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First report of *aspergillus sydowii* ASP17 as a promising biological control agent against soil-borne fungal and fungal-like plant pathogens: A laboratory study

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ABSTRACT- This study aimed to investigate the antagonistic properties of the *Aspergillus sydowii* ASP17 isolate against soil-borne fungal and like-fungal pathogens including *Rhizoctonia solani*, *Phytophthora nicotianae*, and *Pythium aphanidermatum* under in vitro experiments. The results showed that *A. sydowii* ASP17 inhibited 57.4% growth of *R. solani*, 45.4% of *P. nicotianae*, and 53.3% of *P. aphanidermatum* in a dual culture test. In the second experiment, *A. sydowii* ASP17 showed inhibition rates of 72.7% for *R. solani*, 45.9% for *Ph. nicotianae*, and 56% for *P. aphanidermatum*. The highest efficacy in the dual culture test was observed against *R. solani*, but in the volatile organic compounds test, the highest efficacy was against *P. aphanidermatum*. The ASP17 isolate acted by antibiosis by forming inhibition zones caused by the antifungal substances without contact with the pathogen. This study also investigated the production of volatile organic compounds, hydrogen cyanide, and the cell wall-degrading enzymes protease and lipase, which may be related to the biocontrol activity of the isolate. The production of hydrogen cyanide was also examined in the *A. sydowii* ASP17 isolate. These data could support the potential use of *A. sydowii* ASP17 as a biocontrol agent in agriculture, especially in environments where the use of chemical fungicides is undesirable.

INTRODUCTION

Soil-borne pathogens such as *Pythium* spp., *Phytophthora* spp. and *Rhizoctonia* spp. are responsible for major agricultural issues related to soil-borne diseases. These organisms are capable of greatly reducing crop yield. These pathogens inhabit the soil environment, where they can survive for years in organic matter, plant debris, or resistant structures like spores and sclerotia (Katan, 2017). In extensive agricultural systems, a variety of factors can increase the spread of pathogens and the severity of soil-borne diseases, including continuous cropping and excessive tillage (Katan, 2017; Katan, 2000). Species of the fungus-like genera *Pythium* and *Phytophthora* are known to cause root rot and damping-off diseases, especially in seedlings (Erwin and Ribeiro, 1996). *Rhizoctonia* spp. are known to cause stem canker and damping-off in young and mature plants (Baker, 1974). Managing soil-borne diseases is a complex process and must be approached in an integrated manner. This includes crop rotation and resistance cultivars, using soil health management practices that reduce pathogen populations and increase plant resilience (Katan, 2017; Katan, 2000). Once the biology and ecology of such pathogens are properly understood, effective strategies can be planned to mitigate their impacts on crop production. The high prevalence of soil-borne fungal and fungal-like pathogens, including *Pythium*, *Phytophthora*, and *Rhizoctonia* species, represents a major problem in global agriculture. This leads to enormous crop losses and food insecurity. Chemical control procedures have been employed thus far. However, the use of chemicals is associated with concerns about environmental sustainability and the development of resistant pathogen isolates. As a result, interest in biological control strategies, particularly fungal antagonists such as *Trichoderma* spp., has increased significantly, as they have an incredible potential to suppress these pathogens through various mechanisms. The use of biological control agents is emerging as a promising strategy for managing soil-borne fungal diseases, apart from cultural practices (Köhl et al., 2011). Biological control agents are living organisms such as bacteria and fungi that can suppress or inhibit the growth and development of plant pathogens (Pal and Gardener, 2006). The effectiveness of biological control agents against diseases can be further enhanced by combining them with other techniques, such as resistant cultivars and soil amendments (Larkin and Fravel, 1998). However, successful biological control requires a good understanding of the interactions among the biological control agent, pathogen, plant, and the environmental factors that influence its performance. Testing fungi in vitro against different soil-borne diseases is one of the most important stages of effective biological control. To assess the antagonistic properties of various microorganisms against phytopathogens such as *Pythium* spp., *Phytophthora* spp., *Rhizoctonia* spp., and *Fusarium* spp., the test was performed in vitro. In vitro assays usually involve dual culture techniques on agar plates, where potential biocontrol agents are grown along with target soil-borne pathogens. This

