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Research article

Amylolytic and antibacterial activity of filamentous fungi isolated from the rhizosphere of different plants grown in the Tamanghasset region

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ABSTRACT

In the present study, we were interested in studying the amylolytic and antibacterial activity of some filamentous fungi isolated from the rhizosphere of cultivated plants in Tamanghasset region. Consequently, 11 pure strains belonging to the different fungal genera were isolated *Alternaria, Aspergillus, Cladosporium, Curvularia, Fusarium, Mucor* and *Penicillium*. Positive result of amylolytic activity was revealed on all the isolated strains, with important hydrolysis zones of 54.33 ± 1.15 mm, 54.00 ± 3.61 mm, 52.00 ± 6.08 mm and 51.33 ± 15.01 mm for *Aspergillus* sp.1, *Curvularia* sp., *Fusarium* sp.2 and *Mucor* sp. respectively. In addition, analysis of variance (*ANOVA*) of the means of hydrolysis zones diameters shows that the values linked by the same letter do not show any significant difference at $P < 0.05$. Antibacterial activity of the isolated fungal was demonstrated by the agar cylinder technique against four pathogenic bacterial strains. The results showed a variability of the inhibition zones, thus the most important results were recorded against *S. aureus*, *E. coli* and *K. pneumonia* for all fungi which produced inhibition zones ranging from 15.33 ± 0.00 to 23.66 ± 1.71 mm. while all isolate had the lowest inhibition zone against *P. aeruginosa*. In conclusion, the obtained results indicated the isolated filamentous fungi have the potential to inhibit the four pathogenic bacterial strains, *S. aureus, E. coli, K. pneumonia* and *P. aeruginosa*, while simultaneously showed significant amylolytic activity.

1. Introduction

A large number of microorganisms such as bacteria, viruses, fungi and protozoans inhabit the rhizosphere, so it is considered as one of the richest narrow regions of soil abutting the roots where microorganisms are stimulated by their activities. Indeed, since antiquity, humans have used microorganisms for the preparation of traditionally fermented products [1,2]. Filamentous fungi are important actors in the microbial world. Moreover, with the development and evolution of research methods, the exploitation of filamentous fungi has undoubtedly become an indispensable tool for the living, due to their considerable importance in the production of a large number of bioactive substances namely: antibiotics, enzymes, organic acids and alkaloids. These substances are economically very

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important [3,4] considering their uses in many industrial fields such as: production of fermented foods, therapeutic substances, biological fertilizers, etc [5,6].

For this purpose, particular attention to bioactive substances isolated from filamentous fungi has been the subject of several studies [7,8]. As an example among the molecules synthesized by fungi we find the amylases, responsible for the hydrolysis of starch into simple sugars (glucose and dextrin) [9,10]. In fact, the first enzyme industrially produced was an amylase of fungal origin in 1894 [11]. Currently, these amylases are used in several sectors (food, detergents, textiles, etc.) [12,13].

Moreover, most of the pharmaceutical industries have concentrated their research on controlling the phenomenon of bacterial resistance to antibiotics and the search for new antibacterial substances synthesized by fungi [14,15]. Indeed, a very large number of antibacterial metabolites have been approved as drugs, such as cephalosporins and fusidic acid, these metabolites are identified in many species of fungi around the world [16]. However, in recent decades antibiotic resistance has been widely recognized as one of the main global health problems, that is responsible for more than 700,000 deaths per year [17–19].

Algeria is one of the largest North African countries, with very diverse ecosystems (marine, desert, mountain, steppe …), indeniably, each ecosystem characterized by a remarkable microbial flora. In light of this, this study aimed to examine the efficacy of certain fungal strains, isolated from the rhizosphere of cultivated plants in Tamanghasset region, southern Algeria, in inhibiting the growth of four pathogenic bacterial strains, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* and to screen them for the amylase production. This work will help to reveal if fungi producing bioactive molecules are present in the soil and could be exploited by the pharmaceutical or food industry.

2. Material and methods

2.1. Soil samples collection and isolation of fungi

Assessment of the amylolytic and antibacterial activity of some filamentous fungi, were performed *in vitro* at the Biology Department, Faculty of Science and Technology, Tamanghasset University, Algeria.

