> Fs1	<i>F. sambucinum</i> Fs2	<i>F. sambucinum</i> Fs3
<i>T. hamatum</i> ÖT16	VSH*	VSH	VSH
<i>T. virens</i> KB31	H***	H	H
<i>T. viride</i> VG19	SH**	H	SH
<i>T. viride</i> VG18	SH	VSH*	SH
<i>T. harzianum</i> LO52	SH	SH	SH
<i>T. harzianum</i> TUZ16	H	SH	H
<i>T. strigosum</i> LO8	H	H	H
<i>T. inhamatum</i> KEB12	SH	SH	H
<i>T. asperellum</i> ÖT1	SH	VSH	SH
<i>T. gamsii</i> VG47	VSH	SH	SH
<i>T. tomentosum</i> VG2	H	H	WH****
<i>T. neokoningii</i> A15	H	H	WH
<i>T. atroviride</i> VG3	VSH	VSH	VSH
<i>T. strigosum</i> LO43	H	SH	SH
<i>T. gamsii</i> VG48	VSH	VSH	VSH

VSH\*: Very strong mycoparasitism: Antagonist completely covers the colony of the pathogen  
SH\*\*: Strong mycoparasitism: Antagonist shows a strong improvement over the pathogen colony  
H\*\*\*: Moderate mycoparasitism: The development of the antagonist on the pathogen colony is easily discernible  
WH\*\*\*\*: Poor mycoparasitism: Very poor development of antagonist on the pathogen colony is noticed

*Trichoderma* isolates showed hyperparasitic character and inhibited the pathogen colony by growing over them (Table 1). Some antagonist isolates have also completely covered the pathogen isolates and they have demonstrated very strong hyperparasitic (VSH) features (Table 1). Some *Trichoderma* isolates showed very strong hyperparasitic (VSH) or strong hyperparasitic (SH) features, while some isolates were only moderately hyperparasitic (H) and they were less effective. The efficiency of antagonists showing strong hyperparasitic features were approximately close to each other against three isolates of *F. sambucinum* (Fs1, Fs2, Fs3). For example, *T. atroviride* VG3, *T. gamsii* VG47, *T. gamsii* VG48 and *T. hamatum* ET16 antagonists were the fastest and the most effective mycoparasites against three isolates of the pathogen. Images of antagonist growth on *F. sambucinum* isolates in PDA medium according to the duel culture method were shown Figure 1.



**Figure 1.** Images of some antagonist growth on *F. sambucinum* isolates in PDA medium according to the duel culture method [*T. gamsii* VG47- *F. sambucinum* 2 (VSH: Very strong mycoparasitism); *T. hamatum* ÖT16 - *F. sambucinum* 3 (VSH: Very strong mycoparasitism); *T. atroviride* VG3- *F. sambucinum* 2 (VSH: Very strong mycoparasitism); *T. virens* KB31- *F. sambucinum* 2 (H: Moderate mycoparasitism); A: Antagonist, P: Pathogen

### Evaluation of tuber applications

The efficacy of *Trichoderma* species on tubers against *F. sambucinum* isolates (Fs2, Fs3, Fs4) was presented in Table 2.

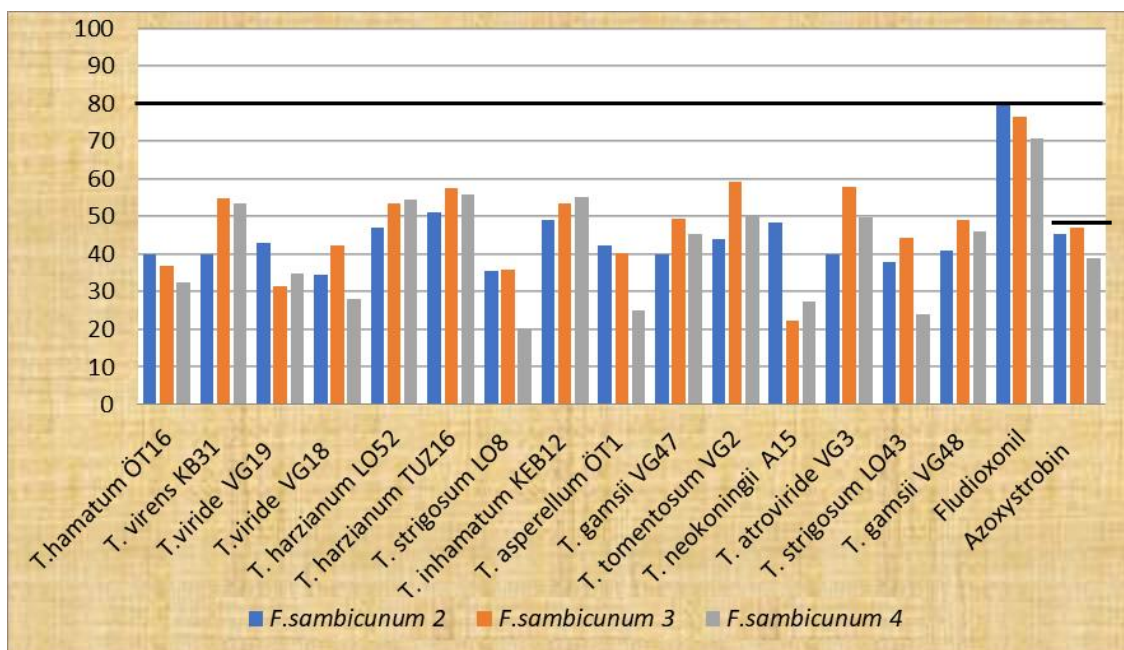
**Table 2.** The extent of suppressing (mm) and grouping of *F. sambucinum* isolates (Fs2, Fs3, Fs4) on potato tubers of some fungicides and *Trichoderma* species

