Phylogenetic diversity and antioxidant activity of selected fungi from ethno-medicinal plants and soil

Rahul Chandra Mishra 1,2 · Colin J. Barrow 2 · Rishu Kalra 1 · Neeraj Dwivedi 1 · Sunil K. Deshmukh 1 · Mayurika Goel 1

Received: 16 June 2021 / Revised: 20 December 2021 / Accepted: 21 December 2021
© German Mycological Society and Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract
Endophytic fungi are prolific generators of bioactive metabolites with natural antioxidant properties that have a range of potential uses. In the present work, the antioxidant activity and phylogenetic diversity of endophytic fungi obtained from semi-arid terrestrial plants and the fungi obtained from the mangrove soil samples are investigated. The fungal isolates were identified by employing molecular characterisation and phylogenetic analysis by internal transcribed spacer (ITS) sequences. Fungi belonging to six taxonomic orders of Ascomycota associated with nine genera were identified, these being Diaporthales, Eurotiales, Hypocreales, Onygenales, Pleosporales and Xylariales. The antioxidant activity of the methanolic extracts of the fungal isolates was determined using modified 2,2'-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) methods. Amongst the fungal isolates, Aspergillus sp. AREF023 and Neocosmospora sp. AREF014, obtained from different parts of Datura metel, displayed the highest level of radical inhibition (80.76% and 70.62% respectively). GC/MS analysis of the active isolates confirmed the presence of known antioxidant phenolic compounds, including 2-tert-Butyl-5-hydroxymethyl-5-methyl-[1,3]dioxolan-4-one and 2,5-ditert-butylphenol, in the their crude extracts. Our findings suggest that fungal isolates from D. metel can be a sustainable resource for natural antioxidants.

Keywords Molecular characterisation · Bio-autography · Datura metel · Aspergillus sp. · Neocosmospora sp

Introduction
Endophytic fungi are diverse filamentous fungi that grow within plant tissues without causing any immediate harmful effects (Hyde and Soytong 2008; Debbage et al. 2013), These endophytic fungi are prolific producers of secondary metabolites and can serve as a valuable reservoir of lead molecules in the hunt for drug candidates against infectious diseases, cancer and many other disorders (Rana et al. 2020; Mishra et al. 2021a, b). Curvularia sp. G6-32, an endophytic isolate of medicinal plant Sapindus saponaria, produces asperpentyn, an epoxyquinone, which is a potential antioxidant (Polli et al. 2020). In addition, Diaporthe prunorum, an endophytic fungus obtained from Hypericum ascyron, produces terpenes and isocoumarins that have potential antimicrobial activity (Qu et al. 2020). The unique phenomenon of cross-talk existing between the colonizing endophytic fungi and the plant host results in the production of related and often the same bio-actives as originally produced by the host plant (Caruso et al. 2020). Endophytic fungi are novel source of fungal metabolites that sustainably help in the discovery of bioactive natural products.

The present study screened endophytic fungi isolated from medicinal plants and mangrove soil from two diverse regions for antioxidant activity. Firstly, the semi-arid region characterised by its intermediate climatic conditions between the desert and humid regions, secondly the semi-arid land forms, comprising of succulent, non-succulent and xerophytic perennials (Yadav et al. 2020). Secondly, mangrove sediments are complex as compared to plant ecosystems in terms of a characteristic marine biome. The vegetation is well equipped to survive saline stress (Guillén-Navarro et al. 2015). There is no up to date published study that describes the phylogenetic study of endophytic fungi and soil fungi

Mayurika Goel mayurikagoel@gmail.com; mayurika.goel@teri.res.in

1 TERI-Deakin Nano Biotechnology Centre, The Energy and Resources Institute (TERI), TERI GRAM, Gurgaon 122001, India
2 Centre for Chemistry and Biotechnology, School of Life and Environmental Sciences, Deakin University, Waurn Ponds, VIC 3220, Australia
obtained from these two sources in India. With this rationale, the ethno-medicinal plants, *D. metel*, *B. monnieri*, *C. citratus* and *O. tenuiflorum*, and soil sediments from marine ecosystem were selected for the isolation of fungi. In the current study, we have identified, established the genetic relationship and screened the antioxidant activity of endophytic fungi isolated from some semi-arid terrestrial plants and the fungi obtained from the soil of the mangrove region of a marine area. Several of these endophytic and soil fungal isolates were found to be significantly active by inhibiting the DPPH and ABTS radicals and showed active spots on the DPPH bioautography assay. These bioactive endophytic and soil fungi isolates were further subjected to molecular characterisation and their chemical constituents were investigated using GC-MS.

**Materials and method**

**Sampling sites and collection of samples**

**Terrestrial plants**

One each healthy terrestrial plant parts, i.e. stem, leaves and roots of four species, *Bacopa monnieri*, *Cymbopogon citratus*, *Datura metel* and *Ocimum tenuiflorum*, was collected from Aravalli Bio-diversity Park (28°28’59.9″N 77°06’34.7″E) Gurgaon, Haryana, India. The identification and validation of plant species were done as previously described (Gamble 1925), and samples were preserved in our laboratory. Plant parts such as leaf, stem and roots were randomly cut with an ethanol-disinfected sickle and placed independently in sterile polythene bags. Endophytic fungi were isolated within 48 h of collecting the samples.

**Soil samples**

Nine mangrove soil samples were collected randomly in July 2018 from the marine area of Alibaugh (18°39’23.9544″N, 72°52’47.5248″E) Maharashtra, India. The identification and validation of plant species were done as previously described (Gamble 1925), and samples were preserved in our laboratory. Plant parts such as leaf, stem and roots were randomly cut with an ethanol-disinfected sickle and placed independently in sterile polythene bags. Endophytic fungi were isolated within 48 h of collecting the samples.

**Isolation of fungal samples**

**Endophytic fungi**

Isolation of the fungal isolates was done using a previous method of Qadri et al. (2013) with some modifications. Briefly, mature and excided small plant parts (leaves, stems and roots) were used for isolation of the fungal endophytes. The samples were washed for 1 min with 70% ethanol (v/v) and disinfected for 2 min using 2% sodium hypochlorite solution (v/v) followed by 20 s of brief rinsing with 70% (v/v) ethanol. Later they were rinsed with sterile distilled water. These plant parts were placed on potato dextrose agar (PDA) plate amended with 50 μg/ml chloramphenicol. PDA plates containing three pieces of plant material were incubated for 7–10 days at 28 °C. Plating was done in triplicates.