Soil samples were collected in sterile polythene bag under aseptic conditions from 2 agricultural regions in the wilaya of Tamanghasset, in southern Algeria, and then immediately transported to the Laboratory, where they were analyzed. For each sample, 1 g of soil was introduced into a test tube containing 9 mL of sterile distilled water. After then, the mixture was agitated for 3 min, and serial of decimal dilutions of each sample was prepared ranging from 10^{-1} to 10^{-5} [20]. Then, 0.1 mL of each sample was spread on Petri dishes previously poured with potato dextrose agar medium (PDA) [21,22].

A second isolation technique consists in dispersing a very small quantity of each soil sample (5 mg) directly on the surface of the PDA medium (supplemented with gentamicin 40 mg/mL) by disinfected hands, to ensure rapid growth of fungal colonies [23,24]. All these dishes were incubated for 2–7 days at 27 °C. At the end of the incubation period, the developing fungi that appeared in the plate were then isolated in a new plate to be purified, and each single colony was stored on Synthetischies Nahrstoffarmer Agar (SNA) slants at 4 °C [6].

The identification and characterization of the selected fungal strains were realized at the genus level, based on macroscopic and microscopic characters, following [25–28].

2.2. Study of amylolytic activity

Study of amylolytic activity of the isolated fungal strains was carried out on the PDA culture medium supplemented with 1% soluble starch [$(C_6H_{10}O_5)_n$; GRM3029-500 g, India] according to the method described by Refs. [29,30]. Indeed, fungal discs for each strain were prepared, then, a single disc was placed in the center of a Petri dish containing the previous medium. After, these dishes were incubated for 3–5 days at 30 °C.

After incubation, the dishes were flooded with Gram's iodine for 1 min, and then they were rinsed with distilled water three times to eliminate traces of iodine. Amylolytic activity was determined by the formation of a clear zone around the fungal growth colony, indicates hydrolysis of starch by enzyme producing strain, on the other hand, absence of this zone was revealed by the presence of blue color around the growth colony indicates negative result. Experiments were run in triplicate for each fungal strain and the zone of amylolytic activity was measured for each colony. The negative control group was plain agar disc only [[29–31]].

2.3. Screening the isolated fungi for antibacterial activity

Four test bacterial strains were used for this study to examine the potency of the isolated fungi for antibacterial activity. Three Gram-negative bacteria (*E. coli, P. aeruginosa* and *K. pneumonia*) and one Gram-positive bacteria (*S. aureus*), originally isolated from the internal bacteriology laboratory of the Mesbah Baghdad hospital in Tamanghasset, Algeria.

The antagonism test consists in researching the biological activity of fungal strains obtained against these pathogenic bacteria. This test was performed using the agar cylinder technique described by Ref. [33]. Each bacterium was seeded on nutrient agar to obtain young culture. Then bacterial suspensions were prepared to produce a suspension of about 10^6 CFU mL^{-1} and inoculate 1 mL of each suspension on the Mueller-Hinton Agar (MHA) medium then, fungal discs were taken using a sterile loop and placed in the inoculated dishes, so experiments were run in triplicate for each fungal strain. The dishes were incubated at 37 °C for 24–48 h [34].

The antibacterial activity was revealed after incubation by the formation of an inhibitory zone, which was seen around the fungal discs and the results were presented as mean values of the diameters of the three measurements calculating using a ruler in millimetres

(mm) [5,35].

2.4. Data collection analysis

All data collected from laboratory experiments were analyzed by JMP SAS Pro software (JMP®, Version<15>.

3. Results and discussion

3.1. Isolation and identification of fungi

In the present study, we have performed preliminary the isolation of filamentous fungi from collected soil samples. Different fungi were obtained and purified from the samples (Fig. 1), these fungi were represented by 11 purified strains belonging to the genera *Alternaria* sp., *Aspergillus* sp., *Cladosporium* sp., *Curvularia* sp., *Fusarium* sp., *Mucor* sp. and *Penicillium* sp. Indeed, according to Ref. [36], the diversity of strains developed on culture media is generally due to the richness of soil by different microorganisms. However, for maximum fungal growth, PDA agar is considered a more favorable medium [4,37].

Isolated pure cultures of fungal were further cultured on Petri dishes on PDA medium and left to grow for 5–7 days to get maturation stage. At this stage, microscopic and macroscopic characteristics were observed for morphological identification.

