

ORIGINAL ARTICLE

Effect of *Fusarium* and *Rhizoctonia* isolates on the pre-attachment stage of *Phelipanche aegyptiaca*

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Abstract

Phelipanche aegyptiaca is an obligate root-parasitic plant of the Orobanchaceae family that comprises the most threatening weed for major crops in regions with a Mediterranean climate zone, as well as in Asia, southern and Eastern Europe. The pre-attachment stage is crucial for its successful attachment and parasitism, making this phase a key target for biological control strategies. In this study, the effect of 79 fungal isolates, including 49 *Fusarium* and 30 *Rhizoctonia*, on the pre-attachment stages of *P. aegyptiaca* were investigated. According to the experimental results, the *Fusarium* isolates inhibited the seed germination by the rate of 17.9 to 97.1%, while the disease severity values were between 21.3 and 99.8%. Approximately 40% of the 49 *Fusarium* isolates exhibited high inhibition effects (70–100%) and around 70% of the isolates caused high disease severity (70–100%) on the radicle. A positive correlation was observed between the inhibition rate and disease severity for the *Fusarium* isolates. Moreover, 15 *Fusarium* isolates displayed high effects in both parameters simultaneously. In contrast, the *Rhizoctonia* isolates exhibited seed inhibition rates ranging from 1.2 to 37.6% and disease severity values between 0 and 66.0%, with none showing high effects on any pre-attachment stages. These findings suggest that the *Fusarium* isolates have a great potential to use biocontrol agents against *P. aegyptiaca*, while the *Rhizoctonia* isolates have less, which may be due to differences in their secondary metabolite profiles or other pathogenicity-related traits. These findings pave the way for future research to optimize *Fusarium*-based biocontrol strategies and better understand the broader potential of these fungal isolates in managing *P. aegyptiaca*.

Keywords: biocontrol, Egyptian broomrape, *Fusarium* and *Rhizoctonia* isolates, parasitic weed, *Phelipanche aegyptiaca*

Introduction

Broomrapes (*Orobanche* spp. and *Phelipanche* spp.) are root holoparasitic weeds that threaten major crops in regions with a Mediterranean climate, as well as in Asia, southern and Eastern Europe. Tomato (*Solanum lycopersicum* L.) is particularly susceptible to *Phelipanche aegyptiaca* (Pers.) Pomel, which is known to cause significant damage and yield losses (Joel *et al.* 2007). Approximately 7% of the world's tomato production comes from Turkey, making it the third-largest producer globally, after China and India, with an area of 158 thousand hectares and 13 million tons of

production (FAOSTAT 2022). Depending on host sensitivity, environmental factors, and infestation density, broomrapes cause yield losses between 5 and 100% (Parker 2012). *Phelipanche aegyptiaca* and *P. ramosa* serve as the main limiting factors in both processing and table tomato production in Turkey, with a reported yield loss of 24% attributed to broomrape infestations (Aksoy and Uygur 2008). At maturity, a single plant can release thousands of microscopic seeds, leading to excessive soil seed bank accumulation (Delavault *et al.* 2017). The germination of these obligate parasites

requires the precise recognition of germination stimulants exuded by the host. Broomrapes are extremely sensitive to strigolactones (SLs) secreted by plants into the rhizosphere at picomolar concentrations (Brun *et al.* 2018).

The life cycle of broomrapes consists of two main phases: the pre-attachment (preparasitic) stage and the post-attachment (parasitic) stage. The pre-attachment stage, which involves seed conditioning and germination, is a critical phase, during which the parasitic plant seeds detect and respond to chemical signals emitted by host plant roots (Bouwmeester *et al.* 2003; Cardoso *et al.* 2011). The parasitic stage begins when the broomrape attaches to the host's vascular system through the haustorium, siphoning water and nutrients, which ultimately leads to reduced crop yields (Yoshida *et al.* 2016). After attachment, the parasitic plant forms a tubercle, from which shoots develop. These shoots emerge above the soil, bloom and produce seeds (Rispaill *et al.* 2007). The predominantly underground life cycle of broomrapes, combined with their prolific seed production, long-term soil persistence, and ease of spreading to uninfected areas, makes their management particularly challenging (Goldwasser and Rodenburg 2013).

Various control methods, including cultural, physical, chemical, and biological approaches, as well as the development of resistant varieties, can be employed to manage broomrapes (Fernández-Aparicio *et al.* 2016; Eizenberg and Goldwasser 2018). Among these, the pre-attachment stage is of particular interest, as disrupting this phase can significantly reduce the parasite's ability to establish and develop (Misra *et al.* 2019).

Over the past 70 years (from 1954 to the present), studies conducted across 25 countries on four continents have reported 104 fungal species from 42 genera associated with five main broomrape species, highlighting the extensive diversity of fungi interacting with these parasitic plants (Başbağcı *et al.* 2024). In these studies, *Fusarium* species have been extensively studied due to their ability to produce secondary metabolites with phytotoxic properties, which exhibit herbicidal effects on the various plant species, including parasitic weeds (Xu *et al.* 2021; Macías-Rubalcava and Garrido-Santos 2022). Around 183 phytotoxic fungal secondary metabolites have been identified, classified into five major groups: polyketides, terpenoids, nitrogenous metabolites, phenols, and phenolic acids, originating from 37 fungal genera (Bendejacq-Seychelles *et al.* 2024). Among these, *Fusarium* species stand out for their herbicidal effects against *P. aegyptiaca* and other parasitic weeds (Capasso *et al.* 1996; Dor *et al.* 2007). However, most of these studies focus on the general herbicidal properties of *Fusarium*

metabolites and their effect on the post-attachment stage rather than the specific pre-attachment stage that disrupts parasitic weed germination and radicle elongation. This represents a critical knowledge gap, as the targeted disruption of the pre-attachment stage could provide a more effective and sustainable control strategy.