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technique allows us to observe the interactions between fungi in terms of competition for nutrients, space, and the production of inhibitory compounds (D'Ambrosio et al., 2022). For example, certain *Trichoderma* species effectively suppress the growth of *Fusarium* spp. and *Rhizoctonia* spp. through the production of antifungal metabolites and competition with these pathogens (Thambugala et al., 2020). Laboratory screening of effective biological control agents in controlled experiments is necessary before conducting actual field trials. However, it should be remembered that results obtained in vitro cannot always be directly extrapolated to field conditions because of complex interactions in natural ecosystems (Fisher et al., 2012). Although in vitro tests remain an important tool in the development of biocontrol strategies, they must be combined with greenhouse and field studies for validation in real-life scenarios. The genus *Aspergillus* is widely distributed and common in different environments as it is cosmopolitan (Samson et al., 2014). Several species of the genus *Aspergillus* are commercially used due to their ability to synthesize and secrete a wide range of enzymes, antibiotics, and other metabolites, including mycotoxins (Volke-Sepulveda et al., 2016). *A. sydowii* (Bainier & Sartory) Thom & Church is a common saprotrophic fungus in the soil (Rypien and Andras, 2008). The soil isolates of this species are generally considered non-pathogenic. However, several studies reported the involvement of marine isolates in epizootic diseases in sea fans (Alker et al., 2001; Marfenina et al., 2013; Yarden, 2014). Some isolates of the fungus *Aspergillus*, mostly belonging to the section *Versicolores*, represent opportunistic pathogens in humans and animals (Siqueira et al., 2016). The ecologically versatile *A. sydowii* is a well-known species that grows in various habitats, including decaying organic matter, and is known for its agricultural benefits. Recent studies have shown that it can solubilize phosphorus (an important plant growth-promoting nutrient), thereby increasing its availability and uptake in crops such as maize (Baron et al., 2018). The biocontrol potential of *A. sydowii* against some important soil-borne diseases, such as those caused by *Pythium*, *Phytophthora*, and *Rhizoctonia* spp., has been poorly studied. Considering the economic significance of these pathogens and capacity of *A. sydowii* to generate diverse enzymes and metabolites, there appears to be ample justification for exploring the potential of this fungus in biological control.

MATERIALS AND METHODS

Fungal isolates

Gharin (2023) isolated *Aspergillus sydowii* from arugula (*Eruca sativa* Mill.) vermicompost. The isolate was identified using internal transcribed spacer (ITS) region sequencing and *Aspergillus* morphological keys (Raper and Fennel, 1965; Klich and Pitt, 1988). Furthermore, a study conducted by Gharin (2023) revealed that *A. sydowii* ASP17 has promising potential for promoting plant growth. Because of its favorable characteristics, the ASP17 isolate was deemed suitable for additional research in the field of biocontrol. The isolates *Rhizoctonia solani* J.G. Kühn, *Phytophthora nicotianae* Breda de Haan, and *Pythium aphanidermatum* (Edson) Fitzp used in this study are part of the Fungal Collection of the Department of Plant Protection, School of Agriculture, Shiraz University, Shiraz, Iran. The isolate of *Trichoderma harzianum* used in this study was obtained from the market under the brand name Trichomix® and served as a positive control for the experiments.

Dual culture test

All fungal isolates used in this study were from preserved cultures on potato dextrose agar (PDA) plates and incubated at 28 °C in the dark for five days. Mycelial plugs with a diameter of five mm were taken from the periphery of the colonies to serve as inocula for the experiments. ASP17 was tested against each pathogenic fungus. In each 9-cm Petri dish containing PDA, a mycelial plug of isolate ASP17 was placed on one side of the Petri dish, and the plug of the pathogenic fungus was placed on the opposite side. The control experiment was performed by placing a mycelium plug of each pathogen on one side of a Petri dish and a block of PDA medium on the other side (Campanile et al., 2007). A combination of the *A. sydowii* isolate and pathogenic fungi was prepared using three replicates in two experiments. All plates were kept in the dark at 28 °C and were observed daily. Observations were performed when the growth of the pathogenic fungus ceased in the dual cultures or when the colony spread to cover the entire plate in the control setup. The width of the inhibition zone and the radii of colonies for both fungi were measured in the opposite direction. The interaction between the isolate and the pathogens was evaluated in vitro according to the method described by Mejía et al. (2008). Colony growth inhibition rates for pathogenic fungi were calculated using the formula (CGIR = (RCK (Radius of the control colony) − Rtest (Radius of pathogen colony)) / RCK) × 100) given by Landum et al., 2016.

