Journal of Plant Protection Research eISSN 1899-007X

ORIGINAL ARTICLE

The activity of *Trichoderma* spp. culture filtrate to control *Phelipanche aegyptiaca* infection in tomato

Farnaz Jalali[1], Saeed Abbasi[1]*, Hooman Salari[2]

[1] Plant Protection Department, Razi University, 6714414971, Kermanshah, Iran

[2] Plant Production and Genetics Department, Razi University, 6714414971, Kermanshah, Iran

Vol. 64, No. 2: 189–199, 2024

DOI: 10.24425/jppr.2024.150252

Received: January 14, 2024
Accepted: February 27, 2024
Online publication: June 03, 2024

*Corresponding address:
abbasikhs@yahoo.com

Responsible Editor:
Łukasz Sobiech

Abstract

Two independent pot experiments were conducted to evaluate the potential of two different application methods for culture filtrates of 10 *Trichoderma* spp. strains to reduce infection of *Phelipanche aegyptiaca* in tomatoes. In the first method (foliar spray), seedlings were foliar sprayed three times with culture filtrate of each *Trichoderma* spp. strains pre- and post-transplanting. In the second method (soil drench), these culture filtrates were incorporated into the top 5 cm of the soil surface during transplantation. Foliar sprays of T33, T60, and T36 significantly reduced the progression of *P. aegyptiaca* infection throughout the growing period (85 days). The number of *P. aegyptiaca*'s aboveground stalks and underground juveniles was also significantly reduced (83 and 66%) in T33-treated plants at the end of the experiment, while the fresh and dry weights of tomato fruits was significantly increased (86% and 90%). In the second approach, T66, T33, T35, T36, and T67 strains caused a significant reduction in the progression of *P. aegyptiaca* infection on tomatoes during the same period. The fresh and dry weights of stalks and attached juveniles of *P. aegyptiaca* in T66-treated plants were significantly reduced by 77, 52, 75, and 49%, respectively, compared to the control. The conclusion showed that *T. virens* T33 culture filtrate as foliar spray through some kind of induced systemic resistance (ISR) and *T. brevicompactum* T66 culture filtrate as soil drench can be used as natural bioherbicides to control *P. aegyptiaca* in tomatoes.

Keywords: biocontrol, beneficial fungi, broomrape, mycoherbicides, parasitic plants, weed management

Introduction

Parasitic plants, are some of the most serious problems in agriculture (Hatcher and Froud-Williams 2017). Infestation by some of them directly decreases crop biomass through a parasitic strategy of accumulation of the plant with the parasite, activity of the parasite sink and reduction of nutrient uptake or further by changes in the photosynthetic system and hormonal balance which lead to noteworthy losses in many monocots or dicots (Fernández-Aparicio *et al.* 2016; Cartry *et al*. 2021). *Orobanchaceae* is the major family of parasitic plants (Joel 2013) which consists of *Alectra*, *Orobanche*, *Phelipanche,* and *Striga* genera. These genera have 12 major species of weeds. Broomrapes that are well-known holoparasites in the Mediterranean basin, central and eastern Europe and all over Asia belong to *Orobanche* and *Phelipanche* genera (Parker 2013). *O. crenata*, *O. cernua*, *O. cumana*, *O. foetida*, *O. minor*, *P. aegyptiaca,* and *P. ramosa* are the seven species infesting crop plants and pose problems for agriculture (Parker 2009).

Tomato (*Lycopersicon esculentum* L.) is one of the main vegetables in Iran. According to the latest report provided by Iran's Ministry of Agriculture, the total tomato planting area is about 80,000 hectares, with

a yield of more than 370,000 tons. In Kermanshah province, which is located in the western part of Iran, *Phelipanche aegyptiaca* is one of the harmful agents that causes great damage to tomato production. The rate of infestation in some fields has reached such levels that it has become uneconomical to grow tomatoes on them.

Currently, according to available reports, there is no effective treatment for controlling broomrapes without affecting the host because they are host-attached parasites. Crops are abandoned, quarantined or replaced to prevent parasite infestation. However, infestation can still occur due to the establishment of a bank of seeds which is difficult to eliminate due to the prolific production of seeds, their easy dispersion, physiological dormancy, seed durability, and germination coordinated with a specialized range of hosts (Fernández-Aparicio *et al.* 2016; Haring and Felsner 2018).

It has been shown that soil fungi contribute to the decrease in the weed seed bank due to their enzymes. Additionally, microbial phytotoxins have been found in the spermosphere of cultivated seeds (Schiltz *et al.* 2015). Although there is no information on the effects of these compounds on parasitic plant seeds, some of them may have a negative impact on the survival of them. The radicles of parasitic weeds that grow from seeds and carry haustoria are also attacked by these compounds. This method results in killing the seedlings before they attach to the roots and prevents parasitism (Fernández-Aparicio *et al.* 2016).

Another possibility of microorganisms interfering with parasitism is through induced systemic resistance (ISR) in the host. Some of these biological inducers have been successful against broomrape parasitism under experimental conditions (Fernández-Aparicio *et al.* 2016). *Pseudomonas* isolates induce ISR and are marketed as Proradix, which reduces *P. ramosa* parasitism by 80% in susceptible varieties of hemp and tobacco without toxic effects on the plant (Gonsior *et al.* 2004). However, the role of jasmonic acid-dependent ISR against broomrape is unclear. Likewise, *Rhizobium leguminosarum* induces the phenylpropanoid pathway and leads to the development of defense mechanisms against *O. crenata* penetration (Mabrouk *et al.* 2007a, b, c, 2010).

Trichoderma spp. are among the most successful biological control fungi (BCF) in agriculture. They have many direct and indirect beneficial effects on plants (Tyśkiewicz *et al.* 2022), including promotion of plant growth (Hermosa *et al.* 2012), ISR to plant disease (Mathys *et al.* 2012), tolerance to abiotic stresses (Zhang *et al.* 2016; Jalali *et al.* 2017) and antagonism against plant pathogens, mainly fungi, bacteria and nematodes (Zaidi *et al.* 2014).

Abdel-Kader and co-workers reported that isolates of *Trichoderma* spp. could colonize *O. crenata* juveniles at different levels under laboratory conditions. In other studies, to find an economical and appropriate method to obtain the maximum positive effect when using *T. harzianum* (T1 and T3) and *T. viride* (T2) mycoherbicides against *P. ramosa* infections, various methods were used in pea, faba bean, and tomato fields (Abdel-Kader and El-Mougy 2007, 2009). The inhibitory effect of crude extracts of *T. harzianum* on the germination of *P. ramosa* seeds under *in vitro* conditions has also been reported (Yahia *et al.* 2015). Considering that there are currently no studies on the effects of *Trichoderma* spp. culture filtrate in reducing broomrape infection on plants, our study aimed to evaluate the potential of application of *Trichoderma* spp. culture filtrates to control *P. aegyptiaca* infection in tomato.

