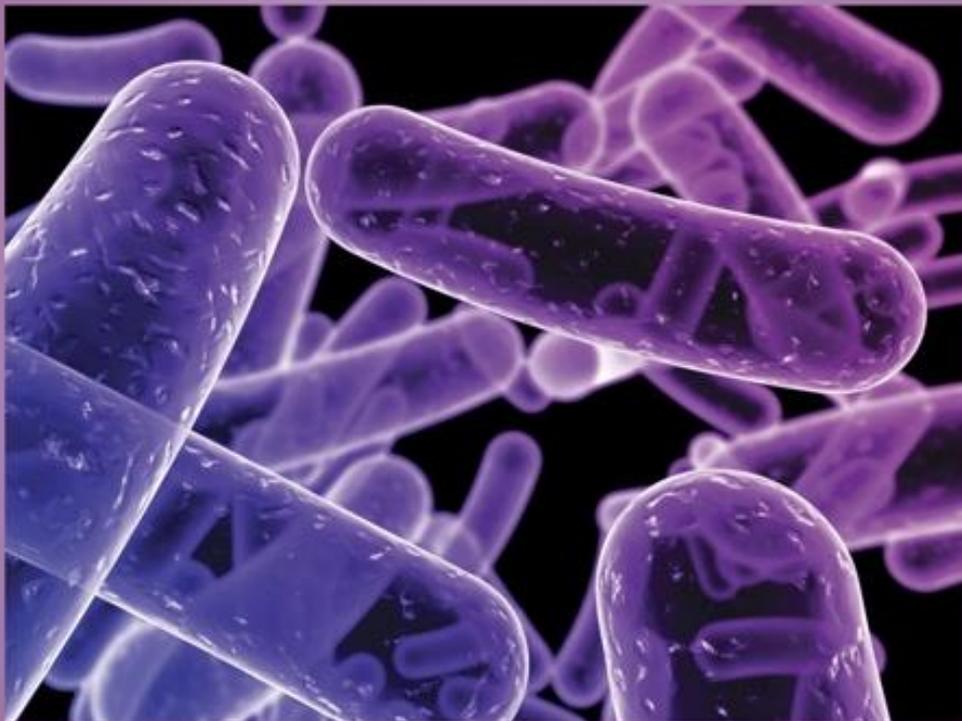




EGYPTIAN ACADEMIC JOURNAL OF  
**BIOLOGICAL SCIENCES**  
MICROBIOLOGY

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ISSN  
2090-0872

[WWW.EAJBS.EG.NET](http://WWW.EAJBS.EG.NET)

**Vol. 15 No. 2 (2023)**



## GC-MS Analysis, Antifungal, and Antioxidant Activity of Rice Straw-Based Extract Produced by *Aspergillus tubingensis* AUMC 15759 in Solid State Fermentation

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### ARTICLE INFO

Article History

Received: 11/8/2023

Accepted: 19/9/2023

Available: 23/9/2023

#### Keywords:

*Aspergillus*;  
Antifungal;  
Antioxidant;  
Metabolites;  
Wastes.

### ABSTRACT

Black aspergilli are excellent candidates for a variety of secondary metabolites. In this investigation, *Aspergillus tubingensis* AUMC 15759 produced certain bioactive compounds in solid-state fermentation (SSF) utilizing some agricultural wastes, namely bagasse, barley bran, bean hay, date palm leaves, flax seeds, orange peels, rice straw, soybean, and wheat bran. Rice straw extract yielded the highest inhibition zones of  $14.1 \pm 0.2$ ,  $13.6 \pm 0.85$ ,  $12.0 \pm 0.9$ , and  $15.0 \pm 0.9$  mm, against *Candida albicans*, *Candida glabrata*, *Candida krusei*, and *Saccharomyces cerevisiae*. The rice straw extract demonstrated impressive antioxidant activity (%DPPH) that was nearly similar to that of ascorbic acid exhibiting  $IC_{50}$  of  $1.2 \pm 0.2$  mg/ml. The liquid/liquid fractionation approach of the rice straw crude extract's liquid yielded five fractions, with the dichloromethane fraction being the most effective. GC-MS analysis disclosed the identification of nine substances linked to phenolic chemicals (3,4,5-trimethoxy phenol, 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol, Phi. -Aspidinol, m-Aminophenylacetylene, and 1-Phenyl-2-propene), fatty acids (Palamitic acid and cis,cis-Linoleic acid), quinone derivative (2,6-Dimethyl-3-(methoxymethyl)-p-benzoquinone), and alkaloid (N-Didehydrohexacarboxyl-2,4,5-trimethylpiperazine). The dichloromethane fraction displayed potent antifungal activity. The MIC was 6.25 mg/ml, with inhibition zones of  $14.0 \pm 0.4$ ,  $15.5 \pm 0.7$ ,  $14.0 \pm 0.4$ , and  $14.2 \pm 0.2$  mm against the investigated fungi, respectively. It also had the lowest  $IC_{50}$  ( $1.4 \pm 0.2$ ) when compared to the remaining fractions of *n*-butanol ( $1.8 \pm 0.4$ ), ethyl acetate ( $1.5 \pm 0.6$ ), *n*-hexane ( $11.5 \pm 0.2$ ), and water ( $13.5 \pm 1.1$ ), or ascorbic acid ( $10.64 \pm 0.84$ ) mg/ml. Finally, this fungus displayed antifungal and antioxidant activities when grown on agricultural wastes. It can be concluded that the tested strain of *A. tubingensis* displayed antifungal and antioxidant activities when grown on agricultural wastes, particularly rice straw.

## INTRODUCTION

Fungi are a diverse group of microorganisms that play a number of roles in human life. Antibiotics, antimicrobials, antioxidants, antiviral, anti-inflammatory, enzyme inhibitors, cytotoxic, hypercholesteremic, immunomodulators, and immunosuppressive medicines have primarily been examined in fungi (Naher *et al.*, 2021; Conrado *et al.*, 2022). Secondary metabolites, such as aromatic compounds, alkaloids, amino acids, fatty acids, phenolic compounds, and quinone derivatives have been recorded to be produced by fungi in several kinds of literature (Brambilla *et al.*, 1988; Nishina *et al.*, 1991; Cole *et al.*, 2003; Leyden *et al.*, 2007; Dhahir *et al.*, 2012; Adedoyin *et al.*, 2013; Zabka and Pavela, 2013; Evidente *et al.*, 2014; Li *et al.*, 2014; Dheeb, 2015; Kareem *et al.*, 2015; Chandra and Arora, 2016; Gao *et al.*, 2016; Mollavali *et al.*, 2016; He *et al.*, 2017; Pathak *et al.*, 2018; Chen *et al.*, 2021; Widjajanti *et al.*, 2022).

Species of *Alternaria*, *Aspergillus*, *Claviceps*, *Fusarium*, and *Penicillium* are the most active fungi producing approximately 15,600 fungal metabolites (Sulyok *et al.*, 2020). Black aspergilli are excellent candidates for various secondary metabolite production, including N-Ps, aflatoxins, fumonisins, cyclopiazonic acid, ophiobolins, and others. Many of these active compounds have remarkable biological properties, such as antibacterial, antioxidant, anticancer, anti-hyperuric, anti-HIV, and antitubercular properties (Choque *et al.*, 2015; Siddiquee, 2018).

Each year, agriculture produces five billion metric tons of lignocellulosic biomass, which includes barley bran, barley hay, bean straw, date palm leaves, rice bran, rice husk, sugarcane bagasse, wheat bran, wheat straw, leftover fruits and vegetable wastes, corn cobs, cassava stems, peanut shells, coconut shells, leaves, and leftover cotton leaf scraps (Abou Hussein and Sawan, 2010; Khanh and Thanh, 2010; Ismail *et al.*, 2018; AL-Kolaibe *et al.*, 2021). Rice straw has a high potential

as a biomass source since it is high in cellulose (39%), hemicellulose (22%), lignin (16%), and ash (18%) (Sarnklong *et al.*, 2010; Minu *et al.*, 2012), as well as high silica content (El Safty, 2020).

Consequently, this research was put forward in order to use some agricultural wastes as sustainable sources for the production of secondary metabolites. Therefore, the ability of *Aspergillus tubingensis* AUMC 15759 to create certain active metabolites that have antifungal and/or antioxidant activity, using the rice straw waste in solid-state fermentation was assessed.

## MATERIALS AND METHODS

### 1. Strain Isolation and Identification:

The *Aspergillus* isolate used in this study was recovered from a soil sample close to the Sohag-Qena Road, Sohag Governorate, Egypt, using the dilution plate method (Harris and Sommers, 1968). Appropriate dilution of the soil suspension was transferred to Petri plates containing Czapek's-Dox agar (CzA). The plates were then incubated at 25°C for 10 days. The developed culture was then purified on CzA using the single spore isolation technique (Choi *et al.*, 1999), and preserved in Assiut University, Mycological Centre's culture collection as AUMC 15759. This isolate was selected for evaluation of its ability to utilize some agricultural wastes to produce bio-active metabolites that may be beneficial as antifungal and/or antioxidant compounds.

### 2. Morphological Identification of the *Aspergillus* Isolate:

The *Aspergillus* isolate used in this study was morphologically identified based on its macroscopic and microscopic characteristics using some relevant references (Raper and Fennell, 1965; Moubasher, 1993). Growth rates of the new species were studied on Czapek's agar (CZ; (Raper and Fennell, 1965), malt extract agar (MEA; (Samson *et al.*, 2010), Czapek's yeast Autolysate agar (CYA; (Pitt, 1979). Inoculations were made from spore suspensions prepared in a 0.2% agar and 0.05% Tween 80 solution (Samson *et al.*, 2014). Plates were inoculated in a three-point

pattern using a micropipette and inoculum size of 1.0  $\mu\text{L}$ /spot, and the plates were then incubated in the dark at 25°C for 7 days. The microscopic characteristics were examined from the MEA culture.

