Antibacterial and antioxidant activities of endophytic fungi and nettle (Urtica dioica L.) leaves as their host

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Abstract: Nettle (Urtica dioica L.), as a plant rich in biologically active compounds, is one of the most important plants used in herbal medicine. Studies have shown that this plant has antioxidant, antiplatelet, hypoglycemic and hypocholesterolemic effects. In this study, we characterized three Alternaria endophytic fungi isolated from their host U. dioica. We hypothesized that these endophytic fungi can produce new bioactive metabolites, which may possess the bioactive property with potential application in the medical and pharmaceutical industries. The antibacterial activity was evaluated against reference and isolated strains, including Methicillin-Resistant Staphylococcus aureus. A wide range of antimicrobial activities similar to those measured in nettle leaves was detected especially for Alternaria sorghi. Furthermore, the highest antioxidant activity detected with DPPH free radical scavenging was measured for A. sorghi and nettle leaves ethyl acetate extracts. In addition, whereas catalase activity was similar in the three isolated fungi and nettle leaves, total thiol content and superoxide dismutase activity were significantly higher in leaves. A. sorghi showed the best activities compared to other isolated fungi. The characterization and further production of bioactive compounds produced by this endophyte should be investigated to fight bacteria and especially those that develop drug multi-resistance.

Key words: Antibacterial activity; Endophytic Alternaria species; Oxidative stress; Superoxide dismutase; Thiols.

Introduction

The emergence of antibiotic multi-resistant bacteria and new diseases for which modern medicine remains powerless led to a renewed interest in traditional medicine based on a solid and ancient knowledge of medicinal plants and their potential hosts. Indeed, medicinal plants produce thousands of molecules from secondary metabolism with therapeutic potential. Moreover, these plants often contain in their tissues or organs so-called endophytic organisms that offer their host resistance to biotic and abiotic stresses (1, 2). Furthermore, these endophytic fungi are known to promote the growth of the host plant and help to defend itself against pathogens (2, 3). Thus, these fungi are able to produce various molecules with medical and pharmaceutical great interests such as antioxidant, anticancer, antiviral, immunomodulatory or molecules against tuberculosis or plant or animal parasites (1, 2, 4). Moreover, endophytic fungi were also shown to increase the activity of ROS detoxification enzymes such as catalase (CAT), superoxide dismutase (SOD) and peroxidases (POD), which promote the host plant's resistance to various stresses such as drought and soil salinity (5, 6).

Our study was focused on the world's widespread and medicinal Nettle (Urtica dioica L.) that also shelters endophytic fungi. To our knowledge, this is the first study of its kind to have studied both the biological activities of Urtica dioica leaves and endophytic colonizing fungi. This study aims to evaluate the antibacterial and antioxidant activity, the content of phenolic compounds and total thiols as well as the activity of ROS detoxifying enzymes (CAT and SOD).

Materials and Methods

All chemicals were purchased from Sigma-Aldrich (Germany), Biochem Chemopharma (Quebec), Bio-Red (France), Liofilchem (Italy) or Biokar (France).

Plant material and leaf extract preparation

Nettle (Urtica dioica L.) was identified and collected from Larba Nath Irathen (Tizi-Ouzou, Algeria). Fresh leaves were immediately washed with water. The leaves were shade-dried at 40°C for two to three days and powdered. Then, 20g powder was mixed with 200 mL of water or ethyl acetate for 24h. The extracts were filtered on Whatman No.1 paper. The aqueous filtrate was frozen and lyophilized at a pressure of 5 mmHg at -50°C. The ethyl acetate filtrate was concentrated using a rotary vacuum evaporator at reduced pressure at 40°C. The nettle leaves extracts were then stored at -20°C until used.

Isolation of endophytic fungi

Healthy leaves were carefully selected for the isola-
tion of endophytic fungi. Leaves samples were washed thoroughly to remove adhered dust and debris. Under aseptic conditions, the leaf surfaces were sterilized by macerating in 96% ethanol for 1 min, followed by soaking in 10% sodium hypochlorite for 4 min and subsequently rinsed with ethanol for 30s. They were then washed in sterile distilled water and blot dried on sterile filter paper (7).