Microscopic characteristics such as hyphal color and structures, shape and size of conidia and conidiophores, whereas macroscopic evaluation involves observing texture, reverse and color of colony, they were described in detail in Table 1, according to Refs. [38,39]. These 11 isolated fungi being morphologically distinct, they present a variation in the color and of the colony including whitish, olive green, green, black, Grey, white with pink etc.

Regarding the structures, such as conidiophore, conidia and hyphae they were different according to genera.

3.2. Study of amylolytic activity

After 5 days of incubation, all strains revealed a positive result, with the appearance of clear zones (clear halo) around each fungal colony (Fig. 2), this indicates that the strains tested produce amylase responsible for the hydrolysis of starch according to Ref. [30].

The values represented in Table 2 shown important amylolytic activity in strains *Aspergillus* sp.1, *Curvularia* sp., *Fusarium* sp.2 and *Mucor* sp. expressed by the diameters (average 54.33 ± 1.15 mm, 54.00 ± 3.61 mm, 52.00 ± 6.08 mm, 51.33 ± 15.01 mm) respectively. Moreover, these values were linked by the same letter so don't present a significant difference at $P < 0.05$, therefore the amylolytic activity in these strains was similar or very near. The lowest was observed in the *Alternarai* sp.1 strain with an average of 18.67 ± 1.53 mm. These results were in accordance with those published by Refs. [32,40,41] who showed amylolytic activities between 22 and 38 mm of diameter in different species of *Aspergillus, Penicillium, Fusarium, Mucor, Alternaria, Cladosporium* and *Curvularia.*

Further, a zone of hydrolysis of more than 50 mm was formed around of 4 fungal colonies isolated from soil samples from collected different sites of Jalandhar, Punjab, India [13].

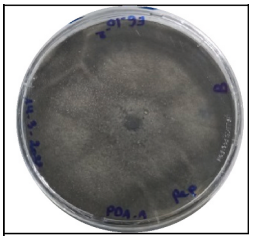
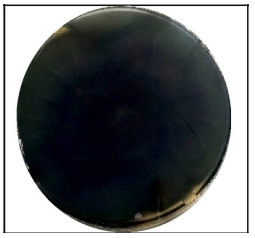
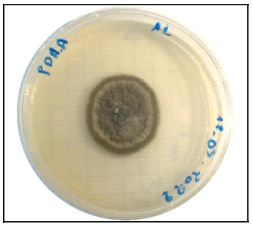
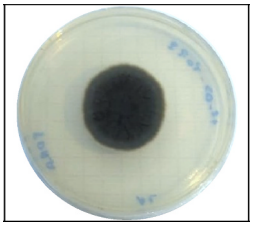
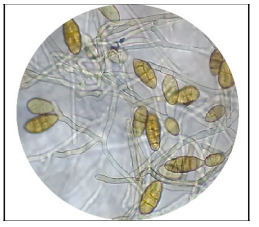
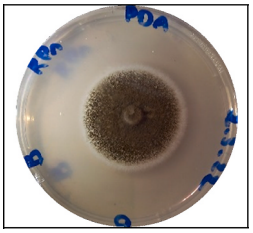
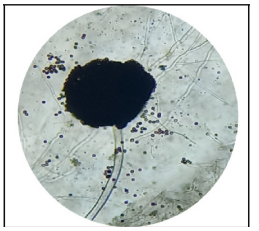
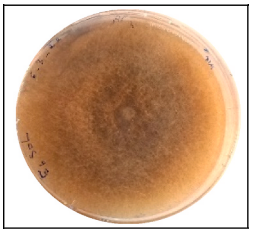
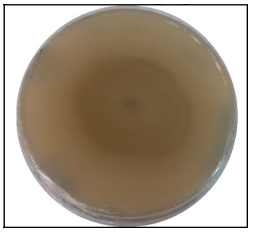
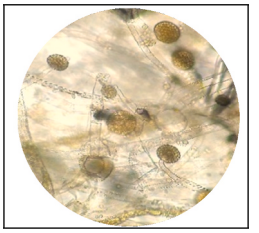
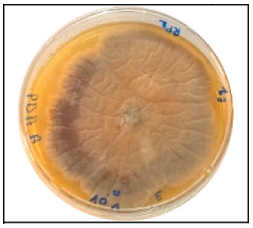
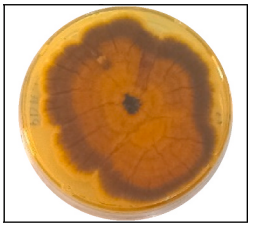
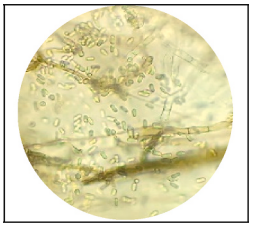
In addition, several authors showed that the majority of *Aspergillus*, *Trichoderma* and *Penicillium* species isolated from agricultural soils have the ability to produce amylases with variable activity depending on the growth conditions and the type of substrate used [11, 36,42].