### 2.1. Molecular Identification of the *Aspergillus* Isolate:

For molecular identification of such isolate, DNA was extracted at the Molecular Biology Unit, Assiut University, Assiut, Egypt following CTAB method described by Moubasher *et al.* (2019). PCR was conducted at SolGent Co., Ltd. (Yuseong-Gu, 34014, Daejeon, South Korea), using SolGent EF (Al-Bedak and Moubasher, 2020), and the universal primer pair Bt2a and Bt2b were used for amplification of the *beta-tubulin* gene (Glass and Donaldson, 1995).

### 3. Phylogenetic Analysis:

Using the DNASTAR (version 5.05), a contiguous sequence of the *Aspergillus* isolate AUMC 15759 was produced. Sequences that were most similar to the *Aspergillus* isolate AUMC 15759's *Beta-tubulin* sequence, were all downloaded from the GenBank database. All sequences in this analysis were aligned by MAFFT (version 6.861b) (Kato and Toh, 2010). The alignment gaps and weak uninformative characters were optimized by BMGE (Criscuolo and Gribaldo, 2010). MEGA X (version 10.2.6) was used to conduct the maximum-likelihood (ML) and maximum-parsimony (MP) phylogenetic analyses (Kumar *et al.*, 2018), and the robustness of the most parsimonious trees was evaluated by 1000 replications (Felsenstein, 1985). Utilizing Modeltest 3.7's Akaike information criterion (AIC), the optimum nucleotide substitution model for ML analysis was identified (Posada and Crandall, 1998). The resulting tree was edited and saved in TIF format (Al-Bedak *et al.*, 2020).

### 4. Agricultural Wastes:

Nine agricultural wastes, namely bagasse (B), barley bran (BB), bean hay (BH), date palm leaves (DPL), flax seeds (FS), orange peels (OP), rice straw (RS), soybean (SB), and wheat bran (WB), were used in this study. All substrates were acquired from local

markets in Assiut Governorate, Egypt. Prior to use, the wastes were prepared following Ramadan *et al.* (2023).

### 5. Solid-state Fermentation (SSF):

The agricultural wastes under test were separated into 10 gm portions and placed into 500 ml Erlenmeyer conical flasks individually. After that, citrate buffer (pH 5.0) was used to further wet the leftovers until they contained 80% moisture. A 5.0 ml spore suspension of *Aspergillus* sp. AUMC 15759, grown for seven days, was produced and added to each flask at a concentration of  $1.5 \times 10^8$  spores/ml. The incubation period was seven days at a static temperature of 30°C.

### 6. Harvesting of the Crude Extracts:

Following the incubation time, the flask contents underwent a 48 h oven drying process at 60°C. The mycelial mat and solid substrate were homogenized and stirred in 50 ml of methanol for 2 h at 180 rpm in each flask. The clear supernatant was obtained after centrifugation (10,000 rpm at 4°C for 10 min). The methanol extract was evaporated (Heidolph: Model reddot winner 2020; Germany). The sample was lyophilized using a freeze drier (VirTis: Model 6 KBTES-55, NY; USA).

### 7. Determination of the Antifungal Activity:

*Candida albicans* AUMC 13415, *Candida glabrata* AUMC 13412, *Candida krusei* AUMC 13420, and *Saccharomyces cerevisiae* AUMC 13515 were obtained from Assiut University Mycological Centre (AUMC)'s culture collection for use in this assay. The antifungal activities of crude extracts, derived from *Aspergillus* sp. AUMC 15759's fermentation of nine agricultural wastes were evaluated using the well diffusion method (Magaldi *et al.*, 2004; Valgas *et al.*, 2007). The tested fungi were grown in Sabouraud's dextrose broth for 24 h before being used to inoculate 9-cm-diameter Petri plates with a spore suspension containing  $1.5 \times 10^8$  spore/ml (= 0.5 McFarland standard solution). Dimethyl sulfoxide (DMSO) was utilized to make 20 mg/ml solutions of the nine extracts. A 50  $\mu\text{l}$  of each extract solution (20 mg/ml) was separately transferred to a 5-mm well in the

Sabouraud's dextrose agar (SDA). As positive antifungal reference drug, fluconazole (15 mg/ml) was used.

### 8. Antioxidant Activity:

The crude extracts were employed at concentrations of 20, 10, 5, 2.5, 1.25, 0.62, 0.31, and 0.15 mg/ml in methanol solution. A 40 µl aliquot of the sample-containing methanol solution was mixed with 3 ml of 0.004% freshly-prepared 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Yen and Duh, 1994). The mixture was then kept in the dark for 15 min. At 515 nm, the absorbance was determined (Model: Spectronic 1201, Milton Roy; Canada). Until the absorbance stabilized, the decline in absorbance was constantly observed, and data was gathered for 16 min at 1-minute intervals. Ascorbic acid was used as a standard. The 50% inhibitory concentration (IC<sub>50</sub>), the concentration required to inhibit DPPH radical by 50%, was estimated from graphic plots of the dose-response curve. The experiment was conducted in triplicates. The percentage inhibition (PI) of the DPPH radical was estimated using equation (1):

$$PI = (AC - AT) \times 100 \quad (1)$$

Where AC = control absorbance at zero time; AT = sample absorbance + DPPH at 16 min.

### 9. Liquid/liquid Fractionation of the Crude Extracts:

Using the liquid-liquid fractionation technique (Colgrove and Svec, 1981), the crude extract powder (18 g) was suspended in

200 ml of distilled water in a separating funnel. 200 ml of each of *n*-hexane, dichloromethane, ethyl acetate, and *n*-butanol, were sequentially added to the extract. The mixture was vigorously shaken for 10 min, and the process was repeated three times. Each solvent fraction was then collected, pooled and dried under reduced pressure, yielding five fractions, namely *n*-hexane, dichloromethane, ethyl acetate, *n*-butanol, and aqueous fractions. The antifungal activity of the five fractions produced was tested at concentrations of 25, 12.5, 6.25, 3.75, 1.85, and 0.9 mg/ml. Nystatin (50 mcg/disc) was used as a positive control. Antioxidant activity was carried out as stated previously. The most active portion was analyzed using Gas Chromatography-Mass Spectroscopy (GC-MS).

### 10. GC-MS Analysis:

The Analytical Chemistry Unit (ACAL), Faculty of Science, Assiut University, Egypt, conducted this analysis to determine the active components of the dichloromethane fraction. A 0.5 gm of the tested fraction's residue was dissolved in 5 ml methanol and then centrifuged for 15 min (10,000 rpm at 5°C). A 10 µl of the tested fraction was submitted to a GC-MS instrument (7890A-5975B; Thermo-Scientific GC/MS; type ISQ; USA). The following GC-MS conditions were used in this study (Table 1).

**Table 1.** Parameters and process conditions employed for the GC-MS analysis of the dichloromethane fraction produced by *Aspergillus* isolate AUMC 15759 utilizing rice straw in SSF.

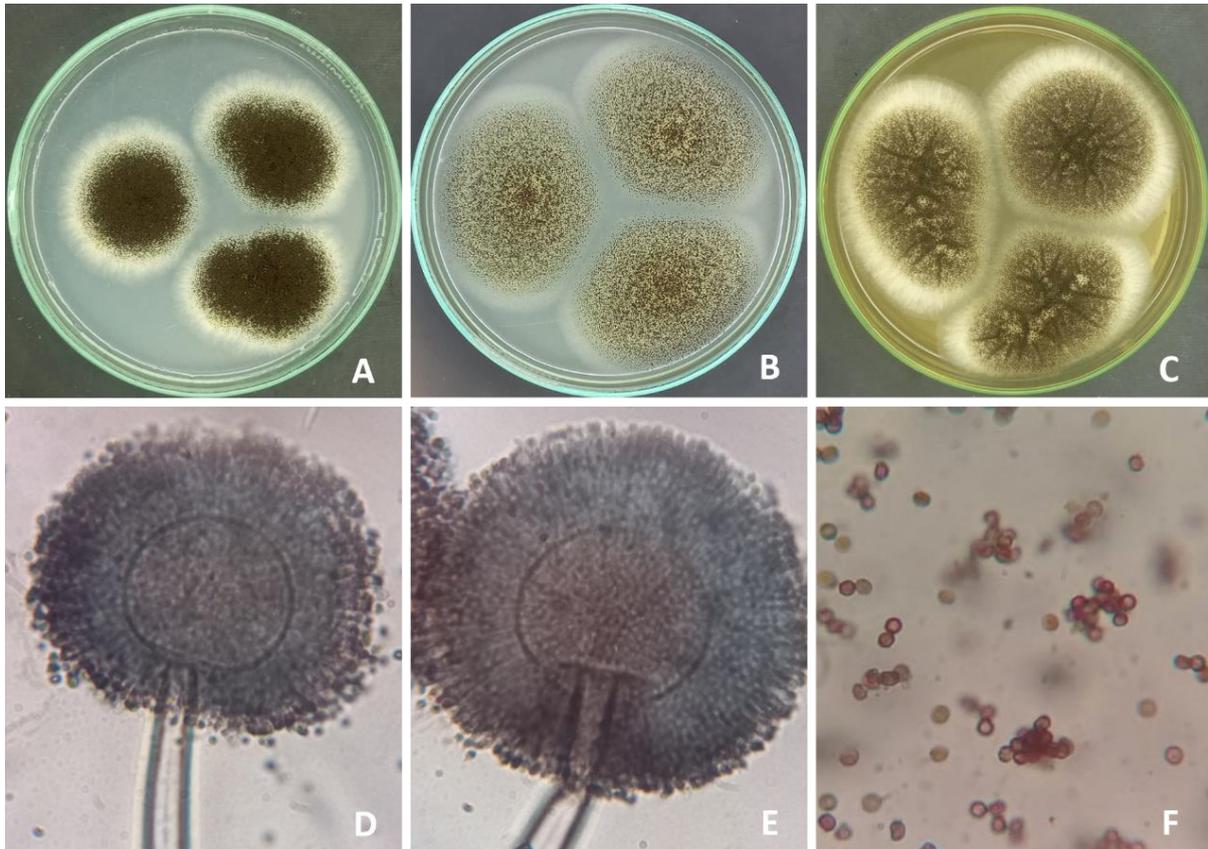
Parameter	Specifications and process conditions
Column type	HP-5MS Capillary Standard non-polar
Column dimensions	30 mm × 0.25 mm × 0.25 µm film
Equilibration time	0.5 min
Max temperature	280°C
Oven program	at 120°C for 5 min, 30°C/min rose to 265°C for 25 min; then 50°C/min rose to 280°C for 5 min
Run time	48 min
Post run	260°C for 2 min
Flow program	0.5 ml min for 10.9 min and then 1 ml min for 30 min
MS source	230–250°C
MS Quad	150–200°C

## RESULTS

### 1. Morphological identification of the *Aspergillus* isolate AUMC 15759:

*Aspergillus* isolate AUMC 15759 showed identical morphological characteristics to *Aspergillus tubingensis*. The

fungus has moderate growth and sporulation on MEA after 7 days at 25°C. Conidiophore pale brown, brown, smooth. Vesicle biserial, spherical, 40-80 µm. conidia globose to subglobose, 3-5 µm. Sclerotia is absent (Fig. 1).



