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Optimization of different growth parameters for maximum production of bioactive crude metabolites by *Aspergillus fumigatus*

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Abstract: *Aspergillus fumigatus* is a green echinulate with greenish phialides and 2.5-3 mm conidia. The diverse biological functions of *A. fumigatus* secondary metabolites make them interesting. The ethyl acetate extract of *A. fumigatus* was tested for antibacterial activity. Culture media, temperature, incubation and pH were optimized for *A. fumigatus* growth. Continuous 150rpm agitation incubated the fungus at 28°C for 10 days. Potato Dextrose Broth at 28°C in shaking incubator at pH 04 produced the most biomass and secondary metabolites. Metabolite antibacterial activity was tested. *Salmonella flexneri* had the greatest zone of inhibition at 100µl (25.66mm) while *Staphylococcus aureus* had the least (16.33mm). At 75µg/mL, *S. flexneri* showed 23.66mm activity and *S. typhi* 14.66mm. At 50µg/mL, *S. flexneri* was 21.33mm and *S. typhi* 12.33mmMBC was 0.01µg/µl and MIC50 varied. At 100µg/mL, the metabolites showed antifungal efficacy against *Penicillium chrysogenum* (26.33mm) but not *A. flavus* (21.33mm). *A. oryzae* was significantly inhibited at 75µg/mL (26.33mm) and 50µg/mL (20.33mm). 1000µl demonstrated 100% phytotoxicity, 100µl 60%, and 10µl 50%. *Bactrocera cucurbitae, Sitotroga cerealella* and *Callosobruchus maculatus* were killed at 150, 100, and 75µl. Metabolites and antibiotics synergized well. Metabolites have alkanes, esters and ethers in their infrared spectra.

Keywords: Echinulate, ethyl acetate, *Aspergillus fumigatus*, secondary metabolites.

INTRODUCTION

Fungi are indeed a distinct kingdom of organisms, separate from plants and animals. They play crucial roles in various ecosystems and have significant economic importance. Despite their importance, the study of fungi lags behind that of plants and animals. The estimated number of fungal species is quite vast, ranging from 700,000 to 5 million species worldwide. However, only a fraction of these species, approximately 100,000, have been formally described and classified by scientists. This indicates that there is still much to learn about fungi, their diversity and their ecological and economic significance. Further research is needed to explore and understand the vast world of fungi (Gautam, *et al.*, 2022). Fungi are found in a wide range of environments, including soil, water, air and living organisms. They play important roles in decomposing organic matter and recycling nutrients in ecosystems. Some fungi form mutualistic relationships with plants and help them absorb nutrients from the soil, while others are pathogens that can cause diseases in plants, animals and humans (Dix, 2012).

Fungi play a crucial role in the natural ecosystem as decomposers, breaking down dead plant and animal matter and recycling nutrients back into the soil. Some fungi also form symbiotic relationships with other organisms, such as mycorrhizal fungi that form associations with plant roots to exchange nutrients. However, there are also many pathogenic fungi that cause diseases in plants, animals and humans. For example, the fungus *Fusarium oxysporum* can cause wilt disease in a wide range of crops, including corn and wheat, leading to significant losses in agricultural productivity (Rogers 2010). These fungi form symbiotic associations with plant roots and are known as mycorrhizae. There are two types of mycorrhizae: end mycorrhizae and ectomycorrhizae. End mycorrhizae penetrate the root cells and form arbuscules, while ectomycorrhizae form a sheath around the roots and penetrate between cells. Both types of mycorrhizae help plants absorb nutrients from the soil, especially phosphorus, which is often limiting for plant

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growth. In exchange, the fungi receive carbohydrates from the plant. This mutually beneficial relationship is essential for many plants to grow and survive in nutrient-poor soils (Garg & Chandel, 2011). Additionally, some soil microorganisms produce antibiotics and other compounds that inhibit the growth of fungal pathogens. Therefore, maintaining a healthy soil microbiome is important for preventing soil-borne fungal diseases and promoting plant health (Lugtenberg *et al*., 2016).

Endophytic fungi can produce a diverse array of secondary metabolites with potential applications in medicine and agriculture. These bioactive compounds have been found to have various biological activities, including antitumor, antiviral, antifungal and antibacterial properties. Some of these compounds have already been developed into drugs, such as the anticancer drug taxol, which is produced by an endophytic fungus. The potential for discovering new bioactive compounds from endophytic fungi is vast, as only a small percentage of these fungi have been studied for their secondary metabolites (Shi *et al*., 2015). Secondary metabolites produced by fungi have diverse biological activities and play important roles in the ecological interactions between fungi and other organisms.