No	Treatments	<i>F.sambucinum</i> Fs2	<i>F.sambucinum</i> Fs3	<i>F.sambucinum</i> Fs4	Mean
1	<i>T. hamatum</i> ÖT16	11.50 kt	12.63 jm	16.06 fg	13.40 c
2	<i>T. virens</i> KB31	11.50 kt	9.06 vx	11.06 mu	10.54 fg
3	<i>T. viride</i> VG19	10.94 mu	13.75 hj	15.50 fh	13.40 c
4	<i>T. viride</i> VG18	12.50 jn	11.56 ks	17.13 ef	13.73 bc
5	<i>T. harzianum</i> LO52	10.13 rw	9.31 ux	10.81 nv	10.08 gh
6	<i>T. harzianum</i> TUZ16	9.38 u	8.50 wy	10.50 pv	9.46 h
7	<i>T. strigosum</i> LO8	12.31 jo	12.88 il	19.00 bd	14.73 b
8	<i>T. inhamatum</i> KEB12	9.75 tx	9.31 ux	10.69 ov	9.92 gh
9	<i>T. asperellum</i> ÖT1	11.06 mu	12.00 jp	17.88 ce	13.64 c
10	<i>T. gamsii</i> VG47	11.75 kr	10.13 rw	13.00 ik	11.63 de
11	<i>T. tomentosum</i> VG2	10.69 ov	8.19 xy	11.81 kr	10.23 gh
12	<i>T. neokonigii</i> A15	9.88 sx	15.56 fg	17.25 df	14.23 bc
13	<i>T. atroviride</i> VG3	11.50 kt	8.44 wy	11.94 kq	10.63 eg
14	<i>T. strigosum</i> LO43	11.88 kr	11.19 lt	18.06 ce	13.71 bc
15	<i>T. gamsii</i> VG48	11.31 kt	10.19 qw	12.88 il	11.46 df
16	Fludioxonil	3.69 z	4.69 z	7.00 y	5.12 i
17	Azoxystrobin	10.44 pv	10.63 ov	14.56 g1	11.87 d
18	Control (+)	19.13 bc	20.06 b	23.81 a	21.00 a
19	Control (-)	0.00	0.00	0.00	0.00
Mean		11.07 b	11.00 b	14.39 a	12.16
CV%		10.56			
LSD <sub>0,05</sub>		Treatment; 1.03**	Fs; 0.42**	Treatment x Fs 1.79**	

\*\*P < 0.01. Levels not connected by same letter are significantly different

The effects of fungicides and isolates of *Trichoderma* on *F. sambucinum* isolates showed significant differences in terms of individual and mean values (P < 0.01). The differences between the treatments were grouped by the LSD (LSMeans Differences Student's t) test (Table 2). The disease was observed at all tubers in replications inoculated with Fs2, Fs3, and Fs4 isolates of *F. sambucinum* and treated with antagonists and certain fungicides. However, the levels of influence were different. The disease severity in the positive control of Fs2, Fs3 and Fs4 isolates was the highest and it was 19.13, 20.06 and 23.81, respectively. Fludioxonil, a fungicide, was the most inhibitory treatment (3.69, 4.69 and 7 mm) on growth of all three isolates investigated (Fs2, Fs3 and Fs4). The inhibitory effect of Azoxystrobin followed the Fludioxonil as 10.44, 10.63 and 14.56 mm. The most effective of *Trichoderma* species on Fs2 isolate was *T. harzianum* TUZ16 with 9.38 mm. *T. tomentosum* VG2 was effective on Fs3 isolate with 8.19 mm and *T. harzianum* TUZ16 was effective on Fs4 isolate with 10.50 mm. Fludioxonil was the most inhibitory fungicide on growth of all three isolates with an average of 5.12 mm. Average growth of isolates with Azoxystrobin treatment was 11.87. *Trichoderma harzianum* TUZ16 (9.46 mm) was the most inhibitory species of