Materials and Methods

Fungal strains

During the winter season of 2020–2021, tomato fields around the tomato paste manufacturer of Rojin Taak Agro-industries Company, Kermanshah, Iran were surveyed for isolation of *Trichoderma* spp. The soil samples were collected from 75 locations. Each point was randomly sampled at 5–10 cm depth. The samples were stored in plastic bags and were subjected to isolation of *Trichoderma* spp. using the spreading method. Briefly, one gram of powdered soil of each sample was spread onto four spots on Petri dishes containing amended TSM (trichoderma selective sedia). The medium contained: 0.2 g $MgSO_4 \cdot 7H_2O$, 0.9 g K_2HPO_4, 0.15 g KCl, 1.0 g NH_4NO_3, 3.0 g glucose, 0.15 g rose Bengal, 20 g agar, 0.25 g chloramphenicol, 0.2 g captan, and 0.2 g pentachloronitrobenzene (Elad and Chet 1983). Without chloramphenicol, pentachloronitrobenzene and captan were dissolved in 1000ml distilled water and autoclaved at 121°C for 20 min. Then these three components were added into the solution at 45°C.

The plates containing soil samples were incubated in the dark for 48 hours and then were transferred to 12 h day/12 h night conditions. *Trichoderma* colonies were isolated after 5 days of incubation at 25°C. These colonies were purified by transferring to water agar (WA) medium in one step and then transferring their hyphal tips to potato dextrose agar (PDA) media. Pure fungal cultures were maintained on PDA slants at 4°C for further studies.

Plant materials

In greenhouse experiments, the tomato cultivar Karoon (Falat Company, Iran) was used as one of the common tomato cultivars in Iran. Seeds of *P. aegyptiaca* were collected from a broomrape-infected field of

tomato in Kermanshah, Iran. The seeds were cleaned and stored in a refrigerator until use (about 1 year later).

Preparation of fungal culture filtrates

Among the obtained *Trichoderma* isolates, 10 distinct strains were selected based on the differences in colony morphology and location and used in greenhouse experiments. PDB (potato dextrose broth) medium was used for the cultivation of selected *Trichoderma* strains.

Growth suspensions of *Trichoderma* strains were prepared for use with mycoherbicidal methods. Two discs of each fungal strain (collected by cork borers with a diameter of 5 mm) obtained from 7-day-old cultures propagated on PDA medium were inoculated into individual Falcon tubes (15 ml) containing 10 ml of PDB medium. The Falcon tubes were incubated at 27±2°C for 14 days and shaken every 3 days to promote fungal growth. Fungal growth was then isolated by filtration through sterile gauze and then through a microbial filter (0.2 µm) to obtain the culture filtrate. These culture filtrates were transferred immediately to spray bottles and used in greenhouse experiments.

Greenhouse experiments

The experiment was conducted in the summer and autumn of 2022 in the research greenhouse of the Department of Plant Pathology, Faculty of Agriculture, Razi University, Kermanshah, Iran. Two independent pot experiments were conducted to evaluate the potential of the culture filtrates produced by the tested fungi.

The following procedure for preparing pots was carried out in both experiments: Pots (25 cm diameter, 25 cm deep) were filled with autoclaved soil to a depth of 10 cm and artificially infested with *P. aegyptiaca* seed bank (10 mg) into a circle with a 15 cm diameter. The pots were again filled with autoclaved soil to more than 5 cm and artificially infested with an equal amount of *P. aegyptiaca* into a 10 cm diameter circle.

Experiment 1: Foliar spray

Tomato seedling preparation

Tomato seeds were sown into polystyrene foam cells containing coco-peat soil. For each treatment, a separate polystyrene foam unit was considered. Seedlings were kept at 25°C, 16 h day/8 h night for 8 weeks. Irrigation was done regularly from the bottom of the polystyrene foam unit.

Five weeks after germination, 3 ml of the culture filtrate of the corresponding treatment was sprayed on the aerial part of each seedling in each individual polystyrene foam cells with spray bottles. Spraying was repeated twice in the 7th week (1 week before transferring to the pot), and once in the 9th week (1 week after transferring to the pot). Eight-week-old tomato seedlings were transferred to each pot (one seedling per pot). Then pots were filled with sterilized soil to the appropriate height and irrigated thoroughly.

Experiment 2: soil drench

The preparation of tomato seedlings was the same as in the previous experiment, except that they did not receive any treatment until they were transferred to the pot. When the seedlings were transplanted, 5 ml associated with each specific treatment was incorporated into the planting holes in the pots. Other details remained the same as in the previous trial.

Both experiments consisted of 12 treatments, including seedlings treated with culture filtrates of 10 strains of T26, T33, T35, T36, T40, T46, T60, T66, T67 and T72 that would be infected with *P. aegyptiaca* and untreated controls with and without *P. aegyptiaca*. The pots associated with both experiments were maintained at 28-32°C and 16-h day/8-h night and received the usual irrigation.

Experimental design and data analysis

Treatments were arranged according to a completely randomized design (CRD), with four replicates (pots) of each specific treatment, and the experiment was repeated twice independently, for a total of eight replicates. Statistical analysis was performed on the obtained data using SPSS software (version 16.0). Data were submitted for Analysis of variance (ANOVA). The significance of the treatment effects used was determined at the 5% probability level (p = 0.05), and treatment means were separated by Duncan's multiple range test. Excel software was used to draw charts and calculate the area under the chart.

Accurate identification of effective *Trichoderma* strains

Among the *Trichoderma* strains tested, T33 and T66 strains, which had significant effects on *P. aegyptiaca*, were accurately identified based on morphological and molecular data.

Morphological characterization

The colony growth patterns of *Trichoderma* strains of T33 and T66 cultured on PDA, malt extract agar (MEA), and corn meal dextrose agar (CMD) media

and the micro-morphological characteristics of conidia, conidiophores, phialides, and chlamydospores were observed according to the description of Gams and Bissett (2002).

DNA extraction and polymerase chain reaction

DNA extraction of the selected strains was done using the DNA extraction kit manufactured by Denzaist Asia Company, according to the instructions recommended by the manufacturer. A portion of the translation-elongation factor 1 alpha tef1 gene was amplified using the primer pairs tef1fw (5'- GTGAGCGTGGTATCACCATCG-3') and tef1rev (5'-GCCATCCTTGGAGACCAGC -3') (Kullnig-Gradinger *et al*. 2002) and The ITS-rDNA region (ITS1-5.8 S-ITS2) was amplified using the primer pairs ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al*. 1990). The reaction mixture in the final volume of 50 ml included 25 ml of 2X PCR Master Mix (Sinaclon Company, Iran), 10 µM of each primer and about 50 ng of genomic DNA.

The cycling conditions for the tef1 gene included: initial denaturation at 94°C for 1 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 59°C for 1 min, extension at 74°C for 50 s, and final extension at 74°C for 7 min. For the ITS-rDNA region cycling conditions consisted of an initial denaturation at 90°C for 2 min, 35 PCR cycles of denaturation at 95°C for 40 s, annealing at 57°C for 40 s and extension at 72°C for 50 s. These were followed by a final extension at 72°C for 10 min. The PCR reactions were performed in a Biometra thermocycler (Tpersonal, Germany). The amplicons were separated on a 1% agarose gel. The gel was stained with Red Gel and visualized under UV to confirm DNA amplification. The PCR products were purified and sequenced by Macrogen, Inc. (South Korea).