**Fig. 1.** *Aspergillus tubingensis* AUMC 15759. (A–C), Seven-day-old colonies on Cz, MEA, and CYA at 25°C (D–E), Conidiophore and biserial conidial heads (F), Conidia.

### 2. Molecular Studies and Phylogenetic Analysis:

Based on a megablast search on NCBI's database using the  $\beta$ -tubulin gene sequence of the *Aspergillus* isolate AUMC 15759 in this study, the closest hits are *Aspergillus tubingensis* QF 05 [(GenBank accession number KY608858; identities = 542/543 (99.82%); Gaps = 1/543 (0%)], *Aspergillus tubingensis* NBRC 31125 and NBRC 4407 [(GenBank accession numbers LC573675 and LC573673; identities = 541/542 (99.82%); Gaps = 1/542 (0%)].

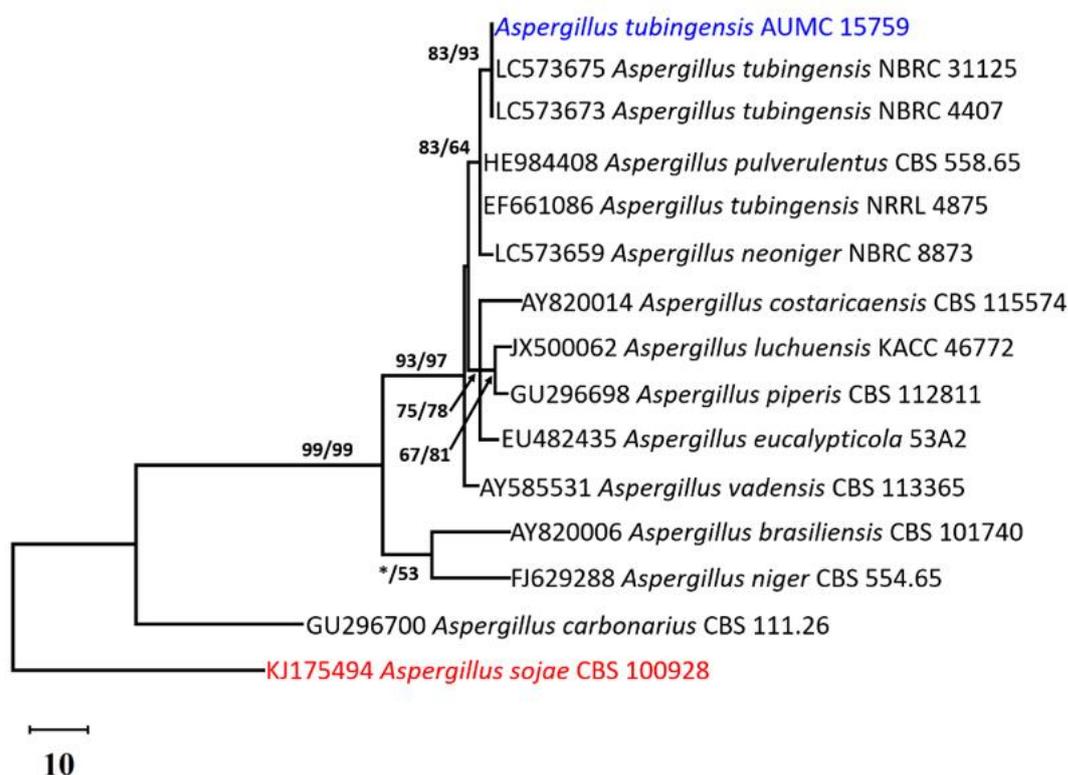
>*Aspergillus tubingensis* AUMC 15759's *Beta-tubulin* sequence:

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CGGTGCTGCTTTCTGGTACGTA
TTCAC TGCCACTGGATTGGGGATGGA
ACATCATCTCTCAAGCTATCTTAGCTT
GAGTTCAGATGTTATCCATCGGGGAT
ATAGCTACGGTTAAGAACACGTCTAA
CAACTCAACAGGCAGACCATCTCTGG
CGAGCACGGCCTTGACGGCTCCGGTG
TGTAAGTACAAC TTTTCACACCTCTC
AATTGGTCAACAATGTGGAAAGGATT
GGGTTTCCTGACGCGCAGGATAGTTA
CAATGGCACCTCCGACCTCCAGCTGG
AGCGCATGAACGTCTACTTCAACGAG
GTTAGATCACACCGTCCCTGAGTTTTT
TCACGACAATATCATCAATGTCCTGA
CCACTTCAGCAGGCTAGCGGTAACAA
```

GTATGTCCCCCGTGCCGTCCTCGTCTCGA  
TCTCGAGCCCGGTACCATGGACGCCG  
TCCGTGCCGGTCCCTTCGGCCAGCTCT  
TCCGCCCCGACAACCTTCGTCTTCGGCC  
AGTCCGGTGCTGGTAACAACCTGGGCC  
AAGGGTCACTAACTGAGGGT

For molecular identification of the *Aspergillus* isolate AUMC 15759 in this study,  $\beta$ -tubulin-based phylogenetic analysis was conducted. The final dataset, which contained 15 sequences, produced 522 characters, of which 338 characters could align unambiguously, 101 were counted as variable (29.9% of complete), and 34 as informative characters (10.1%). Kimura 2-parameter modeled by using a discrete Gamma distribution (K2+G) was the perfect model to

represent the relationship between taxa. Maximum parsimony analysis produced 9 trees, the most parsimonious of which with a tree length of 211 steps, - log-likelihood of 1684.58, consistency index of 0.746667, retention index of 0.762500, and composite index of 0.569333, is presented to depict the evolutionary history of the *Aspergillus* isolate in this study (Fig. 2). In the phylogenetic analysis, the isolate in this study was grouped with two *Aspergillus tubingensis* strains, *A. tubingensis* NBRC 31125 and *A. tubingensis* NBRC 4407, endorsing a very strong support value of 83% ML/93% MP. As a result, the isolate in this study was identified as *Aspergillus tubingensis*.



**Fig. 2.** The most parsimonious phylogenetic tree using a heuristic search (1000 replications) of *A. tubingensis* AUMC 15759  $\beta$ -tubulin sequence (blue) compared to most similar  $\beta$ -tubulin sequences of *Aspergillus*: section Nigri. ML/MP  $\geq 50\%$  are indicated close to respective nodes. The tree is rooted to *Aspergillus sojae* CBS 100928 as out-group (red).

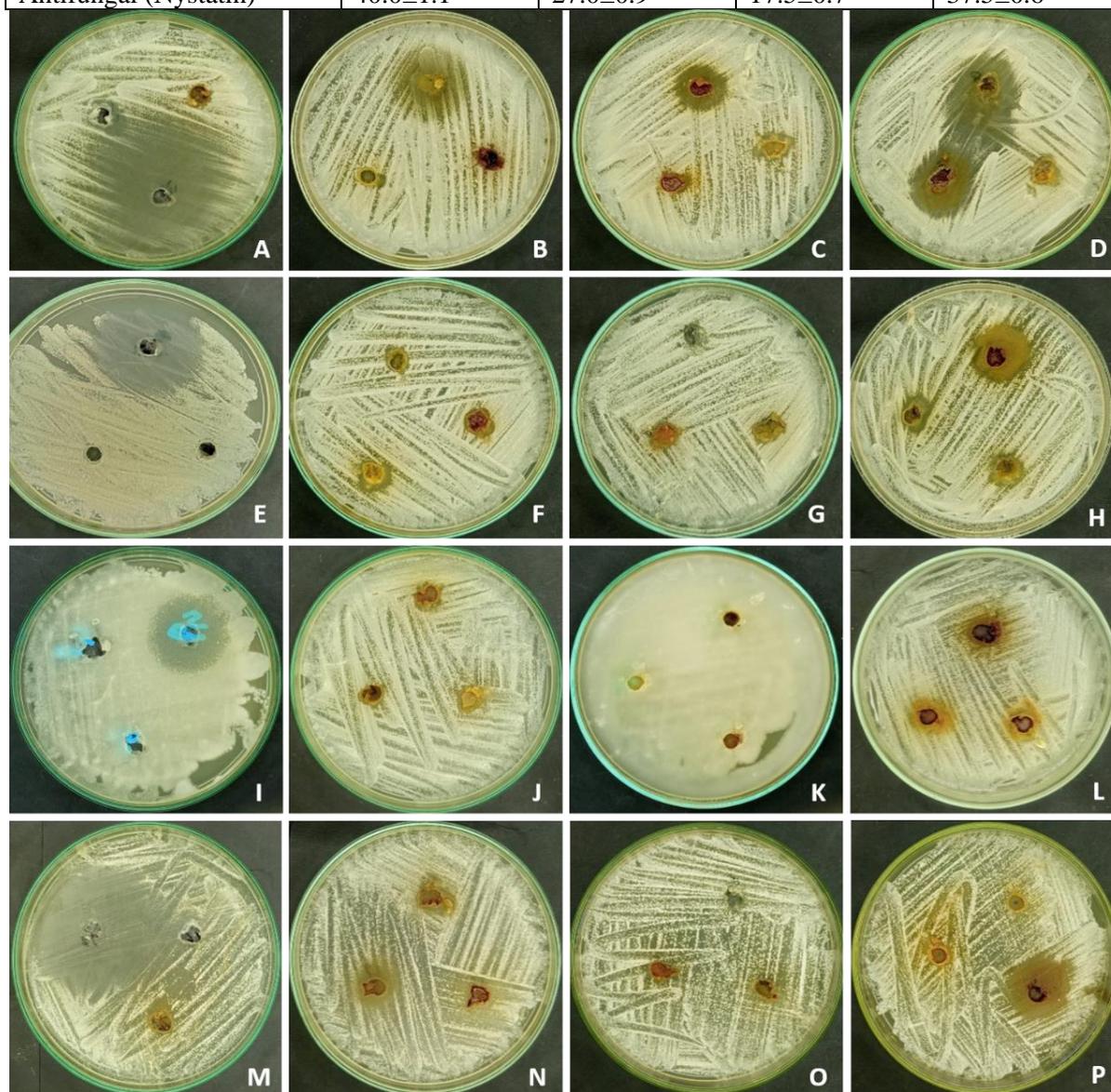
### 3. Production of Secondary Metabolites by *Aspergillus tubingensis* AUMC 15759 Utilizing Agricultural Wastes:

Rice straw's crude extract was the most effective compared to the remaining

extracts exhibiting the highest inhibition zones of  $14.1 \pm 0.2$ ,  $13.6 \pm 0.85$ ,  $12.0 \pm 0.9$ , and  $15.0 \pm 0.9$  mm against the four tested fungi, *C. albicans*, *C. glabrata*, *C. krusei* and *S. cerevisiae*, respectively (Table 2 and Fig. 3).

**Table 2.** Antifungal activity (mean±SD;  $n=3$ ), represented in mm of inhibition zones, of the crude extracts of different agricultural wastes fermented by *A. tubingensis* AUMC 15759 in SSF. The antifungal drug nystatin was used as a positive control.

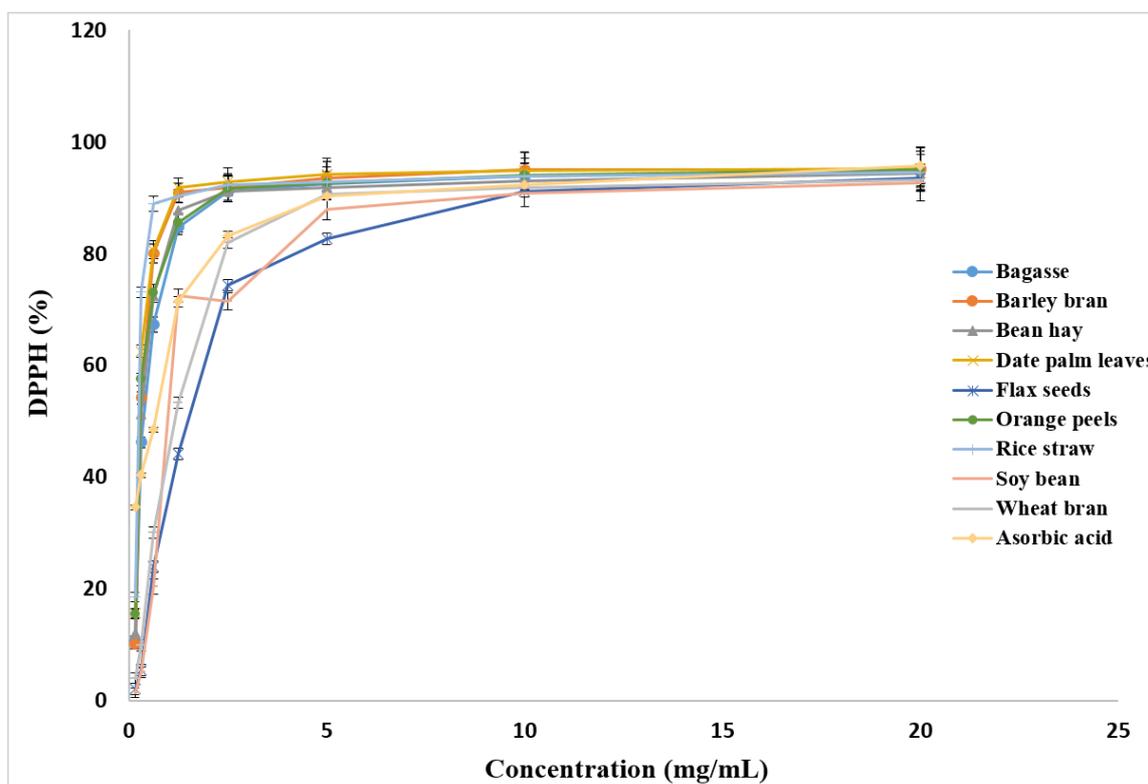
Produced extracts	Inhibition zone (mm)			
	<i>C. albicans</i>	<i>C. glabrata</i>	<i>C. krusei</i>	<i>S. cerevisiae</i>
Bagasse	13.0±0.6	-	-	12.5± 0.4
Barley bran	-	-	-	-
Bean hay	-	-	-	14.0±0.7
Date palm leaves	14.3±0.4	-	-	-
Flaxseeds	-	-	-	-
Orange peels	-	-	-	-
Rice straw	14.1±0.2	13.6±0.8	12.0±0.9	15.0±0.9
Soybean	-	9.5± 0.7	9.6±0.4	-
Wheat bran	13.5±0.7	12.6±0.5	11.9±0.1	-
Antifungal (Nystatin)	40.0±1.1	27.0±0.9	17.5±0.7	37.5±0.6

**Fig. 3.** Antifungal activity of the crude extracts of different agricultural wastes fermented by *A. tubingensis* AUMC 15759 in SSF. (A–D), *C. albicans*; (E–H), *C. glabrata*; (I–L), *C. krusei*; and (M–P), *S. cerevisiae*.

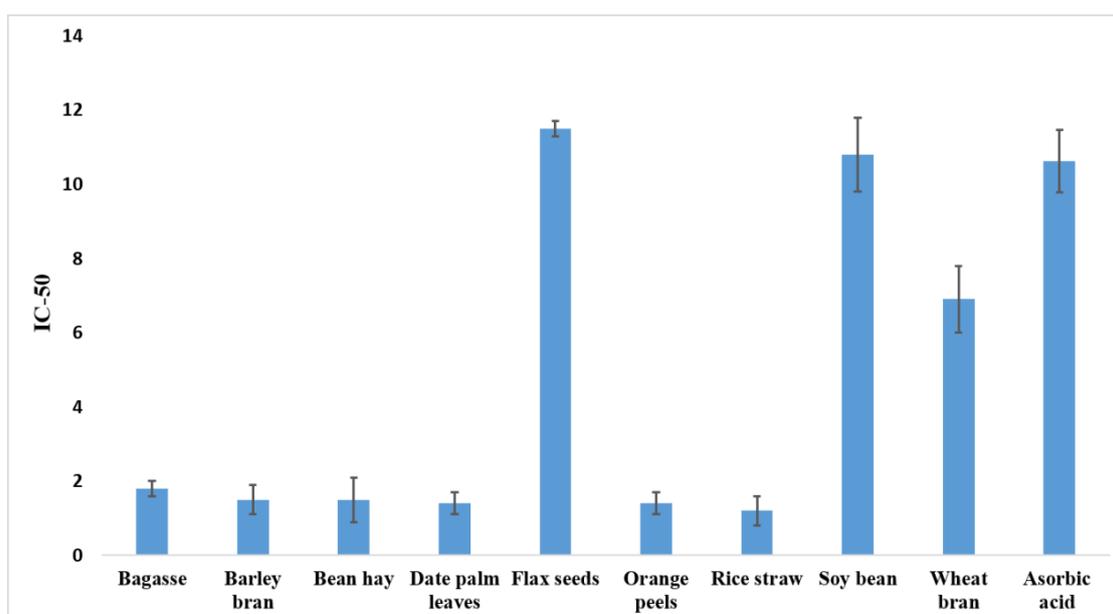
#### 4. Antioxidant Activity of Agricultural Wastes' Crude Extracts Produced by *A. tubingensis* AUMC 15759 in SSF:

The antioxidant potential of different extracts derived from the agricultural wastes,

used in this study, were examined. Rice straw produced the most powerful antioxidant activity exhibiting  $IC_{50}$  of  $1.2 \pm 0.4$  mg/ml (Figs. 4-5).



**Fig. 4.** Antioxidant activity (% DPPH) of different agricultural wastes' crude extracts produced by *A. tubingensis* AUMC 15759 in SSF compared to ascorbic acid as standard.

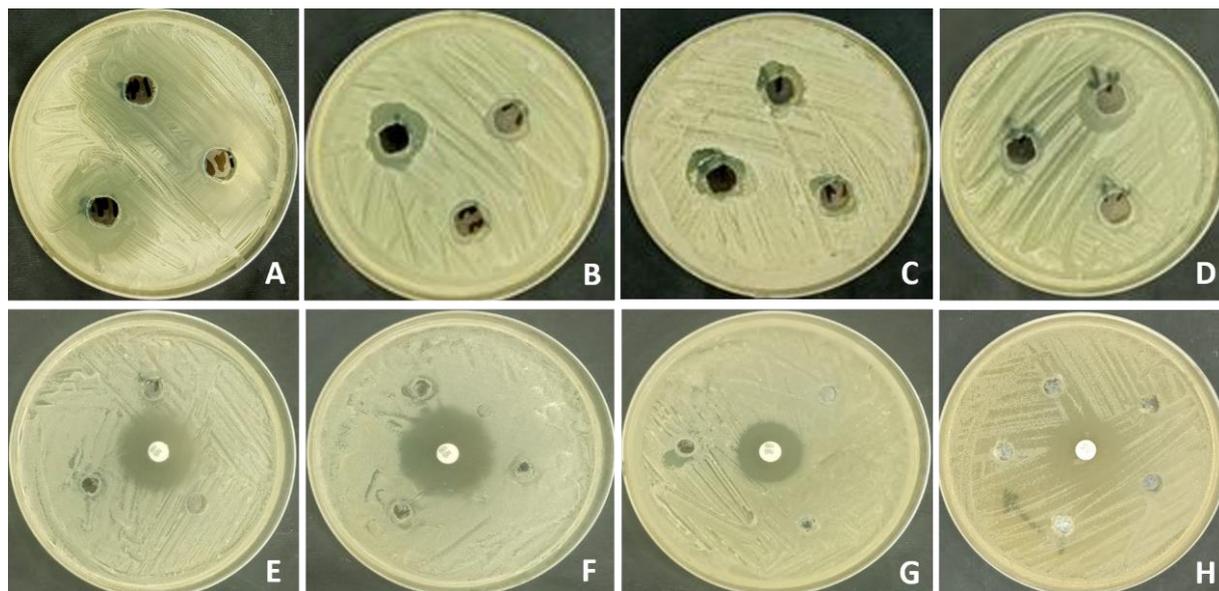


**Fig. 5.**  $IC_{50}$  of different agricultural wastes' crude extracts produced by *A. tubingensis* AUMC 15759 in SSF compared to ascorbic acid as standard.

## 5. Fractionation of the Rice Straw Crude Extract:

It was determined whether the fractions obtained using *n*-hexane, dichloromethane, ethyl acetate, *n*-butanol and water had any antifungal properties. The four examined fungi—*C. albicans*, *C. glabrata*, *C. krusei*, and *S. cerevisiae*—were all unaffected

by aqueous fraction, whereas the fraction obtained by dichloromethane was the strongest one in terms of antifungal activity. The concentration of 6.25 mg/ml was the MIC exhibiting  $14.0\pm 0.4$ ,  $15.5\pm 0.7$ ,  $14.0\pm 0.4$ , and  $14.2\pm 0.2$  mm inhibition zone against the tested fungi, respectively (Fig. 6 and Table 3).



**Fig. 6.** The antifungal potential of the dichloromethane fractions obtained by liquid-liquid fractionation technique on Rice straw extract (A–D), *C. albicans*, *C. glabrata*, *C. krusei*, and *S. cerevisiae*, respectively. (E–H), Effect of nystatin on the tested fungi *C. albicans*, *C. glabrata*, *C. krusei* and *S. cerevisiae*, respectively.

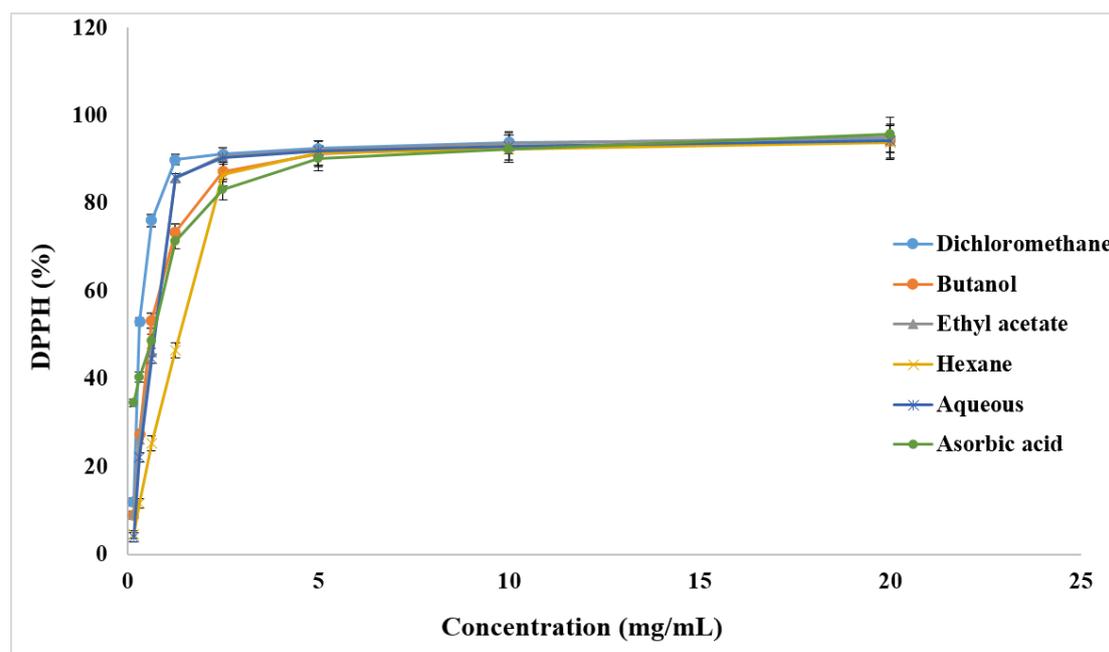
**Table 3.** The antifungal activity of the fractions harvested from rice straw extract produced by *A. tubingensis* AUMC 15759 in SSF compared to nystatin as a positive control.

Fractions	<i>C. albicans</i>				<i>C. glabrata</i>				<i>C. krusei</i>				<i>S. cerevisiae</i>			
	25 mg/mL	12.5 mg/mL	6.25 mg/mL	3.75 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	3.75 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	3.75 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	3.75 mg/mL
Hexane	-	-	-	-	$15.0\pm 0.7$	$14.0\pm 0.3$	$13.0\pm 0.3$	-	$14.0\pm 0.7$	$13.5\pm 0.2$	$13.0\pm 0.1$	-	$14.1\pm 0.7$	$13.2\pm 0.8$	$13.1\pm 0.3$	-
Dichloromethane	$30.0\pm 0.3$	$28.0\pm 0.2$	$14.0\pm 0.4$	-	$16.5\pm 0.5$	$16.0\pm 0.6$	$15.5\pm 0.7$	-	$16.0\pm 0.2$	$15.0\pm 0.1$	$14.0\pm 0.4$	-	$15.2\pm 0.2$	$14.9\pm 0.1$	$14.2\pm 0.2$	-