Bioassay for ASP17 volatile organic compounds (VOCs) against plant pathogens

The bioactivity of ASP17 volatile organic compounds against plant pathogens was assessed in two experiments by evaluating the growth of the test organisms using a dual Petri dish culture method (Dennis and Webster, 1971). In this bioassay method, two 9-cm Petri dishes without lids were placed in opposite directions and sealed with two layers of parafilm. A Petri dish containing PDA was placed on top of this dual culture and inoculated with a 5-mm plug of the pathogenic fungi. The lower dish contained a 4-day-old culture of isolate ASP17 with a colony diameter of 5 cm. The quantification of VOCs was performed in triplicate, and a dual culture system without ASP17 isolate served as a negative control for each pathogen. The cultures were incubated in the dark at 28 °C. Measurements of the growth of the test organisms, including colony morphology, mycelial growth, and inhibition of mycelial growth were taken 5-7 days post-inoculation (DPI) for both fungi and oomycetes (Li et al., 2015).

Secretion of cell wall-degrading enzymes and their capacity to produce hydrogen cyanide

The study of the different types of cell wall-degrading enzymes, including protease and lipase, produced by ASP17

isolates was performed using an agar plate assay using a specific substrate medium described by Deb and Dutta (2021). For the protease production study, a skim milk agar medium was used consisting of 28 g of skim milk powder, 5 g of casein enzyme hydrolysate, 2.5 g of yeast extract, 1 g of dextrose, and 15 g of agar, all dissolved in 1 L distilled water, resulting in a final pH of 7.0 ± 0.2 at a temperature of 25 ± 2 °C. The formation of a transparent halo around the colony indicates positive casein lysis activity. Lipase production was evaluated using a hydrolysis medium containing Tween-80, which consisted of 10 g of peptone, 5 g of sodium chloride, 0.1 g of calcium chloride dihydrate, and 20 g of agar dissolved in 1 L distilled water. A 1% Tween-80 concentration served as the exclusive lipid substrate, and the analysis was based on the observation of visible precipitate formation around the colonies.

Hydrogen cyanide production was assessed using the method described by Kremer and Souissi (2001). Isolate ASP17 was cultured on potato extract culture medium supplemented with agar and 5 g glycine per liter. One day after culturing the isolates, Whatman filter paper No. 1 treated with 2% sodium carbonate in 0.05% picric acid was placed on the lid of the Petri dish and sealed with parafilm. The culture was incubated at a temperature of 28 ± 2 °C. The color change of the filter paper from yellow to orange-brown served as an indicator of hydrogen cyanide production.

Data analysis

The data obtained were statistically analyzed using SAS 9.4. The GLM procedure in SAS was applied to test for significant differences among the groups using variance analysis. In the case of significant differences, the Tukey post hoc test was used at the 0.01 significance level to perform specific group comparisons.

RESULTS AND DISCUSSION

Dual culture test

The results of the variance analysis (Table 1) showed that the main factors, ASP17 and *T. harzianum*, had significant effects on the dual culture test.

The results of an in vitro antagonist test against soil-borne diseases indicated that isolate *A. sydowii* ASP17 inhibited *Rhizoctonia solani* by 57.4%, *Phytophthora nicotianae* by 45.4%, and *Pythium aphanidermatum* by 53.3%. In the second experiment, *A. sydowii* ASP17 inhibited *R. solani* (72.7%), *Ph. nicotianae* (45.4%), and *P. aphanidermatum* (56%). The ASP17 isolate moderately inhibited all three pathogens, and its highest efficacy was against *R. solani* at 57.4% and 72.7% in both experiments (Table 2). However, its performance against *P. nicotianae* was significantly lower, indicating that although it can be used to manage diseases caused by certain pathogens, it may not be suitable for all pathogens. In contrast, *T. harzianum* exhibited higher inhibition rates against *R. solani* (83%), *Ph. nicotianae* (74.1%), and *P. aphanidermatum* (80%). In the second experiment, *T. harzianum* exhibited higher inhibition rates against *R. solani* (80.5%), *Ph. nicotianae* (62.2%), and *P. aphanidermatum* (78%). Although both antagonists show potential, *T. harzianum* more efficiently controlled soil-borne pathogens than the other antagonist (Table 2).