20 plants and 10 leaves per plant that were cut into four segments were placed aseptically on Petri dishes containing potato dextrose agar medium (PDA), supplemented with chloramphenicol (100 µg.mL⁻¹) to prevent bacterial growth. The Petri dishes were incubated at room temperature for 2 months and checked until mycelium or colonies appeared. All fungal endophytes were then isolated and placed in new PDA plates. After morphological and microscopic identification of all isolates, three of the most abundant endophytic fungi were retained for the rest of this study.

Molecular characterization of endophytic fungi
Genomic DNA was extracted from pure mycelia using the Universal Pathogen Kit (Omega Biotek Mag-Bind) and sequenced using the ribosomal internal transcribed spacer (ITS). PCR amplification of the fungal genomic ITS region 1 and 2 was performed using the following primers: ITS1- Forward primer (TCCG TAGGTGAACCTGCGG) and ITS4- Reverse primer (TCCTCCGCTATTGATATGC) according to White et al. (8). The sequences were deposited in the NCBI database and the following accession numbers were obtained: MK880626 (Alternaria sorghi), MK880625 (Alternaria tenuissima) and MK880627 (Alternaria alternata).

Fermentation and extraction of endophytic fungi
Endophytic fungi were cultured on PDA at 28°C for 6 days. Subsequently, each fungus was cultivated by placing agar discs containing an actively growing pure mycelium or colonies appeared. All fungal endophytes were then isolated and placed in new PDA plates. After morphological and microscopic identification of all isolates, three of the most abundant endophytic fungi were retained for the rest of this study.

Determination of antibacterial activity

Bacterial strains
The antibacterial activity of *Urtica dioica* leaves and endophytic fungi extracts were evaluated against reference and isolated strains. All reference strains were obtained from the Laboratory for Food Safety (Maisons-Alfort, France). The isolated strains from raw milk and acidified milk were obtained by Dr. Titouche, University of Tizi-Ouzou, Algeria (Table 1).

Five Gram-positive bacteria species including *Bacillus cereus* ATCC 10876, *Staphylococcus aureus* ATCC 25923 and Methicillin-Resistant *Staphylococcus aureus* (MRSA): *S. aureus* ATCC 43300, *S. aureus* LGA 251, *S. aureus* MU 50 and three Gram-negative bacteria species including *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 4352 and *Citobacter freundii* ATCC 8090 were used.

Agar diffusion method (ADM)
The lyophilized crude extracts of nettle leaves and endophytic fungi were diluted in distilled water (5 mg.mL⁻¹) and the ethyl acetate extracts of both were diluted in dimethylsulfoxide (5 mg.mL⁻¹) (DMSO, diluted to 1/10). Initially, the bacterial strains were inoculated on a sterile Muller-Hinton agar plate and incubated at 37°C for 18 h. For each extract, a sterilized 5 mm diameter disc was saturated with 20 µL of crude extract and placed on the Muller-Hinton agar plates containing bacteria with turbidity adjusted to 0.5 Mc Farland in sterile physiological water. Each experiment was carried out in triplicate.

A filter paper disc saturated only with DMSO or distilled water was used as a negative control. Moreover, chloramphenicol disc was used as a positive control (30 µg/disc). All plates were incubated at 37°C for 18 h. Antibacterial activity was evaluated by measuring the diameters of the growth inhibition zones (mm) of the tested pathogenic bacteria against standards (10).

Minimum inhibitory and minimum bactericidal concentrations (MIC/MBC)
The MIC of extracts was determined using the serial macro-dilution method in Mueller-Hinton broth (11). The concentrated crude extracts were diluted using the two-fold serial dilution method. Each crude extract was diluted in series at 0.19; 0.39; 0.78; 1.56; 3.12; 6.25; 12.50 and 25 mg.mL⁻¹. The inoculates were prepared in the same medium at a density adjusted to a 0.5 Mc Farland turbidity standard (10⁶ colony-forming units (CFU)/mL).

Then, 1 mL of each dilution was added to 1mL of inoculates. Culture medium without samples and other

Table 1. Phenotypic and genotypic characterization of *Staphylococcus aureus* isolates.

| Strain | Sample type | Spa-type | ST | Toxin genes profile | Phenotype of resistance | Antimicrobial resistance genes |
|--------|-------------|----------|----|---------------------|-------------------------|-------------------------------|
| S84    | Rawmilk     | t024     | 8  | seb, sep            | OFX-FOX-TE-NOR-P        | tet (K), blaZ, mecA           |
| S95/S96| Acidifiedmilk| t024     | 8  | seb, sep            | OFX-FOX-TE-OX-NOR-P     | tet (K), blaZ, mecA           |
| S100/S101| Rawmilk   | t024     | 8  | seb, sep            | OFX-FOX-TE-OX-NOR-P     | tet (K), blaZ, mecA           |

