RESEARCH ARTICLE

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Antagonistic activities of mycoparasitic *Pythium* species against *Fusarium oxysporum* f. sp. *lycopersici* and *Botrytis cinerea* on tomatoes

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Abstract
In this study, antagonistic effects of *Pythium acanthophoron*, *P. lycopersicum*, *P. oligandrum* and *P. paroecandrum* against *Fusarium oxysporum* f. sp. *lycopersici* and *Botrytis cinerea* were investigated by *in vitro* and *in vivo* trials. *In vitro* mycoparasitic activities of *Pythium* species were determined by dual culture, inverted plate culture and agar diffusion tests. As a result of dual culture tests, suppressive effects of all mycoparasites were over 70% against mycelial growth of the pathogens. Inverted plate tests showed that antagonistic effects of mycoparasites regarding their volatile compounds were rather low. In the agar diffusion test, all mycoparasites showed antibiosis effect, however *P. lycopersicum* had the highest suppressive effect on both pathogens. In pot trials, mycoparasites were effective to protect tomato seedlings when pathogens were seperately inoculated, and suppressed the symptoms. When two pathogens were inoculated together, *P. paroecandrum* was ineffective against *B. cinerea*, but decreased the severity of wilt symptoms, while other mycoparasites totally inhibited both diseases. Chromatographic analyses made by using leaf samples taken 12, 24, 48 and 72 hours after pathogen inoculation showed meaningful increase on chlorogenic acid, caffeic acid and epicatechine, in the samples taken 48 hours after inoculation. Analyses after the inoculations of tomatoes with the mycoparasites and/or pathogens showed that mycoparasites also caused increase in the amounts of phenolics. This indicated that the mycoparasites could be effective to induce defense mechanisms of tomato plants against pathogens. Among them, *P. oligandrum* can be mentioned as the most effective mycoparasite regarding the induction of phenolics.
Keywords: *Solanum lycopersicum*, Fusarium wilt, Gray mold, Biocontrol, *Pythium* spp.

INTRODUCTION

Root, stem and fruit rots, damping off and wilt diseases caused by soil-borne plant pathogens are among the factors limiting the yield and crop quality. *Fusarium oxysporum* and *Botrytis cinerea* are widespread and important pathogens causing diseases on various crops (Lamichhane et al. 2017). *F. oxysporum* is one of the most common pathogens which has pathogenic forms specialized to 120-150 different host plants (Attitalla et al. 2004; Bogale et al. 2007; Kinyoda et al. 2022). Each form has one or more vegetative compatibility groups and one form generally causes disease on one host plant. There are two known special forms, *Fusarium oxysporum* f. sp. *lycopersici* (FOL) (Sacc.) W. C. Snyder & H. N. Hans and *F. oxysporum* f. sp. *radicis-lycopersici* (FORL) (Jarvis & Shoemaker) causing wilt and, crown and root rot diseases on tomatoes, respectively (Boix-Ruíz et al. 2015). *Botrytis cinerea* Fr., another important fungal agent of tomato, causing gray mold disease, is also a very common and polyphagous pathogen, which is known to

have more than 200 hosts (Panno et al. 2021). It can infect leaves, flowers, stem and fruits of tomatoes and damage all plant in a very short time (Dik and Wubben, 2004). Control of both pathogens is difficult, since they can form resistant structures and survive in soil for many years. Mainly chemical control is used besides cultural methods like crop rotation and use of resistant varieties. However, because of the well-known negative side effects of fungicides on environment and human health (Panth et al. 2020), biological control, which is known to be a safer method, gained importance and various studies were made on the use of different fungal and bacterial agents against plant pathogens (Heydari and Pessarakli 2010; Pandit et al. 2022).