**Fungi from soil**

Soil fungal isolates were isolated on water agar (WA) by employing soil plate method. Incubation was done at 28 °C for 3–5 days, and checked for fungal colonies appearing in culture, and then a single colony was transferred on PDA plate and further incubated for growing pure culture. The cultures in petri dishes were incubated at 28 °C for 7–10 days for observation. Plating of each soil sample was done in triplicate. All the isolates were deposited in the TERI Deakin Nanobiotechnology culture collection at 4 °C.

**Metabolite production**

Potato Dextrose Broth (PDB, Himedia) was used for metabolite production. Three fungal plugs (8–9 mm in diameter) from 10-day-old culture were inoculated in 1000-ml Erlenmeyer flasks containing 250 ml of the medium. The flasks were incubated at 28 °C for 8–10 days at 150 rpm. The growth conditions and time period had been optimised (Fig. S1) based on the assessment of growth kinetics using ergosterol estimation method (Axelsson et al. 1995). Ergosterol is the major sterol present in the cell membranes of filamentous fungi, and monitoring its level is a useful method for estimating growth kinetics.

**Preparation of methanolic extract**

For preparation of the extract, methanol was added slowly in the grown culture (1:1) (to avoid over heating), and kept at shaking for 6 h. The media was then filtered through sterile muslin cloth to separate mycelia. The filtrate obtained was then concentrated to 1/5 volume using rotary evaporator and diluted to 100 ml with distilled water. The diluted solution was then passed through Dianion HP20 column (Merck) and was eluted using 70% methanol. The obtained elute was concentrated in peer shape flasks, diluted with distilled water and lyophilised to obtain a powdered extract.

**Thin layer chromatography (TLC) bio-autography**

Each fungal extract was spotted and eluted with CHCl₃:MeOH (85:15 v/v) on silica 60 F₂₅₄ TLC plate
(Merck), followed by spraying with 0.2% DPPH solution in methanol and incubated for 30 min in dark. Active compounds appeared as yellow spots against a purple background.

**Total antioxidant capacity assay**

**DPPH assay**

DPPH radical scavenging assay was conducted according to the previous method of Blois (Blois 1958) with modifications. DPPH (Sigma) was dissolved in 100 ml of MeOH. The investigated samples were prepared by dissolving 1 ml of 0.2 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol and 0.5 ml of test sample at different concentrations (5 to 100 μg/ml) at room temperature. The absorbance at 517 nm was measured after 30 min versus the blank (0.5 ml of methanol instead of test sample in 1-ml DPPH solution). Positive controls ascorbic acid, tert-butylhydroquinone (TBHQ) and Trolox were also subjected to the same procedure for comparison. The analysis was carried out in triplicate, and the percentage (%) of inhibition was calculated by the following formula:

\[
\text{%inhibition} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100
\]

The DPPH radical scavenging activity of each sample was expressed as Trolox equivalent antioxidant capacity (TEAC) (Shimamura et al. 2007). TEAC was calculated as follows:

\[
\text{TEAC} = \frac{\text{IC}_{50} \text{ of Trolox (μg ml}^{-1})}{\text{IC}_{50} \text{ of sample (μg ml}^{-1})}
\]

**ABTS assay**

The ABTS radical scavenging assay of the fungal crude extracts performed in accordance with the method reported by Re et al (Re et al. 1999). Briefly, 2.45 mM potassium persulfate and 7 mM ABTS aqueous solution were mixed to generate ABTS⁺ radical. The initial absorbance mixture solution was adjusted to 0.70 ± 0.02 at 745 nm. Different sample concentrations (5 to 100 μg/ml) and standard Trolox (1 to 50 μM) with ABTS⁺ working solution were adjusted to a final volume of 1 ml and reacted at room temperature (25 °C) for 6 min. The decrease in absorbance at 745 nm was recorded and calculated as half maximal inhibitory concentration (IC₅₀) for test samples and Trolox was calculated by plotting the scavenging capacity against the concentration. Trolox standard solution (final concentration 0–50 μM) was used. Results were expressed in terms of TEAC (Shimamura et al. 2007). TEAC was calculated as follows:

\[
\text{TEAC} = \frac{\text{IC}_{50} \text{ of Trolox (μg ml}^{-1})}{\text{IC}_{50} \text{ of sample (μg ml}^{-1})}
\]

**Molecular identification of the fungal isolates**

**Genomic DNA extraction**

Only the isolates which displayed optimum antioxidant activity were further subjected to sequencing and identification. For that, the pure culture obtained after isolation of the fungal isolates was grown in 250-ml Erlenmeyer flasks containing 50 ml of the medium, incubated at 28 °C for 8–10 days at 150 rpm. The fungal mycelia obtained were freeze-dried and the genomic DNA was extracted by the CTAB (cetyl trimethylammonium bromide) method (Brent et al. 1995).

**Phylogenetic analysis**

Phylogenetic analyses of the endophytes were carried out by the acquisition of the ITS1-5.8s ITS2 ribosomal gene sequencing. ITS region of rDNA was amplified and sequenced with universal primers ITS4 (5′-TCCT CCGCTTATTGATATGC-3′) and ITS1 (5′-CCGT AGGTGAACCTGCGG-3′). PCR amplification was performed according to the methods of Rajendran et al (Rajendran et al. 2017). The amplicons obtained were gel purified by amicon ultra columns (Millipore, USA) and 20–40 ng was used for sequencing at Eurofins Laboratory Pvt, Ltd. (Bengaluru, India). Finch TV software (http://www.geospiza.com/Products/finchtv.shtml) was used for assembling sequences and homology was determined using BLASTn against the NCBI Gen Bank database (Altschul et al. 1997). A phylogenetic tree was constructed using the sequences displaying maximum homology. CLUSTAL W was employed for multiple sequence alignment and MEGA X was used for phylogenetic and molecular evolutionary analyses (Kumar et al. 2018). Following the Tamura-Nei model and maximum likelihood algorithm, phylogenetic reconstruction was done with bootstrap values calculated from 1000 replicate runs (Tamura and Nei 1993).

**GC/MS analysis**

The volatiles and major chemical components of the fungal extracts were analysed using Agilent 6890 GC-MS coupled to Agilent 5873 (EI mode, 70 eV). The GC conditions were as follows: 1-min split less time, helium carrier at 1.0 ml/min, oven temperature from 70 to 135 °C at 2 °C/min, for 10 min; then to 220 °C at 4 °C/min, 10 min; and finally to 270 °C at 3.5 °C/min, for 20 min. For analysis HP-5MS capillary column (0.32 mm × 30 m × 0.25 μm), GC injector with
temperature set at 280 °C and MS transfer line temperature at 290 °C were used. The detected compounds were compared with the mass spectra from the NIST library for their identification (Proestos et al. 2006).