Table 1. Mean square of source of variation in the first and second experiments in the dual culture test

Source of variation	df	*Rhizoctonia solani*	*Phytophthora nicotianae*	*Pythium aphanidermatum*
First experiment				
Fungi	2	5420**	4188**	4978**
Error	6	24.2	21.2	3.11
CV (%)	-	10.5	11.5	3.96
Second experiment				
Fungi	2	5920**	3122**	4852**
Error	6	4.3	5.5	10.6
CV (%)	-	4.06	6.51	7.31

** 0.01 significance level

Table 2. The inhibitory effects of the *Aspergillus sydowii* ASP17 isolate on three pathogenic fungi compared with *Trichoderma harzianum*

Treatment	*Rhizoctonia solani*	*Phytophthora nicotianae*	*Pythium aphanidermatum*
First experiment			
Control	0	0	0
ASP17	57.4^{b}	45.4^{b}	53.3^{b}
T. harzianum	83.0^{a}	74.1^{a}	80.0^{a}
SE	2.84	2.65	1.01
Second experiment			
Control	0^{c}	0^{c}	0^{c}
ASP17	72.7^{b}	45.9^{b}	56.0^{b}
T. harzianum	80.5^{a}	62.2^{a}	78.0^{a}
SE	1.19	1.35	1.88

The antagonistic activity of the ASP17 isolate against the three pathogenic fungi was classified as shown in Fig. 1: Antibiosis: The colony of the pathogenic fungus stopped growing without the colonies on both sides meeting each other and formed an inhibition band between the two colonies due to some antifungal substances produced by the ASP17 isolate. *T. harzianum* as a positive control showed different mechanisms of antagonism against the pathogenic fungi as follows: mycoparasitism: the growth of *T. harzianum* on the colony of *P. aphanidermatum* and *R. solani* resulted in partial or complete coverage of the colony of the pathogenic fungus; competition for substrate: the growth of both colonies of *T. harzianum* and *P. nicotianae* came to a stop after contact and ended in a stalemate (Fig. 1).

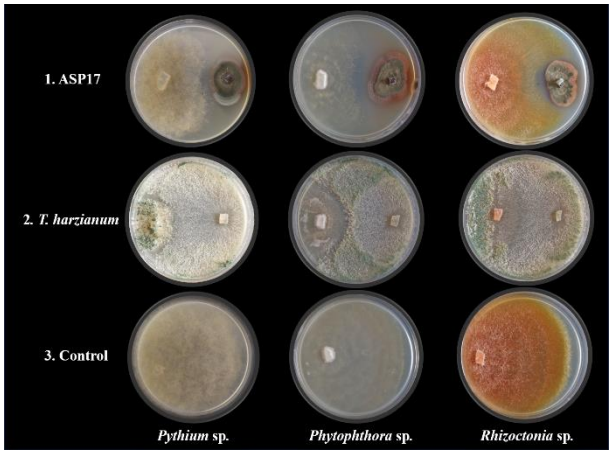

Fig. 1. Inhibitory spectrum of *Aspergillus sydowii* ASP17. first row: co-cultures of ASP17 and plant pathogens; second row: positive control of *Trichoderma harzianum* and plant pathogens; third row: plant pathogens used as control.