The sequences were manually edited using the BioEdit software. Edited sequences were submitted to the GenBank (www.ncbi.nlm. nih.gov/genbank).

Results

Survey of soils and isolation of *Trichoderma* spp. strains

Nearly 100 *Trichoderma* spp. isolates were obtained from 36 sampling sites. These isolates belonged to at least 10 different morphotypes. Therefore, we selected 10 strains of each type obtained from different locations for our greenhouse experiments.

Greenhouse experiments

Experiment I: Effects of foliar spray of tomato seedlings with *Trichoderma* culture filtrates on inducing a possible systemic resistance against *Phelipanche aegyptiaca* infection

Daily monitoring of the appearance of aboveground stalks of *P. aegyptiaca* on tomato plants for 85 days after transplantation showed that foliar spray of tomato seedlings with filtrates of three *Trichoderma* spp. strains of T33, T60 and T36, could effectively inhibit the progress of *P. aegyptiaca* infection on tomato plants compared to the positive control by 78, 63 and 51%, respectively (based on the area under the graph) (Fig. 1A, 1B). At the end of the experiment, the number of *P. aegyptiaca* aboveground stalks and underground juveniles attached per host plants was significantly reduced in plants treated with T33 (83 and 66%) and T60 (65 and 72%) culture filtrates compared to the positive control (Table 1).

Furthermore, fresh and dry weights of tomato fruits were significantly increased in plants treated with T33 culture filtrate compared to the positive control (86% and 90%, respectively). The dry weight of tomato fruits in plants treated with T33 culture filtrate was not significant compared to the negative control (Table 2).

Experiment II: Effects of soil drench with *Trichoderma* spp. culture filtrates on *Phelipanche aegyptiaca* populations and parameters and tomato plant growth parameters

Daily monitoring of the appearance of *P. aegyptiaca* aboveground stalks on tomato plants for 85 days after transplantation showed a reduction in the progression of *P. aegyptiaca* infection on tomato plants throughout the growth period by 77, 60, 60, 53 and 53%, respectively, compared to the positive control (based on area under the chart) (Figs. 2a, 2b). At the end of the experiment, the number of aboveground stems on a single tomato plant treated with T66, T33, T35, T67, and T72 culture filtrates was significantly reduced by 71, 58, 53, 47 and 43%, respectively, (85 days after transplantation) compared to the positive control (Table 3).

Although no significant differences were observed in the number of aboveground stalks and attached juveniles, the fresh and dry weights of stalks and attached juveniles of *P. aegyptiaca* were significantly reduced when treated with T66 culture filtrate compared to the positive control by 77, 52, 75 and 49%, respectively (Table 3). Furthermore, the root dry weight of tomato plants treated with T35, T33, and T66 culture filtrate significantly increased by 60, 53 and 48%, respectively, compared to the positive control (Table 4).

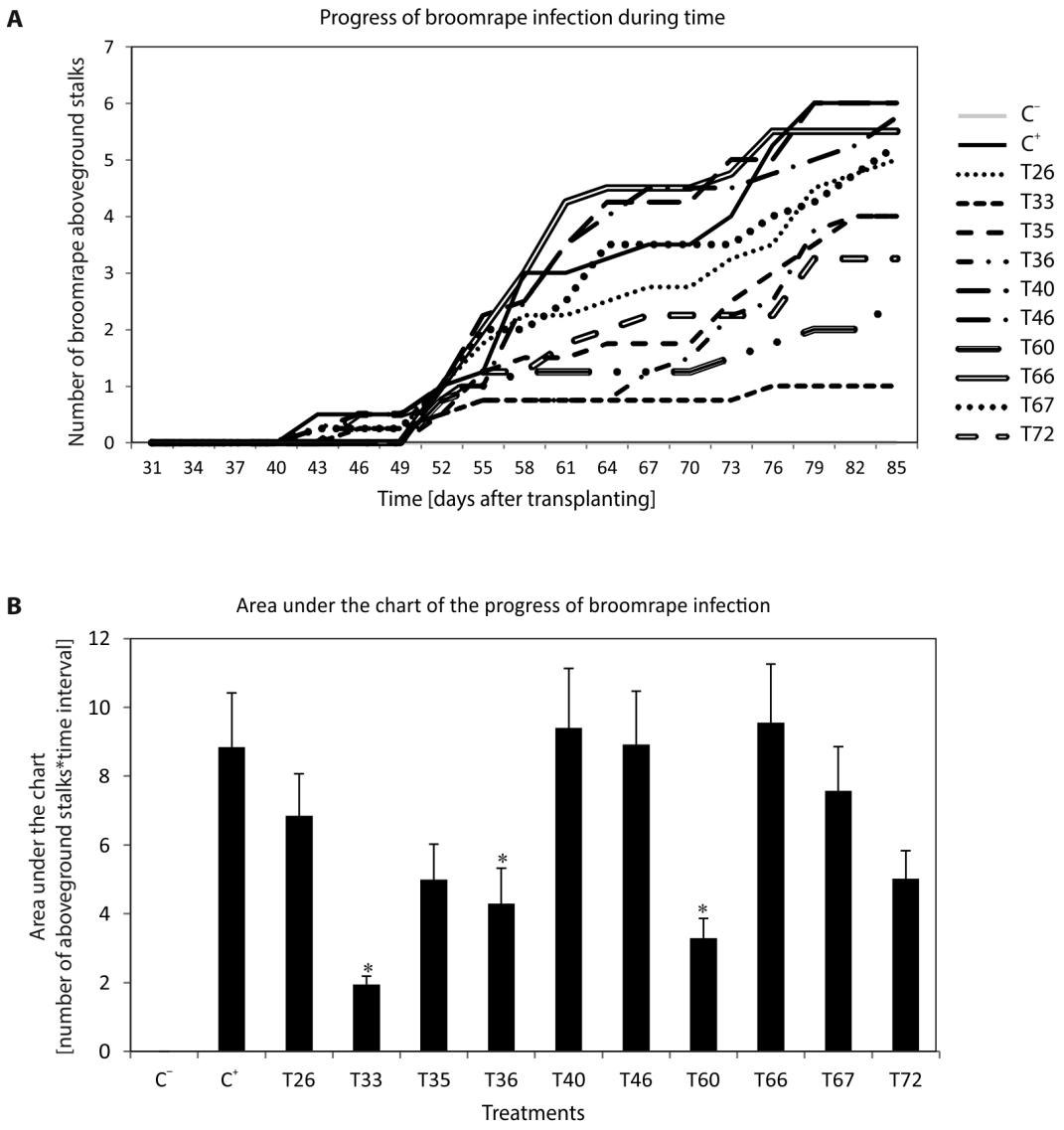


Fig. 1A. The progress chart of the appearance of *Phelipanche aegyptiaca* stalks on tomato plant cultivar, Karoon, artificially infested with *Phelipanche aegyptiaca* in response to foliar spray with culture filtrates of *Trichoderma* strains
C^- – negative control, C^+ – positive control, T26–T72 – *Trichoderma* strains

