



# 6th International Electronic Conference on Medicinal Chemistry

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## Screening of endophytic actinomycetes for antifungal potential and cytotoxicity

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# Screening of endophytic actinomycetes for antifungal potential and cytotoxicity

## Graphical Abstract



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## Abstract:

Many diseases in humans and animals are caused by toxins produced by fungus. The most common disease of the respiratory tract is caused by many fungal species and *Aspergillus fumigatus* is the most prominent and primary pathogen among them. Due to the emergence of multiple drug resistance in the last years, the treatment of pathogenic fungi has become quite difficult. Therefore, the need to find out new biologically active and novel compounds has become a necessity. Medicinal plants of Pakistan such as *Ocimum tenuiflorum* (Tulsi) and *Azadirachta indica* (Neem) are well known for their medicinal uses. Endophytic actinomycetes are known for producing a variety of bioactive metabolites that are beneficial for plant health. The purpose of our study was to check the antifungal potential and to carry out the cytotoxicity profiling of endophytic actinomycetes residing within these plants. Agar plug method was used for preliminary screening and 2 isolates with antifungal activity were observed. Further minimum inhibitory concentration (MIC) was done of their concentrated broth and they were observed to be potent against *A. fumigatus* strains with a MIC value of 16 $\mu$ g/ml. Interestingly, cytotoxicity profiling of endophytic actinomycetes on baby hamster kidney (BHK-21) cell line showed that the concentrated broth of endophytic actinomycetes was most potent at a concentration of 100mg/ml. Our study concludes that endophytic actinomycetes, inhabiting tulsi, and neem plants have potent antifungal activity against *A. fumigatus* along with cytotoxic activity.

**Keywords:** Actinomycetes; Endophytes; *Aspergillus fumigatus*



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# Introduction

- Traditional Medicine
  - Essential role in the healthcare systems
  - In Asian countries
    - 80% of the population is dependent on medicinal practices [1]
- Medicinal Plants
  - Attractive targets for discovering novel therapeutic agents [1]

[1] Miller KI, Ingrey SD, Alvin A, Sze MYD, Roufogalis BD, Neilan BA. Endophytes and the microbial genetics of traditional medicines. *Microbiology Australia*. 2010;31(2):60-



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## Introduction(Cont.)

- ***Ocimum teniflorum***
- Locally known as ‘Tulsi’
- Widespread throughout the Southeast Asian tropics[2]
- Clinical pharmacological properties of oils and extracts from leaves
  - Antibacterial, anti-inflammatory, anti-pyretic, anti-diabetic, antifungal and anti stress effects [3]



*Ocimum teniflorum* growing in the nursery of University of Veterinary and Animal Sciences (UVAS), Lahore Pakistan. Photo courtesy: Ms. Ezza Ashraf

[2] Warriar P, Nambiar V, Ramankutty C. Indian Medicinal Plants: A Compendium of 500 Species, vol. III, Orient Longman Pvt. Ltd, Anna Salai, Chennai, India. 1995:38-42.

[3] Singh E, Sharma S, Dwivedi J, Sharma S. 2012. Diversified potentials of *Ocimum sanctum* Linn (Tulsi): An exhaustive survey. J Nat Prod Plant Resour. 2(1): 39-48.



## Introduction(Cont.)

- ***Azadirachta indica***
- Locally known as ‘Neem’
- Native to India, Pakistan, Bangladesh, Burma, Malaysia and Sri Lanka [4]
- Clinical pharmacological properties
  - Antibacterial, anti inflammatory, antiulcer, antiviral, anti-fungal, anti-diabetic and sedative properties [5]



*Azadirachta indica* growing in the nursery of University of Veterinary and Animal Sciences (UVAS), Lahore Pakistan. Photo courtesy: Ms. Ezza Ashraf

[4] Babu KS, Naik VKM, Latha J, Ramanjaneyulu K. 2016. Pharmacological review on natural products (*Azadirachta indica* Linn). IJCS. 4(5): 01-04.

[5] Agrawal D. Medicinal properties of neem: new findings. History of Indian science and. 2001.



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## Introduction (Cont.)