Results

Isolation of endophytic fungi, fungal isolates from soil and screening for antioxidant activity

In total, 43 fungal isolates were obtained from different parts of the plant and mangrove soil. From them, 17 antioxidant-related fungal isolates (10 endophytic fungi and 7 soil fungi) obtained after initial screening of the pure isolates are illustrated in Table S1. Methanol extracts of these fungal isolates were screened for antioxidant potential using two assays. Extract antioxidant activity was tested at a concentration of 1000 µg/ml.

TLC bio-autography analysis

Amongst the various solvent elution tested, chloroform/methanol/acetic acid (8.5:1.5 by volume) gave the best separation of compounds from the 70% methanol extracts with retention factor values ($R_s$) ranging from 0.15 to 0.87. The existence of strong adsorbing sites, as depicted on TLC plate (Fig. S2), confirms the suitability of this solvent system for separation and mobility of the fungal extracts. The baseline of all the methanolic fractions indicated a range of deep to light yellow colour, indicating the presence of antioxidant constituents in varying amounts. The spots of $R_s$ 0.87–0.71 displayed bright yellow spot on the TLC plate confirming the presence of antioxidant compounds in these extracts. Radical scavenging activity of methanol extracts from all fungal isolates against DPPH and ABTS$^+$ radical is shown in Table 1. The IC$_{50}$ values were obtained in the range of 33–616 µg/ml for the DPPH assay and 14–167 µg/ml for ABTS assay. The lower the IC$_{50}$ and the higher the TEAC value, the greater is the antioxidant activity.

Amongst the 17 fungal crude extracts, isolate AREFF023, identified as Aspergillus sp. obtained from the internal tissues of roots of D. metel, exhibited promising DPPH scavenging activity (IC$_{50}$ 33.49 µg/ml, TEAC of 0.3 and 80.76% of DPPH radical inhibition) and also against ABTS$^+$ (IC$_{50}$ 14.33 µg/ml and 75.18 % of ABTS$^+$ radical inhibition). The second most potent isolate was Neocosmospora sp. AREF014, which showed moderate antioxidant activity (IC$_{50}$ 35.39 µg/ml against DPPH and TEAC of 0.28; and IC$_{50}$ 33.50 µg/ml against ABTS$^+$).

Phylogenetic diversity of cultivable fungi

Amongst the seventeen isolates, eleven (Fig. 1) displaying commendable antioxidant activities were subjected to sequencing for their molecular characterisation. A further categorisation of these isolates was carried out into nine genera, namely Alternaria, Aspergillus, Auxarthron, Curvularia, Diaporthe, Monascus, Neocosmospora, Talaromyces and Xylaria which belong to six orders Diaporthales, Eurotiales, Hypocreales, Onygenales Pleosporales and Xylariales, found in phylum Ascomycota. Amongst the isolated endophytic fungi Alternaria and Aspergillus was the predominant genus, with relative frequency of 18.18%, while the remaining genera Auxarthron, Neocosmospora, Monascus, Talaromyces, Xylaria, Curvularia and Diaporthe were found with relative frequency of 9.99%.

The phylogenetic tree (Fig. 2) displays three large clades containing several subclades. First clade contained five subclades each of them containing one endophytic isolate which cluster with three sequences of Aspergillus, Monascus, Talaromyces and Auxarthron sp. acquired from NCBI with 98 to 100% bootstrapping. The second clade consisted of three subclades each one having

| Sample | DPPH (IC$_{50}$ µg ml$^{-1}$) | ABTS (IC$_{50}$ µg ml$^{-1}$) | TEAC |
|--------|-----------------------------|-----------------------------|------|
| Ascorbic acid | 5.33 | 4.37 | 1.90 |
| TBHQ | 9.95 | 3.83 | 1.01 |
| Trolox | 10.13 | 7.42 | 1.00 |
| AREF001 | 273.96 | 144.28 | 0.03 |
| AREF004 | 171.6 | 18.51 | 0.05 |
| AREF007 | 210.35 | 26.15 | 0.04 |
| AREF010 | 71.2 | 33.90 | 0.14 |
| AREF011 | 171.7 | 63.24 | 0.05 |
| AREF014 | 35.36 | 33.50 | 0.28 |
| AREF015 | 97.4 | 55.95 | 0.10 |
| AREF016 | 183 | 59.10 | 0.05 |
| AREF017 | 302.37 | 125.11 | 0.03 |
| AREF018 | 357.05 | 167.63 | 0.02 |
| AREF019 | 149.44 | 63.47 | 0.06 |
| AREF020 | 222.89 | 116.72 | 0.04 |
| AREF021 | 80.36 | 51.71 | 0.12 |
| AREF023 | 33.49 | 14.33 | 0.30 |
| AREF026 | 124.26 | 36.70 | 0.08 |
| AREF029 | 134.26 | 29.59 | 0.07 |
| AREF030 | 616 | 39.53 | 0.01 |
one newly identified endophytic isolate with three sequences of *Xylaria*, *Neocosmospora* and *Diaporthe* sp. obtained from NCBI. Third and the last clade had two subclades in which one endophyte sequence clustered with three sequences of *Curvularia* sp. and other sub cluster contained two endophyte isolates sequences clustered with *Alternaria* sp. Characterisation data from this study have been submitted in the NCBI GenBank, accession codes MT013399 to MT013409 (Tables 2 and 3).

**Detection of bioactive metabolites of the methanol extracts by GC-MS analysis**

Considering the highest antioxidant activity obtained from the methanol extracts of *Aspergillus* sp. AREF023 and *Neocosmospora* sp. AREF014, a tentative identification of the metabolites from these two isolates was carried out using GC-MS. Chemical diversity was observed in the constituents of both the extracts, belonging to diverse classes of alkenes, di-ethers, esters, fatty acids, phenols, polysaccharides, organic acids and hydrocarbons. A total of 20 compounds constituting 96.3% of the relative area in the methanol extract and 95% similarity with the standard mass spectra were revealed from the GC-MS analysis of the AREF023 extract (Fig. 3a) (Table 4). The methanol extract of AREF023 indicated the presence, amongst others, of phenolics, including 2-tert-butyl-5-hydroxymethyl-5-methyl-[1,3]dioxolan-4-one (8.97%), a common antioxidant molecule which is used in a range of applications. Similarly, analysis of AREF014 showed the presence of 7 compounds, having resemblance almost 97% with the standard masses in the library and representing 100% of the relative area in the extract (Fig. 3b) (Table 5). The extract clearly showed the presence of the phenolic compound, 2,5-ditert-butylphenol (36.45%), which is widely used as a dietary antioxidant compound.