Butanol	-	-	-	-	$15.0\pm 0.5$	$14.0\pm 0.9$	$13.5\pm 0.4$	-	$15.5\pm 0.3$	$15.0\pm 0.1$	$14.5\pm 0.4$	-	$21.0\pm 0.2$	$16.0\pm 0.1$	$15.0\pm 0.3$	-
Ethyl acetate	$10.0\pm 0.1$	-	-	-	$13.5\pm 0.4$	$13.1\pm 0.7$	$13.0\pm 0.4$	-	$13.0\pm 0.1$	$12.9\pm 0.2$	$12.5\pm 0.4$	-	18.5	$18.0\pm 0.3$	$16.9\pm 0.4$	-
Aqueous	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nystatin	$27.0\pm 0.3$				$25.0\pm 0.7$				$30.2\pm 0.5$				$33.2\pm 0.9$			

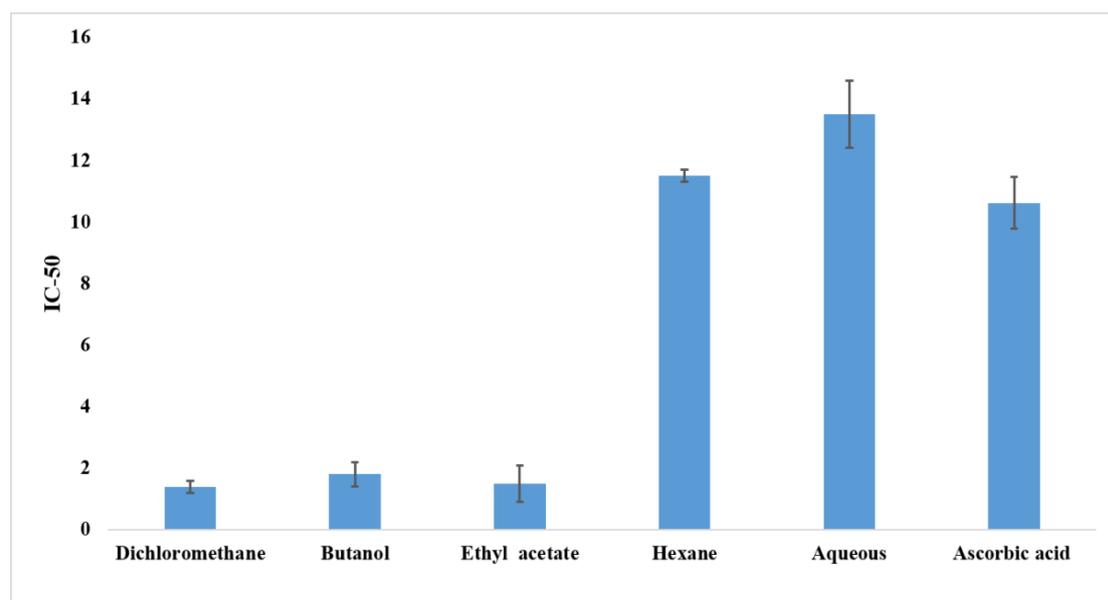
## 6. Antioxidant Activity:

The dichloromethane fraction showed remarkable antioxidant activity of 95.0% of DPPH activity, which is almost similar to that of ascorbic acid (95.6%). The fractions of *n*-butanol, ethyl acetate, *n*-hexane and aqueous showed a narrower spectrum of DPPH

activity ranging from 90% to 93% (Fig. 7). Dichloromethane fractions exhibited the lowest  $IC_{50}$  ( $1.4\pm 0.2$ ) compared to the remaining fractions of *n*-butanol ( $1.8\pm 0.4$ ), ethyl acetate ( $1.5\pm 0.6$ ), *n*-hexane ( $11.5\pm 0.2$ ), aqueous ( $13.5\pm 1.1$ ), and ascorbic acid ( $10.64\pm 0.84$ ) mg/ml (Fig. 8).



**Fig. 7.** Antioxidant activity (% DPPH) of different fractions obtained from the rice straw extract produced by *A. tubingensis* AUMC 15759 in SSF compared to ascorbic acid as standard.



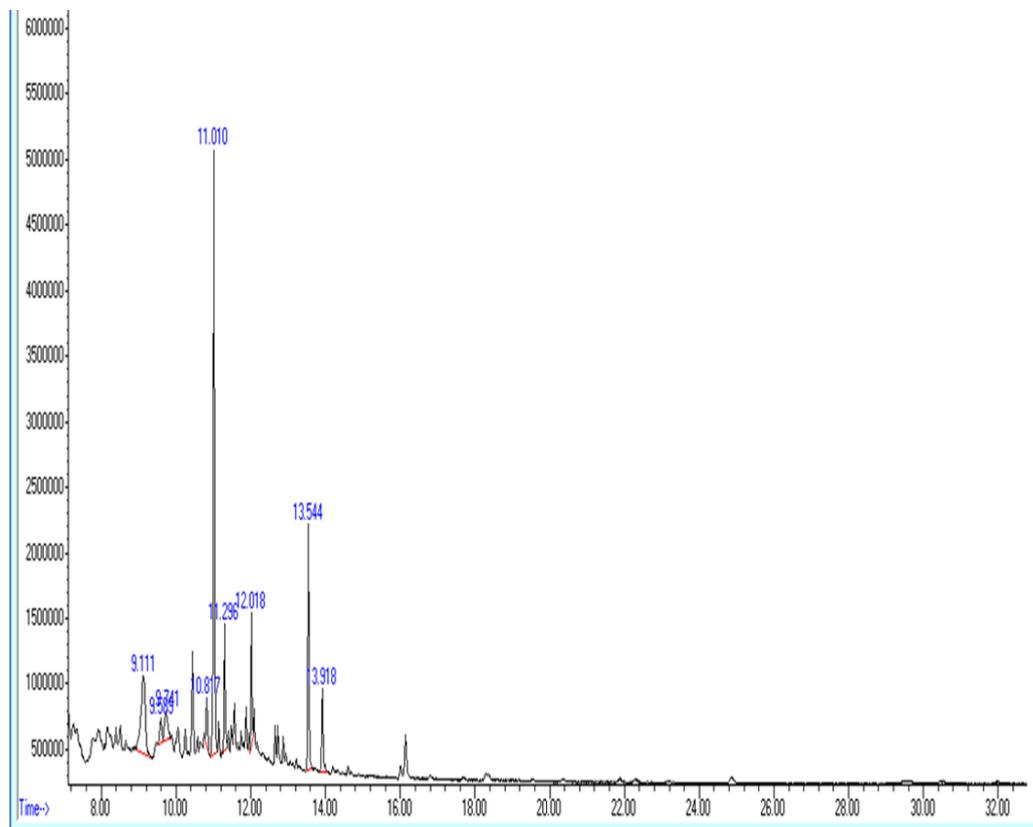
**Fig. 8.** IC<sub>50</sub> of different fractions obtained from the rice straw extract produced by *A. tubingensis* AUMC 15759 in SSF compared to ascorbic acid as standard.

### 7. GC-MS Analysis:

The GC-MS analysis of the dichloromethane fraction (the most active fraction) was carried out for the detection of its components that have antifungal and/or antioxidant activities. The current results revealed the identification of nine peaks (Fig. 9), based on retention time, molecular weight, and fragmentation pattern of the most

prominent chemicals. The most prevalent compounds were five phenolic compounds (3,4,5-trimethoxy phenol, 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol, Phi.-Aspidinol, m-Aminophenylacetylene, and 1-Phenyl-2-propene) which comprised 50% of the total and two fatty acids (Palamitic acid and cis,cis-Linoleic acid) constituted 20%. Quinone derivatives (2,6-Dimethyl-3-

(methoxymethyl)-p-benzoquinone), and alkaloids (N-Di-dehydrohexacarboxyl-2,4,5-trimethylpiperazine) were also detected comprising 10% each. The detected compounds were found to have antioxidants, antibacterial, cytotoxic, antitubercular, antifungal, anti-acne, and anti-inflammatory activities (Table 4).



**Fig. 9.** GC-MS chromatogram of the dichloromethane fraction produced by *Aspergillus tubingensis* AUMC 15759 in SSF.

**Table 4.** GC-MS spectral analysis of the chemical compounds detected in the dichloromethane fraction derived from the rice straw extract produced by *Aspergillus tubingensis* AUMC 15759 in SSF.

RT (min)	Compound name	Compound group	Biological activity	Molecular weight	Molecular formula
9.596	2,6-Dimethyl-3-(methoxymethyl)-p-benzoquinone	Quinone derivatives	Antioxidants, antibacterial, cytotoxic, antitubercular, antifungal	180	C <sub>10</sub> H <sub>12</sub> O <sub>3</sub>
9.743	3,4,5-trimethoxy phenol	Phenolic compound	Antioxidants, antifungal	184	C <sub>9</sub> H <sub>12</sub> O <sub>4</sub>
10.438	4-((1E)-3-Hydroxy-1-propenyl)- 2-methoxyphenol	Phenolic compound	Antioxidants, antifungal	180	C <sub>10</sub> H <sub>12</sub> O <sub>3</sub>
10.819	Phi.-Aspidinol	Phenolic compound	Antibacterial, anti-acne, anti-inflammatory	224	C <sub>12</sub> H <sub>16</sub> O <sub>4</sub>
11.016	N-Di-dehydrohexacarboxyl-2,4,5-trimethylpiperazine	Alkaloids	Antioxidants, antifungal	294	C <sub>13</sub> H <sub>22</sub> N <sub>2</sub> O <sub>4</sub>
11.299	Palamitic acid	Fatty acid	Cytotoxicity, antioxidant, antimicrobial	256	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>
12.018	cis,cis-Linoleic acid	Fatty acid	Cytotoxicity, antioxidant, antimicrobial	280	C <sub>17</sub> H <sub>30</sub> O <sub>2</sub>
13.549	m-Aminophenylacetylene	Phenolic compound	Antioxidants, antifungal	117	C <sub>8</sub> H <sub>7</sub> N
13.918	1-Phenyl-2-propene	Phenolic compound	Antioxidants, antifungal	150	C <sub>9</sub> H <sub>10</sub> S

## DISCUSSION

Five billion metric tons of agricultural wastes, including wheat bran, cotton leaf scraps, sugarcane bagasse, rice bran, rice straw, and fruit and vegetable waste, are produced annually from agriculture worldwide. About half of the plant matter consists of lignocellulose, which is the most abundant green organic matter on earth (Singh *et al.*, 2012). It is made up of non-covalent and covalent connections holding cellulose (35–50%), hemicellulose (20–35%), and lignin (15–25%) in close proximity to one another (Limayem and Ricke, 2012). Therefore, it is crucial to transform these wastes into beneficial products with industrial and commercial value while also minimizing their detrimental impact on the environment (Wang *et al.*, 2016; AL-Kolaibe *et al.*, 2021). The most effective use of agricultural output requires an integrated and sustainable strategy. Rice straw contains significant amounts of cellulose (39%), hemicellulose (22%), lignin (16%), and ash (18%), and it has a high potential as a biomass source (Sarnklong *et al.*, 2010; Minu *et al.*, 2012; El Safty, 2020).

Fungi have been extensively studied for the production of several extracellular metabolites such as antibiotics, antimicrobials, antioxidants, antiviral, anti-inflammatory, enzyme inhibitors, cytotoxic, hypercholesteremic, immunomodulators, and immunosuppressive medications (Choque *et al.*, 2015; Siddiquee, 2018; Naher *et al.*, 2021; Conrado *et al.*, 2022; Ramadan *et al.*, 2023). Thus, in this study, *A. tubingensis* AUMC 15759 was used to ferment nine agricultural wastes under solid-state fermentation conditions to identify which of them is deemed a valuable substrate for the synthesis of bioactive metabolites. The crude methanol extract of rice straw was shown to be the most effective in terms of antifungal and antioxidant properties. It showed a significant positive impact on the tested fungi, *C. albicans*, *C. glabrata*, *C. krusei*, and *S. cerevisiae*, over the remaining crude extracts.