Fig. 1B. Mean values of area under the chart of the progress of the appearance of *Phelipanche aegyptiaca* stalks on tomato plants in response to foliar spray with culture filtrates of *Trichoderma* strains
C^- – negative control, C^+ – positive control, T26–T72 – *Trichoderma* strains (Number of replicates, $n = 8$). Error bars represent the standard deviation of the mean.
*indicates significance compared to the positive control using Duncan's multiple range test ($p < 0.05$)

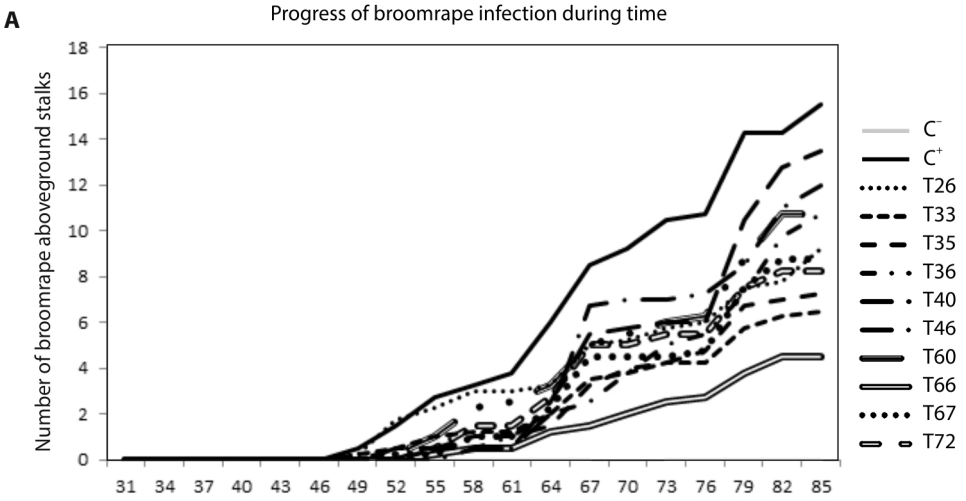
Table 1. Growth parameters of parasitic *Phelipanche aegyptiaca* on tomato plants in response to foliar spray with *Trichoderma* culture filtrates under greenhouse conditions (mean ±SE)

Treatments	Broomrape growth parameters					
	TNAS	TNUAJ	FWAS [g]	FWUAJ [g]	DWAS [g]	DWUAJ [g]
C^-	0.0 a	0.0 a	0.0 a	0.0 a	0.0a	0.0 a
C^+	6.0 ± 0.9 d	14.3 ± 2.4 cd	31.3 ± 3.2 bcd	59.2 ± 9.5 bcdef	5.7 ± 0.8bcd	11.1 ± 1.0 bcd
T26	5.0 ± 1.1 d	10.5 ± 2.5 bcd	51.0 ± 8.0 d	71.3 ± 4.8 ef	10.3 ± 2.1d	12.7 ± 1.7 bcd
T33	1.0 ± 0.4 ab	5.0 ± 1.1 ab	24.4 ± 8.9 bc	49.5 ± 12.5 bcde	3.8 ± 1.5ab	10.4 ± 3.7 bcd
T35	4.0 ± 1.3 cd	16.3 ± 2.3 d	16.1 ± 5.1 ab	75.2 ± 5.8 f	4.7 ± 0.7bc	14.3 ± 0.4 cd
T36	4.0 ± 0 cd	8.5 ± 1.4 bcd	27.1 ± 9.6 bc	45.9 ± 7.7 bcd	5.6 ± 1.5bc	12.5 ± 1.4 bcd
T40	6.0 ± 0.4 d	8.3 ± 4.4 bcd	31.7 ± 1.8 bcd	37.7 ± 6.6 bc	6.5 ± 1.1bcd	9.4 ± 0.9 bc

Table 1. Growth parameters of parasitic *Phelipanche aegyptiaca* on tomato plants in response to foliar spray with *Trichoderma* culture filtrates under greenhouse conditions (mean ±SE) – continuation

Treatments	Broomrape growth parameters					
	TNAS	TNUAJ	FWAS [g]	FWUAJ [g]	DWAS [g]	DWUAJ [g]
T46	5.8 ± 0.7 d	14.5 ± 2.5 cd	35.7 ± 4.2 bcd	69.9 ± 7.3 def	8.2 ± 0.9bcd	14.9 ± 1.8 d
T60	2.0 ± 0.6 abc	4.0 ± 0.8 ab	29.2 ± 4.9 bc	35.4 ± 8.6 b	4.6 ± 1.3bc	8.6 ± 0.7 b
T66	5.5 ± 1.0 d	9.5 ± 2.2 bcd	41.0 ± 4.6 cd	60.8 ± 4.0 cdef	8.6 ± 0.8cd	14.9 ± 0.4 d
T67	5.3 ± 0.4 d	11.3 ± 0.9 bcd	43.4 ± 6.9 cd	74.5 ± 4.7 f	9.1 ± 2.7cd	11.5 ± 2.0 bcd
T72	3.3 ± 1.2 bcd	6.5 ± 3.4 abc	36.6 ± 4.6 cd	59.8 ± 7.2 cdef	6.1 ± 0.3bcd	14.7 ± 0.5 cd

TNAS – total numbers of aboveground stalks; TNUAJ – total numbers of underground attached juveniles; FWAS – fresh weight of aboveground stalks; FWUAJ – fresh weight of underground attached juveniles; DWAS – dry weight of aboveground stalks; DWUAJ – dry weight of underground attached juveniles. C^- – negative control, C+ – positive control, T26–T72 – *Trichoderma* strains. Mean values within columns followed by the same letter are not significantly different ($p \leq 0.05$)



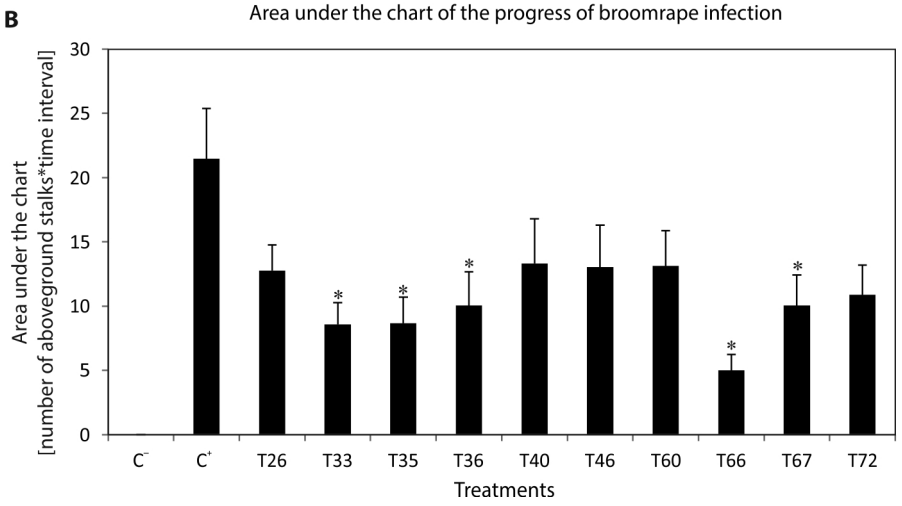


Fig. 2A. The progress chart of the appearance of *Phelipanche aegyptiaca* stalks on the tomato plant cultivar, Falat Karoon, artificially infested with *Phelipanche aegyptiaca* in response to soil drench with culture filtrates of *Trichoderma* strains
C^- – negative control, C^+ – positive control, T26–T72 – *Trichoderma* strains