**Discussion**

According to the antioxidant assays, amongst all the isolates found, *Aspergillus* sp. AREF023 displayed the highest antioxidant activity, followed by *Neocosmospora* sp. AREF014, comparable to that of the standards used, Trolox, TBHQ and ascorbic acid. Scavenging activity is directly proportional to the concentration of extracts and varies in their activity because of the varying metabolite
This is the first report of the antioxidant activities of endophytic fungi identified as *Aspergillus* sp. AREF023 obtained from *D. metel*. Selected for their higher antioxidant capacity, 11 fungal isolates (08 endophytic and 03 soil fungi) representing the phylum *Ascomycota* were identified. The phylogenetic tree composition. This is the first report of the antioxidant activities of endophytic fungi identified as *Aspergillus* sp. AREF023 obtained from *D. metel*.

**Table 2**  Isolated and identified endophytes, including genus or species, and their identity percentage found in the NCBI (National Centre for Biotechnology Information) website

| Sl. No | Endophytic isolate | Phylum | Order | Genera | Description | Accession | Identity (%) |
|--------|--------------------|--------|-------|--------|-------------|-----------|--------------|
| 01.    | AREF001            | Ascomycota | Onygenales | Auxarthron | Auxarthron sp. | MT013399 | 98.28        |
| 02.    | AREF004            | Ascomycota | Pleosporales | Alternaria | Alternaria sp. | MT013400 | 98.93        |
| 03.    | AREF007            | Ascomycota | Pleosporales | Alternaria | Alternaria sp. | MT013401 | 96.27        |
| 04.    | AREF010            | Ascomycota | Eurotiales | Aspergillus | Aspergillus sp. | MT013402 | 99.62        |
| 05.    | AREF014            | Ascomycota | Hypocreales | Neocosmospora | Neocosmospora sp. | MT013403 | 99.81        |
| 06.    | AREF019            | Ascomycota | Hypocreales | Monascus | Monascus sp. | MT013404 | 99.48        |
| 07.    | AREF020            | Ascomycota | Eurotiales | Phomopsis | Phomopsis sp. | MT013405 | 99.01        |
| 08.    | AREF023            | Ascomycota | Xylariales | Xylaria | Xylaria sp. | MT013406 | 99.52        |
| 09.    | AREF023            | Ascomycota | Eurotiales | Aspergillus | Aspergillus sp. | MT013407 | 99.82        |
| 10.    | AREF029            | Ascomycota | Pseudosphorales | Curvularia | Curvularia sp. | MT013408 | 100          |
| 11.    | AREF030            | Ascomycota | Diaporthales | Phomopsis | Phomopsis sp. | MT013409 | 99.44        |
developed in this study represents the relations amongst the isolated fungal samples (Fig. 2) (Table 3).

When the methanolic extracts of the isolates with the highest antioxidant activity were subjected to GC-MS analysis, different bioactive compounds, with various pharmaceutical applications, such as antibacterial, antioxidant, anticancer activities, including some used industrially, were detected in the present study. For example, the compound diethyl phthalate is known to have antimicrobial, acetylcholinesterase and neurotoxic activity (Velanganni et al. 2011). Phthalates are known to have antagonism against pathogens and other pharmacological activities. The active isolates Aspergillus sp. AREF023 and Neocosmospora sp. AREF014 confirmed the presence of phenolic compounds 2-tert-Butyl-5-hydroxymethyl-5-methyl-[1,3]dioxolan-4-one and 2,5-ditert-butylphenol, respectively. Plant phenolic compounds are well known to have potent antioxidant activity (Heendeniya et al. 2020; Khalil et al. 2020; Xiu-Qin et al. 2009; Adhami et al. 2021; Mishra et al. 2020). In another study carried out on a species of Xylaria isolated from Gingko biloba, several phenolic compounds, including 2-hydrazino-8-

### Table 3 DNA reference sequences used in this study, including original references (where applicable) and GenBank accession numbers