In contrast to *Fusarium*, the potential of *Rhizoctonia* species as biocontrol agents against parasitic weeds remains largely unexplored. *Rhizoctonia* species are ubiquitous and highly versatile fungi that inhabit a wide range of soil environments (Adams 1996). Many *Rhizoctonia* isolates are known for causing an extensive range of plant diseases, particularly those that affect the root systems of crops (Ajayi-Oyetunde and Bradley 2018; Basbagci *et al.* 2019; Bhuiyan *et al.* 2024). Additionally, some *Rhizoctonia* isolates form beneficial relationships as mycorrhiza with plants (Andersen and Rasmussen 1996). However, certain isolates of these fungi have shown potential for biological control, as they inhibit plant growth. While *Rhizoctonia* isolates are well-documented for their roles in plant diseases and as biocontrol agents against pathogens in various crops (Sneh *et al.* 2013; Ajayi-Oyetunde and Bradley 2018), there is a notable absence of studies investigating their potential against parasitic weeds such as *P. aegyptiaca*. The possibility of these fungi to target and interfere with the early stages of parasitic plant development, including the pre-attachment stage of *P. aegyptiaca*, represents a promising avenue for integrated parasitic weed management.

This study aimed to evaluate the effect of the *Fusarium* and *Rhizoctonia* isolates on seed germination and the radicle of *P. aegyptiaca* seed.

Materials and Methods

Fungal and plant material

The *Fusarium* and *Rhizoctonia* isolates utilized in this study were originally obtained from infected tissues of *P. aegyptiaca* plants collected in the Western Mediterranean Region of Turkey during an earlier study (Basbagci *et al.* 2023). The *P. aegyptiaca* seeds used in the experiments were sourced from a prior study conducted by Cignitas and Kitis (2022).

Preparation of fungal inoculum

In the assays, liquid spore solution was prepared for *Fusarium* isolates according to the protocol of Pitrat *et al.* (1991). The isolates were grown on potato dextrose agar (PDA) at $23 \pm 1^\circ\text{C}$ for 14 days. Then, 4 mm in diameter mycelial discs were taken from the edge of

the cultures and inoculated in 500 ml flasks containing the liquid synthetic media. The flasks were placed on an orbital shaker at 50 rpm and $25 \pm 1^\circ\text{C}$ for 2 weeks. Afterward, the fungal solutions were filtered through Miracloth and diluted to a spore concentration of 1×10^5 conidia $\cdot \text{ml}^{-1}$ using a hemocytometer. For the *Rhizoctonia* isolates, 4 mm in diameter mycelial discs of 7-day-old fungal cultures were used as an inoculum source.

Seed preparation and germination stimulation

Phelipanche aegyptiaca seeds were surface sterilized by sequentially dipping in 1% sodium hypochlorite (NaOCl) and 75% ethanol for 3 min each, followed by six rinses with sterile distilled water (Bai et al. 2020). Approximately 100 seeds were then transferred onto 2% water agar in 60 mm Petri plates, which were sealed with parafilm and incubated in darkness at $21 \pm 1^\circ\text{C}$ for 1 week to pre-condition the seeds. To stimulate germination and radicle elongation, 20 μl of GR24 (10^{-5} M, >99% purity; www.strigolab.eu), a synthetic strigolactone, was applied to each plate after pre-conditioning.

Seed inhibition assay

The seed inhibition assay was designed to determine the effect of the isolates on the seed germination of *P. aegyptiaca*. For the *Fusarium* isolates, 1 ml of the spore solutions were dripped simultaneously with GR24 onto each Petri plate. For the *Rhizoctonia* isolates, the mycelial discs were placed in the center of the plates. The control plates contained 1 ml of sterile distilled water for the *Fusarium* isolates and a sterile PDA disc for the *Rhizoctonia* isolates. All plates were incubated at $21 \pm 1^\circ\text{C}$ for 7 days. Ten replicates were conducted for each treatment and each plate contained ~150 seeds. The seeds which formed radicles were considered as germinated.

The inhibition effects of the isolates on seed germination were evaluated by comparing germinated and non-germinated seeds between the treatments. Germinated seeds were identified as those with visible radicle formation under a stereo microscope (Leica, LAS EZ4) at 20 \times magnification. The germination inhibition rate (IR) was calculated using Abbott formula:

$$IR [\%] = \frac{(A - B)}{A} \times 100,$$

where: A – the percentage of germinated seeds on the control plate, B – the percentage of germinated seeds on the treated plate (Abbott 1925).

Radicle necrosis assays

The radicle necrosis assay was designed to determine the disease severity on the radicles of the germinated seeds. To allow seed germination, the plates containing seeds treated with GR24 were incubated at $21 \pm 1^\circ\text{C}$ for 4 days. Then, 1 ml of spore solutions were dripped onto the seeds for the *Fusarium* isolates, while mycelial discs were placed in the center of the plates for the *Rhizoctonia* isolates. The control plates contained 1 ml of sterile distilled water for the *Fusarium* isolates and a sterile PDA disc for the *Rhizoctonia* isolates. All plates were incubated at $21 \pm 1^\circ\text{C}$ for 12 days. The necrotic area on the radicles was evaluated according to a 0–4 scale (0 = healthy seed, 1 = 1–25% necrosis of radicle, 2 = 26–50% necrosis of radicle, 3 = 51–75% necrosis of radicle, 4 = 76–100% necrosis of radicle) (Cignitas et al. 2024). Ten replicates were conducted for each treatment and 10 seeds were scored for each plate. The percentage of disease severity (DS) was calculated with the Townsend-Heuberger formula:

$$DS [\%] = \sum \frac{(n \times v)}{(Z \times N)} \times 100,$$

where: n – the number of seeds in the disease scale, v – a numerical value of the disease score, Z – the highest score value, and N – the total number of seeds (Townsend-Heuberger 1943).