3.3. Screening the isolated fungi for antibacterial activity

The results of this test showed the appearance of transparent halos around the different discs of the fungi tested, these are the zones of inhibition, corresponding to the fungal antagonistic action over the tested bacteria (Fig. 3). This means that these fungal strains produced bioactive compounds with antibacterial activities against the bacterial strains tested as indicated in Ref. [43]. According to previous research, filamentous fungi isolates have antimicrobial activity [44]. In addition, the production of bioactive substances, such

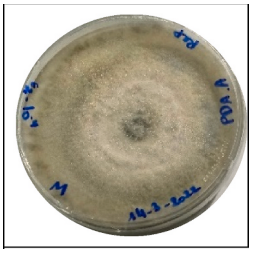
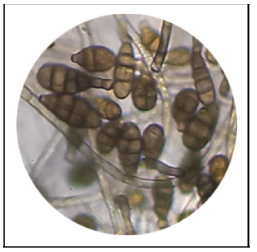
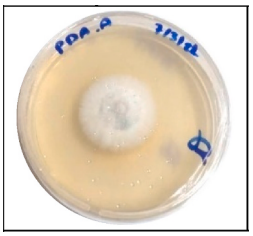
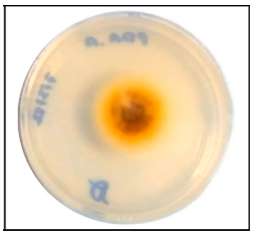
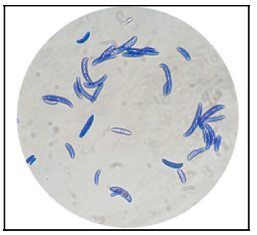
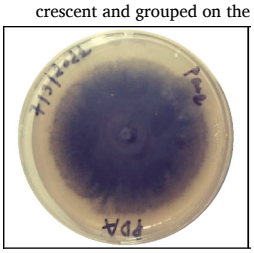
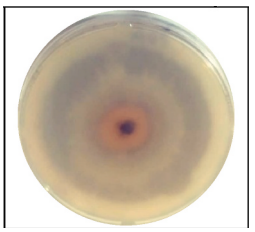
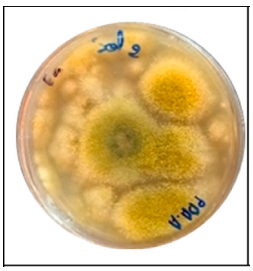
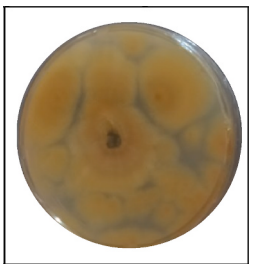
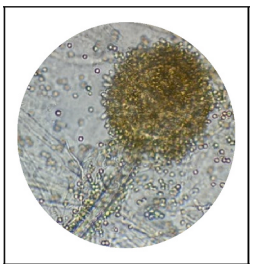
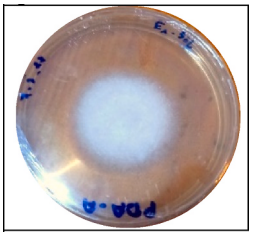
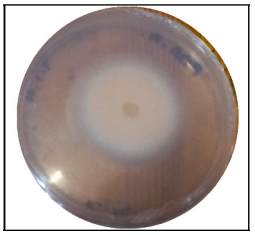
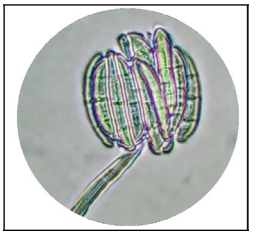
Fig. 1. Isolation of fungi from soil samples on PDA medium.