Fig. 2B. Mean values of area under the chart of the progress of the appearance of *Phelipanche aegyptiaca* stalks on tomato plants in response to soil drench with culture filtrates of *Trichoderma* strains
C^- – negative control, C^+ – positive control, T26–T72 – *Trichoderma* strains (Number of replicates, $n = 8$). Error bars represent the standard deviation of the mean.
*indicates significance compared to the positive control using Duncan's multiple range test ($p < 0.05$)

Table 2. Growth parameters of tomato plants in response to foliar spray with culture filtrates of *Trichoderma* strains and infection with *Phelipanche aegyptiaca* under greenhouse conditions (mean ±SE)

Treatments	Growth parameters of tomato plants					
	SFW [g]	FFW [g]	RFW [g]	SDW [g]	FDW [g]	RDW [g]
C^-	109.8 ± 4.7 ab	72.3 ± 4.7 a	49.9 ± 4.2 a	29.2 ± 0.5 a	6.3 ± 0.2 a	12.0 ± 1.4 a
C^+	106.3 ± 13.0 abcd	4.8 ± 1.8 c	39.9 ± 10.1 ab	17.4 ± 2.5 bc	0.4 ± 0.1 c	8.6 ± 2.6 ab
T26	117.0 ± 6.1 a	5.9 ± 2.5 c	30.8 ± 1.8 ab	14.3 ± 0.5 c	0.5 ± 0.2 c	8.6 ± 1.8 ab
T33	112.7 ± 6.0 a	33.7 ± 11.0 b	32.8 ± 4.0 ab	20.8 ± 1.9 b	4.3 ± 1.5 b	6.9 ± 2.2 ab
T35	109.3 ± 6.1 abc	10.8 ± 4.0 c	25.8 ± 3.6 b	15.8 ± 0.9 c	1.0 ± 0.4 c	3.4 ± 1.9 b
T36	98.3 ± 3.3 abcd	4.9 ± 3.0 c	33.1 ± 7.3 ab	14.6 ± 0.9 c	0.6 ± 0.4 c	5.6 ± 3.1 b
T40	87.7 ± 6.7 bcd	6.8 ± 2.5 c	17.8 ± 5.7 b	11.8 ± 2.4 c	0.4 ± 0.1 c	2.6 ± 1.1 b
T46	83.6 ± 3.6 d	0.0 c	18.7 ± 6.4 b	14.4 ± 1.1 c	0.0 c	2.4 ± 0.9 b
T60	83.8 ± 8.2 d	8.6 ± 5.3 c	20.8 ± 6.6 b	12.4 ± 1.4 c	0.8 ± 0.5 c	2.1 ± 1.0 b
T66	93.9 ± 4.4 abcd	0.6 ± 0.4 c	30.6 ± 4.9 ab	12.5 ± 0.7 c	0.1 c	6.7 ± 1.8 ab
T67	82.3 ± 10.5 d	0.0 c	20.1 ± 7.5 b	12.6 ± 1.0 c	0.0 c	6.4 ± 2.1 ab
T72	85.9 ± 2.5 cd	0.0 c	22.7 ± 7.7 b	13.0 ± 2.7 c	0.0 c	2.4 ± 0.4 b

SFW – shoot fresh weight; FFW – fruit fresh weight; RFW – root fresh weight; SDW – shoot dry weight; FDW – fruit dry weight; RDW – root dry weight. C^- – negative control, C^+ – positive control, T26–T72 – *Trichoderma* strains. Mean values within columns followed by the same letter are not significantly different ($p \leq 0.05$)

Table 3. Growth parameters of parasitic *Phelipanche aegyptiaca* on tomato plants in response to soil drench with *Trichoderma* culture filtrates under greenhouse conditions (mean ±SE)

Treatments	Broomrape growth parameters					
	TNAS	TNUAJ	FWAS [g]	FWUAJ [g]	DWAS [g]	DWUAJ [g]
C^-	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
C^+	15.5 ± 1.6 e	27.5 ± 7.0 bc	53.8 ± 12.6 c	125.9 ± 4.2 cd	6.5 ± 0.2 b	27.8 ± 3.0 cd
T26	9.3 ± 2.9 bcde	16.3 ± 3.2 bc	37.3 ± 6.5 bc	116.2 ± 11.3 cd	6.6 ± 0.9 b	26.9 ± 2.0 cd
T33	6.5 ± 1.1 bc	15.0 ± 5.4 bc	28.3 ± 8.8 bc	101.4 ± 14.9 bc	6.0 ± 1.1 b	17.3 ± 4.8 bc
T35	7.3 ± 1.0 bcd	23.3 ± 2.4 bc	30.8 ± 13.5 bc	142.9 ± 10.0 d	5.3 ± 1.2 b	27.1 ± 2.0 cd
T36	10.8 ± 3.3 bcde	21.5 ± 2.7 bc	24.2 ± 7.3 abc	106.6 ± 5.5 c	4.8 ± 0.6 b	21.0 ± 0.8 bcd
T40	13.5 ± 1.6 de	22.8 ± 2.6 bc	36.6 ± 5.2 bc	118.8 ± 4.5 cd	5.0 ± 0.7 b	23.3 ± 1.2 bcd
T46	12.0 ± 1.4 cde	28.5 ± 1.6 c	33.8 ± 7.2 bc	127.3 ± 7.6 cd	6.5 ± 0.5 c	27.4 ± 2.7 cd
T60	10.8 ± 2.7 bcde	19.3 ± 3.2 bc	35.3 ± 6.2 bc	124.5 ± 8.4 cd	5.4 ± 1.0 b	28.5 ± 4.3 d
T66	4.5 ± 1.1 ab	13.8 ± 3.5 b	12.5 ± 5.2 ab	59.8 ± 14.1 b	1.6 ± 0.7 a	14.0 ± 3 b
T67	8.8 ± 0.9 bcd	16.8 ± 5.4 bc	28.5 ± 6.4 bc	102.1 ± 3.1 c	5.1 ± 0.3 b	22.9 ± 1.2 bcd
T72	8.3 ± 2.0 bcd	21.8 ± 4.3 bc	26.2 ± 11.8 abc	99.6 ± 16.7 c	5.2 ± 1.4 b	21.0 ± 5.4 bcd