OFX: ofloxacin; Fox: cefoxitin; TE: tetracycline; OX: oxacillin; NOR: norfloxacin; P: penicillin.
ST: sequence-type; Spa-type: staphylococcal protein A-type; tet (k): gene coding for resistance to tetracycline; blaZ: gene coding for resistance to penicillin; mecA: gene coding for methicillin resistance.
media without microorganisms were used as controls in the tests. The tubes were incubated at 37°C for 24 h. Bacterial growth was detected by optical density (OD) at 620 nm. The MIC value was defined as the lowest concentration of extracts preparation in which no bacterial growth occurred and which is expressed in mg.mL⁻¹. All tests were repeated three times and confirmed.

The MBC examination consisted of transferring 100 µL of bacterial culture from each well where no growth was observed into the Müller–Hinton Agar plates. The plates were incubated at 37°C for 24 h. Colony growth on the plates was verified after this period. MBC was defined as the lowest concentration of extract which resulted in complete inhibition of bacterial growth and is expressed in mg.mL⁻¹.

Determination of antioxidant activity

**DPPH radical scavenging activity**

The total antioxidant activity of crude extracts was measured by DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging capacity according to Brand-Williams et al. (12) with modifications. The concentration of leaves and endophytic fungi extracts were diluted in series between 50 and 450 µg.mL⁻¹. Ascorbic acid was used as a positive control. For the negative control and the blank, DPPH solution in methanol and methanol was used respectively. DPPH methanol solutions (0.1 mM, 1.95 mL) were mixed with 50 µL of extracts. The reaction mixture was vortexed and then incubated in dark conditions for 30 min at room temperature. Absorbance was measured using a spectrophotometer at 517 nm. The decrease in the absorbance of the reaction mixture indicated higher free radical scavenging activity, which was calculated using the following equation:

\[
\text{DPPH scavenging capacity (\%)} = \left( \frac{A_o - A_s}{A_o} \right) \times 100\%
\]

where, \(A_o\) is the absorbance of the control and \(A_s\) is the absorbance of the extract.

The antioxidant activity of all extracts was expressed as IC50 value (µg.mL⁻¹) (Inhibition concentration 50) calculated by linear regression analysis.

**Total thiol content**

Total thiol assay was performed according to Issawi et al. (13). Frozen samples of nettle leaves and endophytic were ground in liquid nitrogen and then extracted into 1mL of 0.2N HCl. After centrifugation at 16000 g for 4 min, 500 µL of extracted solution was neutralized with 400 µL NaOH (0.2M) and 50 µL NaH₂PO₄ (0.2M); 0.7mL of 0.12M NaH₂PO₄ (pH 7.5), 6µL of 1M EDTA, 0.1mL of 6mM Dithiobis 2-nitro benzoic acid (DTNB) were added to 0.2 mL extract. For the standards, the extract was replaced by 0; 10; 20; 50 and 100 nmol of reduced glutathione (total volume 1mL). The absorbance at 412 nm was read 5min after the addition of standards or extract.

**Determination of CAT and SOD enzymatic activities**

Frozen leaves and endophytic fungi samples were ground to a fine powder in liquid nitrogen (the endophytic fungi were initially cultured in PDA at 28°C for 6 days). Proteins were solubilized into 1mL of extraction buffer containing 50mM phosphate buffer pH 7.8, 1mM EDTA, 1% (w/v) polyvinylpyrrolidone and 10% (v/v) glycerol and centrifuged at 16000g for 10 min at 4°C. The supernatant was collected and used for protein quantification according to Bradford (14) and then for the assays of enzymatic activities.

Catalase (EC 1.11.1.6) and SOD (EC 1.15.1.1) activities were assayed according to Issawi et al. (13). 40µg of total soluble proteins from each sample were diluted in 50 mM phosphate potassium buffer (pH 6.5). CAT activity was computed by calculating the amount of H₂O₂ with a molar extinction coefficient of 43.6M⁻¹ cm⁻¹. SOD activity was estimated by the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) by SOD. One unit of enzyme activity was taken as that amount of enzyme required to inhibit 50% of the NBT reduction.

