Journal of Umm Al-Qura University for Applied Sciences (2024) 10:301–312
https://doi.org/10.1007/s43994-023-00105-x

ORIGINAL ARTICLE

Effects of temperature and water activity on 25 de novo strains of pathogenic plant fungi in Al-Baha and Baljurashi cities in Saudi Arabia

Bandar Almiman[1]

Received: 2 August 2023 / Accepted: 6 November 2023 / Published online: 12 December 2023
© The Author(s) 2023

Abstract
Fungi inflict a great deal of damage to crops in fields and in storage facilities, causing issues such as leaf spot, wilt, rust, dieback and rot, as well as releasing mycotoxins that taint vegetation. In the current study, 25 de novo fungal isolates were taken from infected plant tissue (leaf, root and fruit), at pre- and post-harvest stages. Isolates were identified using molecular markers; 8 genera and 15 species were determined. The most common species was *Penicillium* spp. (40%), *Aspergillus* spp. (20%), *Fusarium* spp. (16%) and *Alternaria alternata* species (8%). The remaining 16% was comprised of various types of fungi, including *Geotrichum candidum*, *Neofusicoccum parvum*, *Rhizopus stolonifera* and *Mucor fragilis*. Many of these genera are known to cause significant crop damage and are notorious mycotoxin producers. An evaluation of the optimal growth temperature revealed the ideal temperatures were 30 °C for 56% of isolates, 25 °C for 28% of isolates and 20 °C for 16% of isolates. An assessment of water activity showed that 60% of isolates belonged to *Penicillium* and *Aspergillus* spp. and were mesophilic and xerophilic. Another 28% of isolates were *Fusarium* spp., *Geotrichum candidum*, *Neofusicoccum parvum* and *Mucor fragilis*, and hydrophilic. The remaining 12%, representing *Alternaria alternata* and *Rhizopus stolonifera*, were mesophilic. The current study provides accurate eco-physiological response data and molecular information for each isolate. The findings can assist the development of novel approaches to control the expansion of invasive fungal infections and minimise their deleterious consequences.

Keywords Plant diversity · Mycotoxigenic species · Fungal pathogen · Optimal temperature and water activity · ITS rDNA molecular identification · NCBI GenBank

1 Introduction

The climactic conditions and diverse geological features of the Kingdom of Saudi Arabia (KSA) support a diverse range of plant life forms [40]. The nation is home to around 2,300 plant species, belonging to 142 families [12, 19].

The Al-Baha province in southwestern KSA has six major cities: Al-Baha, Alaqiq, Almandaq, Almikhwah, Baljurashi and Qilwah. The capital of this territory is Al-Baha [11]. The region has a diverse ecological topology, with forests, mountains, ravines and areas of abundant vegetation. Al-Baha has more than 320 species of flora, comprising members of 228 genera and 75 families. Among these are many fruits, vegetables, grains and plants with medicinal applications [10]. The region has a long tradition of farming, of both native and introduced crops [19]. Consequently, the presence (or otherwise) of fungi in Al-Baha is an important issue.

Worldwide, fungi routinely induce catastrophic levels of disease in vegetation during growth and in storage. The world's annual maize harvest, for instance, is sometimes halved as a result of fungal infection [23]. In addition, fungal organisms taint plants with mycotoxins, which can harm both animals and humans. Such harm is widely acknowledged. The International Agency for Research has highlighted the tumorigenic potential of mycotoxins [37], and the Food and Agriculture Organization has estimated that approximately half of all crops worldwide are tainted with mycotoxins in a typical year [23].

✉ Bandar Almiman
Balmiman@bu.edu.sa

[1] Department of Biology, Faculty of Science, Al Baha University, Alaqiq, Al Baha 65779-7738, Kingdom of Saudi Arabia

More than 400 mycotoxins are currently known. These include aflatoxins, ochratoxins, trichothecenes and fumonisins [48]. The extent of damage caused by mycotoxins varies by their type: *Aspergillus*, *Penicillium* and *Fusarium* spp. are recognised as causing very extensive harm to the quality and yield of crops, and have been classified among the five most hazardous species in terms of mycotoxin production.

Dates are a major crop in KSA. The country has approximately 28 million palm trees, which annually produce about 14% of the world's dates. Research has found that between 2.7 and 33% of KSA-grown dates are lost or contaminated as a result of fungal infection [22, 52]. Some Saudi date crops have been contaminated with mycotoxin arising from *Fusarium* spp. [7, 8]. Crops imported into KSA have also been found to carry mycotoxins and/or have been affected by fungi. *Aspergillus niger*, *A. flavus*, *Colletotrichum musae* and *Pencillium* species are toxicogenic species that have been identified in imported vegetation [1, 3].

Several nations are considering the banning of various pesticides and fungicides from their markets [21]. This is frequently due to the association of such substances with adverse effects on humans and animals, and their deleterious effects upon advantageous, insects and fungi. Thus, the hunt is now on for alternative approaches that can eliminate or restrict the effects of harmful fungal agents [17]. Some nations have already sought to protect their vegetation from dangerous microorganisms by using more environmentally-friendly options. For example, countries such as Indonesia, Canada and Sweden have deployed various plant extracts and biological and environmental constraints [43, 47].

Fungi are influenced by ambient conditions, which can support or restrict their behaviours. An ability to recognise the conditions that deter problematic phytopathogens is indispensable to those who must protect crops during their growth and storage. The most influential conditions for fungal activity are temperature and the amount of water present. These factors affect the levels of growth, spore formation and mycotoxin synthesis. Water and temperature also influence the ability of crops to fight mycotoxin and fungal infection [7].

The ambient temperature preferences of fungi vary by species and even strains within a species. For example, Hope et al. [27] demonstrated that the preferred temperature for growth of two strains from *Fusarium culmorum* differed by 10°C. However, all fungi have one objective, which is to optimise their biological status and outcomes, for example through sporulation, mycelium growth and the ability to extract maximum benefit from the plants that they target [26, 36].

Water is a key determinant of the chemical and physical properties of crops. Its availability can also be used to control microbial activity. The availability of water, for example, in ambient air, in food or food products, is measured in units of water activity (aw) [16, 50]. Fungi are influenced by water activity, and have been classified into three groups according to their optimal water levels. Hydrophilic fungi favour a level greater than 0.90 aw, whereas the mesophilic group prefers water levels between 0.85 and 0.90 aw. The final group, xerophilic fungi, grow best between 0.65 and 0.85 aw [38]. In favourable water activity conditions, fungal spores thrive, reducing crop yields and contaminating plants with mycotoxins [32, 50].

De novo fungal species and strains are identified precisely using molecular techniques, which is more accurate than the traditional approach of classification based upon of appearance [59]. Molecular identification methods include the use of gel electrophoresis and detecting novel sequences for BLAST analysis. This allows the identification of features that resemble those of species sequences stored in repositories such as UNITE and the *National Centre for Biotechnology Information (NCBI)* [49].

It is generally acknowledged that internal transcribed space (ITS) markers are the gold-standard indicators for the identification of fungi species. The ITS markers used for this purpose operate at the locus between small and large ribosomal RNA sub-units 18S, 5.8S and 28S [62]. These markers have the advantage of specificity,the conserved area within the ITS marker is almost universally seen in fungal genomes. Its polymerase chain reaction (PCR) fragment is within the range of 400–900 base pairs [13, 20]. The use of ITS1 and ITS4 primers to identify fungi is widespread, because there is substantial overlap between the 18S and 28S sub-units, and this is an area in which there is a great deal of heterogeneity among species [64].

By 2012, 175,000 lengths of ITS sequences had been linked to more than 15,000 species of fungi and stored in GenBank [56]. However, five years later the accessible data still limited, less than 1% of more than 5 million fungal species known to exist [49]. There is widespread agreement that ITS markers should generate a sequence homology of between 95 and 100% before de novo isolates be assigned to a category [35].