Statistics and visualization

Statistical analyses were performed in RStudio version 4.2.1 (Core 2015). Visualization was obtained using the ggplot2 package (version 3.5.1) (Wickham 2009). Inhibition rate and disease severity were compared using the Kruskal-Wallis test followed by an arcsine transformation (Breslow 1970). The effects of isolates on seed germination and radicle necrosis were categorized as low (0–40%), medium (40–70%), and high (70–100%). Significant differences between the groups were analyzed using one-way ANOVA at a significance level of $p < 0.05$, followed by Tukey's Honest Significant Difference (HSD) test for pairwise comparisons. Mean values of inhibition rate and disease severity were calculated from raw data, while letters and standard deviations are based on arcsine-transformed data. The relationship between DS and IR values was determined using Pearson's product-moment correlation coefficient, a correlation matrix was visualized using the corrplot package (version 0.95). The significance of correlations was tested at the 0.05, 0.01, and 0.001 levels. Values closer to 1 or –1 indicate strong positive or negative correlations, respectively, while values closer to 0 suggest weak or no correlation.

Results

Effect of the *Fusarium* isolates on *Phelipanche aegyptiaca* seed in vitro

The *Fusarium* isolates significantly affected the pre-attachment stages of *P. aegyptiaca*. The effect of the 49 isolates on seed inhibition varied from 17.9 to 97.1%, with FUS15 showing the lowest inhibition (17.9%) and FUS3 exhibiting the highest inhibition (97.1%). Nineteen isolates exhibited a high inhibition effect on the seed germination, while 24 and 6 of them showed medium and low effects, respectively. According to the radicle necrosis assay, the isolates showed disease severity from 21.3 to 99.8%, with FUS41 showing the

lowest disease severity (21.3%) and FUS25 exhibiting the highest disease severity (99.8%). Thirty-five isolates exhibited high effect, while 10 and four isolates showed medium and low effects on the radicles, respectively (Table 1, Fig. 1).

Based on the 49 *Fusarium* isolates, a positive correlation was observed between the inhibition rate and disease severity ($r = 0.35$, $p < 0.001$). The scatter plot revealed that higher inhibition rates were associated with increased disease severity (Fig. 2).

FUS2, FUS3, FUS7, FUS17, FUS18, FUS21, FUS22, FUS23, FUS25, FUS37, FUS38, FUS42, FUS44, FUS46 and FUS50 isolates exhibited high effects (70-100%) on both inhibition rate and disease severity (Fig. 3).

Table 1. Effect of the *Fusarium* isolates on *Phelipanche aegyptiaca* seed inhibition and disease severity

Isolate	Seed inhibition rate [%]*		Disease severity [%]*	
	Mean \pm SD**	Min–Max**	Mean \pm SD**	Min–Max**
FUS1	38.0 \pm 0.04 j–n	33.3–46.8	65.8 \pm 0.13 c–l	47.5–80.0
FUS2	87.7 \pm 0.11 a–g	76.0–94.7	79.0 \pm 0.27 a–i	57.5–100.0
FUS3	97.1 \pm 0.06 a	91.6–98.9	84.5 \pm 0.08 a–h	77.5–95.0
FUS4	49.9 \pm 0.11 g–n	35.4–65.6	93.0 \pm 0.15 a–f	70.0–100.0
FUS5	55.2 \pm 0.08 f–n	41.6–64.5	92.8 \pm 0.14 a–f	77.5–100.0
FUS6	41.4 \pm 0.08 i–n	28.1–56.2	95.8 \pm 0.15 abc	87.5–100.0
FUS7	76.2 \pm 0.12 a–k	58.3–93.7	79.5 \pm 0.16 a–j	65.0–95.0
FUS8	46.0 \pm 0.10 h–n	29.1–56.2	64.0 \pm 0.14 d–m	47.5–87.5
FUS9	62.8 \pm 0.08 c–m	50.0–76.0	72.8 \pm 0.08 b–l	65.0–82.5
FUS10	58.5 \pm 0.06 e–n	47.9–64.5	82.5 \pm 0.09 a–j	70.0–90.0
FUS11	59.5 \pm 0.06 d–n	48.9–66.6	61.1 \pm 0.07 e–m	47.5–70.0
FUS12	36.5 \pm 0.07 k–n	27.0–45.8	52.6 \pm 0.07 g–m	40.0–60.0
FUS13	41.8 \pm 0.05 i–n	33.3–50.0	89.8 \pm 0.10 a–h	80.0–97.5
FUS14	45.5 \pm 0.06 h–n	36.4–55.2	51.3 \pm 0.10 h–m	32.5–75.0
FUS15	17.9 \pm 0.08 n	10.4–29.1	41.3 \pm 0.06 i–m	30.0–47.5
FUS17	94.3 \pm 0.06 abc	89.5–97.9	81.0 \pm 0.14 a–j	65.0–95.0
FUS18	93.0 \pm 0.02 a–e	90.6–93.7	95.5 \pm 0.08 a–e	92.5–100.0
FUS20	60.7 \pm 0.08 c–m	43.7–68.7	32.3 \pm 0.09 lm	17.5–45.0
FUS21	78.7 \pm 0.09 a–k	62.5–87.5	74.0 \pm 0.13 b–l	60.0–92.5
FUS22	96.1 \pm 0.04 ab	91.6–97.9	94.5 \pm 0.19 a–d	70.0–100.0
FUS23	88.7 \pm 0.07 a–g	81.4–95.3	80.5 \pm 0.14 a–j	67.5–95.0
FUS24	50.8 \pm 0.07 g–n	34.8–59.3	90.0 \pm 0.14 a–g	80.0–100.0
FUS25	79.6 \pm 0.06 a–j	73.2–87.2	99.8 \pm 0.05 a	97.5–100.0
FUS30	55.1 \pm 0.08 f–n	45.3–66.2	72.0 \pm 0.14 b–l	47.5–90.0
FUS31	56.1 \pm 0.06 f–n	47.6–65.1	90.0 \pm 0.06 a–h	85.0–95.0
FUS32	68.1 \pm 0.07 a–l	59.3–74.4	71.3 \pm 0.15 b–l	50.0–92.5
FUS34	59.6 \pm 0.12 c–m	43.0–75.5	66.0 \pm 0.16 c–l	27.5–82.5
FUS35	67.5 \pm 0.06 a–l	59.3–79.0	82.5 \pm 0.15 a–i	60.0–95.0
FUS36	71.7 \pm 0.08 a–l	58.1–82.5	36.0 \pm 0.15 klm	15.0–60.0
FUS37	71.6 \pm 0.09 a–l	58.1–82.5	76.3 \pm 0.22 a–k	32.5–92.5
FUS38	78.2 \pm 0.09 a–k	59.3–86.0	96.8 \pm 0.10 abc	92.5–100.0
FUS40	83.0 \pm 0.04 a–i	77.9–88.3	68.8 \pm 0.22 c–l	30.0–90.0