**Statistical analysis**

All biological experiments were performed at least three times independently. Results were expressed as mean ± SD. Data were analyzed by Student’s t-test and one-way ANOVA using the PAST free software.

**Results**

**Isolation and molecular identification of endophytic fungi**

144 isolates were obtained from nettle leaves with a colonization percentage of 18%, calculated as follows: the number of colonized segments on the total number of segments.

Three isolates were selected for this study because they were considered to be the most abundant after the morphological and microscopic characterization of the different isolates. The molecular identification of the three selected isolates was done by sequencing their ITS regions.

The result of identification showed that all isolates belong to Alternaria genus. Isolate 1 was closely related to A. sorghi, isolate 2 was closely related to A. tenuis-sima and isolate 3 was closely related to A. alternata with 99% or more similarity.

**Antibacterial activity**

The antibacterial activity was evaluated in both water and ethyl acetate extracts of U. dioica leaves and the three selected endophytic fungi against isolated and reference strains. All extracts displayed significant antibacterial activity against the tested bacteria in comparison with the positive control (chloramphenicol) (Table 2).

For water extract, U. dioica leaves exhibited similar activities to endophytic fungi with the inhibition zones ranging between 9.5 to 15.5 mm against Gram-positive bacteria (Table 2). The maximum inhibition zones were observed against the MRSA strain: MU50 and S.95, with the values of 15.5 and 14 mm respectively. A. sorghi exhibited the highest antimicrobial activity compared to the other fungi.

E. coli 25922 was resistant to leaves extract. However, Alternaria sorghi presented the highest inhibition zone against this strain with 12.5 mm (Table 2). Overall, except Alternaria sorghi extract, the antibacterial activity of all extracts against Gram-positive bacteria was more effective than that observed against Gram-negative bacteria.
For ethyl acetate extract, no activity was exhibited by the leaves extract against *K. pneumonia* 4352 and *B. cereus* 10876, while this extract exhibited stronger activity against the MRSA strain *S. aureus* MU50 with 12.16 mm (Table 2). *A. sorghi* generally displayed stronger antimicrobial activity toward bacterial tested than the other fungi, except for the MRSA strain *S. aureus* MU50. Furthermore, the ethyl acetate extracts had a weak inhibitory effect on Gram-negative strains.

MIC and MBC values of leaves and endophytic fungi preparations, water and ethyl acetate extracts were also determined as an evaluation of their activity against selected bacteria (Table 3). For the antibacterial evaluation, all extracts were able to inhibit the growth of Gram-positive and Gram-negative bacteria, with MIC and MBC values ranging from 0.19 to 1.56 mg.mL$^{-1}$. Both extracts of *U. dioica* leaves and endophytic fungi had bacteriostatic and bactericidal activity against all examined bacteria (Table 3).

For water extract, leaves exhibited the highest activity against *S. aureus* MU50 with bactericidal activity with MIC and MBC values of 0.19 and 0.39 mg.mL$^{-1}$ respectively (Table 3). Bactericidal activity against isolated *S. aureus* with MIC and MBC values ranging from 0.39 to 1.56 mg.mL$^{-1}$was also examined. *A. sorghi* extract showed the bactericidal activity against all bacteria tested with MIC and MBC values ranging between 0.39 and 3.12 mg.mL$^{-1}$.

For ethyl acetate extract, *A. sorghi* exhibited the highest antibacterial activities against reference and isolated *S. aureus* strains with MIC and MBC values ranged between 0.39 to 1.56 mg.mL$^{-1}$ (Table 3).

### DPPH free radical scavenging activity

Antioxidant activity monitored as DPPH radical scavenging activity showed that ethyl acetate extract presented the highest antioxidant activity compared to the water extract since its radical scavenging capacity was similar to that of ascorbic acid (Fig. 1).

For water extracts, *U. dioica* leaves extract had
the lower percentage inhibition of DPPH radical than all fungal extracts excepted for *A. alternata*. The free radical scavenging activities of the endophytic fungi extracts were as following: *A. alternata* (72.10 ± 0.60 %), *A. tenuissima* (76.77 ± 0.84%) and *A. sorghi* (83.53 ± 0.70 %) at 450 µg.mL⁻¹ extract. All extracts showed significant difference (p < 0.001) when compared with ascorbic acid (97.30 ± 1.05 %).