*Trichoderma* species and followed by *T. inhamatum* (9.92 mm). Mean values of disease severity with *T. neokoningii* A15, *T. strigosum* LO43, *T. asperellum* ÖT1, *T. hamatum* ÖT16, *T. viride* VG19 and *T. viride* VG18 treatments were 14.23, 13.71, 13.64, 13.40, 13.40 and 13.73, respectively and placed in a separate group. Therefore, *T. Strigosum* LO8 (14.73) and *T. neokoningii* A15 (14.23) were the least effective species. The effect of treatments in preventing the disease was given in *Figure 2*.



**Figure 2.** Effects of antagonists and fungicides on the prevention of disease in experiments with *F. sambucinum* Fs2, Fs3, and Fs4 infected isolates

The influence ratio of treatments, comparing the positive control, ranged from 20.20 to 80.71%. Average of the most effective treatments against three isolates of the pathogen was 75.97% for Fludioxonil, 54.82% for *T. harzianum* TUZ16, 49.21% for *T. inhamatum* KEB12, 51.73% for *T. harzianum* LO52, 51.22% for *T. tomentosum* VG2 and 49.21% for *T. atroviride* VG3, respectively. The appearances of some tubers cut in the evaluation phase after the treatments were shown in *Figure 3*.

Following the evaluation, the tissue pieces were taken from pathologically contaminated tubers that had been treated and had not been treated, and they were left to grow on the PDA medium. The growth of pathogen and *Trichoderma* species were observed.

## Discussion

This study has been carried out in two phases. In the first phase, the interaction of pathogens and antagonists was measured in the PDA medium. In the second phase, the activity of antagonists was investigated with three isolates of pathogen in potato tubers. Fifteen isolates, 10 of which were *Trichoderma* species, were used in the study. Majority of these species have been reported as the first isolation in Turkey (Aydin and Turhan, 2009). Therefore, this type of *Trichoderma* was the first time studied against



dry rot disease of potato caused by *F. Sambucinum*. According to our information, some *Trichoderma* species such as *T. gamsii* have been studied the first time against this pathogen in potato. Previous studies were mostly conducted with *T. harzianum*, *T. viride* and *T. virens* (Daami-Remadi et al., 2006; Sadfi et al., 2001; Ayed et al., 2006a; Cherif et al., 2001; Ru and Di., 2012; Wharton and Kirk, 2014).



**Figure 3.** Appearance of disease developments in potato tubers, treated with antagonist and *F. sambucinum* isolates. Above: *T. harzianum* TUZ16- *F. sambucinum* 3 (the most inhibitory antagonist); *T. inhamatum* KEB12- *F. sambucinum* 4 (strong inhibitory antagonist). Below: *T. viride* VG18- *F. sambucinum* 4 (least inhibitory antagonist); *T. neokoningii* A15- *F. sambucinum* 4 (least inhibitory antagonist)