These results indicate that the primary mode of action of the ASP17 isolate against pathogenic fungi is through antibiosis. The production of antifungal substances completely inhibited pathogenic fungi growth, stopping their growth without physical contact. This is supported by previous studies showing that antifungal compounds can significantly reduce the growth rates of pathogenic fungi, further supporting the potential of the ASP17 isolate for integrated pest management strategies (Li et al., 2015). In contrast, *T. harzianum* directly exploits mycoparasitism by physically invading and covering the fungal colonies of *P. aphanidermatum* and *R. solani* to ensure that no growth occurs. In addition, competition for the substrate is used against *Ph. nicotianae*; when both fungi meet, a stalemate in growth occurs. Due to these multiple effects, *T. harzianum* can adapt its mechanisms to the pathogenic threat, making it a biocontrol agent (Poveda, 2021). Overall, the contrasting strategies of ASP17 isolate and *T. harzianum* emphasize the importance of understanding the ecological interactions between beneficial and pathogenic fungi. These findings can contribute to the development of integrated pest management strategies that utilize natural antagonistic relationships to improve the sustainability of agriculture. Research has shown that certain representatives of the genus *Aspergillus*, such as *A. candidus* Link and *A. montenegroi* Y. Horie, Miyaji & Nishim, have high antifungal activity against many phytopathogens, including *Fusarium* spp. and *Phytophthora* spp. They produce a wide variety of secondary metabolites that include compounds with antibacterial or general antimicrobial activities, including sphaeropsidin and formosusin A, which have already been shown to be very effective against tomato late blight and wheat leaf rust, respectively (Ngo et al., 2021). In vitro tests have demonstrated that these compounds can reduce the development of pathogens, which opens enormous potential for the use of *Aspergillus* spp. as biocontrol agents in agriculture. Several species, including *A. chevalieri* (L. Mangin) Thom & Church and *A. egyptiacus* Moub. & Moustafa, protect crops from fungal attacks. These species are not only able to suppress the growth of pathogens, such as *Alternaria* sp., but they can also promote plant growth by producing hydrogen cyanide, indole-3-acetic acid, and siderophore, provide nutrients and induce defense mechanisms in plants (Daigham et al., 2023). Among the biological control agents that have been studied for their effectiveness against soil-borne fungi, *Trichoderma* species is one of the best known; it includes filamentous fungi that efficiently colonize plant roots and outcompete pathogens for food and space (Harman et al., 2004). *Trichoderma* species have been shown to produce antifungal compounds, parasitize other fungi, and induce systemic resistance in plants, which has made these species a versatile tool in controlling a wide spectrum of diseases caused by *Pythium* spp., *Phytophthora* spp., *Rhizoctonia* spp., and *Fusarium* spp. (Howell, 2003; Mukherjee et al., 2013).

Bioassay for ASP17 VOCs against plant pathogens

The results of the variance analysis (Table 3) showed that the main factors, ASP17 and *T. harzianum*, had significant effects on bioassay for ASP17 VOCs against plant pathogens.

The maximum inhibition of *Rhizoctonia solani* and *Pythium aphanidermatum* was achieved in the treatment with isolate ASP17: 29.1% and 32.2%, respectively (Fig. 2). For the second time, the maximum inhibition of R. *solani* and *P. aphanidermatum* was achieved in the treatment with isolate ASP17: 29.5% and 37.8%, respectively (Table 4). It could be interpreted that the volatile compounds produced by this isolate have some bioactive properties responsible for the suppression of these pathogens, making it a potential candidate for application of the biological control strategy in agriculture. The isolate ASP17 showed no inhibition of *Ph. nicotianae* (Fig. 2). This means some of those pathogens could be susceptible to the volatile compounds, while others may not be affected, demonstrating specificity.

Table 3. The mean square of the source of variation for the first and second experiments in the bioassay for *Aspergillus sydowii* ASP17 volatile organic compounds against plant pathogens

Source of variation	df	*Rhizoctonia solani*	*Pythium aphanidermatum*
First experiment			
Fungi	1	1270**	1552**
Error	4	0.105	0.541
CV (%)	-	2.22	4.57
Second experiment			
Fungi	1	1305**	2143**
Error	4	0.45	1.81
CV (%)	-	4.57	7.12

** 0.01 significance level

Table 4. The inhibitory effect of the *Aspergillus sydowii* ASP17 isolate with volatile compounds on three plant pathogens

Treatment	*Rhizoctonia solani*	*Phytophthora nicotianae*	*Pythium aphanidermatum*
First experiment: Inhibition with volatile compounds (%)			
Control	0^{b}	0	0^{b}
ASP17	29.1 ± 0.18^{a}	0	$32.2\ a \pm 0.42^{a}$
Second experiment			
Control	0^{b}	0	0^{b}
ASP17	29.5 ± 0.39^{a}	0	$37.8\ a \pm 0.77^{a}$

There was no inhibition against *Ph. nicotianae* (Table 4). This observation raises important questions concerning the specificity and efficacy of the volatile compounds produced by *A. sydowii*. This may be related to the specificity of biochemical pathways and/or resistance mechanisms specific to a particular pathogen. It is important to understand such interactions when developing effective biocontrol measures, as not all pathogens respond similarly to the same biocontrol agent. The results also highlight the need for further investigation into the mechanisms underlying the bioactivity of *A. sydowii* volatile compounds. Identification of the major compounds responsible for the inhibitory effect could help in the development of more targeted and effective biopesticides. In addition, studies on the synergistic effects of *A. sydowii* in combination with other biocontrol agents could increase efficacy against a wider spectrum of pathogens. Inhibition of soil-borne pathogens by fungal VOCs has already been shown. As these volatiles diffuse in the soil and interact with plant roots, they could mediate plant responses and increase resistance to pathogens (Razo-Belmán et al., 2023).