In this context, it should be noted that while contemporary research has done much to characterise regional species and to trace phylogenetic links with reference sequence isolates, it has rarely focused on the conditions required to promote or restrict the growth and behaviour of fungi. The current paper therefore seeks to fill this gap in the literature and to contribute to the understanding of this aspect.

2 Materials and methods

2.1 Sample collection sites and sampling

Samples were collected from 25 crop production and storage sites in the cities of Al-Baha and Baljurashi. Al-Baha is

situated 2400 m above sea level, at longitudinal 41° 28′ 4 E and latitude 20° 0′ 46 N [11]. Baljurashi (19° 51′ N, 41° 33′ E) is 2000 m above sea level (Fig. 1). Consent for access was secured from all site owners. Multiple fungal samples were taken from the sites. The aim was to identify the predominant fungal pathogen at each of the 25 sites. Great care was taken to avoid the inadvertent inclusion of transient isolates, such as surface spores carried by wind onto soil or plant parts.

2.2 Isolation of fungi and preparation of stock culture

The gathered samples were rinsed in tap water, surface disinfected for 2 min in 1% sodium hypochlorite solution, then rinsed thoroughly in distilled water. The surfaces of the infected tissues (leaf, root and fruit) were scraped with a sterilised loop, to facilitate the transfer of spores and mycelia to a potato dextrose agar (PDA) medium. The spores and mycelia were cultured in PDA for 5 days at 25 °C. After this period, a 0.5 mm-sized inoculum was extracted from the edge of the culture and transferred to a fresh plate containing PDA medium, which was then sub-cultured to attain a pure isolate [1, 54]. Stock cultures of the de novo isolates were made up and kept in the stock culture bank et al.-Baha University. Table 1 describes the new isolates.

2.3 DNA extraction

Sub-culture of the colonial mycelia was carried out in potato dextrose broth to provide a greater yield of DNA. Tissue was then pulverised into a powder with a pestle and mortar using liquid nitrogen. A 150–200 mg sample was placed in a 2-ml Eppendorf tube and DNA extraction was performed using GeneJET Plant Genomic DNA Purification (Thermo Fisher Scientific®) in accordance with the manufacturer's recommendations. The DNA was stored at − 20 °C until required.

2.4 Molecular identification and constructing phylogenetic tree

The 20 µl and 50 µl PCR protocols were established using forward (ITS1: TCCGTAGGTGAACCTGCGG) and reverse (ITS4: TCCTCCGCTTATTGATATGC) primers. The following conditions were applied: 35 cycles of initial denaturation, 95 °C for 3 min; denaturation, 94 °C for 1 min;

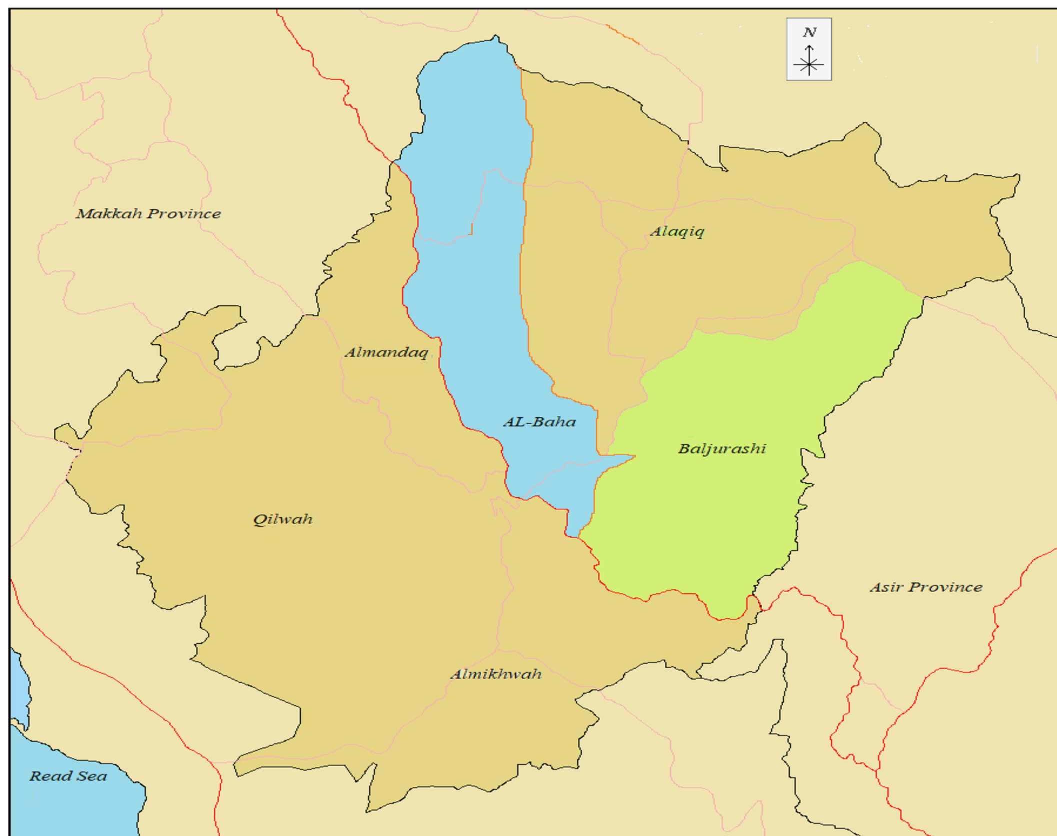

Fig. 1 Sampling sites for the 25 novel fungal isolates, taken from infested crops at post and pre-harvest conditions in Al-Baha (17 isolates) and Baljurashi (8 isolates)

Table1 Details of the 25 new isolates, with code from Al-Baha University culture collection and their NCBI accession numbers

	Fungal species	Host	Tissue part	Location	Code	Origin	NCBI accession number
1	*Penicillium expansum*	*Punica granatum*	Fruit	Storage	BHU143A	Al-Baha	OQ357555.1
2	*Penicillium expansum*	*Ocimum basilicum*	Leaf	Storage	BHU150	Al-Baha	OQ359096.1
3	*Rhizopus stolonifera*	*Punica granatum*	Root	Field	BHU143B	Al-Baha	OQ371313.1
4	*Penicillium glabrum*	*Psidium guajava*	Fruit	Storage	BHU110	Al-Baha	OQ357606.1
5	*Penicillium glabrum*	*Psidium guajava*	Fruit	Storage	BHU134	Al-Baha	OQ359094.1
6	*Alternaria alternata*	*Punica granatum*	Leaf	Field	BHU114	Al-Baha	OQ357616.1
7	*Alternaria alternata*	*Solanum lycopersicum*	Fruit	Field	BHU163	Al-Baha	OQ360641.1
8	*Fusarium verticillioides*	*Psidium guajava*	Fruit	Field	BHU135	Al-Baha	OQ359095.1
9	*Aspergillus flavus*	*Ficus palmata*	Leaf	Field	BHU161	Al-Baha	OQ360639.1
10	*Fusarium equiseti*	*Solanum melongena*	Root	Field	BHU165	Al-Baha	OQ360646.1
11	*Geotrichum candidum*	*Morus rubra*	Fruit	Storage	BHU162	Al-Baha	OQ360640.1
12	*Neofusicoccum parvum*	*Persea americana*	Leaf	Field	BHU167	Al-Baha	OQ360655.1
13	*Mucor fragilis*	*Phaseolus vulgaris*	Fruit	Storage	BHU170	Al-Baha	OQ368728.1
14	*Penicillium crustosum*	*Daucus carota*	Fruit	Storage	BHU171	Al-Baha	OQ368729.1
15	*Fusarium oxysporum*	*Allium fistulosum*	Fruit	Field	BHU164	Al-Baha	OQ360645.1
16	*Penicillium commune*	*Punica granatum*	Fruit	Storage	BHU074B	AL-Baha	OR046062
17	*Fusarium oxysporum*	*Prunus persica*	Root	Field	BHU007	Al-Baha	ON845734
18	*Aspergillus niger*	*Vitis vinifera*	Fruit	Storage	BHU012	Baljurashi	OR046054
19	*Penicillium glabrum*	*Solanum melongena*	Fruit	Storage	BHU137	Baljurashi	OR047862
20	*Penicillium glabrum*	*Punica granatum*	Fruit	Storage	BHU112	Baljurashi	OR046540
21	*Aspergillus flavus*	*Eriobotrya japonica*	Fruit	Storage	BHU120	Baljurashi	OR046564
22	*Aspergillus nidulans*	*Malus*	Fruit	Storage	BHU121	Baljurashi	OR048817
23	*Aspergillus flavus*	*Prunus armeniaca*	Fruit	Storage	BHU130	Baljurashi	OR047841
24	*Penicillium glabrum*	*Prunus persica*	Fruit	Storage	BHU131	Baljurashi	OR047842
25	*Penicillium glabrum*	*Prunus persica*	Fruit	Storage	BHU132	Baljurashi	OR047843