Endophytic actinomycetes strain EH-11 isolated from *Ocimum teniflorum* (Tulsi).  
Photo courtesy: Ms. Ezza Ashraf

- Actinomycetes
  - Greek 'aktis' (a ray) and 'mykes' (fungus)
  - Producers of a broad array of secondary metabolites
    - Useful applications in veterinary and human medicine [6]
- Endophytic Actinomycetes
  - Starting platform
  - Antibiotics, enzyme, anticancer agents, immunomodulators, anthelmintic agents,
  - Long-held alliance, plants and endophytic microorganisms develop good information transfer [7]

[6] Janso JE, Carter GT. 2010. Biosynthetic potential of phylogenetically unique endophytic actinomycetes from tropical plants. *J Appl Environ Microbiol* 76(13): 4377-4386.

[7] Zhao K, Penttinen P, Guan T, Xiao J, Chen Q, Xu J, Lindström K, Zhang L, Zhang X, Strobel GA. 2011. The diversity and anti-microbial activity of endophytic actinomycetes isolated from medicinal plants in Panxi plateau, China. *Curr Microbiol.* 62(1): 182-190.



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## Introduction (Cont.)

- *Aspergillus fumigatus* in poultry feed
  - *Aspergillus fumigatus* is a saprophyte and opportunistic fungal pathogen
  - In poultry, it causes brooder pneumonia
- Pathogenic Member of *Aspergillus*
  - Of particular concern is *Aspergillus fumigatus*





# Results and discussion

- Total 12 endophytic actinomycetes were isolated from a previous study [8]
- Selected isolates were sub-cultured on Glucose yeast extract malt extract (GYM) agar plates

## Endophytic actinomycetes from medicinal plants of Punjab

Groups	No. of Isolates
<i>Azadirachta indica</i> (Neem)	4
<i>Ocimum teniflorum</i> (Tulsi)	8

[8] Tanvir, R., I. Sajid, and S. Hasnain, *Screening of endophytic Streptomyces isolated from Parthenium hysterophorus L. against nosocomial pathogens*. Pak J Pharm Sci, 2013. 26: p. 277-283



## Results and discussion (Cont.)

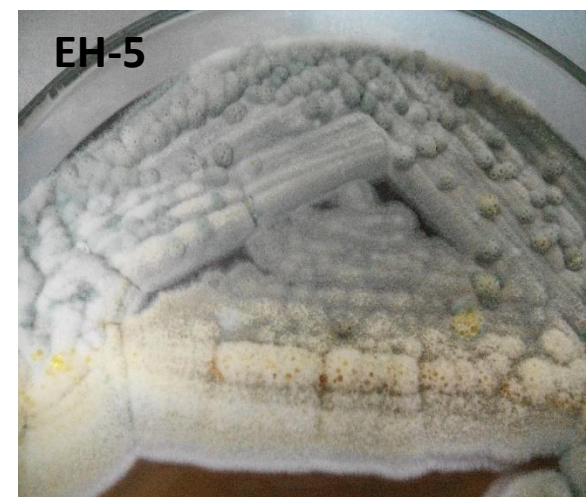
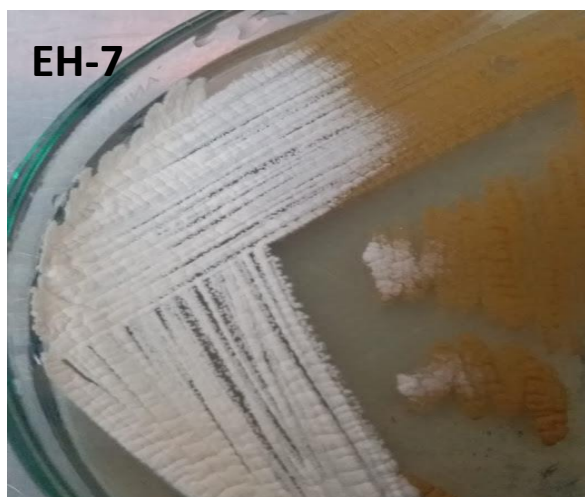
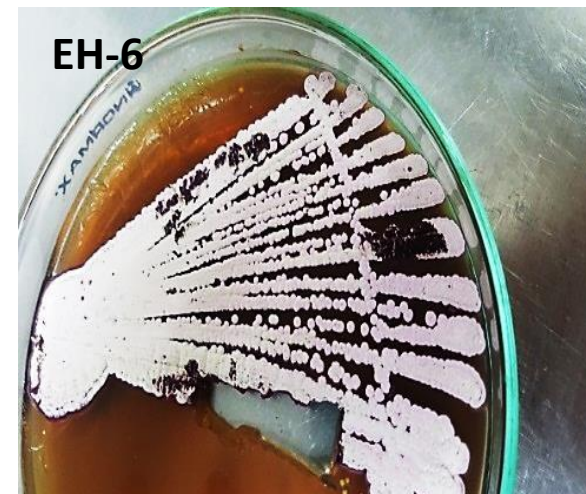
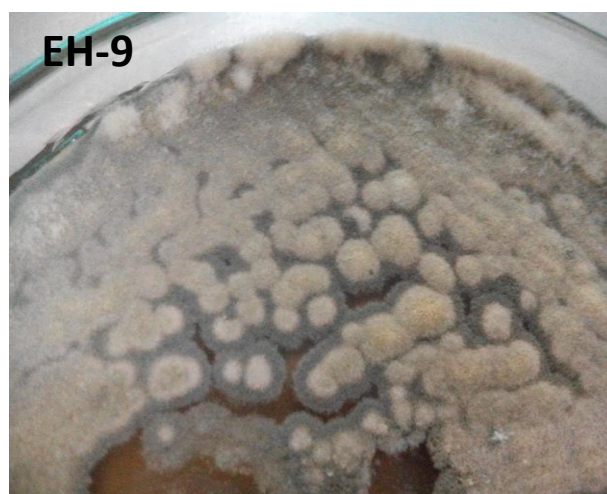