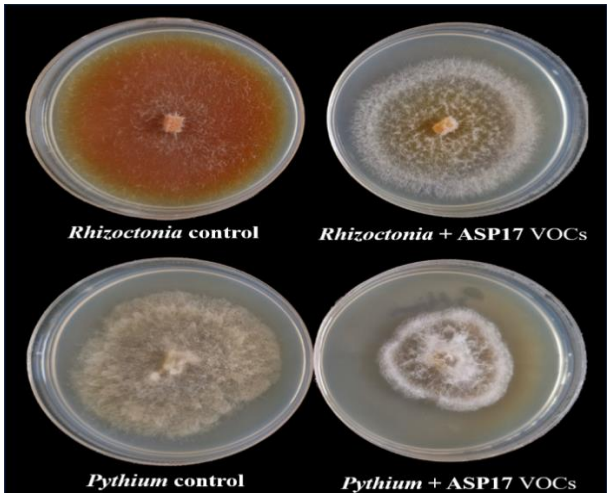

Fig. 2. The inhibitory effect of *Aspergillus sydowii* ASP17 volatile organic compounds on plant pathogens.

This specificity underlines the importance of understanding which VOCs are effective against pathogens and how they can be applied in agricultural practices. The interactions of fungal VOCs with plants occur via various signaling pathways. Plants have evolved mechanisms to sense these volatiles, which can trigger defense responses and alter metabolic pathways to increase resistance to fungal infections (Wonglom et al., 2020). For example, it has been reported that the VOCs released from the biofertilizer fungus *Trichoderma* species trigger a priming effect in plants against pathogen defense genes (Esparza-Reynoso et al., 2021; Sarkar and Sadhukhan, 2023). Fungal VOCs are very promising, and such applications in agriculture are only evident in developing environmentally friendly biocontrol methods. In addition, technological development in controlled release through microencapsulation could make the application of VOCs in agriculture more realistic (Razo-Belmán et al., 2023). Overcoming these challenges will open new avenues in the fight against soil-borne diseases and further lead to more sustainable agricultural practices.

Cell wall-degrading enzyme secretion and the capacity to produce hydrogen cyanide

In the present study, the ASP17 isolate was shown to be able to produce protease and lipase enzymes, as indicated by the halo formation around the colony due to the substrate dissolution in the culture plate test (Fig. 3). In addition, the incubation time was related to this secretion: the highest protease secretion was reached on the 5th day, while the maximum lipase secretion was reached on day 3. The ASP17 isolate was proved to secrete both protease and lipase, which probably makes it a biocontrol agent. Protease can degrade the cell wall proteins of the pathogenic fungi, ultimately leading to the

destabilization of the cell wall with subsequent lysis (Schild et al., 2011; Jashni et al., 2015). Similarly, lipases can hydrolyze the lipids in the fungal cell membranes, helping to disrupt the integrity of the fungus (Kumar et al., 2022). This fact further enhances the potential efficacy of the ASP17 isolate as a biocontrol agent, as it has a dual mechanism of action against the targeted soil-borne pathogens. The ASP17 isolate thus holds enormous ecological significance. In natural soil ecosystems, competition between microbial populations is intense. The ability of the ASP17 isolate to grow on organic substrates is due to the secretion of proteolytic and lipolytic enzymes, which can potentially place it as a competitor against pathogenic fungi (Whipps, 2001). By degrading organic matter and acting against pathogenic fungi, this ASP17 isolate can create conditions for a healthy microbiome in the soil. In turn, this would promote the health and resilience of plants.

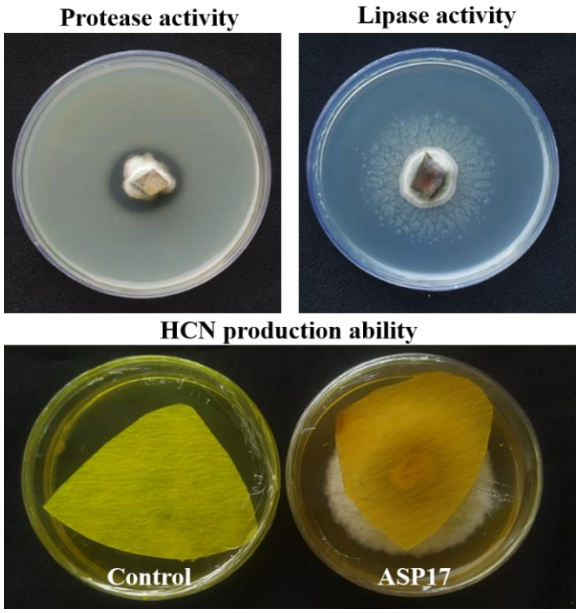

Fig. 3. Cell wall-degrading enzyme secretion and the capacity to produce hydrogen cyanide in *Aspergillus sydowii* ASP17.