Table 1. Effect of the *Fusarium* isolates on *Phelipanche aegyptiaca* seed inhibition and disease severity – continued

Isolate	Seed inhibition rate [%]*		Disease severity [%]*	
	Mean ± SD**	Min–Max**	Mean ± SD**	Min–Max**
FUS41	31.2 ± 0.13 lm	19.7–53.4	21.3 ± 0.10 m	10.0–30.0
FUS42	92.2 ± 0.03 a–f	89.5–94.1	98.0 ± 0.11 ab	95.0–100.0
FUS44	94.4 ± 0.03 a–d	93.0–96.5	90.8 ± 0.09 a–h	82.5–97.5
FUS46	70.7 ± 0.11 a–l	46.2–81.7	89.5 ± 0.06 a–h	82.5–95.0
FUS47	43.6 ± 0.18 i–n	11.8–61.2	77.5 ± 0.07 b–k	65.0–87.5
FUS49	64.2 ± 0.11 b–m	46.2–87.1	71.8 ± 0.07 b–l	62.5–82.5
FUS50	78.3 ± 0.09 a–k	66.6–90.3	90.3 ± 0.06 a–h	82.5–92.5
FUS51	57.7 ± 0.14 e–n	29.0–69.8	79.3 ± 0.12 a–k	62.5–92.5
FUS53	84.8 ± 0.03 a–h	80.6–87.1	57.3 ± 0.18 f–m	25.0–85.0
FUS54	55.2 ± 0.14 f–n	24.7–75.2	81.0 ± 0.15 a–j	60.0–97.5
FUS55	75.7 ± 0.10 a–k	62.3–89.2	65.3 ± 0.16 c–m	32.5–82.5
FUS56	57.9 ± 0.15 e–n	37.6–86.0	90.0 ± 0.05 a–h	85.0–92.5
FUS58	53.2 ± 0.20 g–n	22.5–84.9	86.0 ± 0.09 a–h	70.0–92.5
FUS60	22.1 ± 0.09 mn	13.9–35.4	39.8 ± 0.18 j–m	20.0–80.0
FUS62	39.3 ± 0.23 j–n	6.45–63.44	83.3 ± 0.12 a–i	67.5–95.0
FUS63	42.0 ± 0.20 i–n	13.9–67.7	84.5 ± 0.19 a–h	45.0–97.5
FUS69	52.5 ± 0.20 g–n	18.2–74.1	86.5 ± 0.10 a–h	75.0–95.0

*Values are presented as means ± standard deviation (SD) along with the range (Min–Max)

**Different letters within the same column indicate significant differences (Tukey test, $p < 0.05$)

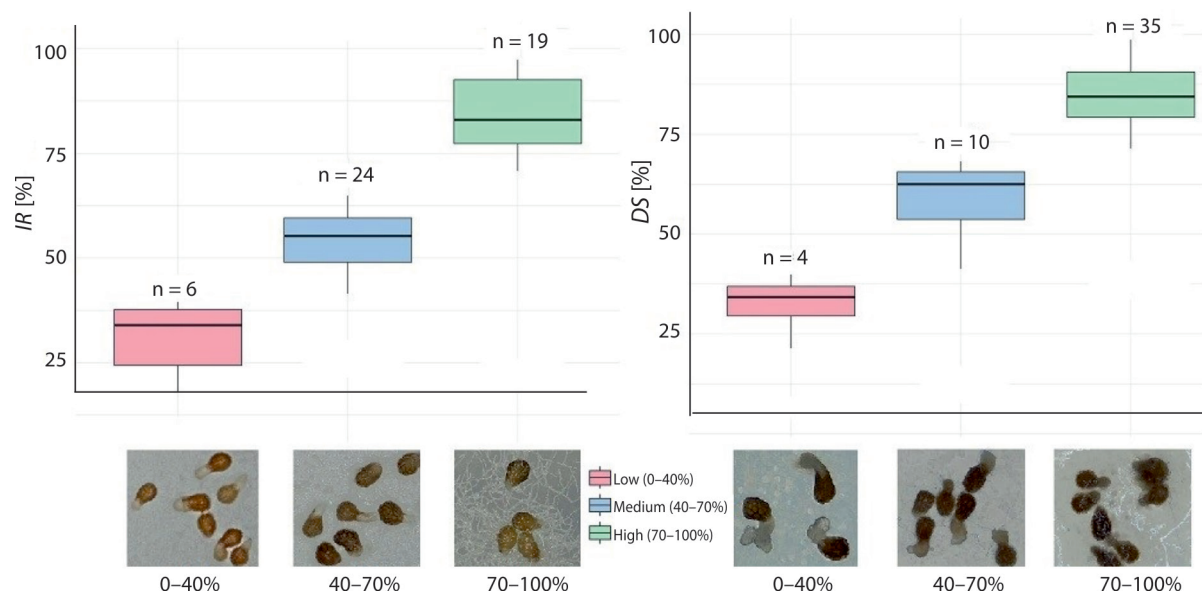


Fig. 1. The box plot analysis of the *Fusarium* isolates on the pre-attachment stage of *Phelipanche aegyptiaca*. The graphs show the inhibition rate (IR, %) and disease severity (DS, %) in low, medium, and high categories, with the number of isolates (n) indicated above the plots. The images represent the microscopic observation of the seeds based on IR and DS categories