Mycoparasitic *Pythium* species are among the biological agents used against different plant pathogens (Paulitz and Baker, 1987; Martin and Hancock, 1987; Lodha and Webster, 1990; Ağaner et al. 2021). *Pythium oligandrum* Drechsler (Deacon, 1976), *P. acanthicum* Drechsler (Deacon and Henry, 1978), *P. nunn* Lifshitz, Stanghellini & Baker (Lifshitz et al. 1984), *P. periplocum* Drechsler (Hockenhull et al. 1992), *P. acanthophoron* Sideris and *P. mycoparasiticum* Deacon, S.A.K. Laing & L.A. Berry (Jones and Deacon, 1995) were the first *Pythium* species determined to have mycoparasitic activity. *P. radiosum* Paul (Paul, 1999), *P. contiguanum* Paul (Paul, 2000), *P. canariense* Paul (Paul, 2002), *P. bifurcatum* Paul (Paul, 2003), *P. paroecandrum* Drechsler (Abdelghani et al. 2004), *P. citrinum* Paul (Paul, 2006), *P. lycopersicum* Karaca, Tepedelen Belgouthi and Paul (Karaca et al. 2008), and *P. amasculinum* Yu (Tepedelen, 2008) were subsequently described as mycoparasites. *P. oligandrum* is the most common and known mycoparasite and detailed studies were made on its mycoparasitic activity against different plant pathogens on various crops (Al-Hamadani and Cooke, 1983; Abdelzaher et al. 1997; Benhamou et al. 1997; Al-Rahawi and Hancock, 1998; Madsen and Neegaard, 1999; Picard et al. 2000a; Takenaka et al. 2003; Bělonožníková et al. 2022). However, there is less work on antagonism mechanisms of other *Pythium* species. The aim of this research is to determine *in vitro* and *in vivo* antagonistic activities of *P. acanthophoron*, *P. lycopersicum*, *P. oligandrum* and *P. paroecandrum,* isolated from Mediterranean region of Türkiye, against two important tomato pathogens; F. *oxysporum* f. sp. *lycopersici* and *B. cinerea*.

MATERIALS AND METHODS

Determination of the *in vitro* antagonistic activities of the mycoparasitic *Pythium* species

In the study, *F. oxysporum* f. sp. *lycopersici* (FOL) isolate obtained from Western Mediterranean Agricultural Research Institute (Antalya, Türkiye) and *B. cinerea* isolated from diseased tomato plants taken from greenhouses in Antalya were used as pathogens and *P. lycopersicum* (AD7-6), *P. acanthophoron* (IS7-2), *P. pareocandrum* (MKB2-1) and *P. oligandrum* (AK4T-1) isolates obtained from soil samples of different crops in the Mediterranean region of Türkiye, were used as mycoparasites. *B. cinerea* was isolated from the stem sections of tomato plants showing gray mold symptoms. Stem pieces including healthy and diseased tissue were surface sterilized with 1% NaOCl solution, blotted dry and transferred to Potato Dextrose Agar (PDA) plates. Growing hyphal tips were transferred to obtain pure cultures after incubation at 21°C for a few days. Mycoparasites were isolated from soil by using surface soil dilution plate (SSDP) method (Tepedelen, 2008).

In vitro antagonistic activities of mycoparasitic *Pythium* species were investigated by dual culture, inverted plates and agar diffusion methods. To determine the parasitic activities of *Pythium* species against pathogens, dual culture plate method was used. Mycelial disks of each pathogen and mycoparasite, taken from the growing edge of agar plates, were transferred to opposite sides of 9 cm diameter Petri plates with PDA and incubated at 25°C for 4 days. Plates inoculated only with pathogens served as controls and 3 replicate plates were used for each application. Radial growth of pathogen colonies were compared with controls and rate of inhibition (%) was calculated (Elshahawy and El-Mohamedy, 2019).

Effects of the possible volatile metabolites of the mycoparasites were investigated by inverted plate culture technique. Seperate PDA plates were inoculated with a single agar plug of pathogens and mycoparasites. After removing the lids, plates with mycoparasites were inverted over plates with pathogens and covered by parafilm. Similar plates without mycoparasites served as controls. After 4 days incubation at 25°C, growth inhibition was calculated (Pavitra et al. 2022).

Agar diffusion test was used to determine the possible antibiotic production of the mycoparasites. Spore suspensions of the pathogenic fungi (10^6 spores/ml) were spread over the plates with PDA. Sterilized culture filtrates (100 µl) of the mycoparasites grown in liquid medium were aseptically pipetted to 5 mm diameter wells made by a sterile cork borer. Plates were incubated at 25°C for 4 days and diameter of the inhibition zones around the wells were measured (Karunasinghe et al. 2020).