Photo courtesy: Ms. Hafsa Shahzadi



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## Results and discussion (Cont.)

- Cultivation of *Aspergillus fumigatus* strains
  - 05 fungal isolates (*A. fumigatus*) were isolated and characterized, from a previous study



Photo courtesy: Ms. Ezza Ashraf



## Results and discussion (Cont.)

- Screening of endophytic actinomycetes
  - Concentrated Broth culture [9]
    - 100 mg/ml concentration
    - Extracts stored in 0.1% DMSO
- Biological screening
  - Agar plug method [10]
  - Agar well diffusion method [11]
    - *Aspergillus fumigatus*

[9] Tanvir, Rabia, Imran Sajid, Shahida Hasnain, Andreas Kulik, and Stephanie Grond. "Rare actinomycetes *Nocardia caishijiensis* and *Pseudonocardia carboxydivorans* as endophytes, their bioactivity and metabolites evaluation." *Microbiological Research* 185 (2016): 22-35.

[10] Balouiri M, Sadiki M, Ibsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis*. 2016;6(2):71-9.

[11] Gebreyhannes, Gebreselema, Feleke Moges, Samuel Sahile, and Nagappan Raja. "Isolation and characterization of potential antibiotic producing actinomycetes from water and sediments of Lake Tana, Ethiopia." *Asian Pacific Journal of Tropical Biomedicine* 3, no. 6 (2013): 426-35.



## Results and discussion (Cont.)

- *In vitro* antifungal activity testing of endophytic actinomycetes by agar plug method
  - 2 strains showed broad spectrum antifungal activity
  - Maximum zones of inhibition of 11mm