Effect of the *Rhizoctonia* isolates on *Phelipanche aegyptiaca* seed in vitro

The effect of the *Rhizoctonia* isolates on pre-attachment stages of *P. aegyptiaca* was limited. Seed

inhibition rate of the isolates was between 1.2 and 37.6%, with RH2 showing the lowest inhibition (1.2%) and RH29 exhibiting the highest inhibition (37.6%). All the *Rhizoctonia* isolates exhibited a low effect on the seed germination. According to the radicle

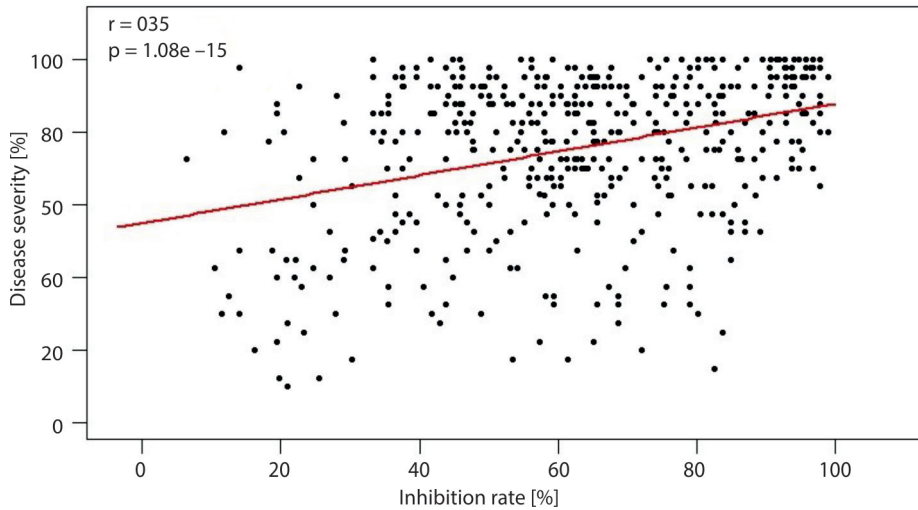


Fig. 2. The correlation between the inhibition rate (*IR*) and disease severity (*DS*) of the *Fusarium* isolates on *Phelipanche aegyptiaca* seed. The red line represents the linear regression fit, degrees of freedom (*df*) = 488

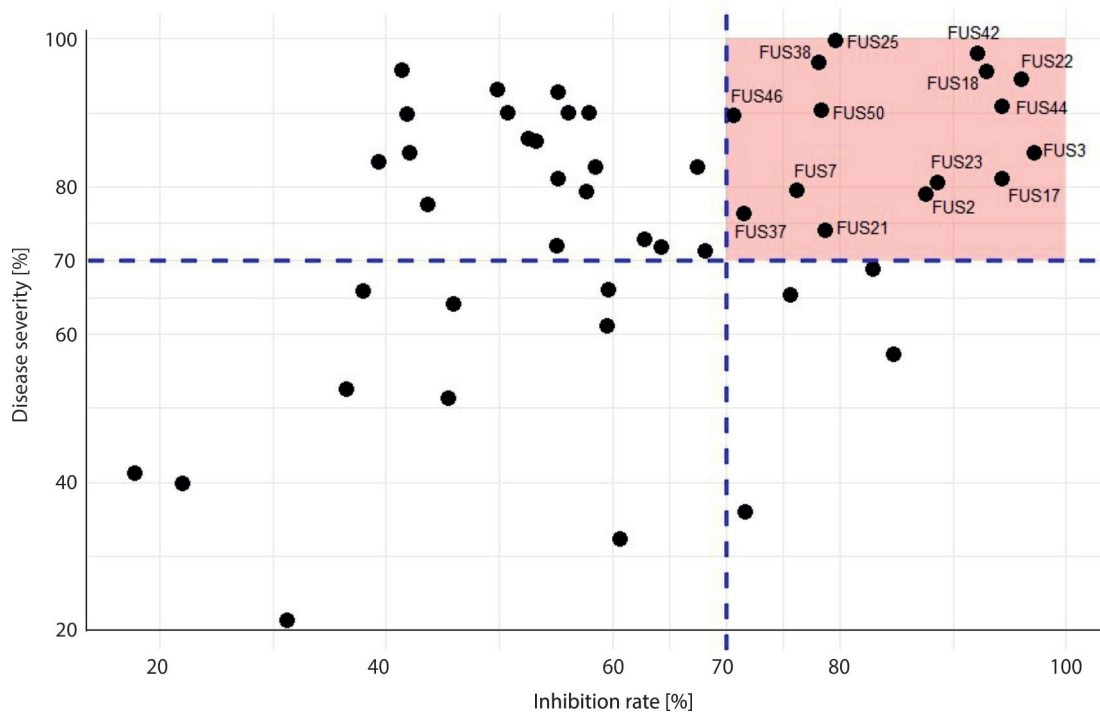


Fig. 3. Inhibition rate (*IR*) versus disease severity (*DS*) of *Fusarium* isolates (mean value of 10 replicates per isolate). The graph displays both parameters simultaneously, with the red-shaded area (70–100%) indicating high effects in both measurements (blue dashed lines mark the 70% threshold). Fifteen isolates fall within this high-effect zone

necrosis assay, the disease severity values of the isolates varied between 0.0 and 66.0%, with RH11, RH12, RH13, RH14, RH15 showing the lowest disease severity (0.0%) and RH30 exhibiting the highest disease

severity (66.0%). Twenty-six isolates exhibited a low effect, while four isolates exhibited a high effect on the radicle (Table 2, Fig. 4).