Determination of the *in vivo* biocontrol activities of the mycoparasitic *Pythium* species

At first, virulence of the mycoparasitic *Pythium* isolates were investigated and compared by the pathogenic species *P. deliense,* isolated from diseased tomato seedlings. Sterile sand, water and cornmeal mixture in 9:2:1 (v:v:v) rates were

used to obtain *Pythium* inoculum. Four weeks old tomato seedlings (cv. Caroca F1) were used in the pathogenicity tests. Tomato seedlings were transferred to the pots containing sterile soil mixture with *Pythium* inoculum (>200 propagules/g). Inoculum concentrations were checked by SSDP method given above. Pots without inoculum were used as controls and three replicate pots each with one seedling were used for each application. Virulence of the isolates was evaluated when severe symptoms on the *P. deliense* inoculated seedlings were observed. Disease severity was determined by using 0-3 scale modified from Botha et al. (1992), where; 0=no symptom, 1=small lesions on the roots or crown, slight wilting, 2=larger lesions on the stem, moderate wilting, 3=lesions girdling the stem, severe wilting or dead seedling.

Regarding the investigation of the interactions among mycoparasites and pathogens, tomato plants (cv. Caroca F1) were inoculated with pathogens with or without mycoparasites and plant growth and disease symptoms were evaluated. Mycoparasitic *Pythium* inoculum was incorporated into soil mixture as mentioned above. Roots of the tomato seedlings were wounded by cutting and inoculated by dipping in FOL spore suspension with a concentration of $2x10^7$ spores/ml for 30 minutes. *B. cinerea* inoculation was performed by spraying tomato leaves with the spore suspension of the pathogen in same concentration. Three replicate pots were used and pots without mycoparasite inoculum were used as controls. Plants were incubated in a climatic room with 22˚C temperature, 12h: 12h light and dark conditions and watered when necessary. After two weeks incubation, plants were observed for disease symptoms and evaluated by using special scales for each disease. For FOL, 0-3 scale modified from Ozbay and Steven (2004) where; 0=no visible symptom, 1=slight color change on vascular bundles, 2=severe color change on vascular bundles, slight wilting of leaves, 3=severe wilting, dried or dead plant, was used. Similarly, 0-3 scale modified from Lou et al. (2011) where; 0= no visible symptom, 1=less than 25% of leaf area was affected, 2=less than 50% of leaf area was affected, 3=more than 50% of leaf area was affected, was used for *B. cinerea*.

For the determination of the effects of mycoparasites on the resistance mechanisms of tomato plants, amounts of the phenolic compounds in tomato leaves; 12, 24, 48 and 72 hours after mycoparasite and/or pathogen inoculation, were determined by chromatographic analyses. High performance liquid chromatography (HPLC) method was used to detect and quantify the phenolic compounds of the leaf samples taken from the tomato plants inoculated with pathogens and/or mycoparasites. Two tomato cultivars, one of them known as relatively susceptible (cv. H-2274) and the other more tolerant (cv. Newton) to the diseases, were used in the experiment. Analyses were performed in the YETEM-Innovative Technologies Application and Research Center of Süleyman Demirel University, by using Shimadzu model HPLC (Shimadzu Corp., Kyoto, Japan) with a SCL-10A vp system controller, a LC-10AD vp pump, Diode Array Detector with wavelength at 278 nm, a SIL10AD vp autosampler, CTO-10 A vp column heater and a DGU-14a degasser and Agilent Eclipse Zorbax C18 column. Standarts of 12 phenolic compounds (gallic acid, catechin, chlorogenic acid, caffeic acid, epicatechin, syringic acid, *p*-coumaric acid, ferulic acid, cinapic acid, *o*-coumaric acid, cinnamic acide and quercetin) were obtained from Sigma (St. Louis, MO, USA).

All trials were repeated two times and SPSS 23® program was used for statistical anayses. Data were subjected to analyses of variance and means were compared by Tukey's test ($P \leq 0.05$).