Table 1
Macroscopic, microscopic characteristics and isolated genus names.

Macroscopic aspect		Microscopic aspect G x40	Identified Genera	References
Recto	Verso			
			*Curvularia* sp.	[28,49]
Macroscopic: Color: black**; Texture:** downy**; Border:** regular**; Reverse of colony:** dark brown to black. **Microscopic: Hypha:** branched and septate; **Conidiophores:** simple and septate**; Conidia:** phragmospores, multicellular.				
			*Alternerai* sp.2	[28,50,51]
Macroscopic: Color: olive green; **Texture**: cottony; **Boder**: regular; **Reverse of colony**: Blacky with a clear colored margin. **Microscopic: Hypha**: Brown septate; **Conidiophores:** septate; **Conidia**: golden brown, arranged in chains with transversal and longitudinal septate.				
			*Aspergillus niger*	[49,52,53]
Macroscopic: Color: Black with a clear margin**; Texture:** granulous**; Boder:** regular**; Reverse of colony:** clear yellow **Microscopic: Hypha:** large and septate; **Conidiophores:** Very long, not septed**; Conidia:** are small globulose grouped in a black conidial head; presence of a large globose vesicles.				
			*Mucor* sp.	[49,54]
Macroscopic: Color: gris; **Texture**: important aerial development; **Bodre**: regular; **Reverse of colony**: griy with a clear margin **Microscopic**: **Hypha:** no septate; **Conidia:** round; **Sporangiophores** are brownish not branched; and sporangium are Brown in color and spherical in shape				
			*Cladosporium* sp.	[28,55,56]
Macroscopic: Color: bleached brunette**; Texture:** velvety**; Bodre:** irregular**; Reverse of colony:** clear brown center with a dark brown margin**.** **Microscopic: Hypha**: septate; **Conidiophores**: branched and elongated; **Conidia:** small, oval and grouped in a chain.				

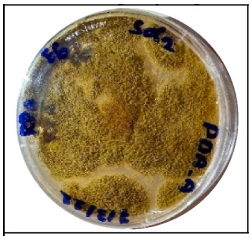
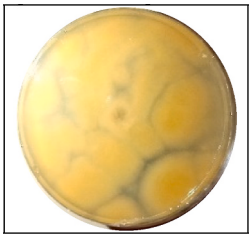
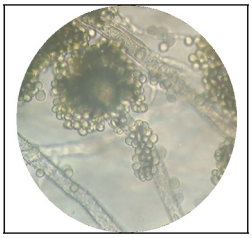
(*continued on next page*)

Table 1 (*continued*)

Macroscopic aspect		Microscopic aspect G x40	Identified Genera	References
Recto	Verso			
			*Alternerai* sp.1	[50,51,57]
Macroscopic: Color: clear green with a white center**; Texture:** cottony**; Border:** regular**; Reverse of colony:** Brown center with a clear margin. **Microscopic: Hypha**: brown with septate; **Conidiophores:** brown, septeted; **Conidia**: are large brown in color with both longitudinal and transverse septa, arranged in chains and a beak at the apical extremity (conical narrowing)				
			*Fusarium* sp.2	[26,58,59]
Macroscopic: Color: blanchâtre**; Texture:** cottony; **Border:** regular; **Reverse of colony**: yellow. **Microscopic: Hypha:** septed**; Conidiophores:** simple**; Conidia:** presence of abundant microconidia, uni- or bicellular and macroconidia in the form of a crescent and grouped on the phialides.				
			*Penicillium* sp.	[28,53,60]
Macroscopic: Color: clear green**; Texture:** powdery**; Border:** regular**; Reverse of colony**: White center with a clear green margin. **Microscopic: Hypha:** septed**; Conidiophores:** branched**; Conidia:** round in chains forming a brush.				
			*Aspergillus* sp.1	[52,53]
Macroscopic: Color: yellow green**; Texture:** cottony**; Border:** irregular**; Reverse of colony:** yellow **Microscopic: Hypha:** septed and et branched**; Conidiophores:** simple not septed with smooth-walled, ending with globose vesicles**; Conidia:** Globulose				
			*Fusarium* sp.1	[58–60]
Macroscopic: Color: whitish**; Texture:** cottony**; Border:** regular**; Reverse of colony:** clear pink **Microscopic: Hypha:** septed**; Conidiophores:** simple and branched**; Conidia:** abundant microconidia, fusiform, septate with 3–4 septa grouped on phialides and presence of somme microconidia uni- or bicellular. Inhibition				