| Species                        | Strain     | GenBank accession numbers — ITS | References                                      |
|--------------------------------|------------|---------------------------------|------------------------------------------------|
| Auxarthron ostraviense         | CCF 4241   | NR_121474                       | (Schoch et al. 2014)                           |
| Auxarthron umbrinum            | AU-BRK4    | MH424172                        | (Angelone and Bidochka 2018)                   |
| Auxarthron conjugatum          | KRP24-4    | HM036583                        | (Menkis and Vasaitis 2011)                     |
| Alternaria brassicace          | M1         | JN108903                        | (Sharma et al. 2013)                           |
| Alternaria brassicace          | W8         | MF356599                        | (Zhao et al. 2018)                             |
| Alternaria alternata           | Cc-01      | MG722823                        | (Zhang et al. 2018)                            |
| Alternaria alternata           | YZU 191238 | MN615420                        | (Zhang et al. 2020)                            |
| Alternaria alternata           | ZmH31      | MG228423                        | (Xing et al. 2018)                             |
| Alternaria alternata           | ZmH30      | MG228422                        | (Xing et al. 2018)                             |
| Aspergillus nidulans           | NA         | MG459155                        | (Jedidi et al. 2018)                           |
| Aspergillus nigér              | DF1        | LT745387                        | (Sivaraman et al. 2018)                        |
| Aspergillus nidulans           | UOA/HCPF 10647 | GQ461904 | (Arabatzis et al. 2011)               |
| Neocosmospora falciformes      | UOA/HCPF 14802 | KP132214 | (Irinyi et al. 2015)                     |
| Neocosmospora solani           | F174       | MF401578                        | (Moumni et al. 2020)                           |
| Neocosmospora solani           | 28         | KY318489                        | (Manganyi et al. 2018)                         |
| Monascus kaoliang              | NA         | AB477250                        | (Shinzato et al. 2009)                         |
| Monascus purpureus             | NA         | AB477247                        | (Shinzato et al. 2009)                         |
| Monascus kaoliang              | NA         | AB477252                        | (Shinzato et al. 2009)                         |
| Talaromyces assistitensis      | KK         | KR909179                        | (Travadon et al. 2016)                         |
| Talaromyces assistitensis      | CBS 118440 | JN999320                        | (Samson et al. 2011)                           |
| Talaromyces trachyspermus      | CBS 373.48 | MH856401                        | (Vu et al. 2019)                               |
| Xylaria palmicola              | 604        | GU322436                        | (Hsieh et al. 2010)                            |
| Xylaria psidii MF773655        | AT_L13_E2  | MF773655                        | (Maduranga et al. 2018)                        |
| Xylaria oxyacanthae            | YM1 1551   | MF773432                        | (Ju et al. 2018)                               |
| Aspergillus terreus            | JAS 1      | JO361749                        | (Silambaram and Abraham 2013)                  |
| Aspergillus terreus            | NTOUTL11   | MN592939                        | (Pang et al. 2019)                             |
| Aspergillus terreus            | NTOU4989   | MN592937                        | (Pang et al. 2019)                             |
| Curvularia sp.                 | MR-2019p   | MN215713                        | (Raza et al. 2019)                             |
| Curvularia lunata              | CBS 730.96 | NR_138223                       | (Amaradasa et al. 2014)                        |
| Curvularia lunata              | NA         | HF934911                        | (Amaradasa et al. 2014)                        |
| Diaporthe detusa              | CBS 109770 | KC343061                        | (Gomes et al. 2013)                            |
| Diaporthe eres                 | FPH2017229 | MH003633                        | (Lin et al. 2018)                              |
| Diaporthe sp.                  | AHGB02_1A  | MH267914                        | (Skaltsas et al. 2019)                         |
hydroxy-4-phenylquinoline, 3, 4-dimethoxy-phenol, 2,4-bis(1,1-dimethylethyl)-phenol, 3,4-dihydro-8-hydroxy-3-methyl-isocoumarin and ferruginol were reported. Antioxidant compounds have previously been shown to be present in some endophytic fungi. Luteolin was obtained from the ethyl acetate fractions of the endophytic fungus *Aspergillus fumigatus* obtained from the roots of Pigeon pea (*Cajanus cajan*). It displayed a scavenging potential of IC$_{50}$ — 16.38 μg/ml against the DPPH radical (standard luteolin — IC$_{50}$ — 10.03 μg/ml), in comparison to BHT — 29.94 μg/ml (Zhao et al. 2014). Shikonin (5,8-Dihydroxy-2-[(1R)-1-hydroxy-4-methyl-pent-3- enyl]naphthalene-1,4-dione) was isolated from endophytic fungus *Neocosmospora tricinctum*, displaying scavenging activity in the DPPH radical scavenger assay with an IC$_{50}$ value of 56.3 μg/ml, whereas it showed an IC$_{50}$ value of 1.93 μg/ml against the ABTS radical (Moussa et al. 2019).

**Conclusion**

The results in this study have established that the endophytic fungal isolates, *Aspergillus* sp. AREF023 and *Neocosmospora* sp. AREF014 contain metabolites rich in phenolics and flavonoids which are the major contributors to antioxidant activities in extracts from these strains. The extract with the highest phenolic content displayed the highest activity in the DPPH radical scavenger assay with an IC$_{50}$ value of 56.3 μg/ml, whereas it showed an IC$_{50}$ value of 1.93 μg/ml against the ABTS radical (Moussa et al. 2019).
Table 4  Chemical constituents and their % content from the methanol extract of *Aspergillus* sp. AREF023 as determined using GC/MS

| Peak No. | Identified compound name (IUPAC)                        | Compound class | CAS No.          | PubChem CID | Molecular weight | Molecular formula | Area % | RT | % identity |
|----------|----------------------------------------------------------|----------------|------------------|-------------|------------------|-------------------|--------|----|------------|
| 1        | Methyl 3,3-dimethoxypropionate                           | Methyl ester   | 7424-91-1        | 81924       | 148.16           | C₆H₁₂O₄          | 12.09  | 5.316 | 90         |
| 2        | Pentanoic acid, 4-oxo-, methyl ester                     | Methyl ester   | 0624-45-3        | 69354       | 130.14           | C₆H₁₀O₃          | 3.38   | 5.649 | 94         |
| 3        | 1-((1-Butoxypropan-2-yloxy)propan-2-yl acetate          | Acetates       | 1000367-08-4     | 21226488    | 232.32           | C₁₂H₂₄O₄         | 1.39   | 6.09  | 38         |
| 4        | Butanedioic acid, dimethyl ester                         | Methyl esters  | 106-65-0         | 7820        | 146.14           | C₆H₁₀O₄          | 21.7   | 6.887 | 90         |
| 5        | 2-Pentene, 2-methoxy-                                   | Alkenes        | 061142-47-0      | 5363459     | 100.16           | C₆H₁₃O            | 3.1    | 7.081 | 47         |
| 6        | Hexanoic acid, 4-oxo-, methyl ester                      | Carboxylic esters | 2955-62-6   | 546293      | 144.17           | C₇H₁₂O₃          | 1.16   | 8.597 | 94         |
| 7        | 2-(1-Butyl-5-hydroxymethyl-5-methyl-[1,3]dioxolan-4-one| Oxaaldinone    | 1000187-75-0     | 554337      | 188.22           | C₉H₁₆O₄          | 8.97   | 8.67  | 43         |
| 8        | Hexanoic acid, 5-oxo-, methyl ester                      | Methyl ester   | 13984-50-4       | 84129       | 144.17           | C₇H₁₀O₃          | 10.93  | 8.966 | 80         |
| 9        | Monomethyl malonate                                      | Carboxylic esters | 16695-14-0       | 538366      | 118.09           | C₄H₈O₄          | 1.54   | 10.18 | 25         |
| 10       | Pentanedioic acid, dimethyl ester                        | Methyl esters  | 1119 – 40-0      | 14242       | 160.17           | C₇H₁₃O₄          | 5.99   | 10.718| 59         |
| 11       | Octanal dimethyl acetal                                 | Di-ethers      | 10022-28-3       | 61431       | 174.28           | C₁₀H₂₂O₂         | 7.43   | 11.636| 53         |
| 12       | (1-Ethylvinylax)trimethylsilane                          | Organosilicon  | 6651-40-7        | 554663      | 144.29           | C₉H₁₆OSi         | 2.02   | 12.736| 25         |
| 13       | Pentanoic acid, 5,5-dimethoxy-, methyl ester            | Methyl esters  | 23068-91-9       | 551444      | 176.21           | C₆H₁₀O₄          | 2.69   | 13.388| 37         |
| 14       | Hexanedioic acid, dimethyl ester                         | Methyl esters  | 6293-90          | 12329       | 174.19           | C₆H₁₃O₄          | 3.37   | 15.95 | 91         |
| 15       | Nonanal dimethyl acetal                                 | Di-ethers      | 18824-63-0       | 87813       | 188.31           | C₁₁H₂₂O₂         | 2.08   | 18.808| 27         |
| 16       | Heptanedioic acid, dimethyl ester                        | Methyl esters  | 1732-08-7        | 74416       | 188.22           | C₈H₁₆O₄          | 1.95   | 21.557| 95         |
| 17       | Octanedioic acid, dimethyl ester                         | Methyl esters  | 1732-09-8        | 15611       | 202.25           | C₁₀H₁₈O₄         | 1.34   | 27.388| 96         |
| 18       | Diethyl Phthalate                                        | Esters        | 84-66-2          | 6781        | 222.24           | C₁₂H₁₄O₂         | 3.97   | 35.871| 97         |
| 19       | Hexadecanoic acid, methyl ester                          | Methyl ester   | 112-39-0         | 8181        | 270.5            | C₁₇H₃₄O₂         | 3.04   | 55.308| 98         |
| 20       | Methyl stearate                                          | Fatty acid     | 112-61-8         | 8201        | 298.5            | C₁₉H₃₈O₂         | 1.85   | 60.921| 99         |
antioxidant capacity. The endophytic fungi *Aspergillus* sp. and *Neocosmospora* sp. are excellent sources of naturally derived antioxidants. However, the toxicity of these fungal extracts having antioxidant activity needs further investigation, particularly in regard to their applicability as nutraceutics and pharmaceutics. In addition, the mechanisms of antioxidant activity of these extracts required investigation in order to gain more insight into their scavenging behaviour in food and biological systems.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11557-022-01776-2.