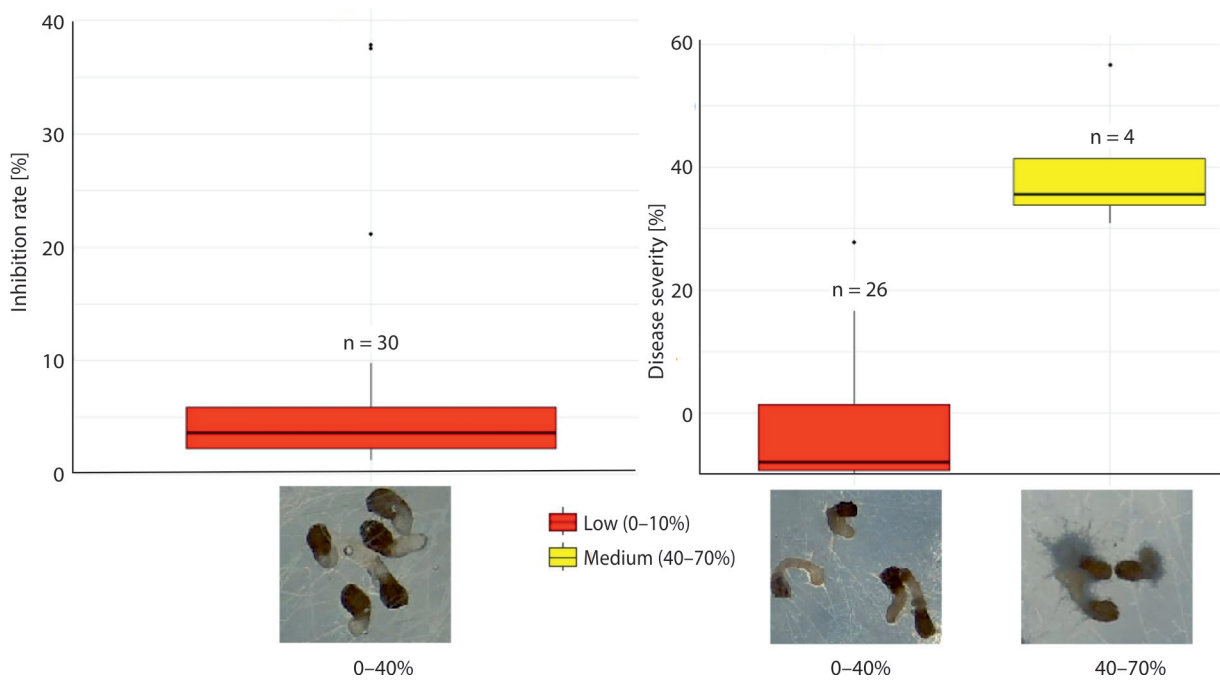


Fig. 4. The box plot analysis of the *Rhizoctonia* isolates on the pre-attachment stage of *Phelipanche aegyptiaca*. The graphs show the inhibition rate (*IR*, %) and disease severity (*DS*, %) in low and medium categories, with the number of isolates (*n*) indicated above the plots. The images represent the microscopic observation of the seeds based on *IR* and *DS* categories

Table 2. Effect of the *Rhizoctonia* isolates on *Phelipanche aegyptiaca* seed inhibition and disease severity

Isolate	Seed inhibition rate [%]*		Disease severity [%]*	
	Mean ± SD**	Min–Max**	Mean ± SD**	Min–Max**
RH1	2.5 ± 0.07 d–g	0.0–6.6	1.2 ± 0.10 kl	0.0–5.0
RH2	1.2 ± 0.07 fg	0.0–3.3	9.7 ± 0.10 f–i	2.5–17.5
RH3	1.4 ± 0.07 fg	0.0–3.3	26.5 ± 0.13 cde	10.0–45.0
RH4	1.6 ± 0.08 fg	0.0–3.3	14.7 ± 0.09 e–h	5.0–25.0
RH5	8.5 ± 0.08 cd	2.2–17.6	46.5 ± 0.04 b	40.0–52.5
RH6	21.1 ± 0.08 b	13.2–36.3	37. ± 0.24 bcd	7.5–67.5
RH7	5.4 ± 0.07 c–g	1.1–11.0	6.7 ± 0.14 h–k	0.0–17.5
RH8	5.9 ± 0.07 c–f	1.1–9.9	8.5 ± 0.15 g–j	0.0–27.5
RH9	4.4 ± 0.06 c–g	1.1–7.7	0.0 ± 0.0 l	0.0–0.0
RH10	1.6 ± 0.11 g	0.0–9.9	0.5 ± 0.07 l	0.0–2.5
RH11	1.6 ± 0.07 fg	0.0–3.3	0.0 ± 0.0 l	0.0–0.0
RH12	2.7 ± 0.08 d–g	0.0–6.6	0.0 ± 0.0 l	0.0–0.0
RH13	3.7 ± 0.10 c–g	0.0–12.1	0.0 ± 0.0 l	0.0–0.0
RH14	3.4 ± 0.06 c–g	1.1–6.6	0.0 ± 0.0 l	0.0–0.0
RH15	7.3 ± 0.08 cde	1.1–12.1	0.0 ± 0.0 l	0.0–0.0
RH16	7.1 ± 0.07 cde	2.2–12.1	21.0 ± 0.21 efg	0.0–40.0
RH17	5.9 ± 0.14 c–g	0.0–14.3	1.0 ± 0.08 kl	0.0–2.5
RH18	2.0 ± 0.09 e–g	0.0–6.6	2.5 ± 0.12 jkl	0.0–10.0
RH19	3.5 ± 0.11 d–g	0.0–9.9	1.2 ± 0.11 l	0.0–7.5
RH20	2.5 ± 0.07 d–g	0.0–6.6	2.7 ± 0.10 i–l	0.0–7.5
RH21	1.6f ± 0.08 g	0.0–4.4	1.0 ± 0.09 kl	0.0–5.0
RH22	2.7 ± 0.09 d–g	0.0–6.6	0.5 ± 0.07 l	0.0–2.5
RH23	2.2 ± 0.08 e–g	0.0–6.6	0.7 ± 0.08 l	0.0–5.0
RH24	9.8 ± 0.07 c	5.5–18.7	11.7 ± 0.11 fgh	2.5–25.0