annealing, 60 °C for 1 min; extension, 72 °C for 1 min; final extension, 72 °C for 5 min. The final product was stored at 16 °C until required.

For the 50 µl PCR, 2.5 µl DNA, 2.5 µl of 20 µM forward primer, 2.5 µl of 20-µM reverse primer, 5 µl 10× reaction buffer, 1 µl dNTPs, 3 µl $MgCl_2$ and 0.25 µl Taq DNA polymerase were made up to 50 µl with free DNAase and RNAase water. The 20 µl PCR used the same combination, but in modified quantities. To create a negative control, the DNA was substituted with free DNAase and RNAase water. Once the PCR cycles had concluded, 5× loading dye was admixed with the PCR yield.

Then, 1 mg agarose powder was made up to a solution with 100 ml 1× TAE buffer, and heated for 2 min in a microwave oven; 5 µl of 10-mg/ml ethidium bromide was admixed. The resulting material was decanted onto a gel tray to cool and to form a solid agarose gel. The latter was then added to a gel electrophoresis tank containing 1× TAE buffer. This was connected to 90 V power for an hour (Fig. 2).

The kite protocol (Qiagen QIAquick PCR Purification Kit) was applied to purify the amplicon products. These were then dispatched to an external sequencing service (Macrongen Inc.). Sequence dimensions and quality were verified using the trace information; ambiguous bases were discarded. With the resultant data in the FASTA format, species were identified using BLAST analysis on NCBI or UNITE [49]. Any GenBank sequences exhibiting a similarity ≥ 96% were downloaded as a reference sequence for the phylogenetic element of the research. Table 2 provides details of the reference isolates [35]. Geneious Prime software, version 2022.2 (developed by Biomatters) was used

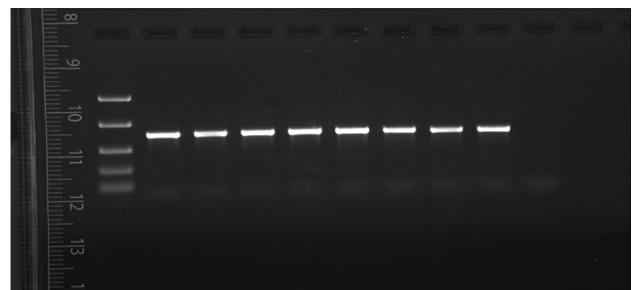

Fig. 2 Gel electrophoresis showing PCR products that confirm the isolates' fungal identity. Based on the DNA ladder, the amplicons are approximately 600–750 bp in length

to create a phylogenetic tree from the multiple sequence alignments generated by the package's MUSCLE alignment tool, and derived from the original and reference sequences (Fig. 3). The Geneious tree builder algorithm was applied to create a phylogenetic tree founded on distance tree and neighbour joining. The Tamura-Nei model provided the genetic distance of the tree. To attain a consensus phylogenetic tree, analysis was carried out based on parsimony with a bootstrap support value set within the range 70–100%. To achieve best fit, the general time-reversible evolutionary model, including 500 bootstrap applications, was applied [6, 29].

The 25 new sequences identified in this research were placed in the NCBI GenBank. All isolates were assigned their own accession number (Table 1). These sequences were uploaded following verification of sequence quality and quantity, confirmation that the submission parameters had been met, and filing of the apposite data for the respective sequences individually.

2.5 Evaluation of growth at three temperatures

The 25 new isolates were each grown in three ambient temperatures. These were 20 °C, 25 °C and 30°C. The objective was to establish each strain's preferred conditions. To begin isolation of the colony cultures and subcultures, 9-cm PDA Petri dishes were used, and 5-mm inoculum discs were placed at the centre point of fresh PDA Petri dishes. Five replicates for each isolate were cultured in an incubator at the given temperatures for 6 days. At that point, the radial growth dimensions as mm/day were evaluated for each sample. Figure 4 presents the growth data as a bar chart; replicate variation is indicated by the error bar [26, 36].

2.6 Growth evaluation at four points of water activity

Four sets of PDA media were prepared by adding a suitable amount of glycerol (Sigma Aldrich G9012) to create simulated media for four different water activities. These conditions were 0.995, 0.99, 0.90 and 0.85 aw. The amount of glycerol was added to 1 L of PDA medium as follows; 22.08 ml for 0.995 aw, none for 0.99 aw (as supplied by PDA manufacturer; this was used as a control), 404.8 ml for 0.90 aw and 625.6 ml for 85 aw.

Testing was conducted with an AQUALAB water activity meter. The media were poured into 9 cm petri dishes and inculcated with a 5 mm disc of fresh growth culture. Five replicates for each water activity were made for each of the 25 isolates. Each isolate was incubated at its optimal growth

Table 2 The NCBI GenBank reference sequences information

	Fungal species	Host	Code	Origin	NCBI accession number
1	*Penicillium expansum*	*Malus domestica*	P36	Qatar—Doha	KJ933300.1
2	*Penicillium expansum*	*Pyrus communis*	YC-IK11	China—Beijing	MK850332.1
3	*Penicillium expansum*	*Vitis vinifera*	PUCV1024	Chile—Quillota	MT218335.1
4	*Rhizopus stolonifera*	*Pyrus communis*	AR7	Indonesia—Jakarta	MF461026.1
5	*Penicillium glabrum*	*Allium cepa*	ORAM981	Turkey—Antakya	MT956624.1
6	*Penicillium glabrum*	Quercus robur	11.23	Croatia—Zagreb	MW540449.1
7	*Alternaria alternata*	*Manilkara zapota*	F10	India—Maharashtra	OL711959.1
8	*Alternaria alternata*	*Capsicum annuum*	BHU115	Saudi Arabia—Al-Baha	ON844336.1
9	*Fusarium verticillioides*	*Annona squamosa*	FLZ-3	China—Liuzhou	ON329035.1
10	*Aspergillus flavus*	*Rosa damascene*	RDE120	India—Kashmir	OP225474.1
11	*Fusarium equiseti*	*Solanum lycopersicum*	PAK-16	Northern Pakistan	MH054926.1
12	*Geotrichum candidum*	*Fragaria ananassa*	20–50	USA—Florida	MT353978.1
13	*Neofusicoccum parvum*	*Mangifera indica*	CMW28426	South Africa—Limpopo	KU997426.1
14	*Mucor fragilis*	*Nicotiana tabacum*	7P1	China—Guizhou	MZ413231.1
15	*Penicillium crustosum*	*Juniperus communis*	TUEF19	Saudi Arabia—Taif	HG798727.1
16	*Fusarium oxysporum*	*Solanum tuberosum*	AM7	Russia—Kazan	MN560044.1
17	*Penicillium commune*	*Ageratina Adenophora*	DCS12	China—Chengdu	MZ047535.1
18	*Aspergillus niger*	*Allium sativum*	BERG-11	Pakistan	MW411863.1
19	*Aspergillus flavus*	*Sorghum bicolor*	Beca_60	Australia—Perth	KY234272.1
20	*Aspergillus nidulans*	*Oryza sativa*	PANCOM1	India—Cuttack	MT007526.1
21	*Fusarium oxysporum*	*Allium cepa*	OLAM3	Turkey—Antakya	MT967273.1
22	*Penicillium glabrum*	*Punica granatum*	BHU068	Saudi Arabia—Al-Baha	ON843628.1
23	*Aspergillus flavus*	*Lycium barbarum*	GFR04	China—Gansu	MT447509.1

temperature. Radial growth measurements were taken as described above [6, 34].