The liquid/liquid fractionation of rice straw's crude extract resulted in five different

fractions, the most effective of which was the dichloromethane fraction in terms of antifungal activity, and it showed remarkable antioxidant activity of 95.0%, which was almost identical to that of ascorbic acid (95.6%). Based on the retention time, molecular weight, and fragmentation pattern of the most significant compounds, the current results revealed the identification of five phenolic chemicals (3,4,5-trimethoxyphenol, 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol, Phi.-Aspidinol, m-Aminophenylacetylene, and 1-Phenyl-2-propene), which accounted for 50%, and two fatty acids (Palamitic acid and cis,cis-Linoleic acid) accounted for 20%, were detected. Quinone derivative (2,6-Dimethyl-3-(methoxymethyl)-p-benzoquinone), and alkaloid (N-Di-dehydrohexacarboxyl-2,4,5-trimethylpiperazine) were also found comprising 10% each. Fungi have been found to create secondary metabolites such as aromatic chemicals, alkaloids, amino acids, fatty acids, phenolic compounds, and quinone derivatives (Brambilla *et al.*, 1988; Nishina *et al.*, 1991; Cole *et al.*, 2003; Leyden *et al.*, 2007; Dhahir *et al.*, 2012; Adedoyin *et al.*, 2013; Zabka and Pavela, 2013; Evidente *et al.*, 2014; Li *et al.*, 2014; Dheeb, 2015; Kareem *et al.*, 2015; Chandra and Arora, 2016; Gao *et al.*, 2016; Mollavali *et al.*, 2016; He *et al.*, 2017; Pathak *et al.*, 2018; Chen *et al.*, 2021; Widjajanti *et al.*, 2022). Species of *Alternaria*, *Aspergillus*, *Claviceps*, *Fusarium*, and *Penicillium* are the most active fungi producing approximately 15,600 fungal metabolites (Sulyok *et al.*, 2020).

The components of the dichloromethane fraction obtained in this study were found to have an effect on the examined fungi, *C. albicans*, *C. glabrata*, *C. krusei*, and *S. cerevisiae*. Regarding the active metabolites produced by *A. tubingensis*, the active metabolite biosynthesized by *A. tubingensis* AHS4, anofinic acid (2,2-dimethyl-2H-1-benzopyran-6-carboxylic acid), was isolated from ethyl acetate fraction. It had potent antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus*

*aureus*, *Escherichia coli*, *Candida albicans*, and *Bacillus subtilis*, which are all human pathogenic microorganisms. *A. tubingensis* has been also documented to excrete asperazine, funalenone, malformins, naphtho- $\gamma$ -pyrones (including aurasperone B), pyranonigrin A, tensidol A & B, (nigragillin) (Samson *et al.*, 2007). The liquid culture of *Chaetomium globosum* showed potent *in vitro* antibacterial activity against *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas fluorescens*. It also recorded significant antifungal activity against *Candida albicans*, *Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani* and *Pythium ultimum* (Awad *et al.*, 2014).

On the other hand, the fungus has also been reported to transform deoxynivalenol (He *et al.*, 2008), cause keratitis in humans (Kredics *et al.*, 2009), degrade the polyurethane polyester (Khan *et al.*, 2017), produce ochratoxin A and zearalenone on sorghum grains (Lahouar *et al.*, 2017), cause primary skin infection (Frías-De-León *et al.*, 2018), and pomegranate fruit rot in China (Guo *et al.*, 2021).

The dichloromethane fraction showed 95.0% of DPPH activity, which almost similar to that of ascorbic acid (95.6%), and it exhibited the lowest IC<sub>50</sub> (1.4±0.2) compared to the remaining fractions of *n*-butanol (1.8±0.4), ethyl acetate (1.5±0.6), *n*-hexane (11.5±0.2), aqueous (13.5±1.1), and ascorbic acid (10.64±0.84) mg/ml. The petroleum ether and ethyl acetate extracts of the liquid culture of *Chaetomium globosum* showed potent *in vitro* antioxidant activity, and the ethyl acetate extract showed the presence of prenissatin, chrysophanol, chrysazin,

#### REFERENCES

- Abou Hussein, S. D., & Sawan, O. M. (2010). The utilization of agricultural waste as one of the environmental issues in Egypt (a case study). *Journal of Applied Sciences Research*, 6(8), 1116-1124.
- Adedoyin, B., Okeniyi, S., Garba, S., & Salihu, L. (2013). Cytotoxicity, antioxidant and antimicrobial activities of essential oil extracted from *Euphorbia heterophylla* plant. *Topclass Journal of Herbal Medicine*, 2, 84-89.
- Al-Bedak, O., & Moubasher, A. (2020). *Aspergillus gaarensis*, a new addition to section Circumdati from soil of Lake El-Gaar in Wadi-El-Natron, Egypt. *Studies in Fungi*, 5(1), 59-65.
- Al-Bedak, O. A., Teama, E. A., Ali, E., Said, M., Shalaby, E., & Moharram, Z. A.

chaetoviridin A and B (Awad *et al.*, 2014). Finally, utilizing agricultural wastes by this fungus demonstrated antifungal and antioxidant activity. More research is required before it can be applied in agriculture and/or medicine.

#### Conclusion

*Aspergillus tubingensis* AUMC 15759 utilizes agricultural wastes to produce some bioactive metabolites in SSF. Rice straw produced the most active crude extract in terms of antifungal and antioxidant activity. The liquid/liquid fractionation technique resulted in five fractions, the most effective of which was the dichloromethane fraction. GC-MS analysis revealed the identification of nine compounds related to phenolic chemicals (5 compounds), fatty acids (2), quinone derivatives, and alkaloid (one compound each). The dichloromethane fraction displayed the highest antifungal against *C. albicans*, *C. glabrata*, *C. krusei*, and *S. cerevisiae*. It also showed remarkable antioxidant activity which was almost identical to that of ascorbic acid.

**Author Contributions:** All authors participated equally in data analysis, authoring, and revising the article. The final version of the manuscript has been reviewed and approved by all authors.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** All data related to this manuscript is incorporated in the manuscript only.

**Conflicts of Interest:** The authors declare no conflict of interest.

- (2020). Impact of fumigation with phosphine on viability of wheat grains stored for six months at two levels of moisture content, in addition to description of four new records of associated fungi and assessment of their potential for enzymatic production. *Journal of Basic & Applied Mycology (Egypt)*, *11*, 77-97.