TNAS – total numbers of aboveground stalks; TNUAJ – total numbers of underground attached juveniles; FWAS – fresh weight of aboveground stalks; FWUAJ – fresh weight of underground attached juveniles; DWAS – dry weight of aboveground stalks; DWUAJ – dry weight of underground attached juveniles; C^- – negative control; C^+ – positive control, T26–T72 – *Trichoderma* strains. Mean values within columns followed by the same letter are not significantly different ($p \leq 0.05$)

Table 4. Growth parameters of tomato plants in response to soil drench with culture filtrates of *Trichoderma* strains and infection with *Phelipanche aegyptiaca* under greenhouse conditions (mean ±SE)
Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$)

Treatments	Tomato growth parameters					
	SFW [g]	FFW [g]	RFW [g]	SDW [g]	FDW [g]	RDW [g]
C^-	157.7 ± 3.0 ab	162.0 ± 21.7 a	40.1 ± 1.6 a	36.5 ± 1.8 a	9.4 ± 1.3 a	7.9 ± 1.2 a
C^+	129.1 ± 15.1 ab	8.9 ± 5.4 b	27.4 ± 5.0 a	18.0 ± 0.4 b	0.5 ± 0.3 b	4.0 ± 0.7 c
T26	144.1 ± 9.2 ab	7.5 ± 4.6 b	45.3 ± 9.0 a	18.8 ± 0.6 b	0.4 ± 0.2 b	4.5 ± 0.4 bc
T33	143.3 ± 6.9 ab	28.8 ± 17.6 b	40.6 ± 6.2 a	21.9 ± 1.2 b	1.8 ± 1.1 b	6.1 ± 1.1 ab

Table 4. Growth parameters of tomato plants in response to soil drench with culture filtrates of *Trichoderma* strains and infection with *Phelipanche aegyptiaca* under greenhouse conditions (mean ±SE)
Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$) – continuation

Treatments	Tomato growth parameters					
	SFW [g]	FFW [g]	RFW [g]	SDW [g]	FDW [g]	RDW [g]
T35	145.3 ± 2.7 ab	13.8 ± 8.4 b	33.5 ± 2.8 a	19.0 ± 0.2 b	0.9 ± 0.5 b	6.4 ± 0.6 ab
T36	160.2 ± 15.4 ab	0.0 b	38.5 ± 8.0 a	20.6 ± 0.6 b	0.0 b	4.8 ± 0.6 bc
T40	151.2 ± 8.7 ab	0.0 b	33.6 ± 1.0 a	19.2 ± 0.6 b	0.0 b	4.9 ± 0.3 abc
T46	152.2 ± 2.5 ab	0.0 b	36.1 ± 2.1 a	20.4 ± 0.6 b	0.0 b	4.6 ± 0.4 bc
T60	169.8 ± 18.2 a	13.9 ± 5.2 b	37.9 ± 5.1 a	19.3 ± 0.8 b	0.8 ± 0.5 b	5.0 ± 0.9 bc
T66	127.4 ± 20.0 b	33.3 ± 7.7 b	38.8 ± 3.7 a	21.8 ± 2.4 b	2.1 ± 0.5 b	5.9 ± 0.8 ab
T67	153.1 ± 6.6 ab	9.3 ± 5.7 b	35.7 ± 5.1 a	20.9 ± 1.2 b	0.5 ± 0.3 b	4.4 ± 0.3 bc
T72	141.9 ± 7.4 ab	15.3 ± 9.4 b	32.8 ± 3.1 a	19.4 ± 1.0 b	0.9 ± 0.5 b	4.9 ± 0.2 abc

SFW – shoot fresh weight; FFW – fruit fresh weight; RFW – root fresh weight; SDW – shoot dry weight; FDW – fruit dry weight; RDW – root dry weight; C^- – negative control; C+ – positive control, T26–T72 – *Trichoderma* strains. Mean values within columns followed by the same letter are not significantly different ($p \leq 0.05$)

Accurate identification of effective *Trichoderma* strains

The colony growth rate of T33 on MEA-containing Petri dishes was fast and reached 9 cm in diameter after 3 days at 30°C. The colony growth rate on PDA-containing Petri dishes was moderate. Sporulation was scattered through the whole medium area and the color of the colony became green soon after sporulation. Conidia were subglobose to ellipsoidal, dark green, 3.7–4.3 × 2.8–3.2 µm. Conidia from adjacent phialides formed spherical masses. The base of conidiophores was sterile and had no branches but was fertile at apices. Phialides were 3–6 often ampulliform 8.8–9.2 × 3.1–3.9 µm with closely appressed which only form at apices of branches. Clamidospores formed in aged cultures, 6.9–9.1× 5.8–8.2 µm and had smooth walls.

The colony growth rate of T66 on MEA-containing Petri dishes was also fast and reached 9 cm in diameter after 4 days at 30°C. Colonies had compact conidiophores in olive green to blue-green or gray olive groups. Conidia were subglobose with smooth walls, 2.7–3.3 × 2.1–2.7 µm. Phialides were long and ampulliform with 2–5 in each group, 8.9–14.2 × 2.1–2.8 µm. Clamidospores in aged cultures were solitary in the middle or end of the hypha and were almost colorless and oblong to oval or pear shaped, 7.3–9.8 × 4.9–7.5 µm.

Through nucleotide sequencing analysis of the tef1, ITS1, and ITS4 regions of the genomes of T33 and T66 strains, and based on morphological characteristics, these strains were identified as *T. virens* and *T. brevicompactum*, respectively.

Discussion

Agriculture faces new challenges due to variables such as climatic conditions, pest and disease management methods, health issues and socio-economic benefits. Some common methods from the past may no longer apply. For example, the use of synthetic pesticides for management purposes is receiving more criticism than in the past (Cartry *et al.* 2021). In addition to health concerns, widespread pesticide use can lead to the development of resistance in target pests over time (Baucom 2019). Therefore, there is a need to use new ecological methods such as biological control to meet agricultural needs including parasitic weed management.

In this study, for the first time, we attempted to investigate the potential of metabolites in 14-day culture filtrates of some *Trichoderma* spp. strains on control of *P. aegyptiaca* infection in tomato plants. We chose culture filtrates of *Trichoderma* strains because based on a series of *in vitro* and greenhouse experiments conducted by Hassan *et al.* (2019) to study the effectiveness of the fungus *T. harzianum* on *Striga hermonthica* germination and sorghum infestation, various kinds of *T. harzianum* inoculum (autoclaved, culture, and filtrate) significantly ($p \leq 0.05$) reduced germination rates, but germination rates were minimized using *T. harzianum* culture filtrate compared to control and other inocula.