For the ethyl acetate extracts, the nettle leaves and *A. sorghi* exhibited the greatest antioxidant activity with DPPH free radical scavenging values of 94.87 ± 1.48 % and 96.88 ± 1.15 %, respectively, while *A. tenuissima* and *A. alternata* gave 87.98 ± 0.64 % and 70 ± 1.23%, respectively. The low IC50 value indicated a high antioxidant activity, thus indicating higher antioxidant activity of the nettle leaves (104.63± 3.33 µg.mL⁻¹) than *A. sorghi* (139.27±1.56 µg.mL⁻¹).

**Total thiol content and ROS detoxification enzymes**

This study also focused on the quantification of total thiol compounds, considered as principal antioxidant molecules and investigated ROS detoxification enzyme activities such as catalases and superoxide dismutases, to assess the potential role of endophytic fungi in the regulation of abiotic and biotic stresses in the plant. Therefore, these enzymes and compounds were measured in nettle leaves and a 6-day old mycelium.

Unexpectedly, all fungi showed nearly the same amount of total phenolic compounds as nettle leaves. However, this was not the case for total thiols; hence, nettle leaves exhibited the highest level as the content of thiols was approximately two-fold more than that of fungi (Fig. 2).

Surprisingly, catalase demonstrated no significant variation between nettle leaves and all fungi. Nevertheless, nettle leaves which had the same SOD activity as *Alternaria alternata* showed significantly increased SOD activity than *Alternaria tenuissima* as well as *Alternaria sorghi* (Fig. 3).

**Discussion**

The antibacterial activities of the leaves of *Urtica dioica* and its three endophytic fungi were carried out using two types of extraction: water and ethyl acetate.
This work is reported to be the first of its kind to focus on foliar endophytic fungi of *Urtica dioica*, however, many studies report the predominance of *Alternaria* in different host plants (3, 15). It is true that *Alternaria* species have been described as plant pathogenic fungi, referred to as saprophytes or plant parasites. However, *Alternaria* *sp.* has been recorded as endophytic fungi colonizing several host plants including medicinal plants (16–19). In addition, Tsai et al (20) showed that endophytic fungi have pathogenic fungi as their ancestors, which have a very different life cycle.

All extracts have interesting inhibition zones against the majority of the bacteria tested, but they remain less than those obtained by the positive control, chloramphenicol. The abundance of molecules of all kinds in the extracts and the interactions that result from them could undoubtedly explain this difference. Gram-positive bacteria are more sensitive to extracts than Gram-negative bacteria. This difference could be attributed to the difference in membrane composition, Gram-negative bacteria, unlike Gram-positive bacteria, have a phospholipid outer membrane containing lipopolysaccharide as a major structural component, making the cell wall more impermeable to antibacterial agents (21).

The results showed that *E. coli* ATCC 25922 strain was resistant to water extract from *Urtica dioica* leaves. Gülcin et al. (22) showed low bacterial activity of water extracts of the same species with an inhibition zone of 8 mm while those of Salinas-Sánchez et al. (23) reported the inefficiency of ethanol extracts from nettle leaves against *E. coli* ATCC 9837 strain. The ethyl acetate extract of nettle leaves was also inactivated against *K. pneumonia* ATCC 4352. This result is consistent with the results reported by Körpe et al. (24), where extracts of *U. dioica* and *U. pilulifera* leaves showed no activity against *K. pneumonia*. However, we showed that the water extract of *A. sorghi* showed an interesting activity against *E. coli* ATCC 25922.

Endophytic fungi and especially *Alternaria* *sp.* can produce new bioactive compounds such as paclitaxel, podophyllotoxin and camptothecin (25). Moreover, *Aspergillus parasiticus* could transform α-santonin into 3,4-epoxy-1α-santonin, with high antibacterial activity (26).

A satisfactory antibacterial activity was also observed during this analysis for the extracts studied against reference MRSA strains or isolated from raw or acidified milk. The water extracts showed the most important activity against *S. aureus* MU50 and *S. aureus* S95 strains with inhibition zones of 15.5 and 14.05 mm respectively. The water extract of *A. sorghi* showed bactericidal activity against strains of *S. aureus* ATCC 43300 (CMI 0.39 mg.mL⁻¹; CMB 0.39 mg.mL⁻¹) and *S. aureus* LGA 251 (MIC 0.78 mg.mL⁻¹; CMB 0.78 mg.mL⁻¹). These results are in good agreement with Gülcin et al. (22) and Tasdelen Fisgin et al. (27) studies and are particularly interesting as clinical isolates of *S. aureus*, especially those resistant to Mëthicilllin (28).