(*continued on next page*)

Table 1 (*continued*)

Macroscopic aspect		Microscopic aspect G x40	Identified Genera	References
Recto	Verso			
			*Aspergillus* sp.2	[52,54,61]

Macroscopic: Color: yellow green**; Texture:** granular**; Border:** irregular**; Reverse of colony:** golden yellow.
Microscopic: Hypha: septed**; Conidiophores:** longs, not septed**; Conidia:** Globulose grouped in a conidial head, presence of small hemispherical vesicles and phialides

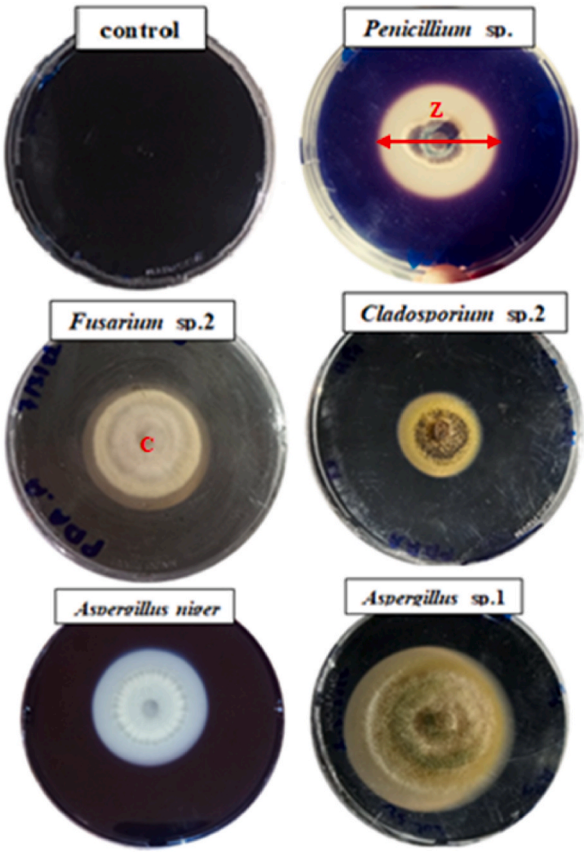


Fig. 2. Results of amylolytic activity of some tested fungi on PAD medium supplemented with 1% starch: Z = hydrolysis zone; C = fungal colony.

as bactericides, fungicides and organic acids by several species of *Aspergillus* was reported by Refs. [34,45].

According to Table 3, all the fungi tested have a remarkable zone of inhibition against the four bacterial strains with variable diameters. While *Fusarium* sp.1 shows no inhibition of the growth of these strains with null diameter.

Significant values of zones of inhibition and effective in eliminating *S. aureus, E. coli* and *K. pneumonia* were observed in all isolated fungi with diameters values ranged between 15.33 ± 0.00 and 23.66 ± 1.71 mm.

Whereas, for *P. aeruginosa* the lowest inhibition zones recorded in all strains tested with diameters ranging from 09.00 ± 0.00 to12.71 ± 0.87 mm. Similar results were found by Ref. [46]. For *P. aeruginosa*. These results also showed that the Gram-negative bacterium (*P. aeruginosa*). In contrast, antibacterial activities of fungi vary from 6.00 to 15.33 ± 3.32 mm were recorded by Ref. [47] against several pathogenic bacterial strains such as *S. aureus*. In addition, the work of [48] showed an important antibacterial activity of 10 species of the genus *Aspergillus* isolated from soil samples against *S. aureus* with the diameter of inhibition zones ranged

Table 2
Comparison of the mean diameters of the starch hydrolysis zones, according to the Student test.