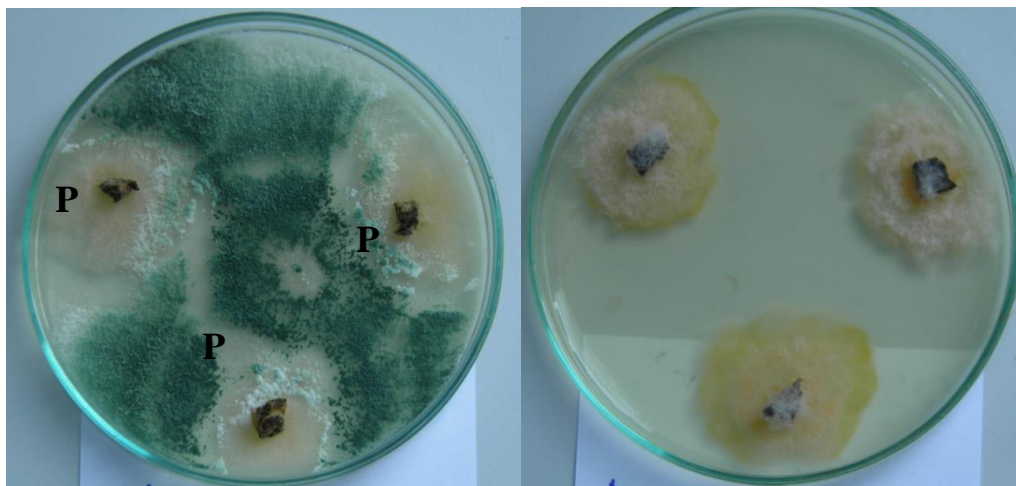
The results showed that some *Trichoderma* species reduced the dry rot disease that occurs in the tubers. The results of our study are in good agreement with some of previous studies. Daami-Remadi et al. (2006) reported that *T. harzianum* and *T. viride* control the disease at a certain rate. The efficiency of isolates from the same species on pathogen was close to each other both in nutrient medium and tubers. For example, *T. harzianum* TUZ16 and *T. harzianum* LO52 isolates obtained from different ecologies had SH and H values in the in vitro study (Table 1). The tuber study revealed that these two isolates of *T. harzianum* were effective in the penetration that ranged from 10.08 to 9.46 mm and at a rate between 54.82 and 51.73%, respectively. The results indicated the differences between the species in activity against the pathogen, but the difference was little in *Trichoderma* isolates of the same species. The efficiency of *T. hamatum* ÖT16, *T. viride* VG18, *T. viride* VG19 and *T. asperellum* ÖT1 isolates against pathogen isolates was high (SH and VSH) in duel cultures and PDA medium due to the mycoparasitism, antibiotic and enzyme production. However, this effect has not sufficiently occurred in vivo conditions. The opposite situation can also occur. A *Trichoderma* isolate that is not sufficiently successful in laboratory conditions may act in vivo. Temperature and some other environmental factors play a great deal of influence on the antagonist activity. This may not adversely affect the biocontrol activity of *Trichoderma* species; but it may reduce the effectiveness over time.

However, the most suitable environment to sustain the activity of a *Trichoderma* species should have similar temperature, moisture and nutrient values to the environment that the species isolated (Samuels, 2006).

*T. gamsii* VG47, *T. gamsii* VG48 and *T. atrovide* VG3 were successful in preventing the pathogen in both experiments. Our findings are in agreement with the previous studies (Cherif et al., 2001; Ru and Di, 2012; Wharton and Kirk, 2014). The results suggest that these antagonists have high compliance ability under various conditions. Establishment of field experiments with high performance isolates found in this study may lead to better results in terms of biological control against *F. Sambucinum*.

The efficacy of antagonists on the three isolates of *F. sambucinum* (Fs1, Fs2, Fs3) was not significantly different. The results of all experiments showed that the behavior of antagonists affecting the different isolates of pathogen was not significantly different.

After the evaluation, the tissues treated have been taken from the potatoes and planted on the PDA medium. In particular, *Trichoderma* species effective on pathogen were re-isolated (Fig. 4). This shows settling of antagonists into the tissue and continuing their activity. *Trichoderma* species have been reported to colonize and maintain their activity throughout the production season in various organs of plants (tubers, roots and stolons) (Harman, 2000; Howell, 2003; Aydın and Turhan, 2013).



**Figure 4.** Fungus developing in potato tissues after evaluation. On the left: *T. harzianum* LO52 re-isolation in a treated tuber with green colour. On the right: re-isolation image of *F. sambucinum* in pathogen with salmon colour-infected tuber). A: Antagonist, P: Pathogen

The fungicides with fludioxonil and azoxystrobin effective substances were used in the study. Fludioxonil has been identified as the most effective treatment. The activity of azoxystrobin was found to be low. Fludioxonil is effectively used against *Rhizoctonia solani*, another important tuberous disease of potatoes (Aydın and Turhan, 2009; Aydın et al., 2011). The fludioxonil has also been reported reducing the severity of dry rot disease of potatoes (Al- Mughrabi, 2010).

In contrast to previous studies, multiple isolates which were different from each other in terms of virulence were used in this study. Some *Trichoderma* species have demonstrated efficiency on all pathogen isolates in almost equal proportions.

## Conclusion

Dry rot disease caused by *F. sambucinum* is known an economically important field and a postharvest disease throughout the world. Biological control has recently gained great attention and shown significant potential to control the dry rot disease. The use of some *Trichoderma* such as *T. viride* VG18, *T. asperellum* ÖT1, *T. harzianum* TZ16, *T. virens* KB31 and *T. inhamatum* KEB12 successfully suppressed the dry rot in potatoes at different rates. The efficacies of some *Trichoderma* species and Fludioxonil treatments in preventing dry rot disease were higher compared to Azoxystrobin fungicide. The results obtained in this study showed that the use of *Trichoderma* isolates is an important approach in controlling the dry rot caused by *F. sambucinum* on potato tuber.

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