*Alternaria* species are known as a source of many bioactive substances with very different structures and activities (29, 30). Thus, boric acid D and Alterusin of *Alternaria sp.* showed inhibitory activity against *Staphylococcus aureus* with CMI of 100μg.mL⁻¹ and 25μg.mL⁻¹, respectively (31). In addition, some anthraquinones of *A. solani* and *Alternaria sp.* showed antibacterial activity on *Bacillus subtilis*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Staphylococcus albus*, *Staphylococcus aureus* and Vibrio parahemolyticus (32–34).

Pyrone derivatives of the endophyte fungus *A. tenuissima* of the Chinese medicinal plant, *Salvia przewalskii*, also exhibited antibacterial activity against the following strains: *B. subtilis*, *B. megaterium* and *C. perfringens* (35). Sonaimuthu et al. (4) demonstrated that tenuazonic acid is an active compound of *A. alternata* against *Mycobacterium tuberculosis* H37Rv (CMI 250μg.mL⁻¹).

In this study, the total antioxidant activity of water and ethyl acetate extracts of *A. endophytes* and their host revealed that in general, all the extracts studied have high antioxidant activity. First of all, we performed a DPPH assay that is based on two scavenging mechanisms: electron transfer (ET) and hydrogen transfer (HAT) thus, this assay reflected the typical and global antioxidant capacity (36). Our results showed that the greatest free radical scavenging activity of DPPH was observed with ethyl acetate extracts and particularly those of *A. sorghi* and nettle leaves. Thus, ethyl acetate extracts from the leaves of *U. dioica* and *A. sorghi* exhibited the highest anti-free radical activity with a DPPH trapping rate of 94.86% and 96.87%, respectively. Such results were consistent with extracts of *A. sorghi* and nettle leaves rich in phenolic compounds that are extracted by polar solvents as ethyl ether (data not shown). Our results indicated that nettle leaves and endophytic fungi are an important source of total thiols. As it has been shown that alterations in cell thiol content are the main determinant of changes in the total antioxidant capacity of the cell, which would make it a useful parameter for the evaluation of oxidative stress (37). Thus, the highest rates of total thiols in the nettle leaves indicated that endophytic fungi helped their hosts to increase thiol antioxidants conferring them to withstand biotic and abiotic stresses (38).

This work also focused on ROS detoxification enzymes, including catalase (CAT) and superoxide dismutase (SOD). It is well known that in plants, catalase eliminates H₂O₂ generated during photosynthesis and fatty acid oxidation, it also plays an important role in detoxifying active oxygen species generated by various types of environmental stress (39). Superoxide dismutase catalyses the dismutation of the superoxide anion (O₂⁻) into O₂ and H₂O₂. The oxygen peroxide thus produced will then be eliminated by catalase and/or peroxidases (40). Results showed that while there was no significant variation in catalase activity in nettle and fungi, the SOD dismutation capacity increased markedly in *U. dioica* leaves. Indeed, SOD represents the first line of defense against superoxide anion scavenging and enzymes than catalase such as peroxidases (glutathione peroxidase, ascorbate and guaiacol peroxidases) are involved in the defense against hydrogen peroxide production (41).

However, Bonnet et al. (42) reported the presence of the endophyte *Epichloë lolii* modified the metabolism by promoting catalase catalysis of H₂O₂ and improving the host's defence capacity. Especially since an increase in the activity of ROS detoxification enzymes
has been shown in individuals of *E. dahluricus* exposed to drought (6).

To date, the mechanisms highlighted by endophytic fungi to improve the plant’s defence systems remain unknown. Therefore, the SOD gene was isolated in *E. laclii*, which provides a new approach for studying the role of gene expression in the detoxification capacity of ROS and the interaction mechanism between endophyte and plant. Zhang et al. (5) showed that relative expression of the genes encoding SOD, POD and CAT and their corresponding enzymes increased in wheat seedlings under saline stress when *Trichoderma longibrachiatum* T6 strain was applied, indicating that this strain significantly protected seedlings from salt-related stress.

The study showed that *A. sorghi* presented the best activities compared to other isolated fungi, in fact, it is common for a plant to be colonized by fungi belonging to the same genus or species and only one or more biotypes of a given fungus will produce a biological actives compounds in culture, that will need further investigations to characterize.

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Conflict of interest

The authors declare that they have no conflict of interest.

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