Some secondary metabolites are involved in pathogenic interactions with plants and animals, while others may provide protection against microbial competitors in soil environments. Secondary metabolites produced by endophytic fungi are of particular interest due to their potential for developing new pharmaceuticals, agrochemicals and other biotechnological applications (Correa *et al*., 2022). The discovery of new metabolites from fungi holds great promise for addressing the challenges posed by multi-resistant pathogenic isolates. Traditional antimicrobial agents are becoming less effective against these pathogens, making the search for novel bioactive compounds from fungi even more important.

The Ethyl acetate extract was then tested for its biological activities. Antibacterial activity was evaluated against three bacterial strains, *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* using the disc diffusion method. Antifungal activity was assessed against three fungal strains, *Candida albicans, Aspergillus flavus* and *Fusarium oxysporum* using the same method. Phytotoxic activity was tested against lettuce seeds and insecticidal activity was evaluated against Sitophilus oryzae using a contact toxicity assay. The results showed that the ethyl acetate extract of *A. fumigatus* exhibited significant antibacterial and antifungal activity against all tested strains. Phytotoxicity and insecticidal activity were also observed. Further studies are needed to identify the active compounds responsible for these activities and to investigate their potential applications in various fields.

MATERIALS AND METHODS

Method for collection of soil samples

Soil sampling is an important step in any study that involves the isolation and identification of microorganisms from soil. In this case, the soil samples were collected from various locations in Peshawar and placed in sterilized polythene bags to prevent contamination. The depth of sampling was 15cm, which is considered the topsoil layer where most microorganisms are found. Once in the laboratory, the soil samples were processed to isolate and identify the target microorganism, *A. fumigatus*.

Isolation of fungi from the soil samples

The soil fungi were cultivated using the serial dilution method on several medium, including Potato Dextrose Agar and Sabouraud Dextrose Agar.

Soil dilution plate method

A microbial suspension was made from 1gm of soil in 1mL of distilled water. Isolating fungus required different dilutions. 1mL of each microbial suspension concentration was applied to sterile petri dishes. Sterile SDA filled 20mL petri plates. After three days at 28°C, the petri plates were monitored daily (Uniyal, *et al*., 2018).

Optimization of fungal biomass and secondary metabolites

Potato Dextrose Broth (PDB), Czapek Yeast Extract Broth (CYB), CZDB, CZDM and YEB were utilized to determine the best fungal media for crude metabolite synthesis (Iqbal *et al*., 2014). Formula for biomass and secondary metabolites:

Average weight of sec. metabolites/dry mycelia = Weight of sec. metabolites/dry mycelia in vial -Weight of vial

Optimization of the incubation temperature

The goal of this study was to find the ideal incubation temperature at which the isolated species could create the most physiologically active secondary metabolites.

Shaking/ static conditions

To determine which of two incubation conditions-shaking or static-would promote the greatest growth of *A. fumigatus*, both were offered.

Extraction of secondary metabolites

Secondary metabolites were extracted from optimized culture media at optimized culture conditions.

Protocol

Optimized culture media (PDB) was prepared and autoclaved, the fungal culture was grown in broth media by inoculating the broth with fungal culture grown on PDA. The flasks were incubated at optimized temp; 28°C

with continuous agitation of 100 rpm for 10 days. Then EtOAc was added in a ratio 1:1 of the media to the flask and was stirred properly to degrade the media components. After 2 to 3 hrs. filtration was performed using Wattman filter paper. The mixture was left to settle down for several minutes. The EtOAc phase was recovered in a flask and evaporated through a rotary aspirator at 45°C under vacuum pressure.

Antibacterial activity

The EtOAc extract of fungus was screened against nine human pathogenic bacterial isolates i.e. *Bacillus subtilis, Salmonella typhi, Salmonella paratyphi, Escherichia coli, Klebsiella pneumonia, Pseudomonas aureginosa, Staphylococcus aureus*, *Shigella flexneri* and *Citrobacter freundii* species were used (Katoch *et al*., 2014). The zones of inhibition were determined from the diameter of the wells while percentage inhibition was recorded by the formula given below (Leng, *et al.,* 2017).

$$\%\ \text{inhibition} = \frac{\text{Zone of inhibition of test sample}}{\text{Zone of inhibition of standard}} \times 100$$

Determination of minimum inhibitory and bactericidal concentrations

Different dilutions obtained after serial dilutions were 0.01, 0.02, 0.04, 0.08, 0.16, 0.32 and 0.64µg/µl. After this 18-24 hrs. bacterial culture of the test sample was inoculated to the test tubes containing sterilized medium. Gentamycin powder was used as positive control in DMSO while DMSO less than 1% was used as negative control (Sadananda *et al*., 2013).