**Acknowledgements** The authors are grateful to Ms. Deep Rajni for support with GC-MS facility.

**Author contribution** MG conceptualised and designed the work. RCM performed the experiments, carried out data analysis, manuscript drafting and editing. SKD and ND helped in the identification and phylogenetic analysis of the fungal strains. RK contributed in the GC-MS analysis. MG and CJB contributed toward the critical revision and final approval of the manuscript. All authors contributed to the article and approved the submitted version.

**Funding** The research activities of the authors are supported by The Energy and Resources Institute, India, and Deakin University, Australia. Rahul Mishra (Candidate ID — 217450416) is supported by a Deakin University HDR scholarship.

**Data availability** All data generated or analysed during this study are included in this article (and its supplementary information files). The names of the repository/repositories and accession number(s) can be found in the article/ supplementary material.

**Declarations**

**Conflict of interest** The authors declare no competing interests.

**References**

Adhami S, Farooqi H, Abdin MZ, Prasad R, Malik AA (2021) Chemical profiling of *Chlorophytum comosum* (Thunb.) jaques by GC-MS/ LC-ESIMS and its antiproliferative effects on human carcinoma cell lines. Anti Cancer Agents Med Chem 21(13):1697–1707. https://doi.org/10.2174/1871520620666201123085300

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25(17):3389–3402. https://doi.org/10.1093/nar/25.17.3389

Amaradasa BS, Madrid H, Groenewald JZ, Crous PW, Amundsen K (2014) *Parasospora seminalis* gen. et comb. nov., the causal organism of buffalograss false smut. Mycologia. 106(1):77–85. https://doi.org/10.3852/13-147

Angelone S, Bidochka MJ (2018) Diversity and abundance of entomopathogenic fungi at ant colonies. J Invertebr Pathol 156:73–76. https://doi.org/10.1016/j.jip.2018.07.009

Arabatzis M, Kambouris M, Kyprianou M, Chrysaki A, Foustoukou M, Kanellopoulou M, Kondyi L, Kouppari G, Koutsia-Karouzou C, Lebessi E (2011) Polyphasic identification and susceptibility to
seven antifungals of 102 Aspergillus isolates recovered from immuno-compromised hosts in Greece. Antimicrob Agents Chemother 55(6):3025–3030. https://doi.org/10.1128/AAC.01491-10

Axelsson BO, Saraf A, Larsson L (1995) Determination of ergosterol in organic dust by gas chromatography-mass spectrometry. J Chromatogr B Biomed Appl 666(1):77–84. https://doi.org/10.1016/0378-4347(94)00553-h

Blois MS (1958) Antioxidant determinations by the use of a stable free radical. Nature 181(4617):1199–1200

Brent R, Kingston RE, Moore DD, Seidman J, Smith JA, Struhl K (1995) Short protocols in molecular biology: a compendium of methods from current protocols in molecular biology. Current Protocols.

Caruso G, Abdelhamid MT, Kalisz A, Sekara A (2020) Linking endophytic fungi to medicinal plants therapeutic activity. A case study on Asteraceae. Agriculture 10(7):286. https://doi.org/10.3390/.../0700286

Debbab A, Aly AH, Proksch P (2013) Mango预生 fungal endophytes—a chemical and biological perception. Fungal Divers 61(1):1–27. https://doi.org/10.1007/s13225-013-0243-8

Gomes R, Glinkie C, Videira L, Gontard N, Ribeiro MAdS, Garcia A, Polonio JC, Santos CM, Silva AA, Chalvet-Monlag S, Coussy O, Debbab A, Aly AH, Proksch P (2013) Diaporthe: a genus of endophytic, saprobic and plant pathogenic fungi. Persoonia. 31:1–41. https://doi.org/10.3767/003158513X668844

Guillén-Navarro K, Herrera-López D, López-Chávez MY, Cancino-Gómez M, Reyes-Reyes AL (2015) Assessment of methods to recover DNA from bacteria, fungi and archaea in complex environmental samples. Folia Microbiol (Praha) 60(6):551–558. https://doi.org/10.14318/fm.2015.180

Heendinya SN, Keerthirathna L, Manawadu CK, Dissanayake IH, Ali R, Marshour A, Alzahabri H, Godakumbura P, Boudjelal M, Peiris DC (2020) Therapeutic efficacy of Nyctanthes arbor-tristis flowers to inhibit proliferation of acute and chronic primary human leukemia cells, with adipocyte differentiation and in silico analysis of interactions between survivin protein and selected secondary metabolites. Biomolecules 10(2):165. https://doi.org/10.3390/biom10020165