3 Results and discussion

3.1 Molecular identification using its genetic marker and phylogenetic analysis

In this study, the ITS marker was initially used to confirm that the samples were fungal isolates. This was done by inspecting the PCR products on the electrophoresis gel (Fig. 2). Subsequently, the amplicon sequences for each isolate were used for blast analysis in GenBank NCBI, to identify their fungal species (Table 1).

Phylogeny derived from ITS genetic markers was used to determine the genotypes of 48 isolates. Of these, 25 were de novo and 23 were reference strains. The reference strains represented organisms from a spectrum of hosts and geographic locations (Tables 1 and 2). The topology of the Geneious phylogenetic tree, which was constructed using the neighbour-joining tree builder algorithm, revealed the hierarchical distribution of the 48 isolates that were being investigated. This ranged from 3 nodes to 2 clades to 2 major groups to 2 minor groups, 2 primary subgroups and 3 secondary subgroups (Fig. 3).

Nodes 1, 2 and 3 showed bootstrap support value (BSV) affinity of 99–100%. Both the first and third nodes were monophyletic groups, with the first node being representative of two taxa, BHU143B and AR7, which are members of the species *Rhizopus stolonifera*. The third node had two taxa, BHU170 and 7P1, belonging to the species *Mucor fragilis.* The second node encompassed the majority of taxa (44/48) and was distributed across two clades, A and B. In turn, A and B comprised 2 and 42 taxa, respectively. The majority of taxa represented monophyletic groups (i.e. they shared a common ancestor). Just 11 taxa were polyphyletic (i.e., had no common ancestor).

The first clade, A, comprised two taxa members of the *Geotrichum candidum* species. In contrast, the second clade, B, was composed of two major groups (B1 and B2). The group B1 had four taxa belonging to the *Alternaria alternata*

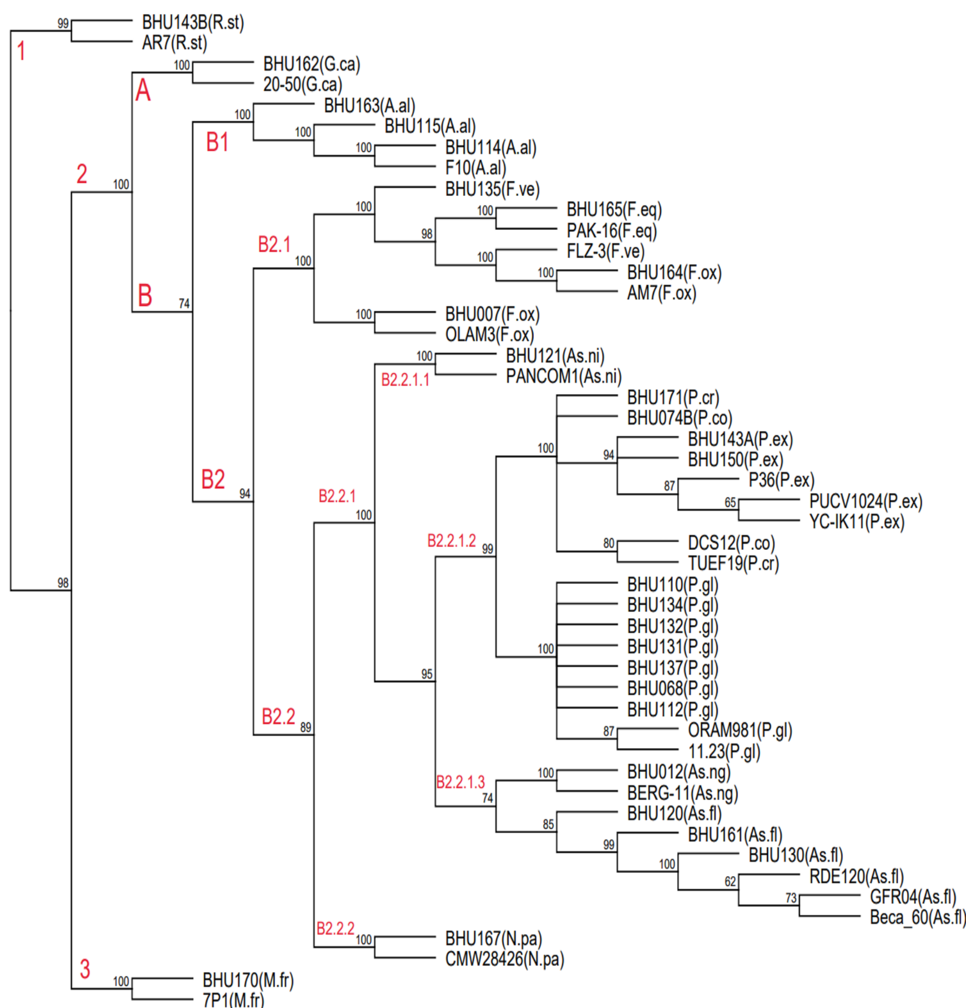

Fig. 3 Neighbour-joining consensus tree using ITS genetic markers. An abbreviation of each species' name is in brackets, followed by the isolate's code

species. The second major group, B2, had the largest number of taxa (38 out of 48).

These were further divided into two minor groups, B2.1 and B2.2. The minor group, B2.1, included 8 taxa representing the three species of the genus *Fusarium* at BSV 98–100%.

The other minor group, B2.2, was divided into two primary subgroups (B2.2.1 and B2.2.2). The first primary subgroup, B2.2.1, generated three secondary subgroups (B2.2.1.1, B2.2.1.2 and B2.2.1.3). The second primary subgroup, B2.2.2, included two taxa belonging to the species *Neofusicoccum parvum.*

The first secondary subgroup (B2.2.1.1) had two taxa belonging to *Aspergillus niger.* The second secondary subgroup (B2.2.1.2) has 18 taxa, with 7 out of 18 being monophyletic and the remaining 11 taxa were polyphyletic. The third secondary subgroup (B2.2.1.3) included eight taxa belonging to *Aspergillus nidulans* and *Aspergillus flavus* species at BSV 73–100% (Fig. 3).

The 15 species and 8 genera were described according to the molecular features and phylogenetic relationships identified through use of the ITS genetic markers (Fig. 3). Although ITS indicators have greatly enhanced scholars' ability to distinguish between isolates from different species, this process may be less effective for differentiating isolates from closely related species. In the present study, most groups, (i.e. 1, 3, A and B1) revealed isolates from different species. Less genetic variation was seen within groups B2.1 and B2.2.1.3, which contained a number of strains from single species, namely *Fusarium verticillioides, Fusarium equiseti, Fusarium oxysporum, Aspergillus niger* and *Aspergillus flavus.*

These findings reflect the previously expressed view that the ITS markers are better for interspecies differentiation than they are for intraspecies differentiation. Several scholars have considered this issue with regard to the species *Fusarium* and *Penicillium* [39, 41, 42, 57]. However, for some species, such as *Alternaria and Aspergillus*, ITS markers offer a precise distinction both between and within species [5, 14, 25, 63].