Antifungal activity

The EtOAc extract of fungus was screened against seven fungal isolates i.e. *A. flavus, Candida albican*, *Alternaria alternata*, *Verticillium chlymadosporium*, *Rhizopus stolonifer*, *Penicillium chrysogenum* and *A. oryzae* (Katoch *et al*., 2014). Fluconazole was used as positive control while DMSO less than 1% was used as negative control (Leng, *et al.,* 2017).

$$\%\ \text{inhibition} = \frac{\text{Zone of inhibition of test sample}}{\text{Zone of inhibition of standard}} \times 100$$

Synergistic activity

Synergistic combinations were prepared using the fungal extract and the antibiotic discs. The synergistic study was carried out in triplicate (Arivudainambi *et al*., 2011). Synergistic effect between fungal extract and different antibiotics i.e. Azithromycin, Amoxicillin, Sulphamethaxazole, Ciprofloxacin and Oxacillin were tested against different bacterial species. Gentamycin was used as positive control while DMSO less than 1% was used as negative control.

Phytotoxic activity

E- Medium was prepared for this activity. Sterile DMSO was used to prepare a stock solution of 1mg/mL. From this stock solution different dose concentrations (i.e. 10, 100 and 1000µg/mL) were then prepared (Khattak *et al*., 2014). Paraquat was used as standard plant growth inhibitor. Then on eighth day the fronds were visually examined and the percent growth inhibition was calculated using the formula by (Leng *et al.,* 2017).

$$\text{Growth inhibition}\ (\%) = 100 - \frac{\text{No of fronds sample}}{\text{No of fronds in control}} \times 100$$

Insecticidal activity

For this activity test insects were collected from Termites lab of Nuclear Institute of Food and Agriculture (NIFA), Peshawar. The insects included *Bactrocera cucurbitae* (*Melon fly*), *Sitotroga cerealella* (*Angoumois grain moth*), *Callosobruchus maculatus* (*Pulse bettle*). To prepare the stock solutions, 10mg of the crude extract was dissolved in 1mL of EtOAc extract of *A. fumigatus* (Han *et al*., 2013). Permithrine (copex) was used as positive control while the organic solvent was used as negative control. The results were recorded after 24 hours by the formula given below (Leng, *et al.,* 2017).

$$\%\ \text{Mortality} = 100 - \frac{\text{No of insect alive in test}}{\text{No of insect alive in control}} \times 100$$

FTIR analysis

FTIR was carried out for the EtOAc extract in the mid – IR region of 400-4000cm^{-1}. Dried sample was mixed with the KBr and the spectrum was noted in the mode of transmittance (Meenambal *et al*., 2012). The model IR Pretige-21 was used for performing FTIR (Shimadzu, Japan) and Infrared radiation (IR) solution software (Chang *et al.*, 2021).

STATISTICAL ANALYSIS

The statistical analysis was performed by using SPSS Software by following the SPSS survival manual. The standard means and deviations were found through the software.

RESULTS

Optimization of growth parameters

Optimization of culture media by determining fungal biomass and secondary metabolites

Five different types of fungal culture media were used to identify the best media for the maximum growth of *A. fumigatus* in two types of incubator i.e. shaking and static at three different temperatures i.e. 25, 28 and 30°C. These media include PDB, CYB, CZDB, CZDM and YEB. From results it was observed that the best medium for the maximum biomass (677.33±2.88mg/250mL) and secondary metabolites (258.66±3.51µg/250mL) was PDB.

Optimization of different temperatures

The growth of *A. fumigatus* was observed at three different temperatures i.e. 25°C, 28°C and 30°C.

Maximum biomass (677.33±2.88mg/250mL) and metabolites (258.66±3.51µg/250mL) was observed at 28°C. It was concluded that *A. fumigatus* grow best at 28°C by using PDB (fig. 1).

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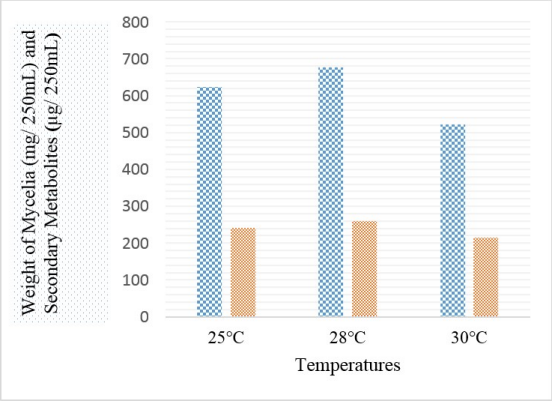