Fungal genera	Diameter of starch hydrolysis zones (mm)
Alternaria sp1.	18.67 ± 1.53 f
Alternaria sp2.	25.67 ± 1.15 ef
Aspergillus niger	37.00 ± 2.00 bcd
Aspergillus sp1.	54.33 ± 1.15 a
Aspergillus sp2.	40.00 ± 0.00 bc
Cladosporium sp.	29.00 ± 5.29 def
Curvularia sp.	54.00 ± 3.61 a
Fusarium sp1.	47.00 ± 8.54 ab
Fusarium sp2.	52.00 ± 6.08 a
Mucor sp.	51.33 ± 15.01 a
Penicillium sp.	33.33 ± 5.77 cde

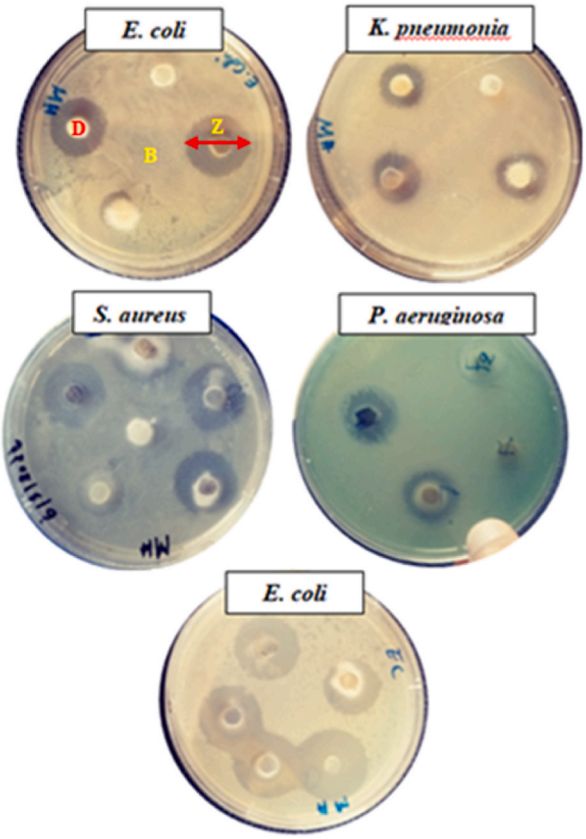


Fig. 3. Antibacterial activity of selected fungi on the growth of the four pathogenic bacteria: Z. Inhibition zone; B. Bacterial mat; D. fungal disc.

Table 3
Antibacterial activity of selected fungi on the growth of the four pathogenic bacteria.

Fungi tested	Inhibition zone (mm)			
	S. aureus	*E. coli*	K. pneumonia	*P. aeruginosa*
***Alternerai* sp.2**	20.66 ± 0.4 a	19.00 ± 0.00a	16.00 ± 0.00b	11.66 ± 5.18de
***Curvularia* sp.**	19.33 ± 3.53a	17.77 ± 0.55b	20.66 ± 0.4 a	12.00 ± 0.38de
Aspergillus niger	20.33 ± 0.77 a	21.00 ± 0.51 a	15.33 ± 0.00c	09.33 ± 3.29f
***Mucor* sp.**	15.33 ± 0.00c	11.33 ± 0.67de	11.33 ± 0.67de	11.66 ± 5.18de
***Cladosporium* sp.**	20.00 ± 0.0 a	19.80 ± 1.15a	15.53 ± 1.53c	12.00 ± 0.38de
***Fusarium* sp.1**	00.00 ± 0.00 g	00.00 ± 0.00 g	00.00 ± 0.00 g	00.00 ± 0.00 g
***Alternerai* sp.1**	22.88 ± 0.00 a	23.66 ± 1.71 a	19.00 ± 2.00a	12.71 ± 0.87de
***Fusarium* sp.2**	16.57 ± 1.61b	16.50 ± 3.71b	15.66 ± 0.00c	09.00 ± 0.00f

For each parameter, the values linked by the same letter show no significant difference at $P < 0.05$.

from 13.00 ± 2.00 to 31.67 ± 1.53 mm.

4. Conclusion

Based on the research carried out, it can be concluded that the fungi isolated from the rhizosphere show antibacterial avtivity against *S. aureus, E. coli, K. pneumonia* and *P. aeruginosa* and important amylolytic activity. Therefore, the bioactive substances of these fungi should be subjected to further research regarding their possible use in the pharmaceutical and food industry.

Declarations

Author contribution statement

SIDAOUI Abouamama: designed the experiments; wrote the manuscript. Bertella Anis: contributed materials and analysis of data. SEMMADI Abir: performed the experiments. HEMDI Maroua: performed the experiments. BAALI Sirine: analyzed and interpreted the data.

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Data availability statement

No data was used for the research described in the article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at [URL].

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