The results of the first experiment indicated that there was sufficient evidence that ISR against *P. aegyptiaca* occurs in tomato plants, at least in the case of *T. virens* T33 culture filtrates. Considering the physical

distance between the application site of *Trichoderma* spp. culture filtrate and where the parasites were present, it seems that reduction in disease progression in plants treated with culture filtrates from five *Trichoderma* strains (Fig. 1A, 1B) may be the result of the ability of these metabolites to reprogram plant gene expression, possibly by stimulating a limited number of common plant pathways, as has been repeatedly reported for *Trichoderma* species and some of its metabolites (Shoresh *et al*. 2010). Furthermore, in tomato plants treated with *T. virens* T33 culture filtrate, a significant reduction in the severity of *P. aegyptica* infection was observed by 83% of stalks and 66% of attached juveniles per host plant compared to the positive control. In other words, according to statistical analysis, tomato plants treated with *T. virens* T33 culture filtrate were identical to the negative control (Table 1). It provides further evidence that spraying three times with foliar spray of *T. virens* T33 culture filtrate throughout the growing season can induce systemic resistance in tomato plants to attack by *P aegyptica* and can significantly reduce its intensity compared to the native range recorded in the positive control. As has been mentioned previously by Cartry *et al*. (2021), defense mechanisms (mechanical barriers) and attack mechanisms (metabolite production) that protect plants from nearby parasitic plants may also be activated in this induced resistance, which requires further investigation. For example, López-Ráez *et al*. (2011) reported that arbuscular mycorrhizal symbiosis reduces strigolactone production in tomato. Such an effect on the degradation of synthetic strigolactones has also been reported for *Trichoderma* spp. metabolites *in vitro* by Boari *et al*. (2016). Significant reductions of 65 and 72% in the number of stalks and juveniles, respectively, were also observed in plants foliar treated with T60 culture filtrate. Despite this, tomato growth parameters did not increase.

Furthermore, the most important growth parameter of the crop is the biomass of reproductive tissues, which is negatively affected by the presence of *P. aegyptiaca* (Fernández-Aparicio *et al*. 2016). Our results also showed that fruit biomass was reduced by 94% in the positive control compared to the negative control. However, foliar spray of *T. virens* T33 culture filtrate can compensate for 90%, with no significant difference from the negative control (Table 2). Since treatment occurs only in the aerial parts, even before exposure to parasites (in two times out of three), the resulting increase in the fruit weight of *T. virens* T33-treated plants compared to the control could be a direct effect of these metabolites on plant growth, or the result of reduced *P. aegyptiaca* infection due to ISR or both. Considering that other growth parameters of tomato plants in the positive control were not significantly affected by the parasite compared to the negative control, this could explain the lack of significant increase in these parameters in plants treated with *T. virens* T33 culture filtrate (Table 2).

In the second experiment, T66, T33, T35, T36, and T67 metabolite treatments resulted in a significant reduction in *P. aegyptiaca* infection progression compared to the positive control (Fig. 2a, 2b). The culture filtrate of *T. brevicompactum* T66 significantly reduced almost all parameters of *P. aegyptiaca*, including the number of total stalks of *P. aegyptiaca* and the fresh and dry weights of stalks and juveniles in pots, compared to the positive control as shown in Table 3. Since these metabolites were applied to sites where seed parasites were encountered, it appeared that these metabolites could affect the seeds or germinating seeds of *P. aegyptiaca*, as well as developing tubercles and juveniles before and after the emergence of stalks on the soil surface. Significant reductions in the total number of *P. aegyptiaca* stalks were also recorded for T33, T35, T67, and T72 (Table 3), which may be part of the reason for the slower progression of *P. aegyptiaca* infection in treated tomato plants compared to the positive control. The dry weight of tomato roots treated with T35, T33, and T66 culture filtrates also increased significantly compared to the positive control (Table 4). Promotion in plant growth, especially root growth and particularly under stress caused by *Trichoderma* spp. and their metabolite interactions with plants have been reported (Jalali *et al.* 2017; Zaidi *et al*. 2014), which may be another reason for the slower progression of infection in treated plants than in positive controls.

In the study of Abdel-Kader and El-Mougy (2007, 2009) to control *O. ramosa* in tomato, pea and faba bean fields, significant reductions in disease incidence (percentage of visual flowering stalks of *Orobanche* around the host plants) and intensity (their numbers attached to the roots of host plants) of *O. ramosa* infection were observed with a combination of mycoherbicide (*T. harzianum* T1 and T3 and *T. viride* T2) and herbicide (glyphosate) treatments. In other studies, the use of combinations of microbial isolates was more effective (Hassan *et al*. 2019; El-Dabaa and Abd-El--Khair 2020). Since an integrated and sustained management plan consisting of multiple control methods acting on different broomrape life stages is well suited to address the broomrape weed problem long term (Fernández-Aparicio *et al.* 2016), and considering our results, it is possible that a combination of foliar spray with *Trichoderma* metabolites (*T. virens* T33, three times) followed by soil drench at the planting site (*T. brevicompactum* T66) may be a better way to control *P. aegyptiaca* infection and severity on tomato. The operational technology needs further research in our future work. Determination of culture filtrate composition and evaluation of its components are some of our other goals to be addressed in further studies. In

summary, our results have promising efficient accessibiliy, the continuation of which may contribute significantly to the development of a new method to control *P. aegyptiaca* in tomato.

Acknowledgements

We wish to thank Dr. Sohbat Bahraminezhad (Razi University) for his assistance with the statistical analysis. Also, we are grateful to members of the Department of Plant Protection, Razi University for all their assistance in the laboratories and for kindly sharing their equipment.

References

Abdel-Kader M.M., El-Mougy N.S. 2007. Applicable control measure against *Orobanche ramosa* in tomato plants. Australasian Plant Pathology 36:160–164. DOI: https://doi.org/10.1071/AP07004

Abdel-Kader M.M., El-Mougy N.S. 2009. Prospects of mycoherbicides for control of broomrapes (*Orobanche* spp.) in Egypt. Journal of plant protection research 49: 64–75. DOI: 10.2478/v10045-009-0009-1

Baucom R.S. 2019. Evolutionary and ecological insights from herbicide resistant weeds: what have we learned about plant adaptation, and what is left to uncover? New Phytologist 223: 68–82. DOI: https://doi.org/10. 1111/nph.15723

Boari A., Ciasca B., Pineda-Martos R., Lattanzio V.M., Yoneyama K., Vurro M. 2016. Parasitic weed management by using strigolactone-degrading fungi. Pest Management Science 72 (11): 2043–2047. DOI: https://doi.org/10.1002/ps.4226

Cartry D., Steinberg C., Gibot-Leclerc S. 2021. Main drivers of broomrape regulation. A review. Agronomy for Sustainable Development 41 (2): 17–39. DOI: https://doi.org/10.1007/s13593-021-00669-0

Elad Y., Chet I., Henis Y. 1981. A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. Phytoparasitica 9 (1): 59–67. DOI: https://doi.org/10.1007/bf03158330

El-Dabaa M.A., Abd-El-Khair H. 2020. Applications of plant growth promoting bacteria and *Trichoderma* spp. for controlling *Orobanche crenata* in faba bean. Bulletin of the National Research Centre 44 (1): 1–10. DOI: https://doi.org/10.1186/s42269-019-0263-y

Fernández-Aparicio M., Reboud X., Gibot-Leclerc S. 2016. Broomrape weeds. Underground mechanisms of parasitism and associated strategies for their control: a review. Frontier in Plant Science 7: 135–158. DOI: 10.3389/fpls.2016.00135

Gams W., Bissett J. 2002. Morphology and identification of *Trichoderma*. p. 3–31. In: "Trichoderma and Gliocladium Volume 1: Basic biology, taxonomy and genetics" (C.P. Kubicek, G.E. Harman, eds.). Taylor and Francis, London, UK.