Fig. 1: Mycelial biomass and crude metabolites at different Temperatures

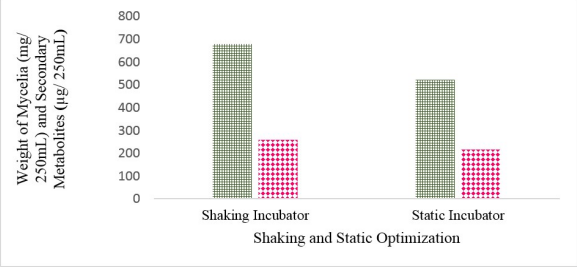

Fig. 2: Mycelial biomass and crude metabolites at static/ shaking condition

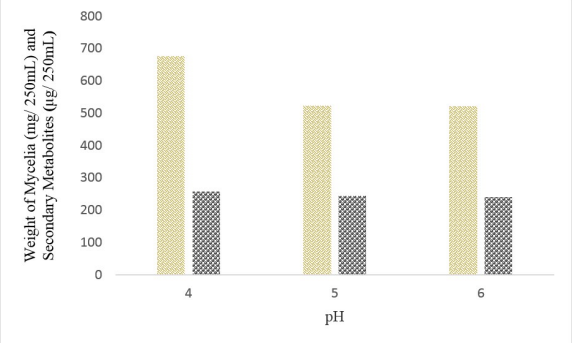

Fig. 3: Mycelial biomass and crude metabolites at different pH

Shaking and static optimization

Maximum biomass (677.33±2.88mg/250mL) and secondary metabolites (258.66±3.51µg/250mL) were obtained at shaking condition (fig. 2)

Optimization of pH

Maximum growth (677.33±2.88mg/250mL) as well as secondary metabolites (258.66±3.51µg/250mL) were produced at the pH of 04 (fig. 3).

Antibacterial activity

The results of antibacterial activity of crude extract at different concentrations are shown in table 2. Similar procedure was used by Swathi *et al*. (2013) and according to their study the crude extract of Microascus spp. showed efficient activity against bacterial and fungal test organisms.

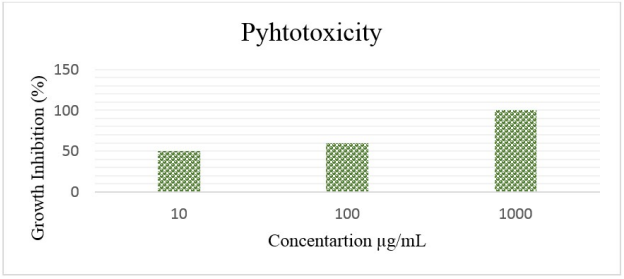

Fig. 4: Phytotoxicity of crude EtOAc extract of *A. fumigatus*

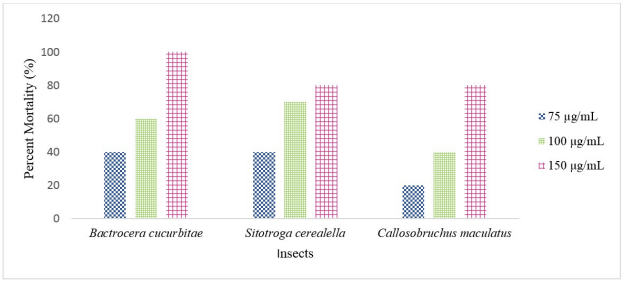

Fig. 5: Insecticidal activity of crude ethyl acetate extract of *A. fumigatus*

Minimum inhibitory and bactericidal concentration measurement

MIC_{50} and MBC values of the crude extract of *A. fumigatus* against tested bacteria were shown in the (table 3).

Antifungal activity

The sample concentration, 10mg of the EtOAc extract per mL of DMSO, showed significant activity in the form of inhibitory zones at the conc. rate of 100µg/mL against *P. chrysogenum* (26.33±0.57mm) and *A. oryzae* (25.66± 0.57 mm. At 75µg/mL, significant result was observed against *A. oryzae* (26.33±0.57mm) and *P. chrysogenum* (24.66±0.57mm. At 50µg/mL, the test sample showed significant activity against *A. oryzae* (20.33±0.57mm) as shown in table 4.

Phytotoxic activity

Phytotoxic screening of crude EtOAc extract of *A. fumigatus* was carried out using *L. minor* plant as a test specie. The results are shown in fig. 4. In a study by

Khattak *et al*., 2014, the crude metabolites of *Penicillium* and *Aspergillus* spp. were tested for phytotoxicity against *L.* minor plant. Different degrees of phytotoxicity were observed on different concentrations of EtOAc and n-hexane fractions of both fungal isolates. Different types of necrotic lesions were observed that varied from reddish brown to dark brown in color.

Three dose concentrations (10, 100 and 1000µg/mL) were used in order to determine the phytotoxic ability of the crude metabolites. The EtOAc fraction of *Aspergillus* sp. showed 0, 30 and 65% mortality respectively.

Insecticidal activity

To the existing chemical treatment methods of biological control of pathogens, microorganisms have been considered a more natural and environmentally conventional alternative (Van *et al.,* 2018). In this study, effective results were observed by the crude extract of *A. fumigatus* against different type of insects used. These observations thus agree with the reports that fungi used in the biological control of pests are able to elaborate metabolites harmful to the pests The insects were monitored and results were noted after 24 hours. After 24 hours percent mortality was determined.