RESULTS AND DISCUSSION

In vitro antagonistic activities of the *Pythium* species

Mycelial growth of *B. cinerea* and FOL was inhibited in different rates by the four antagonistic *Pythium* species used in the study. It was found that the parasitic activities of the antagonists were higher than their antibiosis related effects (Table 1, Figure 1). In the dual plate culture method, all antagonists used in the study clearly inhibited the mycelial growth of both pathogens. The highest rates of inhibition for both pathogens were obtained by *P. lycopersicum*. *P. acanthophoron* showed mean inhibition rate of 80.74% on the mycelial growth of *B. cinerea* and was statistically arranged in the same group with *P. lycopersicum*. *P. oligandrum* and *P. paroecandrum* formed the second statistical group with growth inhibitions over 70%. Similarly, high rates of mycelial growth inhibition were observed for FOL with all mycoparasites. *P. lycopersicum* inhibited the mycelial growth of the pathogen about 83%, whereas other three mycoparasites showed inhibitions over 70%. These findings are coherent with those of El-Katatny et al. (2005) and Attia et al. (2022), who reported that *P. oligandrum* completely overgrew *F. oxysporum* and *P. ultimum* var. *ultimum* in dual cultures. It was determined in the inverted plate method that the inhibitory effects of *Pythium* species related with their volatile components were rather low. Highest inhibitory effect against *B. cinerea* was obtained with *P. oligandrum*, while *P. lycopersicum* showed the highest effect against FOL. *P. acanthophoron* yielded the minimum inhibition rates against both pathogens. Despite our findings, El-Katatny et al. (2005) reported that *P. oligandrum* almost totally inhibited the growth of *P. ultimum* and *F. oxysporum* with its volatile metabolites. Agar diffusion test showed that all four mycoparasitic species produced metabolites with antibiotic activity in varying degrees. Culture filtrate of *P. lycopersicum* caused the widest inhibition zones on the mycelial growth of *B. cinerea* and FOL, while the narrowest zones were obtained by *P. oligandrum* for both pathogens. Previously, *P. oligandrum* was found to have no

antibiotic production as a result of a similar test using two plant pathogenic fungi (El-Katatny et al. 2005). This may because of the differing activities of the isolates or the susceptibility of host organisms.

Table 1. Inhibition of *Botrytis cinerea* and *Fusarium oxysporum* f. sp. *lycopersici* (FOL) mycelial growth caused by the mycoparasitic *Pythium* species in dual plate, inverted plate and agar diffusion tests.

Mycoparasitic *Pythium* species	Mean inhibition rates (%)				Inhibition zone diameter (mm)	
	Dual plate culture		Inverted plate test		Agar diffusion test	
	B. cinerea	FOL	B. cinerea	FOL	B. cinerea	FOL
P. acanthophoron	80.74 a[x]	74.81 b	6.32 c	5.99 c	19.00 b	17.00 bc
P. lycopersicum	84.44 a	82.59 a	6.90 b	7.55 a	25.00 a	21.00 a
P. oligandrum	73.33 b	74.81 b	7.30 a	6.36 bc	17.00 b	15.00 c
P. paroecandrum	78.88 b	72.96 b	6.97 b	7.43 b	23.00 a	19.00 ab

[x]Values in the same column shown by the same letter were statistically not different from each other according to Tukey's test (P≤0.05).

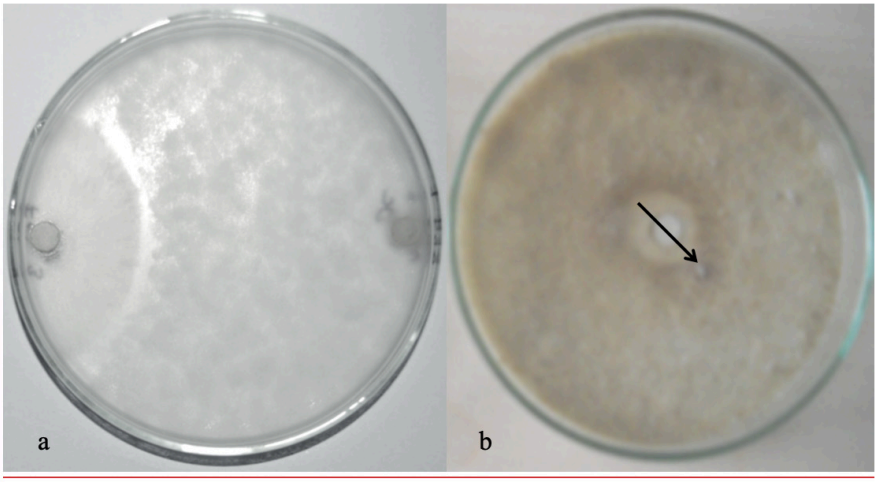

Figure 1. Inhibition of the *in vitro* mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* a) by *P. lycopersicum* (on the right) in dual plate culture and b) by *P. acanthophoron* in agar diffusion test (arrow shows the inhibition zone).