3.2 Optimal temperature for growth of new isolates

The 25 new isolates were representative of 15 species and 8 genera. All were incubated for six days, and their growth activity in three ambient temperatures (20 °C, 25 °C and 30 °C) was quantified. The radial growth was measured for each of the five replicates, to create an error bar chart (Fig. 4 and Table 3).

Each of the new strains was allocated to one of three groups according to their optimal temperature as measured via growth outcomes. The first group comprised species

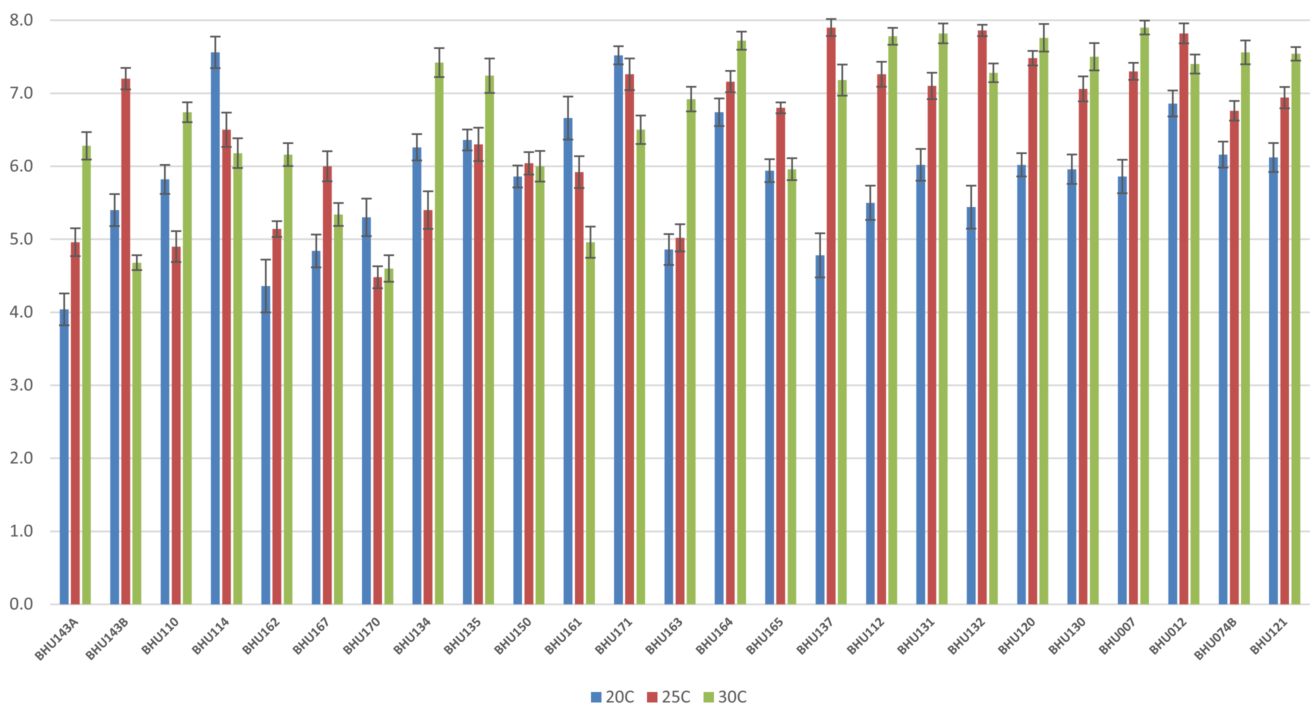

Fig. 4 The growth rates of the new 25 isolates originated from Al-Baha and Baljurashi cities were measured at variable temperatures 20 °C, 25 °C and 30 °C. Five replicates of each sample were exposed to the different temperature and their radial growth was measured. The error bar was initiated for the mean growth of five replicates

isolates that grew optimally at 20 °C, which included *Alternaria alternata* (BHU114), *Aspergillus flavus* (BHU161), *Mucor fragilis* (BHU170) and *Penicillium crustosum* (BHU171). The second group of isolates grew optimally at 25 °C and included *Penicillium expansum* (BHU150), *Penicillium glabrum* (BHU132 and BHU137), *Rhizopus stolonifer* (BHU143B), *Fusarium equiseti* (BHU165), *Neofusicoccum parvum* (BHU167) and *Aspergillus niger* (BHU012). The third group had 14 isolates, which grew optimally at 30 °C. These included *Penicillium expansum* (BHU143A), *Penicillium glabrum* (BHU110, BHU112, BHU131 and BHU134), *Alternaria alternata* (BHU163), *Fusarium verticillioides* (BHU135), *Aspergillus flavus* two strains (BHU120 and BHU130), *Geotrichum candidum* (BHU162), *Fusarium oxysporum* (BHU007 and BHU164), *Penicillium commune* (BHU074B) and *Aspergillus nidulans* (BHU121). Figure 4 and Table 3 give further details.

Table 3 The optimal temperature and water activity of each isolate collected in this study

N	aw_{opt}[b]/ growth rate (mm)	T_{opt}[a] (°C)/ growth rate (mm)	Isolate code	Fungal species
1	0.8513.9	3010.4	BHU143A	*Penicillium expansum*
2	0.8510.7	2510	BHU150	*Penicillium expansum*
3	0.9012.8	2512	BHU143B	*Rhizopus stolonifera*
4	0.8512.7	3011.2	BHU110	*Penicillium glabrum*
5	0.8512	3012.3	BHU134	*Penicillium glabrum*
6	0.9013.3	2012.6	BHU114	*Alternaria alternata*
7	0.9012.1	3011.5	BHU163	*Alternaria alternata*
8	0.99512.6	3012	BHU135	*Fusarium verticillioides*
9	0.9011.8	2011.1	BHU161	*Aspergillus flavus*
10	0.99512	2511.3	BHU165	*Fusarium equiseti*
11	0.99513.5	3010.2	BHU162	*Geotrichum candidum*
12	0.99512.8	2510	BHU167	*Neofusicoccum parvum*
13	0.99513.6	208.8	BHU170	*Mucor fragilis*
14	0.8513.3	2012.5	BHU171	*Penicillium crustosum*
15	0.99513.5	3012.8	BHU164	*Fusarium oxysporum*
16	0.8513.4	2513	BHU012	*Aspergillus niger*
17	0.8514.2	2513.1	BHU137	*Penicillium glabrum*
18	0.8513.3	3012.6	BHU074B	*Penicillium commune*
19	0.8514	3012.9	BHU112	*Penicillium glabrum*
20	0.8514	3012.9	BHU120	*Aspergillus flavus*
21	0.8513.7	3012.5	BHU121	*Aspergillus nidulans*
22	0.8513.5	3012.5	BHU130	*Aspergillus flavus*
23	0.8514.1	3013	BHU131	*Penicillium glabrum*
24	0.8514.1	2513.1	BHU132	*Penicillium glabrum*
25	0.99513.8	3013.1	BHU007	*Fusarium oxysporum*

[a]Optimal temperature (T_{opt})

[b]Optimal water activity (aw_{opt})

It is notable that for 14 of the 25 isolates, the temperature of 30 °C provided optimal growth conditions, while seven others flourished at 25 °C, and only four grew best at 20 °C (Fig. 4 and Table 3). Variation between growth rates appeared most extreme between species, and less diverse within species. The fact that 14 isolates favoured the higher temperature of 30 °C was unsurprising, since this replicates the temperature commonly found in their original geographical habitat.