Table 1: Optimization of fungal biomass and secondary metabolites

S. NO.	Media	Fungal biomass and secondary metabolites	
		Average dry weight of Mycelium (mg/ 250mL)	Average weight of secondary metabolites (µg/ 250mL)
1	PDB	677.33 ± 2.88	258.66 ± 3.51
2	CYB	649.33 ± 2.08	209.66 ± 3.51
3	CZDB	618.33 ± 2.88	232.33 ± 2.51
4	CZDM	607.66 ± 2.51	251.00 ± 3.6
5	YEB	602.33 ± 2.51	168.33 ± 2.8

Table 2: Antibacterial activity of crude extract at different concentrations

S. No.	Bacterial species	ZI (MM)					
		100 (µg/mL)	% Inh.	75 (µg/mL)	% Inh.	50 (µg/mL)	% Inh.
1	*B. subtilis*	18.33 ± 0.57	76.35± 0.57	16.66 ± 0.57	69.41±0.57	14.66± 0.57	61.08± 0.57
2	*C. freundii*	25.33 ± 0.57	97.42± 0.57	22.33 ± 0.57	97.08±0.57	20.66± 0.57	89.82± 0.57
3	*E. coli*	19.66 ± 0.57	78.64± 0.57	17.66 ± 0.57	70.64±0.57	15.33± 0.57	61.32± 0.57
4	*K. pneumonia*	19.66 ± 0.57	78.64± 0.57	16.33 ± 0.57	65.32±0.57	13.66± 0.57	54.64± 0.57
5	*P. aureginosa*	20.66 ± 0.57	93.30± 0.57	18.33 ± 0.57	96.47±0.57	14.33± 0.57	95.53± 0.57
6	*S. aureus*	16.33 ± 0.57	96.05± 0.57	14.66 ± 0.57	97.73±0.57	12.66± 0.57	97.38± 0.57
7	*S. flexneri*	25.66 ± 0.57	98.69± 0.57	23.66 ± 0.57	93.29±0.57	21.33± 0.57	88.87± 0.57
8	*S. paratyphi*	21.66 ± 0.57	86.64± 0.57	16.33 ± 0.57	65.32±0.57	14.66± 0.57	58.64± 0.57
9	*S. typhi*	20.66 ± 0.57	89.82± 0.57	14.66 ± 0.57	63.73±0.57	12.33± 0.57	53.60± 0.57

Table 3: MIC_{50} and MBC of crude extract against different bacteria

S. No	Bacterial species	MIC_{50} (µG/ML)	MBC (µG/ML)
1	*C. freundii*	0.16	0.01
2	*E. coli*	0.32	0.01
3	*K. pneumonia*	0.02	0.01
4	*P. aureginosa*	0.04	0.01
5	*S. aureus*	0.02	0.01
6	*S. flexneri*	0.02	0.01
7	*S. paratyphi*	0.16	0.01
8	*S. typhi*	0.16	0.01

Table 4: Antifungal activity of crude extract at different concentrations

S. No.	Fungal species	ZI (MM)					
		100 (µg/mL)	% Inh.	75 (µg/mL)	% Inh.	50 (µg/mL)	% Inh.
1	*A. alternate*	22.33±0.57	97.08±0.57	18.33±0.57	91.65±0.57	16.66±0.57	87.68±0.57
2	*A. flavus*	21.33±0.57	96.95±0.57	19.33±0.57	96.65±0.57	16.66±0.57	92.55±0.57
3	*A. oryzae*	25.66±0.57	98.69±0.57	26.33±0.57	97.51±0.57	20.33±0.57	92.40±0.57
4	*C. albican*	21.33±0.57	96.95±0.57	18.33±0.57	91.65±0.57	16.66±0.57	92.55±0.57
5	*P. chrysogenum*	26.33±0.57	97.51±0.57	24.66±0.57	98.64±0.57	17.66±0.57	92.94±0.57
6	*R. stolonifer*	24.66±0.57	98.64±0.57	18.33±0.57	91.65±0.57	16.66±0.57	87.68±0.57
7	*V. chlymadosporium*	21.33±0.57	96.95±0.57	19.33±0.57	92.04±0.57	16.66±0.57	98±0.57

Table 5: Antibiogram analysis of different bacteria

S. No.	Bacterial species	ZI of antibiotics (MM)				
		Azithromycin (AZM)	Ampicillin (AMC)	Sulpha-methoxazole (SMZ)	Ciprofloxacin (CRO)	Oxacillin (OX)
1	*B. subtilis*	11.33 ±0.57	-	11.33 ±0.57	16.33 ±0.57	-
2	*C. freundii*	-	-	-	12.33±0.57	-
3	*E. coli*	-	-	-	15.66 ±0.57	-
4	*K. pneumonia*	-	13.33 ±0.57	11.33 ±0.57	16.33 ±0.57	-
5	*S. aureus*	-	-	-	11.33 ±0.57	-
6	*S. flexneri*	-	-	-	14.66 ±0.57	-
7	*V. cholera*	-	-	-	14.66 ±0.57	-