Table 2. Effect of the *Rhizoctonia* isolates on *Phelipanche aegyptiaca* seed inhibition and disease severity – continued

Isolate	Seed inhibition rate [%]*		Disease severity [%]*	
	Mean ± SD**	Min–Max**	Mean ± SD**	Min–Max**
RH25	4.3 ± 0.09 c–g	0.0–8.8	10.2 ± 0.14 ghi	2.5–30.0
RH26	3.2 ± 0.08 c–g	0.0–6.6	18.0 ± 0.10 efg	5.0–30.0
RH27	4.9 ± 0.06 c–g	1.1–7.7	22.0 ± 0.10 def	10.0–32.5
RH28	4.5 ± 0.07 c–g	1.1–8.8	44.7 ± 0.09 b	30.0–60.0
RH29	37.6 ± 0.18 a	7.7–56.0	40.7 ± 0.10 bc	30.0–57.5
RH30	37.2 ± 0.09 a	25.3–53.8	66.0 ± 0.07 a	52.5–72.5

*Values are presented as means ± standard deviation (SD) along with the range (Min–Max)

**Different letters within the same column indicate significant differences (Tukey test, $p < 0.05$)

Discussion

Obligate root parasitic weeds like *P. aegyptiaca* rely on host-derived signals to initiate germination and establish attachment, making their early life stages a critical target for effective control (Vurro *et al.* 2016). Our study demonstrated that *Fusarium* isolates effectively disrupted the pre-attachment stage of *P. aegyptiaca*, as evidenced by significant inhibition of seed germination and by causing necrosis on the radicle. The inhibition rates and disease severity percentages obtained from *Fusarium* isolates highlight their potential as biological control agents. Previous studies have suggested that *Fusarium* species produce phytotoxic compounds that inhibit parasitic weed growth (Watson 2013). The present findings align with this, particularly the high inhibition rates observed in certain isolates. The pre-attachment stage is a critical process for *P. aegyptiaca* to successfully attach to the host plant and perform parasitism (Misra *et al.* 2019) and thus, targeting this stage with *Fusarium* isolates may offer an effective strategy for parasitic weed control. The findings of this study align with previous research that identified *Fusarium* species as effective fungal agents against broomrape species, particularly in inhibiting seed germination and inducing necrosis (Gibot-Leclerc *et al.* 2022). While this study highlighted the substantial variability among *Fusarium* isolates in inhibiting *P. aegyptiaca* seed germination (17.9–97.1%), it is noteworthy that nearly 40% of the 49 isolates demonstrated a high (70–100%) inhibition effect. The observed variability in the inhibition of *P. aegyptiaca* seed germination between different *Fusarium* isolates (ranging from 17.9 to 97.1%) can be attributed to several factors. Firstly, genetic diversity between *Fusarium* isolates likely plays a significant role. Different isolates may produce varying types and concentrations of secondary metabolites, such as mycotoxins, enzymes, or other bioactive compounds, which can differentially

affect seed germination (Desjardins and Hohn 1997). Similarly, another study reported significant variability between *Fusarium* isolates collected from *P. ramosa*, with nearly 80% of the isolates showing strong inhibition effects (Gibot-Leclerc *et al.* 2022). In a related study, 39 *Fusarium* isolates obtained from infected *Orobancha crenata* and *O. ramosa* exhibited significant variability in their ability to inhibit seed germination, with six isolates showing a higher inhibition rate against these parasitic weeds (Abouzeid and El-Tarabily 2010).

In addition to inhibiting germination, *Fusarium* isolates also demonstrated significant activity against germinated seeds by causing necrosis on radicles, with disease severity values ranging from 21.3 to 99.8%. The prevalence of high disease severity within the *Fusarium* isolates underscores their potential as bioherbicides for managing *P. aegyptiaca*. However, the variability in their effects indicates the need for targeted screening to identify the most effective isolates. This is consistent with earlier research, which has pointed out that necrotic activity is influenced not only by the taxonomic classification but also by intraspecific variation (Gibot-Leclerc *et al.* 2022). Notably, approximately 70% of the *Fusarium* isolates in this study, equivalent to 35 out of 49 isolates, exhibited high disease severity (70–100%) on *P. aegyptiaca* radicles. This highlights the originality of this study, as most previous research has focused on necrosis observed on the post-attachment stage of the broomrape. For instance, a study focusing on the post-attachment stage of *Orobancha ramosa* reported that *Fusarium oxysporum* and *F. solani* isolates significantly reduced the number of broomrape shoots and tubercles attached to host roots (Boari and Vurro 2004). Similarly, *F. verticillioides*, which was isolated from *Orobancha cumana* demonstrated high pathogenicity to *O. aegyptiaca*, *O. ramosa*, and *O. cumana* at the post-attachment stage (Dor *et al.* 2009). In contrast, this study emphasized the significance of targeting the pre-attachment stages of *P. aegyptiaca*, a critical phase where the development of a radicle plays

a crucial role in the parasitism process. By focusing on the pre-attachment stage and measuring disease severity through radicle necrosis, this work showed the importance of intervening during this early developmental stage to enhance biocontrol efficacy. This suggests that *Fusarium* isolates may act through multiple mechanisms, including the production of phytotoxic compounds that disrupt radicle viability. In support of this, previous studies have demonstrated that *Fusarium* species are known to produce a variety of secondary metabolites, such as fusaric acid, fumonisins, beauvericin, enniatin, moniliformin, and trichothecenes, many of which exhibit phytotoxic or herbicidal effects (Desjardins and Hohn 1997). Specifically, *F. oxysporum* produces phytotoxins, including fumonisins (Abbas et al. 1995), fusaric acid (Bacon et al. 1996), and protein toxins (Bailey et al. 2000), which not only display herbicidal activity but also help the fungus overcome host defenses and establish itself within the plant tissue (Ghannam et al. 2007). The necrosis observed in radicles during this study may be attributed to the production of these phytotoxins, which can interfere with cellular processes and damage critical radicle structures.