Fungi can grow in wide range of temperatures, mostly between 15 and 42 °C, but most strains favour a temperature between 20 and 30 °C. Optimal growth temperature is largely influenced by the dominant temperature in the fungus's original geographical location [33, 46]. For example, fungal strains in Saudi Arabia prefer a growth temperature range between 25 and 30 °C, which is a moderate to high temperature. In Europe, fungi thrive in temperatures that fall between cool and moderate levels [7]

Several researchers have indicated that the temperature range of 30–35 °C is favoured by *Aspergillus* species (e.g. *A. flavus* and *A. niger*) [5, 25]. Meanwhile, 25 °C has been found to be the ideal temperature for the species *P. camemberti* and *P. roqueforti* [15, 59]. In Europe, temperatures generally range between 15 and 25 °C (i.e. cool to moderate) and are preferred by isolates belonging to the *Fusarium* species within several European habitats [27, 53].

3.3 Optimal water activity for growth

The 25 new isolates were also assessed for their growth response at four water activity points, which were 0.995, 0.99, 0.90 and 0.85 aw, as well as at their optimal temperatures over 6 days of incubation (Fig. 5 and Table 3).

In the current study, 10 strains of *Penicillium* spp. showed optimal growth at 0.85 aw. These were *Penicillium expansum* species (BHU143A and BHU150), *Penicillium glabrum* species (BHU110, BHU112, BHU131, BHU132, BHU134 and BHU137), *Penicillium crustosum* species (BHU171) and *Penicillium commune* (BHU074B). Additionally, five strains of *Aspergillus* spp. also showed optimal growth at 0.85 aw; these were *Aspergillus flavus* species (BHU120, BHU130 and BHU161), *Aspergillus niger* species (BHU012) and *Aspergillus nidulans* species (BHU121). Two strains of *Alternaria alternata* (BHU114 and BHU163) and one strain of *Rhizopus stolonifer* (BHU143B) grew best at 0.90 aw. Meanwhile, four isolates of *Fusarium* spp., *Fusarium verticillioides* (BHU135), *Fusarium equiseti* (BHU165) and *Fusarium oxysporum* (BHU007 and BHU164), achieved their highest growth rates at 0.995 aw. One strain of *Neofusicoccum parvum* (BHU167), one strain of *Mucor fragilis* (BHU170) and one strain of *Geotrichum candidum*

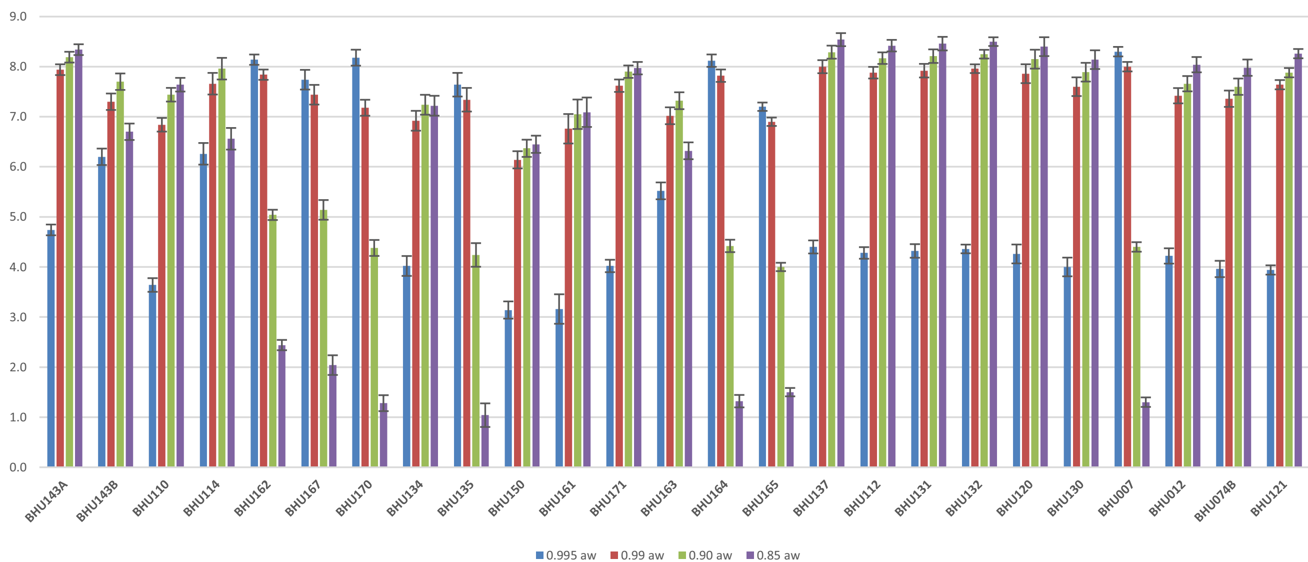

Fig. 5 Growth rates of the new 25 isolates from Al-Baha and Baljurashi, measured at variable water activities 0.995, 0.99, 0.90 and 0.85 aw. Five replicates of each sample were exposed to the different water activities and their radial growth was measured. The error bar was initiated for the mean growth of five replicates

(BHU162) grew best at 0.995 aw. Further details are in Fig. 5 and Table 3.

The growth attainment across all strains indicates substantial variability among isolates from different genera, yet low variability among isolates of the same or related species. In general, a water activity level between 0.90 and 0.99 aw seems to suit most strains, whereas 0.85 aw dramatically restricts growth in some hydrophilic species. For example, the isolates of *Fusarium*, *Neofusicoccum*, *Mucor* and *Geotrichum* genera were classified as hydrophilic because they grew best at the highest level of moisture, at 0.995aw. This finding agreed with several other published works that have found the optimal water activity for growth ranges is between 0.99 and 0.95 aw for hydrophilic fungi, such as *Fusarium* and *Geotrichum* [4, 45, 61]. In contrast, the isolates belonging to *Rhizopus* and *Alternaria* genera were classified as mesophilic because they thrived at a median level of moisture, around 0.90 aw. *Rhizopus stolonifera* and *Alternaria alternata* grew optimally at moderate temperatures and a range of water activity levels from 0.90 aw upwards [51, 66].

Interestingly, most isolates belonging to the *Penicillium* and *Aspergillus* genera, were able to grow at all levels of water activity tested, and they seemed to adapt to low levels of moisture (0.85–0.90 aw). Thus, they can be classified as being between xerophilic and mesophilic. *Aspergillus flavus* and *Penicillium* spp. could grow between 0.85 and 0.90 aw at above 15 °C [2, 24]. The highest extent of water activity had a negative impact on radial growth of *Aspergillus flavu* and *Penicillium* spp., which have been recognised as xerophilic [60]

These findings are compatible with those of other studies. For example, *Fusarium, Trichoderma*, *Neofusicoccum*, *Mucor* and *Geotrichum* species have elsewhere been described as hydrophilic [6, 31, 31, 38]. In other studies, *Rhizopus* and *Alternaria* species have been classified as mesophilic species [44, 55]. Elsewhere, *Penicillium* and *Aspergillus* species have been considered xerophilic [44, 55].

4 Conclusions and future perspectives

The *Penicillium* and *Aspergillus* species accounted for more than half of the 25 strains isolated from various locations in Al-Baha and Baljurashi in the current study. Over quarter of those isolates belonged to the *Fusarium* species, while the remaining isolates corresponded to *Alternaria alternata, Geotrichum candidum*, *Neofusicoccum parvum*, *Rhizopus stolonifera* and *Mucor fragilis.*

The present study notes that *Penicillium* and *Aspergillus* are the species most frequently implicated in harming fruit and vegetable crops in storage conditions. Meanwhile, *Alternaria alternata* and *Fusarium* spp. were found in more than half of the pathogenic field-harvested samples.

The phylogenetic tree, based on ITS markers, has allocated the new isolates to their correct species groups and confirmed their species identification, based on similarity to GenBank reference data. The species recognised as most commonly producing mycotoxigenic effects are *Penicillium, Fusarium, Alternaria* and *Aspergillus* [18, 58].