Hsieh H-M, Lin C-R, Fang M-J, Rodgers JD, Fournier J, Leachat C, Ju Y-M (2010) Phylogenetic status of Xylaria subgenus Pseudoxylaria among taxa of the subfamily Xylariaceae (Xylariaceae) and phylogeny of the taxa in the subfamily. Mol Phylogenet Evol 54(3):957–969. https://doi.org/10.1016/j.ympev.2009.12.015

Hyde K, Soytong K (2008) The fungal endophyte dilemma. Fungal Divers 33(163):e173

Irinyi L, Serena C, Garcia-Hermoso D, Arabatzis M, Desnos-Ollivier M, Vu D, Cardinali G, Arthur I, Normand A-C, Giraldo A (2015) International Society of Human and Animal Mycology (ISHAM)-ITS reference DNA barcoding database—the quality controlled standard tool for routine identification of human and animal pathogenic fungi. Med Mycol 53(4):313–337. https://doi.org/10.1093/mycopathology/mvy008

Jedidi I, Soldevilla C, Lahoura A, Marin P, Gonzalez-Jaen MT, Said S (2018) Mycoflora isolation and molecular characterization of Aspergillus and Fusarium species in Tunisian cereals. Saudi J Biol Sci 25(5):686–874. https://doi.org/10.1016/j.sjbs.2017.11.050

Ju Y-M, Rodgers JD, Hsieh H-M (2018) Xylaria species associated with fallen fruits and seeds. Mycologia. 110(4):726–749. https://doi.org/10.1080/00275514.2018.1469879

Khrali R, Yusuf M, Bassouny F, Gamal A, Madany M (2020) Phytotoxic effect of Allagi maureum on the growth and physiological activities of Pisum sativum L. S Afr J Bot 131:250–258. https://doi.org/10.1016/j.sajb.2020.02.037

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35(6):1547–1549. https://doi.org/10.1093/molbev/msy096

Lin S, Tayor NJ, Peduto Hand F (2018) Identification and characterization of fungal pathogens causing fruit rot of deciduous holly. Plant Dis 102(12):2430–2445. https://doi.org/10.1094/PDIS-02-18-0372-RE

Maduranga K, Attanayake RN, Santhirasegaran S, Weerakoong G, Panaragama PA (2018) Molecular phylogeny and bioprospiccting of endolichenic fungi (ELF) inhabiting in the lichens collected from a mangrove ecosystem in Sri Lanka. PLoS One 13(8):e0200711. https://doi.org/10.1371/journal.pone.0200711

Manganyi MC, Regnier T, Kumar A, Bezuidenhout CC, Ateba CN (2018) Phylogenetic analysis and diversity of novel endophytic fungi isolated from medicinal plant Sceletium tortuosum. Phytochem Lett 27:36–43. https://doi.org/10.1016/j.phytol.2018.06.004

Menkin A, Vasisiti R (2011) Fungi in roots of nursery grown Pinus sylvestris: ectomycorrhizal colonisation, genetic diversity and spatial distribution. Microb Ecol 61(1):52–63. https://doi.org/10.1007/s00248-010-9676-8

Mishra RC, Goel M, Barrow CJ, Deshmukh SK (2020) Endophytic fungi - an untapped source of potential antioxidants. Current Bioactive Compounds 16(7):944–964

Mishra RC, Kalra R, Dwivedi N, Goel M (2021a) Exploring endophytes using “omics”: an approach for sustainable production of bioactive metabolites. Mycoremediation Environ Sustain 3:349–376

Mishra RC, Kalra R, Dilawari R, Deshmukh SK, Barrow CJ, Goel M (2021b) Characterization of an Endophytic strain Talaromyces assimilatus, CPEF04 with evaluation of production medium for extracellular red pigments having antimicrobial and anticancer properties. Front Microbiol 12:1578

Moumni M, Allagui MB, Mancini V, Murolo S, Tarchoun N, Romanazzi G (2020) Morphological and molecular identification of seedborne fungi in squash (Cucurbita maxima, Cucurbita moschata). Plant Dis 104(5):1335–1350. https://doi.org/10.1094/pdis-04-19-0741-re

Moussa M, Ebrahim W, Bonus M, Gohike H, Mándi A, Kurtán T, Hartmann R, Kalscheuer R, Lin W, Liu Z, Proksch P (2019) Co-culture of the fungus Fusarium tricinctum with Streptomyces lividus induces production of cryptic naphthoquinone dimers. RSC Adv 9(3):1491–1500. https://doi.org/10.1039/C8RA09067J

Pang K-L, Guo S-Y, Chen I-A, Burgaud G, Luo Z-H, Dahms HU, Huang J-S, Lin Y-L, Huang J-S, Ho T-W (2019) Insights into fungal diversity of a shallow-water hydrothermal vent field at Kueishan Island, Taiwan by culture-based and metabarcoding analyses. PLoS One 14(12):e0226616. https://doi.org/10.1371/journal.pone.0226616

Polli AD, Ribeiro MAdS, Garcia A, Polonio JC, Santos CM, Silva AA, Orlandelli RC, Castro JC, Abreu-Filho BA, Cabral MR (2020) Secondary metabolites of Curvularia sp. G6-32, an endophyte of Syngonium sanranum, with antioxidant and anticholinesterase properties. Nat Prod Res. 1-6. https://doi.org/10.1080/17486491.2020.1739681

Proestos C, Bozias I, Nychas G-J, Komaitis M (2006) Analysis of flavonoids and phenolic acids in Greek aromatic plants: investigation of their antioxidant capacity and antimicrobial activity. Food Chem 95(4):664–671. https://doi.org/10.1016/j.foodchem.2005.01.049

Qadri M, Johri S, Shah BA, Khajuria A, Sidiq T, Lattoo SK, Abdin MZ, Riyaz-Ul-Hassan S (2013) Identification and bioactive potential of endophytic fungi isolated from selected plants of the Western Himalayas. SpringerPlus 2(1):8

Qu H-R, Yang W-W, Zhang X-Q, Lu Z-H, Deng Z-S, Guo Z-Y, Cao F, Zou K, Proksch P (2020) Antibacterial bisabolane sesquiterpenoids and isocoumarin derivatives from the endophytic fungus Phomopsis prunorum. Phytochem Lett 37:1–4. https://doi.org/10.1016/j.phytol.2020.03.003