Key words: "-"= No growth

Table 6: Synergistic effect of antibiotics and EtOAc extract of *A. fumigatus*

S. No	Bacterial species	Zi of antibiotics & etoac extract of *a. Fumigatus* (mm)				
		(azm) + etoac extract	(amc) + etoac extract	(smz) + etoac extract	(cro) + etoac extract	(ox) + etoac extract
1	*B. Subtilis*	11.33 ±0.57	13.33 ±0.57	13.66 ±0.57	18.33 ±0.57	11.66±0.57
2	*C. Freundii*	11.66 ±0.57	13.33 ±0.57	13.66 ±0.57	20.66 ±0.57	11.66±0.57
3	*E. Coli*	12.66 ±0.57	12.33 ±0.57	13.66 ±0.57	17.33 ±0.57	11.33±0.57
4	*K. Pneumonia*	18.33 ±0.57	16.66 ±0.57	19.66 ±0.57	20.33 ±0.57	12.33±0.57
5	*S. Aureus*	18.66 ±0.57	17.66 ±0.57	16.66 ±0.57	19.66 ±0.57	16.33±0.57
6	*S. Flexneri*	13.66±0.57	16.33 ±0.57	11.66 ±0.57	20.33 ±0.57	14.33±0.57
7	*V. Cholera*	13.66 ±0.57	14.33 ±0.57	13.66 ±0.57	19.66 ±0.57	11.66±0.57

Table 7: Chemical constituents detected in the ethyl acetate extract of *A. fumigatus*

Extract name	IR frequency values CM^{-1}	Chemical constituents
EtOAc	3332	OH- H bonded OH
	2931.80	Alkanes H-C-H
	1732.08	Esters C=O
	1716.65	Esters C=O
	1699.29	Carboxylic acids C=O
	1681.93	Aldehydes C=O
	1616.35	Alkenes C-C=C
	1373.32	Nitro Groups N=O
	1234.44	Ethers C-O
	1201.65	Esters C-O
	1049.28	Ethers C-O
	1033.85	Esters C-O
	1022.27	Esters C-O

Synergistic activity

The structure of secondary metabolites vary immensely. The interest in the secondary metabolites of *A. fumigatus* is due to their wide range of biological activities. The new antifungal antibiotics, fumifungin and synerazol were isolated from the culture broth of *A. fumigatus* (War, *et al.*, 2016). The ethyl acetate extract of fungus was screened against seven human pathogenic bacterial isolates i.e. *B. subtilis, E. coli, K. pneumonia, V. cholera, S. aureus*, *S. flexneri* and *C. freundii* for synergistic activity.

Antibiogram analysis

The results of antibiogram analysis are presented in table 5.

Antibacterial activity of crude extract

The ZI at the concentration rate of 100µg/mL was observed against *S. flexneri* (25.66mm), *C. freundii* (25.33mm), *S. paratyphi* (21.66mm), *S. typhi* (20.66mm), *P. aureginosa* (20.66mm), *E. coli* (19.66mm), *K. pneumonia* (19.66mm) and *B. subtilis* (18.33mm) At the concentration rate of 75µg/mL significant results were observed against *S. flexneri* (23.66mm) and *C. freundii* (22.33mm) while at the concentration rate of 50µg/mL

significant ZI was observed against *S. flexneri* (21.33mm) and *C. freundii* (22.66mm). It showed good activity against *E. coli* (15.33mm), *B. subtilis* (14.66mm), *S. paratyphi* (14.66mm) and *P. aureginosa* (14.33mm) as described in table 2.

Synergistic analysis

Five different antibiotics i.e. Azithromycin (AZM), Amoxicillin (AMC), Sulphamethaxazole (SMZ), Ciprofloxacin (CRO) and Oxacillin (OX) were used in combination with EtOAc extract of *A. fumigatus*. The results are represented in table 6.

FTIR studies

It had been confirmed from spectroscopic analysis that the EtOAc extract of *A. fumigatus* contains various compounds such as alcohol, amines, ether, amides, carboxylic acid etc. The various chemical constituents detected in the extract are given in table 7.

DISCUSSION

Fungi produce a wide range of secondary metabolites with high therapeutic value as antibiotics, cytotoxic substances and insecticidal compounds that promote or inhibit growth, attractor, repellent etc. Fungi produce a wide range of metabolites, many of that appear unnecessary for the primary function of the host. Amrit *et al* (2014) performed a study in which different culture media and environmental factors affecting the growth and sporulation of the tested fungi under different conditions were optimized. For *A. fumigatus,* excellent growth media reported was PDA at 45°C and pH of 4. The results were obtained by using dry mycelia mass while in our study the PDB media was the best for growth after twelve days period at optimum temperature of 28°C at shaking condition.