The present study has shown how ambient conditions, (e.g. temperature and water availability), can affect the

biological activities and virulence of fungi. The growth measured in this investigation is the bedrock for sporulation and mycotoxin production, which in turn correlate with fungal pathogenic activity such as dispersal, colonisation and contamination.

Most of the tested isolates (14 out of 25), favoured a temperature of 30 °C for growth, whereas seven of the remaining isolates showed a preference for 25 °C and four isolates grew best at 20 °C. In terms of water activity's effects on growth, 15 out of 25 isolates belonged to *Penicillium* and *Aspergillus*, and these grew at all water activity levels. However, their highest growth rates were achieved in conditions with low to medium levels of moisture. Three isolates belong to *Rhizopus stolonifera* and *Alternaria alternata* species, grew optimally at moderate levels of moisture, while seven isolates belonging to *Fusarium, Neofusicoccum*, *Mucor* and *Geotrichum* genera preferred high level of moisture for growth.

Table 3 presents integrated data concerning the optimal growth conditions for each of new isolate. This data can contribute to the creation of strategies to mitigate the detrimental consequences of fungal invasion of crops before and after harvest. It will also be useful in the construction of strategies to prevent the spread of fungal infestation across sites [9]. Future studies might helpfully focus on identification of the type and level of mycotoxins produced by the dominant mycotoxigenic species in this work, namely *Penicillium, Aspergillus* and *Fusarium* spp. In addition, scholars might extend the work presented here in the context of exploring or developing biological controls and plant extracts to replace fungicides.

Acknowledgements The author would like to thank the deanship of scientific research in Al-Baha University.

Funding Not applicable for this time.

Data availability The molecular datasets for this work have been deposited in NCBI gene bank and their corresponding accession numbers are available in tables of this work. Also, the datasets for the optimal growth temperatures are available and attached in this work.

Declarations

Conflict of interest The author have no conflicts of interest to disclose.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Abd-Elsalam KA, Roshdy S, Amin OE, Rabani M (2010) First morphogenetic identification of the fungal pathogen *Colletotrichum musae* (Phyllachoraceae) from imported bananas in Saudi Arabia. Genet Mol Res 9:2335–2342
2. Abellana M, Sanchis V, Ramos AJ (2001) Effect of water activity and temperature on growth of three *Penicillium* species and *Aspergillus flavus* on a sponge cake analogue. Int J Food Microbiol 71:151–157
3. Ahmed RA, Mohammed SA (2014) Isolation and classification of fungi associated with spoilage of post-harvest mango (*Mangifera indica* L.) in Saudi Arabia. Afr J Microbiol Res 8:685–688
4. Akbar F, Alam S (2019) Influence of water activity and time duration on *fusarium* mycotoxins production in maize grains during post-harvest storage. Sarhad J Agric 35:1326–1335
5. Alborch L, Bragulat MR, Abarca ML, Cabañes FJ (2011) Effect of water activity, temperature and incubation time on growth and ochratoxin A production by *Aspergillus niger* and *Aspergillus carbonarius* on maize kernels. Int J Food Microbiol 147:53–57
6. Almiman BF (2018) Molecular genetic and genomic characterization of an emerging mycotoxigenic pathogen *Fusarium proliferatum*. Doctoral dissertation, University of Bedfordshire UK.
7. Almiman BF (2023) Identifying phytopathogenic fungi in Albaha province, Saudi Arabia through their molecular and morphological features: an overview. Saudi J Biol Sci 30:103572
8. Almiman BF, Shittu TA, Muthumeenakshi S, Baroncelli R, Sreenivasaprasad S (2018) Genome sequence of the mycotoxigenic crop pathogen *Fusarium proliferatum* strain ITEM 2341 from date palm. Microbiol Resour Announc 7:964
9. Al-Mutarrafi M, Elsharawy NT, Al-Ayafi A, Almatrafi A, Abdelkader H (2019) Molecular identification of some fungi associated with soft dates (*Phoenix dactylifera L.*) in Saudi Arabia. Adv Med Plant Res 7:97–106
10. Al-Namazi AA, Al-Khulaidi AWA, Algarni S, Al-Sagheer NA (2021) Natural plant species inventory of hotspot areas in Arabian Peninsula: Southwest Al-Baha region, Saudi Arabia. Saudi J Biol Sci 28:3309–3324
11. Alshehri KM (2020) Anticancer plants naturally growing in Al-Baha Region, Saudi Arabia. Int J Pharm Res Allied Sci 9:92–101
12. Alzandi AA, Taher EA, Al-Sagheer NA, Al-Khulaidi AW, Azizi M, Naguib DM (2021) Phytochemical components, antioxidant and anticancer activity of 18 major medicinal plants in Al Baha region. Saudi Arab Biocatal Agric Biotechnol 34:102020
13. Arif IA, Bakir MA, Khan HA, Al Farhan AH, Al Homaidan AA, Bahkali AH, Al Sadoon M, Shobrak M (2010) A brief review of molecular techniques to assess plant diversity. Int J Mol Sci 11:2079–2096
14. Armitage AD, Barbara DJ, Harrison RJ, Lane CR, Sreenivasaprasad S, Woodhall JW, Clarkson JP (2015) Discrete lineages within *Alternaria alternata* species group: Identification using new highly variable loci and support from morphological characters. Fungal Biol 119:994–1006
15. Balai LP, Ahir RR (2013) Role of temperature and relative humidity on mycelial growth of *Alternaria alternata* infecting brinjal. Trends Biosci 6:307–308
16. Barbosa-Cánovas GV, Fontana AJ Jr, Schmidt SJ, Labuza TP (2008) Water activity in foods: fundamentals and applications, vol 1. Wiley, Hoboken, pp 15–28
17. Bebber DP, Ramotowski MAT, Gurr SJ (2013) Crop pests and pathogens move poleward in a warming world. Nat Clim Chang 3:985–988
18. Bennett J, Klich M (2003) chotoxins. Clin Microbiol Rev 16:497–516