In our study, it was determined that the inhibitory effects of *Pythium* species showed slight variations and could be changed depending on the pathogens. Similarly, Jones and Deacon (1995) reported that the mycoparasitic performance of *P. acanthophoron* was better than *P. mycoparasiticum*, but lower than *P. oligandrum*. Varying degrees of mycoparasitic activities also mentioned for *P. acanthicum, P. oligandrum* and *P. periplocum* against pathogens under different *in vitro* conditions (Ali-Shtayeh and Saleh, 1999). Thus, it can be mentioned that the mycoparasitic performances of *Pythium* species could be changed according to the isolates, species, host pathogens and conditions during mycoparasitism.

Virulence of the mycoparasitic *Pythium* species on tomato seedlings

In the pathogenicity test, *P. oligandrum* caused no symptoms, while *P. deliense*, used as pathogen control, caused severe root rot and depressed the growth of tomato plants. *P. acantophoron*, *P. lycopersicum* and *P. pareocandrum* caused slight color change on the roots, but they did not cause any disease symptoms on the upper ground parts of the plants (Figure 2).

P. oligandrum was previously isolated from a number of plants in different countries but its virulence was known to be rather low and this species is known as an effective mycoparasite (Plaats-Niterink, 1981; Yu and Ma, 1989). *P. acanthophoron* was originally isolated from *Ananas sativus* and was mentioned as a weak pathogen on this plant by Sideris (Plaats-Niterink, 1981). Later, it was reported to be a mycoparasite (Lodha and Webster, 1990; Jones and Deacon, 1995). Pepper, bean and pine plants were also reported as other hosts of this species (Hall, 1998). *P. paroecandrum* was isolated from various plants and reported to be weakly pathogenic (Plaats-Niterink, 1981). In a study made in Türkiye, it was reported that *P. paroecandrum* caused slight lesions on corn, wheat, bean and cucumber plants, moderate symptoms on tomato and sunflower plants and caused severe post-emergence damping-off symptoms on pepper, tobacco and sugarbeet plants (Hatat, 1995). *P. acanthophoron, P. lycopersicum* and *P. paroecandrum* caused slight decrease in seedling emergence and moderate browning on the roots of tobacco seedlings (Karabuğa, 2011).

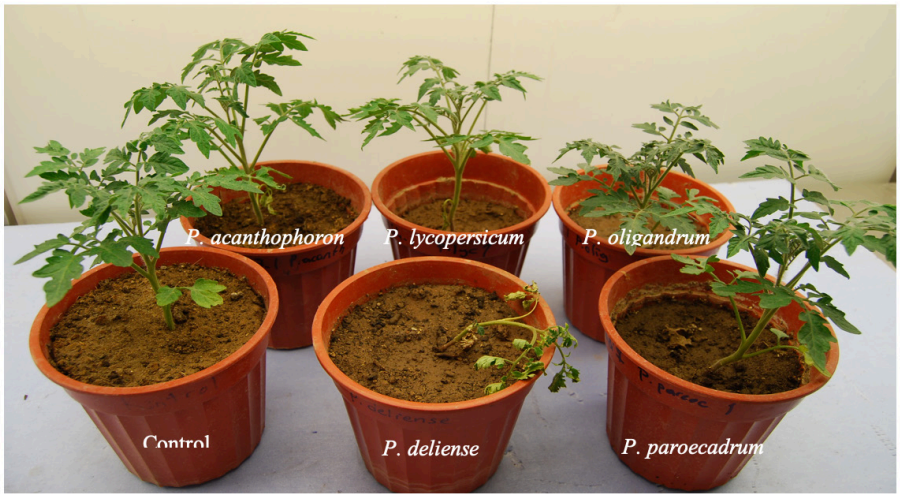

Figure 2. Effects of *Pythium* species on growth of tomato plants.

Biocontrol activities of the mycoparasitic *Pythium* species against two pathogens on tomato plants

In the trials performed to determine the preventive effects of mycoparasitic *Pythium* species against Fusarium wilt and gray mold diseases of tomato, all mycoparasites suppressed FOL and prevented tomato plants from wilting when inoculated with the pathogen, while only FOL inoculated plants showed severe wilting symptoms and dried (Figure 3). Plants inoculated with *B. cinerea* alone showed severe symptoms on the leaves, while those inoculated both with the mycoparasites and the pathogen showed slight symptoms and statistically arranged in the same group with control, except plants inoculated with *P. paroecandrum* which showed slight symptoms. Similarly, on the plants inoculated with both pathogens, again all mycoparasites except *P. paroecandrum* suppressed both pathogens and slight symptoms of both pathogens were observed only on plants inoculated with pathogens and *P. paroecandrum* (Table 2). This showed that *P. pareocandrum* isolate could suppress but not totally inhibit pathogen infections on tomato plants.