The study of Garcia *et al* (2016) revealed the most efficient method for the obtainment of fungal secondary metabolites. The extraction was performed with ethyl acetate, where the results were statistically significant relative to negative control and were obtained for at least one bacterium tested. The obtainment of the metabolites in methanol by incubation of fungal mycelium did not present positive results for none of the isolates while in this study only ethyl acetate extraction was carried out and the results of each activity performed were significant with this respect.

In this study, extraction of secondary metabolites from *A. fumigatus* was carried out. A total of five different types of media were used for growth of *A. fumigatus* i.e. Potato Dextrose Broth (PDB), Czapek Yeast Extract Broth (CYB), Czapek Dox Broth (CZDB), Czapek Dox Minimal Broth (CZDM) and Yeast Extract Broth (YEB). The PDB media was the best for growth after ten (10) days period at optimum temperature of 28°C at shaking condition while in the study of Swathi *et al* (2013), the *Microascus specie* was grown in 1 liter of sterilized PDB media and was incubated for 10 days. After the incubation period, the broth was saturated in ethyl acetate overnight. The ethyl acetate was then separated by rotary evaporator.

The antibacterial and antifungal screening was performed at three different concentrations i.e. 100µl, 75µl and 50µl in triplicates. Agar well diffusion method was used for the screening. The concentration of the sample (crude secondary metabolites) i.e. 10mg/mL of DMSO was screened against nine human pathogenic bacterial isolates and eight fungal species. Similar procedure was used by Swathi *et al*. (2013) and according to that study the crude extract showed efficient activity against bacteria and fungi test organisms. Hence, it was clearly indicated that the crude extract of *Microascus* species have both antibacterial and antifungal activity.

In a study by Khattak *et al*., 2014, the crude metabolites of *Penicillium* and *Aspergillus* spp. were tested for phytotoxicity against *L.* minor plant. Different degrees of phytotoxicity were observed on different concentrations of ethyl acetate fungal isolates. Different types of necrotic lesions were observed that varied from reddish brown to dark brown in color. The ethyl acetate fraction of *Aspergillus* sp. showed 0, 30 and 65% mortality respectively against the *L.* minor plant at dose of 10, 100, and 1000µg/mL, whereas in this study same protocol was followed for the ethyl acetate extract of *A. fumigatus*. At dose of 10, 100 and 1000µg/mL the phytotoxicity observed was 50, 60 and 100%.

To the existing chemical treatment methods of biological control of pathogens, microorganisms have been considered a more natural and environmentally conventional alternative. In this study, effective results were observed by the crude extract of *A. fumigatus* against different type of insects used. These observations thus agree with the reports that fungi used in the biological control of pests are able to elaborate metabolites harmful to the pests.

CONCLUSION

It is concluded from the present study that the best media for the isolation and growth of *A. fumigatus* is PDB at shaking condition with a temperature of 28°C and pH of 04. The ethyl acetate extract of *A. fumigatus* showed significant inhibitory effects on pathogenic bacteria and fungi. The MIC_{50} and MBC values were determined and the extract showed effective results against the tested samples. The extract was used in combination with antibiotics for the determination of synergistic effect and significant effects were observed. The extract of fungal specie has the potential of inhibiting the growth of

Lamina minor plant. Further, it was observed that the extract of *A. fumigatus* has the potential to be used as bio control agent. The FTIR analysis showed the presence of different useful phytochemical compounds in the extract.

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REFERENCES

Abad A, Fernández-Molina JV, Bikandi J, Ramírez A, Margareto J, Sendino J and Rementeria A (2010). What makes *Aspergillus fumigatus* a successful pathogen? Genes and molecules involved in invasive aspergillosis. *Rev. Iberoam. Micol.,* **27**(4): 155-182.

Amrit S and Richa S (2014). Biocontrol and Environmental Studies on Paper Degrading Mycoflora Isolated from Sanganer Area, Jaipur, India. *Int. J. Curr. Microbiol. Appl. Sci.,* **3**(8): 948-956

Arivudainambi USE, Anand TD, Shanmugaiah V, Karunakaran C and Rajendran A (2011). Novel bioactive metabolites producing endophytic fungus *Colletotrichum gloeosporioides* against multidrug-resistant Staphylococcus aureus. *Med. Microbiol. Immunol.,* **61**(3): 340-345.

Correa PA, Nosheen A, Yasmin H and Ansari MJ (2022). Antifungal Antibiotics Biosynthesized by Major PGPR. *In*: Secondary Metabolites and Volatiles of PGPR in Plant-Growth Promotion. Cham: Springer International Publishing, pp.199-247.