Based on the analysis of 49 *Fusarium* isolates, a positive correlation was found between the inhibition rate and disease severity. The higher seed inhibition rates are linked to more disease severity on the radicle of *P. aegyptiaca*. Notably, 15 isolates exhibited high effects in both parameters (70–100%), further emphasizing the potential of these isolates. This correlation indicates that the germination inhibition effects of *Fusarium* isolates are associated with their ability to exacerbate disease severity. This finding is consistent with previous studies that highlight a positive relationship between the inhibition of seed germination and the overall efficacy of *Fusarium* isolates in controlling parasitic weed growth (Abouzeid and El-Tarabily 2010). Therefore, these results further substantiate the potential of *Fusarium* isolates as promising biocontrol agents against *P. aegyptiaca*.

In contrast, *Rhizoctonia* isolates showed limited efficacy in the pre-attachment stage of *P. aegyptiaca*. This lack of activity may be attributed to differences in the pathogenic mechanisms of *Rhizoctonia* isolates compared to *Fusarium* isolates. While they are widely known for their roles in plant diseases, their utility as biocontrol agents against parasitic weeds appears to be restricted. The soil-borne pathogen *Rhizoctonia solani* is generally regarded as a necrotrophic pathogen with a broad host range (Zrenner et al. 2020). Several groups of binucleate *Rhizoctonia* spp. have demonstrated effectiveness in biologically controlling diseases caused by *R. solani* (Herr 1995). *Rhizoctonia* have attracted growing interest in recent years as biocontrol agents, due to their ability to compete for nutrients

with pathogens and promote plant resistance against phytopathogens (Maculewicz 2015). *Rhizoctonia*, particularly binucleate isolates, have been shown to enhance plant growth and stimulate resistance against various pathogens (Lemańczyk et al. 2023; Taheri et al. 2024). However, despite the identification of *Rhizoctonia* isolates on broomrape species in countries such as the USA (Duafala et al. 1976), Nepal (Thomas et al. 1999), Jordan (Hameed et al. 2001), Iran (Karam Pur et al. 2004), Israel (Dor and Hershenhorn 2009), France (Gibot-Leclerc et al. 2022) and Turkey (Basbagci et al. 2023), the information regarding their effects on both pre-attachment and post-attachment stages remains limited. Most studies have focused primarily on the post-attachment stages, with little emphasis on the early stages of parasite development, such as seed germination and radicle necrosis. In contrast to many studies that focus on the post-attachment stages of broomrapes, the present study primarily investigated the effects of *Rhizoctonia* isolates on the pre-attachment stage, specifically seed germination and radicle necrosis. Boyette et al. (1991, 1996) studied a strain of *R. solani* as a biocontrol agent for *Orobancha* species. However, it has not been seriously considered for mycoherbicidal applications due to its inability to produce spores. However, unlike the findings of Hameed et al. (2001), who reported that *Rhizoctonia* species inhibited seed germination of *O. ramosa* by 100% and caused 80–90% disease severity in shoots, this study found that all 30 *Rhizoctonia* isolates showed low germination inhibition rates (0–40%), with only four isolates showing moderate effects (40–70%) on radicle necrosis. Another study showed that *R. solani* could significantly affect broomrape emergence, with an 86.96% suppression rate, but it primarily targeted post-attachment processes (Karam Pur et al. 2004). This discrepancy suggests that while *Rhizoctonia* may have potential as a biocontrol agent, its effectiveness in controlling broomrape during the early stages, particularly pre-attachment, remains limited. Similar to this study, Gibot-Leclerc et al. (2022) determined that *R. solani* and *Rhizoctonia* sp. AG-A isolates did not inhibit the seed germination of *P. ramosa*, showing only 13 and 8% inhibition, respectively. This aligns with the present findings, where the *Rhizoctonia* isolates tested also exhibited limited inhibition on seed germination of *P. aegyptiaca*, further suggesting that the inhibition effects of *Rhizoctonia* isolates on broomrape germination may be relatively less, and that other factors or stages of development might play a more significant role in parasitic suppression.

The pathogenic nature of the *Fusarium* and *Rhizoctonia* species raises concerns about their potential impact on non-target crops. Some strains of these fungi are known to cause diseases in economically important

crops, such as root rot, wilt, and damping-off. To mitigate these risks, it is crucial to select non-pathogenic or host-specific strains for bioherbicide development. Additionally, rigorous host-specificity testing and environmental risk assessments should be conducted before field application.

Conclusions

This study underlined the effectiveness of *Fusarium* isolates in disrupting the pre-attachment stage of *P. aegyptiaca* as biocontrol agents. However, to fully evaluate their application in controlling this parasitic weed, it is essential to identify the species of the *Fusarium* isolates and conduct further greenhouse and field trials to assess their efficacy. While the *Rhizoctonia* isolates showed limited effects, particularly on seed germination, their role in biological control requires further exploration. The differences between *Fusarium* and *Rhizoctonia* isolates suggest that species-specific factors, such as secondary metabolite production and pathogenicity mechanisms, may influence their effectiveness. These findings pave the way for future research to optimize *Fusarium*-based biocontrol strategies and better understand the broader potential of these fungal isolates in managing *P. aegyptiaca*.

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