19. Collenette S (1999) Wild Flowers of Saudi Arabia. National Commission for Wildlife Conservation and Development (NCWCD), Riyadh, pp 703–707
20. Cowan RS, Fay MF (2012) Challenges in the DNA barcoding of plant material. Plant DNA fingerprinting and barcoding: methods and protocols, vol 862. Humana Press, Totowa, pp 23–33
21. Dean R, Van Kan JA, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, Rudd JJ, Dickman M, Kahmann R, Ellis J (2012) The top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathol 13:414–430
22. El-Habbab MS, Al-Mulhim F, Al-Eid S, Abo El-Saad M, Aljassas F, Sallam A, Ghazzawy H (2017) Assessment of post-harvest loss and waste for date palms in the Kingdom of Saudi Arabia. Int J Environ Agric Res 3:1–11
23. Fandohan P, Hell K, Marasas W, Wingfield M (2003) Infection of maize by *Fusarium* species and contamination with fumonisin in Africa. Afr J Biotechnol 2:570–579
24. Garcia-Cela E, Sulyok M, Verheecke-Vaessen C, Medina A, Krska R, Magan N (2022) Interacting environmental stress factors affect metabolomics profiles in stored naturally contaminated maize. Microorganisms 10:853
25. Gasperini AM, Medina A, Magan N (2022) Comparison of growth and aflatoxin B1 production profiles of *Aspergillus flavus* strains on conventional and isogenic GM-maize-based nutritional matrices. Fungal Biol 126:82–90
26. Gock MA, Hocking AD, Pitt JI, Poulos PG (2003) Influence of temperature, water activity and pH on growth of some xerophilic fungi. Int J Food Microbiol 81:11–19
27. Hope R, Aldred D, Magan N (2005) Comparison of environmental profiles for growth and deoxynivalenol production by *Fusarium culmorum* and *F. graminearum* on wheat grain. Lett Appl Microbiol 40:295–300
28. Kalai S, Anzala L, Bensoussan M, Dantigny P (2017) Modelling the effect of temperature, pH, water activity, and organic acids on the germination time of *Penicillium camemberti* and *Penicillium roqueforti* conidia. Int J Food Microbiol 240:124–130
29. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649
30. Koňuchová M, Valík Ľ (2021) Modelling the radial growth of *Geotrichum candidum*: Effects of temperature and water activity. Microorganisms 9:532
31. Latorre B, Díaz Ulloa GA, Reed Vergara MP (2012) Effect water activity on in vitro mycelial growth of Neofusicoccum spp infecting blueberry. Cienc Inv Agr 39:221–228
32. Llorens A, Mateo R, Hinojo M, Valle-Algarra F, Jiménez M (2004) Influence of environmental factors on the biosynthesis of type B trichothecenes by isolates of *Fusarium* spp. from Spanish crops. Int J Food Microbiol 94(1):43–54
33. Ma H, Snook LA, Tian C, Kaminskyj SG, Dahms TE (2006) Fungal surface remodelling visualized by atomic force microscopy. Mycol Res 110:879–886
34. Marín S, Companys E, Sanchis V, Ramos A, Magan N (1998) Effect of water activity and temperature on competing abilities of common maize fungi. Mycol Res 102:959–964
35. Mbareche H, Veillette M, Bilodeau GJ (2021) In silico study suggesting the bias of primers choice in the molecular identification of fungal aerosols. J Fungi 7:99
36. Moore ML, Six DL (2015) Effects of temperature on growth, sporulation, and competition of mountain pine beetle fungal symbionts. Microb Ecol 70:336–347
37. Nelson PE, Desjardins AE, Plattner RD (1993) Fumonisins, mycotoxins produced by *Fusarium* species: biology, chemistry, and significance. Annu Rev Phytopathol 31:233–252
38. Nielsen KF (2003) Mycotoxin production by indoor molds. Fungal Genet Biol 39:103–117
39. O'Donnell K, Ward TJ, Robert VA, Crous PW, Geiser DM, Kang S (2015) DNA sequence-based identification of *Fusarium*: current status and future directions. Phytoparasitica 43:583–595
40. Obaid WA, Boutraa T, Abdel-Hameed UK (2020) Floristics and soil characteristics of Ohud mountain, Al-Madinah Al-Munawarah, Western Saudi Arabia. J Mt Sci 17:2405–2417
41. O'Donnell K, Cigelnik E (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the Fungus *Fusarium* are nonorthologous. Mol Phylogenet Evol 7:103–116
42. O'Donnell K, Cigelnik E, Nirenberg HI (1998) Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. Mycologia 90:465–493
43. Pal KK, Gardener BM (2006) Biological control of plant pathogens. Plant Health Instruct 2:1117–1142
44. Park J, Cox-Ganser JM, Kreiss K, White SK, Rao CY (2008) Hydrophilic fungi and ergosterol associated with respiratory illness in a water-damaged building. Environ Health Perspect 116:45–50
45. Phan LTK, Nguyen HX, De Saeger S, Jacxsens L, Eeckhout M, Devlieghere F (2022) Predictive modelling of the radial growth of *Aspergillus flavus* and *Fusarium proliferatum* on paddy and white rice (Oryza sativa). Int J Food Microbiol 375:109743
46. Pietikäinen J, Pettersson M, Bååth E (2005) Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. FEMS Microbiol Ecol 52:49–58
47. Pimentel D, Hepperly P, Hanson J, Douds D, Seidel R (2005) Environmental, energetic, and economic comparisons of organic and conventional farming systems. Bioscience 55:573–582
48. Rahman HU, Yue X, Ren X, Zhang W, Zhang Q, Li P (2020) Multiplex PCR assay to detect *Aspergillus, Penicillium* and *Fusarium* species simultaneously. Food Addit Contam Part A 37:1939–1950
49. Raja HA, Miller AN, Pearce CJ, Oberlies NH (2017) Fungal identification using molecular tools: a primer for the natural products research community. J Nat Prod 80:756–770
50. Ramirez ML, Chulze S, Magan N (2006) Temperature and water activity effects on growth and temporal deoxynivalenol production by two Argentinean strains of *Fusarium graminearum* on irradiated wheat grain. Int J Food Microbiol 106:291–296
51. Romero Donato CJ, Nichea MJ, Cendoya E, Zachetti VG, Ramirez ML (2023) Interacting abiotic factors affect growth and mycotoxin production profiles of alternaria section *Alternaria* strains on chickpea-based media. Pathogens 12:565
52. Saleh AA, Sharafaddin AH, El-Komy MH, Ibrahim YE, Hamad YK, Molan YY (2017) *Fusarium* species associated with date palm in Saudi Arabia. Eur J Plant Pathol 148:367–377
53. Samapundo S, Devlieghere F, De Meulenaer B, Geeraerd A, Van Impe J, Debevere J (2005) Predictive modelling of the individual and combined effect of water activity and temperature on the radial growth of *Fusarium verticilliodes* and *F. proliferatum* on corn. Int J Food Microbiol 105:35–52
54. Sankar NR, Babu GP (2012) First report of *Fusarium proliferatum* causing rot of garlic bulbs (*Allium sativum*) in India. Plant Dis 96:290
55. Sautour M, Soares Mansur C, Divies C, Bensoussan M, Dantigny P (2002) Comparison of the effects of temperature and water activity on growth rate of food spoilage moulds. J Ind Microbiol Biotechnol 28:311–315
56. Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal Barcoding Consortium (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a

universal DNA barcode marker for Fungi. Proc Natl Acad Sci USA 109:6241–6246

57. Skouboe P, Frisvad JC, Taylor JW, Lauritsen D, Boysen M, Rossen L (1999) Phylogenetic analysis of nucleotide sequences from the ITS region of *Terverticillate Penicillium* species. Mycol Res 103:873–881
58. Solfrizzo M, Girolamo AD, Vitti C, Tylkowska K, Grabarkiewicz-Szczęsna J, Szopińska D, Dorna H (2005) Toxigenic profile of Alternaria alternata and Alternaria radicina occurring on umbelliferous plants. Food Addit Contam 22:302–308.
59. Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC (2000) Phylogenetic species recognition and species concepts in fungi. Fungal Genet Biol 31:21–32
60. Valle M, Van Long NN, Jany J, Bregier T, Pawtowski A, Barbier G, Rigalma K, Vasseur V, Huchet V, Coroller L (2022) Impact of water activity on the radial growth of fungi in a dairy environment. Food Res Int 157:111247
61. Verheecke-Vaessen C, Garcia-Cela E, Lopez-Prieto A, Jonsdottir IO, Medina A, Magan N (2021) Water and temperature relations of *Fusarium langsethiae* strains and modelling of growth and T-2 and HT-2 mycotoxin production on oat-based matrices. Int J Food Microbiol 348:109203
62. White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications, vol 18. Academic Press, New York, pp 315–322
63. Woudenberg J, Groenewald J, Binder M, Crous P (2013) *Alternaria* redefined. Stud Mycol 75:171–212
64. Yan JM, Shi XH, Mei M, Dai HB, Ye HZ (2011) Amplifying and sequencing analysis the internal transcribed spacer (ITS) regions of *Olpidium viciae* Kusano's ribosomal DNA in broad bean. Adv Mater Res 271:507–513
65. Yang R, Su J, Shang J, Wu Y, Li Y, Bao D, Yao Y (2018) Evaluation of the ribosomal DNA internal transcribed spacer (ITS), specifically ITS1 and ITS2, for the analysis of fungal diversity by deep sequencing. PLoS ONE 13:e0206428
66. Yoder JA, Klever LA, Dobrotka CJ (2018) Temperature-water and competitive interactions in vitro of two toxic black molds, *Fusarium oxysporum* and *Rhizopus stolonifer* isolated from a shower setting. Stud Fungi 3:248–255

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.