Rajendran A, Nulit R, Yien CYSYS, Ibrahim MH (2017) Isolation and molecular identification of Colletotrichum gloeosporioides from infected peanut seeds. Int J of Plant Soil Sci. 1-8. https://doi.org/10.9734/IJPSS/2017/35838

Rana KL, Kour D, Yadav N, Yadav AN (2020) Endophytic microbes in nanotechnology: current development, and potential biotechnology
applications. Microbial Endophytes: 231–262. https://doi.org/10.1016/B978-0-12-818734-0.00010-3
Raza M, Zhang Z-F, Hyde KD, Diao Y-Z, Cai L (2019) Culturable plant pathogenic fungi associated in southern China. Fungal Divers 99(1): 1–104. https://doi.org/10.1007/s13225-019-00434-5
Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26(9-10): 1231–1237. https://doi.org/10.1016/s0891-5849(99)00315-3
Samson R, Yilmaz N, Houbraken J, Seifert K, Peterson S, Varga J, Frisvad JC (2011) Phylogeny and nomenclature of the genus Talaromyces and taxa accommodated in Penicillium subgenus Bverticillium. Stud Mycol 70: 159–183. https://doi.org/10.3114/sim2011.70.04
Schoch CL, Robbertse B, Robert V, Vu D, Cardini G, Irinyi L, Meyer W, Nilsson RH, Hughes K, Miller AN, Kim P, Abarenkov K, Aime MC, Arriawanwa HA, Bidartondo M, Boekhout T, Buyck B, Cai Q, Chen J et al (2014) Finding needles in haystacks: linking scientific names, reference specimens and molecular data for fungi. Database 2014: bau061. https://doi.org/10.1093/database/bau061
Sharma P, Deep S, Sharma M, Bhati DS (2013) Genetic variation of Alternaria brassicaceae (Berk.) Sacc., causal agent of dark leaf spot of cauliflower and mustard in India. J Gen Plant Pathol 79(1): 41–45. https://doi.org/10.1016/S0027-0124(11)60073-3
Shimamura T, Matsuura R, Tokuda T, Sugimoto N, Yamazaki T, Sharma P, Deep S, Sharma M, Bhati DS (2013) Genetic variation of Alternaria brassicaceae (Berk.) Sacc., causal agent of dark leaf spot of cauliflower and mustard in India. J Gen Plant Pathol 79(1): 41–45. https://doi.org/10.1016/S0027-0124(11)60073-3
Shinazato N, Namihira T, Tamaki Y, Tsukahara M, Matsuzaki T (2009) Application of random amplified polymorphic DNA (RAPD) analysis coupled with microchip electrophoresis for high-resolution identification of Monosporascus strains. Appl Microbiol Biotechnol 82(6):1187. https://doi.org/10.1007/s00253-009-1937-4
Silambarasan S, Abraham J (2013) Ecofriendly method for bioremediation of chlorpyrifos from agricultural soil by novel fungus Aspergillus terreus JAS1. Water Air Soil Pollut 224(1): 1369. https://doi.org/10.1007/s11270-012-1369-9
Sivaraman G, Visvuvinayagam S, Jha A, Remya S, Renuka V, Ajeej K, Vanik D (2018) Molecular identification and differentiation of Aspergillus species in dry fishes of Gujarat, India. Proc Natl Acad Sci, India, Sect B Biol Sci 88(2): 505–515. https://doi.org/10.1007/s40011-016-0779-y
Skalskas DN, Badotti F, Vaz ABM, da Silva FF, Gazis R, Wurdack K, Castlebury L, Góes-Neto A, Chaverri P (2019) Exploration of stem endophytic communities revealed developmental stage as one of the drivers of fungal endophytic community assemblages in two Amazonian hardwood genera. Sci Rep 9(1): 12685. https://doi.org/10.1038/s41598-019-48943-2
Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10(3): 512–526. https://doi.org/10.1093/oxfordjournals.molbev.a040023
Travador R, Pascall L, Barka D, Daniel P L., David R, Hernán O, Patrice R and Kendra B (2016). Grapevine pruning systems and cultivars influence the diversity of wood-colonizing fungi. Fungal Ecol 24: 82-93. https://doi.org/10.1016/j.funecco.2016.09.003
Velanganni J, Kadamban D, Ramamoorthy D (2011) Phytochemical screening and antimicrobial activity of the stem of Mallotus philippinus (Lam.) Muell. Arg Var Philippensis. Int J Pharm Pharm Sci 3(2): 160e163
Vu D, Groenewald M, De Vries M, Gehrman T, Stielow B, Eberhardt U, Al-Hatimi A, Groenewald J, Cardinalia G, Houbraken J (2019) Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. Stud Mycol 92: 135–154. https://doi.org/10.1016/j.simyco.2018.05.001
Xing HQ, Ma JC, Xu BL, Zhang SW, Wang J, Cao L, Yang XM (2018) Mycobiota of maize seeds revealed by rDNA-ITS sequence analysis of samples with varying storage times. Microbiologyopen. 7(6): e00609. https://doi.org/10.1002/mbo3.609
Xiu-Qin L, Chao J, Yan-Yan S, Min-Li Y, Xiao-Gang C (2009) Analysis of synthetic antioxidants and preservatives in edible vegetable oil by HPLC/TOF-MS. Food Chem 113(2): 692–700. https://doi.org/10.1016/j.foodchem.2008.07.072
Yadav V, Singh A, Mathur N, Yadav R (2020) Isolation and characterization of Alternaria GFAV15, an endophytic fungus from green fruit of Tinospora cordifolia (Willd.) Miers from semi-arid region. S Afr J Bot 134: 343–348. https://doi.org/10.1016/j.sajb.2020.04.003
Zhang P, Qiao YL, Deng JX, Ma D (2020) First report of Alternaria alternata causing leaf spot on Kadsura coccinea in China. Plant Dis 104(11): 1073. https://doi.org/10.1094/PDIS-01-20-0111-PDN
Zhang S, Liang W, Yang Q (2018) First report of Alternaria alternata causing leaf spot on Cotinus coggygria in China. Plant Dis 102(12): 2644. https://doi.org/10.1094/PDIS-05-18-0718-PDN
Zhao D-L, Wang D, Tian X-Y, Cao F, Li Y-Q, Zhang C-S (2018) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26(9-10): 1231–1237. https://doi.org/10.1016/s0891-5849(99)00315-3
Publisher’s note Springer Nature remains neutral with regard to jurisdic- tional claims in published maps and institutional affiliations.