Pythium species can be effective as biological control agents by different mechanisms. Interactions between *P. oligandrum* and *Phytophthora parasitica* cells was observed by electron microscopy and it was determined that mycoparasitism was based on enzyme activity differing from other mycoparasite-pathogen interactions (Picard et al. 2000b). Another study revealed that *P. oligandrum* can affect pathogens by the synthesis of chitinase and β-1,3-glucanase enzymes (El-Katatny et al. 2005).

Figure 3. Preventive effect of *Pythium oligandrum* against *Fusarium oxysporum* f. sp. *lycopersici* (FOL) infection on tomato plants

Table 2. Mean scale values and mean disease severity rates (%) of tomato plants inoculated with the pathogens and/or mycoparasitic *Pythium* species.

Genotypes	Fusarium oxysporum f. sp. lycopersici (FOL)		Botrytis cinerea (BC)	
	Mean scale value	Mean disease severity (%)	Mean scale value	Mean disease severity (%)
Control	0.00[x]b[y]	0.00	0.00[z]c	0.00
FOL	3.00 a	100.00	-	-
BC	-	-	3.00 a	100.00
P. acanthophoron+FOL	0.00 b	0.00	-	-
P. acanthophoron+BC	-	-	0.00 c	0.00
P. acanthophoron+FOL+BC	0.00 b	0.00	0.00 c	0.00
P. lycopersicum+FOL	0.00 b	0.00	-	-
P. lycopersicum+BC	-	-	0.33 bc	11.11
P. lycopersicum+FOL+BC	0.00 b	0.00	0.00 c	0.00
P. oligandrum+FOL	0.00 b	0.00	-	-
P. oligandrum+BC	-	-	0.33 bc	11.11
P. oligandrum+FOL+BC	0.00 b	0.00	0.00 c	0.00
P. paroecandrum+FOL	1.00 ab	33.33	-	-
P. paroecandrum+BC	-	-	1.00 abc	33.33
P. paroecandrum+FOL+BC	1.00 ab	33.33	2.00 ab	66.67

[x] 0-3 scale for FOL; 0=no visible symptom, 1=slight color change on vascular bundles, 2=severe color change on vascular bundles, slight wilting of leaves, 3=severe wilting, dried or dead plant was used.
[y] Means on the same column shown by same letters were not significantly different from each other according to Tukey's test ($p \leq 0.05$)
[z] 0-3 scale for *B. cinerea*; 0= no visible symptom, 1=less than 25% of leaf area was affected, 2=less than 50% of leaf area was affected, 3=more than 50% of leaf area was affected.

Effects of mycoparasitic *Pythium* species and pathogens on phenolic contents of tomato plants

In the preliminary analyses made by using tomato leaf samples taken 12, 24, 48 and 72 hours after inoculation with FOL, 8 of the 12 standard phenolic compounds; gallic acid, catechin, chlorogenic acid, caffeic acid, epicatechin, syringic acid, *p*-coumaric acid and *o*-coumaric acid were detected. It was found that chlorogenic acid, caffeic acid and epicatechin amounts of the leaves showed the most meaningful increases, 48 hours after inoculation. Thus, amounts of caffeic acid, chlorogenic acid and epicatechin of the leaf samples of two tomato cultivars, inoculated with the mycoparasites and pathogens, were evaluated and compared, 48 hours after the inoculations. As a result of the chromatographic analysis, it was found that the inoculations of some of the mycoparasites caused increase on the amounts of phenolics when compared to control plants. However, increase of epicatechin amounts were higher in the leaf samples taken from the resistant cultivar Newton. In addition, inoculations of the both pathogens with mycoparasites caused more prominent increases in the amounts of phenolics, on the same cultivar. Among the mycoparasitic *Pythium* species, *P. oligandrum* inoculated with the both pathogens yielded the highest phenolic amounts (Table 3).