- AL-Kolaibe, A. M., Moharram, A. M., & Al-Bedak, O. A. (2021). Worth while enzyme production and eco-friendly bioconversion of three agricultural residues by *Aspergillus curvatus* and *Aspergillus gaarensis*, promising enzyme-producers isolated from extreme environment. *Journal of Basic & Applied Mycology (Egypt)*, *12*, 1-14.
- Awad, N., Kassem, H., Hamed, M., El-Naggar, M., & El-Feky, A. (2014). Bioassays guided isolation of compounds from *Chaetomium globosum*. *Journal de mycologie medicale*, *24*(2), e35-e42.
- Brambilla, G., Robbiano, L., Cajelli, E., Martelli, A., Turmolini, F., & Mazzei, M. (1988). Cytotoxic, DNA-damaging and mutagenic properties of 2, 6-dimethoxy-1, 4-benzoquinone, formed by dimethophrine-nitrite interaction. *Journal of Pharmacology and Experimental Therapeutics*, *244*(3), 1011-1015.
- Chandra, P., & Arora, D. S. (2016). Production of antioxidant bioactive phenolic compounds by solid-state fermentation on agro-residues using various fungi isolated from soil. *Asian Journal of Biotechnology*, *8*: 8-15.
- Chen, Y.-J., Ma, K.-Y., Du, S.-S., Zhang, Z.-J., Wu, T.-L., Sun, Y., Yan, Y.-F. (2021). Antifungal exploration of quinoline derivatives against phytopathogenic fungi inspired by quinine alkaloids. *Journal of Agricultural and Food Chemistry*, *69*(41), 12156-12170.
- Choi, Y.-W., Hyde, K. D., & Ho, W. (1999). Single spore isolation of fungi. *Fungal Diversity*. *3*: 29-38.
- Choque, E., El Rayess, Y., Raynal, J., & Mathieu, F. (2015). Fungal naphtho- $\gamma$ -pyrones secondary metabolites of industrial interest. *Applied microbiology and biotechnology*, *99*, 1081-1096.
- Cole, J., Jarvis, B., & Schweikert, M. (2003). Secondary Fungal Metabolites: Academic Press, Amsterdam.
- Colgrove, S. G., & Svec, H. J. (1981). Liquid-liquid fractionation of complex mixtures of organic components. *Analytical chemistry*, *53*(12), 1737-1742.
- Conrado, R., Gomes, T. C., Roque, G. S. C., & De Souza, A. O. (2022). Overview of Bioactive Fungal Secondary Metabolites: Cytotoxic and Antimicrobial Compounds. *Antibiotics (Basel)*, *11*(11), 1604.
- Criscuolo, A., & Gribaldo, S. (2010). BMGE (Block Mapping and Gathering with Entropy): a new software for selection of phylogenetic informative regions from multiple sequence alignments. *BMC evolutionary biology*, *10*, 1-21.
- Dhahir, S. A., Aziz, N. M., & Bakir, S. R. (2012). Synthesis, characterization and antimicrobial studies of complexes of some metal ions with 2-[2-amino-5-(3, 4, 5-trimethoxybenzyl)-pyrimidinyl-4-azo]-4-bromo-phenol. *International Journal of Basic & Applied Sciences*, *180*(182), 58-69.
- Dheeb, B. I. (2015). Antifungal Activity of Alkaloids and Phenols Compounds extracted from black pepper *Piper nigrum* against some pathogenic fungi. *Journal of Biotechnology Research Centre*, *9*(2), 46-54.
- El Safty, A. (2020). Environmental and Health Impact of Open Burning

- Rice Straw. *Egyptian Journal of Occupational Medicine*, 44, 679-708.
- Evidente, A., Kornienko, A., Cimmino, A., Andolfi, A., Lefranc, F., Mathieu, V., & Kiss, R. (2014). Fungal metabolites with anticancer activity. *Natural product reports*, 31(5), 617-627.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39(4), 783-791.
- Frías-De-León, M. G., Rosas-de Paz, E., Arenas, R., Atoche, C., Duarte-Escalante, E., de Soschin, D. M., Reyes-Montes, M. (2018). Identification of *Aspergillus tubingensis* in a primary skin infection. *Journal de mycologie medicale*, 28(2), 274-278.
- Gao, C., Guo, N., Li, N., Peng, X., Wang, P., Wang, W., Fu, Y.-j. (2016). Investigation of antibacterial activity of aspidin BB against *Propionibacterium acnes*. *Archives of dermatological research*, 308, 79-86.
- Glass, N. L., & Donaldson, G. C. (1995). Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and environmental microbiology*, 61(4), 1323-1330.
- Guo, M. J., Wang, Q. T., Cheng, Y. H., & Hou, C. L. (2021). Identification of *Aspergillus tubingensis* causing pomegranate fruit rot in China. *Australasian Plant Pathology*, 50, 233-240.
- Harris, R., & Sommers, L. (1968). Plate-dilution frequency technique for assay of microbial ecology. *Applied Microbiology*, 16(2), 330-334.
- He, C., Fan, Y., Liu, G., & Zhang, H. (2008). Isolation and identification of a strain of *Aspergillus tubingensis* with deoxynivalenol biotransformation capability. *International journal of molecular sciences*, 9(12), 2366-2375.
- He, W.-J., Zhou, X.-J., Qin, X.-C., Mai, Y.-X., Lin, X.-P., Liao, S.-R., Wang, J.-F. (2017). Quinone/hydroquinone meroterpenoids with antitubercular and cytotoxic activities produced by the sponge-derived fungus *Gliomastix* sp. ZSDS1-F7. *Natural Product Research*, 31(5), 604-609.
- Ismail, M., Moubasher, A., Mohamed, R., & Al-Beddak, O. (2018a). Agro-industrial residues as alternative sources for cellulases and xylanases production and purification of xylanase produced by *Aspergillus flavus* AUMC 10331 isolated from extreme habitat. *Current Research in Environmental & Applied Mycology*, 8, 313-322.
- Kareem, H. S., Ariffin, A., Nordin, N., Heidelberg, T., Abdul-Aziz, A., Kong, K. W., & Yehye, W. A. (2015). Correlation of antioxidant activities with theoretical studies for new hydrazone compounds bearing a 3, 4, 5-trimethoxy benzyl moiety. *European Journal of Medicinal Chemistry*, 103, 497-505.
- Katoh, K., & Toh, H. (2010). Parallelization of the MAFFT multiple sequence alignment program. *Bioinformatics*, 26(15), 1899-1900.
- Khan, S., Nadir, S., Shah, Z. U., Shah, A. A., Karunarathna, S. C., Xu, J., Hasan, F. (2017). Biodegradation of polyester polyurethane by *Aspergillus tubingensis*. *Environmental pollution*, 225, 469-480.
- Khanh, D., & Thanh, N. H. (2010). *Management of agricultural waste and potential for co-benefits*. Paper presented at the Presentation presented at the Regional Workshop of the Greater Mekong Sub region on the National Strategy of

- Integrated Solid Waste Management.
- Kredics, L., Varga, J., Kocsubé, S., Rajaraman, R., Raghavan, A., Dóczy, I., Venkatapathy, N. (2009). Infectious keratitis caused by *Aspergillus tubingensis*. *Cornea*, 28(8), 951-954.
- Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular biology and evolution*, 35(6), 1547-1549.