Dix NJ (Ed.) (2012). Fungal ecology. Springer Science & Business Media.

García-Torreiro M, López-Abelairas M, Lu-Chau TA and Lema JM (2016). Fungal pretreatment of agricultural residues for bioethanol production. *Ind. Crops Prod*., **89**: 486-492.

Garg N and Chandel S (2011). Arbuscular mycorrhizal networks: Process and functions. *In*: Sustainable Agriculture. Springer, Dordrecht, **2**: 907-930.

Gautam AK, Verma RK, Avasthi S, Bohra Y, Devadatha B, Niranjan M and Suwannarach N (2022). Current insight into traditional and modern methods in fungal diversity estimates. *J. Fungi.,* **8**(3): 226.

Han LR, Wang ZH, Zhang HJ, Xue LS, Feng JT and Zhang X (2013). Isolation of endophytic fungi from *Tripterygium wilfordii* and their insecticidal activities. *Afr. J. Microbiol. Res.,* **7**(9): 771-776.

Iqbal M, Amin M, Iqbal Z, Bibi H, Iqbal A, Din Z, Suleman M and Shah HU (2014). Antimicrobial, cytotoxic and phytotoxic potency of ethyl acetate extract of *Rhizopus stolonifer* culture. *Trop. J. Pharm. Res.,* **13**(1): 87-92.

Katoch M, Singh G, Sharma S, Gupta N, Sangwan P and Saxena AK (2014). Cytotoxic and antimicrobial activities of endophytic fungi isolated from *Bacopa monnieri* (L.) Pennell (Scrophulariaceae). *BMC Complement. Altern. Med.,* **14**(52): 1-8

Khattak SU, Iqbal Z, Lutfullah G, Bacha N, Khan AA, Saeed M and Ali M (2014). Phytotoxic and herbicidal Activities of *Aspergillus* and *Penicillium* species isolated from rhizosphere and soil. Pakistan. *Weed. Res.,* **20**(3): 293-303.

Leng LY, binti Nadzri N, bin Shaari AR and Yee KC (2017). Antioxidant capacity and total phenolic content of fresh, oven-dried and stir-fried tamarind leaves. *Curr. Res. Nutr. Food Sci.,* **5**(3): 282-287.

Lugtenberg BJ, Caradus JR and Johnson LJ (2016). Fungal endophytes for sustainable crop production. *FEMS Microbiol. Ecol.,* **92**(12): fiw194.

Lugtenberg BJ, Caradus JR and Johnson LJ (2016). Fungal endophytes for sustainable crop production. *FEMS Microbiol. Ecol.,* **92**(12):

Meenambal M, Pughalendy K, Vasantharaja C, Prapakaran S and Vijayan P (2012). Phytochemical information from FTIR and GC-MS studies of methol extract of Delonix elat leaves. *Int. J. Chem. Anal. Sci.,* **3**(6): 1446-1448.

Rogers K (Ed.). (2010). Fungi, algae and protists. Britannica Educational Publishing.

Sadananda TS, Govindappa M and Ramachandra YL (2013). Antibacterial activity of *Viscum album* endophytic fungal lectin. *Int. J. Biol. Pharm. Res.,* **4**(12): 1033-1042

Shaaban M, Nasr H, Hassan AZ and Asker MS (2013). Bioactive secondary metabolites from endophytic *Aspergillus fumigatus* (Structural elucidation and bioactivity studies). *Latinoamer. Quím*. **41**(1): 50-60.

Shi YS, Zhang Y, Chen XZ, Zhang N and Yun BL (2015). Metabolites produced by the endophytic fungus *Aspergillus fumigatus* from the stem of *Erythrophloeum fordii* Oliv. *Molecules.* **20**(6): 10793-10799.

Swathi J, Narendra K, Sowjanya KM and Krishna SA (2013). Biological characterisation of secondary metabolites from marine fungi Microascus sps. *Int. J. Res. Pharm. Biomed. Sci.*, **4**(3): 754-759.

Uniyal N, Chauhan A, Sharma K and Mitra D (2018). Plant growth promotion by bacterial inoculum with different conditions and estimation of enzyme activity from germinating seeds and bacterial isolates. *Curr. Trends Biotechnol. Pharm.,* **12**(1): 53-64.

Van Lenteren JC, Bolckmans K, Kohl J, Ravensberg WJ and Urbaneja A (2018). Biological control using invertebrates and microorganisms: Plenty of new opportunities. *BioControl*, **63**(5): 39-59.

War May Zin W, Prompanya C, Buttachon S and Kijjoa A (2016). Bioactive secondary metabolites from a Thai collection of soil and marine-derived fungi of the genera Neosartorya and Aspergillus. *Curr. Drug Deliv.* **13**(3): 378-388.