Our results are consistent with the previous similar studies. Pharand et al. (2002) found that *P. oligandrum* inoculation decreased wilt symptoms on tomato plants by inducing the defense mechanism of the plants against *F. oxysporum* f. sp. *lycopersici*. In another study, it was determined that *P. oligandrum* colonization on tomato roots prevented root rot caused by *Pythium* species and also effective against *B. cinerea* by means of the induction of plant defense (Le Floch et al. 2003). Biochemical analyses made after *P. oligandrum* inoculation of tomato plants showed that amounts of phenolics increased after 3 hours and rishitin synthesis started at 14th hour. Some responses of the host plant showed regular increases during 34 hours (Le Floch et al. 2005).

Table 3. Mean amounts of caffeic acid, chlorogenic acid and epicatechin in the leaf samples of susceptible (cv. H-2274) and tolerant (cv. Newton) tomato plants 48 hours after inoculations of mycoparasites and/or pathogens.

Applications	Mean amounts of phenolics (µg/g)					
	Caffeic acid		Chlorogenic acid		Epicatechin	
	H-2274	Newton	H-2274	Newton	H-2274	Newton
Control	1.9 gh[x]	1.1 h	4.1 cdef	6.8 e	4.6 f	1.7 h
P. acanthophoron	1.3 h	5.3 def	2.5 ef	7.8 de	4.8 ef	18.6 de
P. lycopersicum	1.7 gh	3.9 def	3.4 ef	5.8 e	4.4 f	14.4 ef
P. oligandrum	2.3 gh	5.6 de	4.4 cde	6.6 e	5.4 ef	14.1 ef
P. paroecandrum	2.4 fgh	5.4 def	4.4 cde	8.8 de	8.7 def	17.9 e
BC	1.9 gh	3.0 efgh	4.2 cdef	8.5 de	4.7 ef	3.0 gh
P. acanthophoron+BC	1.5 gh	4.0 def	2.4 f	15.0 bc	5.1 f	1.4 h
P. lycopersicum+BC	1.7 gh	3.0 efgh	2.8 def	17.9 ab	4.6 ef	2.2 h
P. oligandrum+BC	2.4 fgh	5.3 def	4.4 cde	18.6 ab	5.9 ef	3.0 gh
P. paroecandrum+BC	2.5 efgh	3.2 defgh	4.5 bcd	16.2 abc	8.9 def	UD h
FOL	3.6 cd	1.2 gh	6.0 ab	5.3 e	9.6 de	1.7 h
P. acanthophoron+FOL	4.0 bc	3.4 fgh	5.5 abc	12.3 cd	12.9 cd	2.0 h
P. lycopersicum+FOL	4.2 bc	5.5 d	5.2 abcd	19.1 a	17.2 c	2.8 h
P. oligandrum+FOL	3.6 cd	1.1 h	4.6 bcd	7.1 e	17.0 c	UD h
P. paroecandrum+FOL	4.2 ab	3.8 defg	5.4 a	9.0 de	17.7 f	8.4 fg
P. acanthophoron+FOL+BC	2.3 gh	3.9 def	4.3 abcd	UD[y] f	5.7 ef	16.8 e
P. lycopersicum+FOL+BC	2.7 defg	11.1 c	3.9 cdef	14.1 bc	16.3 c	27.0 cd
P. oligandrum+FOL+BC	5.9 a	13.7 b	7.9 a	14.9 bc	28.6 a	36.0 b
P. paroecandrum+FOL+BC	3.4 cdef	18.1 a	4.2 cdef	20.8 a	14.2 c	59.1 a

[x] Means on the same column shown by same letters were not significantly different from each other according to Tukey's test ($p \leq 0.05$).

[y] Under detectable level.

CONCLUSION

All the mycoparasitic *Pythium* species used in this study did not cause serious disease symptom on tomato plants. However, they prevent the plants from diseases when they inoculated together with pathogens. In addition to their suppressive effect on diseases, mycoparasites also induced plant defense. As a result of this study, it was shown that not only *P. oligandrum*, but also the other mycoparasitic *Pythium* species can be effective to suppress plant diseases with different antagonistic mechanisms and can be used as biological control agents against some soil-borne plant pathogens. However, detailed studies should be made to investigate their efficiency under field conditions with different host-pathogen combinations.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declared that they have no conflicts of interest.

Author contribution

Both authors designed the research study, MA performed the research under the supervision of GK. The contribution of the authors on the preparation of the manuscript is equal. The authors read and approved the final manuscript and verify that the text, figures and tables are original.

Ethical approval

Ethics committee approval is not required since the article does not contain studies with human or animals.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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