- Lahouar, A., Marin, S., Crespo-Sempere, A., Saïd, S., & Sanchis, V. (2017). Influence of temperature, water activity and incubation time on fungal growth and production of ochratoxin A and zearalenone by toxigenic *Aspergillus tubingensis* and *Fusarium incarnatum* isolates in sorghum seeds. *International journal of food microbiology*, 242, 53-60.
- Leyden, J. J., Del Rosso, J. Q., & Webster, G. F. (2007). Clinical considerations in the treatment of acne vulgaris and other inflammatory skin disorders: focus on antibiotic resistance. *Cutis*, 79(6), 9-25.
- Li, N., Gao, C., Peng, X., Wang, W., Luo, M., Fu, Y.-j., & Zu, Y.-g. (2014). Aspidin BB, a phloroglucinol derivative, exerts its antibacterial activity against *Staphylococcus aureus* by inducing the generation of reactive oxygen species. *Research in microbiology*, 165(4), 263-272.
- Limayem, A., & Ricke, S. C. (2012). Lignocellulosic biomass for bioethanol production: current perspectives, potential issues and future prospects. *Progress in energy and combustion science*, 38(4), 449-467.
- Magaldi, S., Mata-Essayag, S., De Capriles, C. H., Pérez, C., Colella, M., Olaizola, C., & Ontiveros, Y. (2004). Well diffusion for antifungal susceptibility testing. *International journal of infectious diseases*, 8(1), 39-45.
- Minu, K., Jiby, K. K., & Kishore, V. (2012). Isolation and purification of lignin and silica from the black liquor generated during the production of bioethanol from rice straw. *biomass and bioenergy*, 39, 210-217.
- Mollavali, M., Bolandnazar, S. A., Schwarz, D., Rohn, S., Riehle, P., & Zaare Nahandi, F. (2016). Flavonol glucoside and antioxidant enzyme biosynthesis affected by mycorrhizal fungi in various cultivars of onion (*Allium cepa* L.). *Journal of Agricultural and Food Chemistry*, 64(1), 71-77.
- Moubasher, A. (1993). Soil fungi in Qatar and other Arab countries: *The Centre for Scientific and Applied Research, University of Qatar*, 50, 242.
- Moubasher, A., Ismail, M., Al-Bedak, O., & Mohamed, R. (2019). *Ramophialophora chlamydospora*, a new species from an alkaline lake of Wadi-El-Natron, Egypt. *Asian Journal of Mycology*, 2(1), 110-117.
- Naher, L., Fatin, S. N., Sheikh, M. A. H., Azeez, L. A., Siddiquee, S., Zain, N. M., & Karim, S. M. R. (2021). Cellulase enzyme production from filamentous fungi *Trichoderma reesei* and *Aspergillus awamori* in submerged fermentation with rice straw. *Journal of Fungi*, 7(10), 868.
- Nishina, A., Hasegawa, K., Uchibori, T., Seino, H., & Osawa, T. (1991). 2, 6-Dimethoxy-p-benzoquinone as an antibacterial substance in the bark of *Phyllostachys heterocycla* var. pubescens, a species of thick-stemmed bamboo. *Journal of Agricultural and Food Chemistry*, 39(2), 266-269.
- Pathak, R., Goyal, S., Kumari, A., & Dwivedi, S. K. (2018). Antioxidant

- profiling and fatty acid composition of lipids present in *Hippophae salicifolia* grown in higher altitude of Uttarakhand region. *World Journal of Pharmaceutical Research*, 7(16), 1286-1297.
- Pitt, J. I. (1979). The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*.
- Posada, D., & Crandall, K. A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics (Oxford, England)*, 14(9), 817-818.
- Ramadan, A. M., Shehata, R. M., El-Sheikh, H. H., Ameen, F., Stephenson, S. L., Zidan, S. A., & Al-Bedak, O. A. (2023). Exploitation of Sugarcane Bagasse and Environmentally Sustainable Production, Purification, Characterization, and Application of Lovastatin by *Aspergillus terreus* AUMC 15760 under Solid-State Conditions. *Molecules*, 28(10), 4048.
- Raper, K.B., Fennell, D. I. (1965). *The Genus Aspergillus*. Baltimore; Williams & Wilkins Co: Philadelphia, PA, USA, 686.
- Samson, R., Houbraeken, J., Thrane, U., Frisvad, J., & Andersen, B. (2010). Food and indoor fungi: CBS-KNAW fungal biodiversity centre: Utrecht. 3, 390.
- Samson, R. A., Noonim, P., Meijer, M., Houbraeken, J., Frisvad, J. C., & Varga, J. (2007). Diagnostic tools to identify black aspergilli. *Studies in Mycology*, 59(1), 129-145.
- Samson, R. A., Visagie, C. M., Houbraeken, J., Hong, S.-B., Hubka, V., Klaassen, C. H., Tanney, J. B. (2014). Phylogeny, identification and nomenclature of the genus *Aspergillus*. *Studies in mycology*, 78, 141-173.
- Sarnklong, C., Cone, J. W., Pellikaan, W., & Hendriks, W. H. (2010). Utilization of Rice Straw and Different Treatments to Improve Its Feed Value for Ruminants: A Review. *Asian-Australas J Anim Sci*, 23(5), 680-692.
- Siddiquee, S. (2018). Recent advancements on the role of biologically active secondary metabolites from *Aspergillus* New and future developments in microbial biotechnology and bioengineering, Elsevier, 69-94.
- Singh, R., Kapoor, V., & Kumar, V. (2012). Utilization of agro-industrial wastes for the simultaneous production of amylase and xylanase by *Thermophilic actinomycetes*. *Brazilian journal of microbiology*, 43(4), 1545-1552.
- Sulyok, M., Stadler, D., Steiner, D., & Krska, R. (2020). Validation of an LC-MS/MS-based dilute-and-shoot approach for the quantification of > 500 mycotoxins and other secondary metabolites in food crops: Challenges and solutions. *Analytical and bioanalytical chemistry*, 412, 2607-2620.
- Valgas, C., Souza, S. M. d., Smânia, E. F., & Smânia Jr, A. (2007). Screening methods to determine antibacterial activity of natural products. *brazilian journal of microbiology*, 38, 369-380.
- Wang, B., Dong, F., Chen, M., Zhu, J., Tan, J., Fu, X., Chen, S. (2016). Advances in recycling and utilization of agricultural wastes in China: Based on environmental risk, crucial pathways, influencing factors, policy mechanism. *Procedia environmental sciences*, 31, 12-17.
- Widjajanti, H., Nurnawati, E., & Triuspita, V. (2022). The potency of endophytic fungi isolated from *Hippobroma longiflora* (L) G. Don as an antioxidant sources. Paper presented at the IOP Conference Series: Earth and Environmental Science. 976, 1-10.
- Yen, G. C., & Duh, P. D. (1994). Scavenging effect of methanolic

extracts of peanut hulls on free-radical and active-oxygen species. *Journal of agricultural and food chemistry*, 42(3), 629-632.

Zabka, M., & Pavela, R. (2013). Antifungal efficacy of some natural phenolic

compounds against significant pathogenic and toxinogenic filamentous fungi. *Chemosphere*, 93(6), 1051-1056.