Our results on the production capacity of hydrogen cyanide showed that ASP17 is capable of producing hydrogen cyanide. This fact is evidenced by a significant color change from yellow to orange-brown of the Whatman No. 1 filter paper, which previously served as a clear indicator to produce hydrogen cyanide (Fig. 3). This agrees with the method proposed by Kremer and Souissi (2001) describing glycine supplementation as effective in promoting cyanogenesis in cultures of the bacterium. It is known that hydrogen cyanide is involved in important functions in plant-pathogen interactions and can act in plant defense against microorganisms competing with it (Baker et al., 2020). This means that this ability would enhance the competitiveness of the ASP17 isolate in different ecological niches, mainly in environments containing other microorganisms.

CONCLUSION

The results of the present study provide some useful information on the antagonistic potential of the *A. sydowii* ASP17 isolate against the tested soil-borne pathogens. According to the in vitro antagonist tests, *A. sydowii* ASP17 demonstrated moderate inhibition rates against *Rhizoctonia solani* (57.4%), *Phytophthora nicotianae* (45.4%), and *Pythium aphanidermatum* (53.3%). In the second experiment, *A. sydowii* ASP17 inhibited *R. solani* by 72.7%, *Ph. nicotianae* by 45.9%, and *P. aphanidermatum* by 56%. The highest efficacy in the dual culture test was observed against *R. solani*, but in the volatile compounds test, the highest efficacy was against *P. aphanidermatum*. Although the isolate *A. sydowii* ASP17 has some potential for disease management, its efficacy differs among tested pathogens and shows little effect on the growth of *Ph. nicotianae*. The isolate ASP17 was shown to inhibit the growth of pathogenic fungi by antibiosis, as evidenced by the inhibitory bands formed due to the antifungal substances. This finding provided the first clue that *A. sydowii* ASP17 could be a potential candidate for biocontrol in agriculture, as it produced some volatile compounds with remarkable inhibition of *R. solani* and *P. aphanidermatum*. However, the lack of inhibition against *Ph. nicotianae* alone raises questions about the specificity and efficacy of these compounds. This specificity could be due to the specific biochemical pathways and mechanisms of different pathogens resistance. This would suggest the need for an appropriate biocontrol measure against each pathogen. The ability of the ASP17 isolate to produce protease and lipase enzymes, as well as hydrogen cyanide, is further evidence that it can be used as a biocontrol agent. This research is the first study on the effect of *A. sydowii* against soil-borne pathogens under laboratory conditions. These results show the importance of understanding the interactions between biocontrol agents and pathogens to develop an effective strategy for the management of soil-borne pathogens. Further research should be carried out to explain the mode of action of these fungal isolates and to investigate their application in sustainable agriculture.

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CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Conceptualization: Reza Gharin and Maryam Mirtalebi; Methodology: Reza Gharin and Maryam Mirtalebi; Software: Reza Gharin; Validation: Reza Gharin, Maryam Mirtalebi, and Akbar Karegar; Formal analysis: Reza Gharin; Investigation: Reza Gharin; Resources: Reza Gharin; Data curation: Reza Gharin; Writing—original draft preparation: Reza Gharin; Writing—review and editing: Reza Gharin, Maryam Mirtalebi, and Akbar Karegar; Visualization: Reza Gharin and Maryam Mirtalebi; Supervision: Maryam Mirtalebi and Akbar Karegar; Funding acquisition: Akbar Karegar.

DECLARATION OF COMPETING INTEREST

The authors declare no conflict of interest.

ETHICAL STATEMENT

All authors are aware on the content of the manuscript and consented to submit it to the *Iran Agricultural Research* Journal. We did not send this article to another journal. An ethics statement is not applicable sincee this study is based exclusively on published literature.

DATA AVAILABILITY

The raw data of this research are available at the request of the reviewers and editors.

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