Gonsior G., Buschmann H., Szinicz G., Spring O., Sauerborn J. 2004. Induced resistance – an innovative approach to manage branched broomrape (*Orobanche ramosa*) in hemp and tobacco. Weed Science 52: 1050–1053. DOI: 10.1614/WS-04-088R1

Haring S.C., Flessner M.L. 2018. Improving soil seed bank management: improving soil seed bank management. Pest Managment Science 74: 2412–2418. DOI: https://doi.org/10.1002/ps.5068

Hassan M.M., Azrag M.A., Rugheim A.M., Abusin R.M., Elnasikh M.H., Modawi H.I., Ahmed M.M., Abakeer R.A., Osman A.G., Abdelgani M.E., Babiker A.G. 2019. Potential of *Trichoderma harzianum* as a biocontrol agent against *Striga hermonthica* in sorghum. International Journal of Current Microbiology and Applied Science 8: 195–206.

Hatcher P.E., Froud-Williams R.J. 2017. Weed Research: Expanding Horizons. p 432. John Wiley & Sons, NJ, US. DOI: https://doi.org/10.1002/9781119380702

Hermosa R., Viterbo A., Chet I., Monte E. 2012. Plant-beneficial effects of *Trichoderma* and of its genes. Microbiology 158: 17–25. DOI: https://doi.org/10.1099/mic.0.052274-0

Jalali F., Zafari D., Salari H. 2017. Volatile organic compounds of some *Trichoderma* spp. increase growth and induce salt tolerance in *Arabidopsis thaliana*. Fungal Ecology 29: 67–75. DOI: https://doi.org/10.1016/j.funeco.2017.06.007

Joel D.M. 2013. The haustorium and the life cycles of parasitic Orobanchaceae. p. 21–23. In: "Parasitic Orobanchaceae: Parasitic Mechanisms and Control Strategies" (D.M. Joel, J. Gressel, L.J. Musselman, eds.). Springer, Berlin, Heidelberg.

Kullnig-Gradinger C.M., Szakacs G., Kubicek C.P. 2002. Phylogeny and evolution of the genus *Trichoderma*: a multigene approach. Mycological Research 106: 757–767. DOI: https://doi.org/10.1017/S0953756202006172

López-Ráez J.A., Charnikhova T., Fernández I., Bouwmeester H., Pozo M.J. 2011. Arbuscular mycorrhizal symbiosis decreases strigolactone production in tomato. Journal of Plant Physiology 168: 294–297. DOI: https://doi.org/10.1016/j.jplph.2010.08.011

Mabrouk Y., Mejri S., Hemissi I., Simier P., Delavault P., Saidi M. *et al.* 2010. Bioprotection mechanisms of pea plant by *Rhizobium leguminosarum* against *Orobanche crenata*. African Journal of Microbiology Research 4: 2570–2575.

Mabrouk Y., Simier P., Arfaoui A., Sifi B., Delavault P., Zourgui L., *et al.* 2007a. Induction of phenolic compounds in pea (*Pisum sativum* L.) inoculated by *Rhizobium leguminosarum* and infected with Orobanche crenata. Journal of Phytopathology 155: 728–734. DOI:10.1111/j.1439-0434.2007.01307.x

Mabrouk Y., Simier P., Delavault P., Delgrange S., Sifi B., Zourgui L., Belhadj O. 2007b. Molecular and biochemical mechanisms of defence induced in pea by *Rhizobium leguminosarum* against *Orobanche crenata*. Weed Research 47: 452–460. DOI: 10.1111/j.1365-3180.2007.00583.x

Mabrouk Y., Zourgui L., Sifi B., Delavault P., Simier P., Belhadj O. 2007c. Some compatible *Rhizobium leguminosarum* strains in peas decrease infections when parasitized by *Orobanche crenata*. Weed Research 47: 44–53. DOI: 10.1111/j.1365-3180.2007.00548.x

Mathys J., De Cremer K., Timmermans P., Van Kerckhove S., Lievens B., Vanhaecke M., Cammue B.P.A., De Coninck B. 2012. Genome-wide characterization of ISR induced in *Arabidopsis thaliana* by *Trichoderma hamatum* T382 against *Botrytis cinerea* infection. Frontiers in Plant Science 3: 1–25. DOI: https://doi.org/10.3389/fpls.2012.00108

Parker C. 2009. Observations on the current status of *Orobanche* and *Striga* problems worldwide. Pest Managment Science 65: 453–459. DOI:10.1002/ps.1713

Parker C. 2013. The parasitic weeds of the Orobanchaceae. p. 313–344. In: "Parasitic Orobanchaceae: Parasitic Mechanisms and Control Strategies". (D.M. Joel, J. Gressel, L.J. Musselman, eds.). Springer, Berlin, Heidelberg.

Schiltz S., Gaillard I., Pawlicki-Jullian N. Thiombiano B., Mesnard F., Gontier E. 2015. A review: what is the spermosphere and how can it be studied? Journal of Applied Microbiol 119: 1467–1481. DOI: https://doi.org/10.1111/jam.12946

Shoresh M., Harman G.E., Mastouri F. 2010 Induced systemic resistance and plant responses to fungal biocontrol agents. Annual Review of Phytopathology 48: 21–43. https://doi.org/10.1146/annurev-phyto-073009-114450

Tyśkiewicz R., Nowak A., Ozimek E., Jaroszuk-´Sciseł J. 2022. Trichoderma: The current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. International Journal of Mole-

cular Science 23: 2329–2357. DOI: https:// doi.org/10.3390/ijms23042329

White T.J., Bruns T., Lee S., Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. p. 315–322. In: "PCR Protocols: A Guide to Methods and Applications" (M.A. Innis, D.H. Gelfand, J.J. Sninsky, T.J. White, eds.). Academic Press, London, UK. DOI: https://doi.org/10.1016/b978-0-12-372180-8.50042-1

Yahia M.A., Hassan M.M., Ali T.E., Rugheim A.M., Osman A.G., Abdelgani M.E., Babiker A.E. 2015. Fungal cultures extract as a bio-control agent for suppression of Phelipanche ramosa germination. International Journal of Farming and Allied Sciences 4 (4): 309–313.

Zaidi N.W., Dar M.H., Singh S., Singh U.S. 2014. *Trichoderma* species as abiotic stress relievers in plants. p. 515–523. In: "Biotechnology and Biology of *Trichoderma*" (V.G. Gupta, M. Schmoll, A. Herrera-Estrella, R.S. Upadhyay, I. Druzhinina, M. Tuohy, eds.). Elsevier, Waltham, USA.

Zhang S., Gan Y., Xu B. 2016. Application of plant-growth--promoting fungi *Trichoderma longibrachiatum* T6 enhances tolerance of wheat to salt stress through improvement of antioxidative defense system and gene expression. Frontiers in Plant Science 7: 1405. DOI: https://doi.org/10.3389/fpls.2016.01405