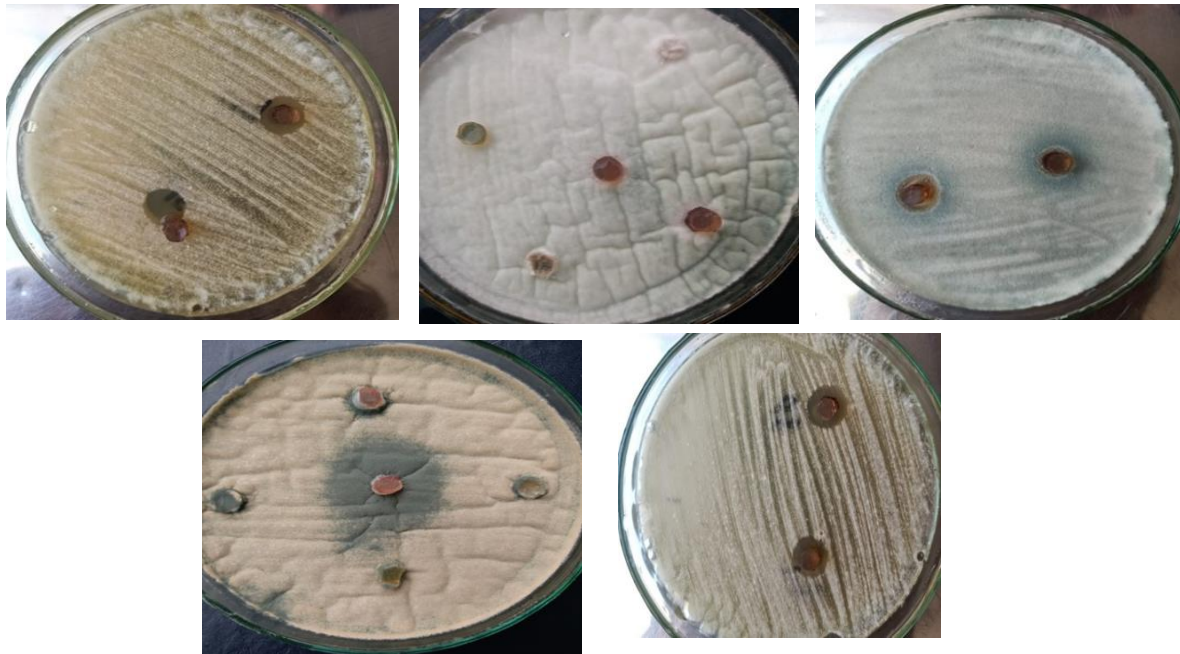


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## Results and discussion (Cont.)

Antifungal activity of endophytic actinomycetes concentrated broth by agar plug method

Endophytic actinomycetes strain code	Zone of Inhibition(mm) against <i>Aspergillus fumigatus</i> strains				
	EF-1	EF-2	EF-3	EF-4	EF-5
EH-1	-	11	9	-	10
EH-2	-	-	-	-	-
EH-3	-	-	-	-	-
EH-4	-	-	-	-	-
EH-5	-	-	-	-	-
EH-6	-	-	-	-	-
EH-7	-	-	-	-	-
EH-8	-	-	-	-	-
EH-9	-	-	-	-	-
EH-10	-	-	-	-	-
EH-11	-	10	9	-	10
EH-12	-	-	-	-	-



## Results and discussion (Cont.)

- Minimum inhibitory concentration (MIC) of endophytic actinomycetes concentrated broth against *Aspergillus fumigatus* strains
- MIC of endophytic actinomycetes was determined for a range of concentrations from 0 to 256 µg/ml.

Endophytic actinomycetes concentrated broth	MIC against <i>A. fumigatus</i> strains		
	EF-2	EF-3	EF-5
EH-1	16	16	16
EH-11	16	16	16



## Results and discussion (Cont.)

- Cytotoxicity results of endophytic actinomycetes EH-1 concentrated broth on BHK-21 cell line
  - O.D was taken at 595 nm, Values are means of triplicate studies

Sr. No	Concentration of EH-1 broth	Mean O.D on BHK-21 cells	Cell survival percentage
1.	100 mg	0.1266	25%
2.	50 mg	0.1307	26%
3.	25 mg	0.1352	28%
4.	12.5 mg	0.1384	29%
5.	6.25 mg	0.1539	36%
6.	3.125 mg	0.1684	40%
7.	1.5625 mg	0.2211	58%
8.	0.7812 mg	0.2312	62%
9.	0.3906 mg	0.2561	70%
10.	0.1953 mg	0.3131	90%
11.	Cells control	0.3410	100%
12.	DMSO control	0.0539	100%





## Results and discussion (Cont.)

- Cytotoxicity results of endophytic actinomycetes EH-11 concentrated broth on BHK-21 cell line
  - O.D was taken at 595 nm, Values are means of triplicate studies

Sr. No	Concentration of EH-11 broth	Mean O.D on BHK-21 cells	Cell survival percentage
1.	100 mg	0.1262	25%
2.	50 mg	0.1400	29%
3.	25 mg	0.1711	41%
4.	12.5 mg	0.2009	51%
5.	6.25 mg	0.2081	54%
6.	3.125 mg	0.2271	60%
7.	1.5625 mg	0.2515	69%
8.	0.7812 mg	0.2592	72%
9.	0.3906 mg	0.3021	86%
10.	0.1953 mg	0.3289	96%
11.	Cells control	0.3410	100%
12.	DMSO control	0.0539	100%



# Conclusions

- Endophytic actinomycetes from *Ocimum teniflorum* (Tulsi) and *Azadirachta indica* (Neem)
  - Rarely studied before
- Our study
  - Diversity of actinomycetes inhabits different plant parts
  - Biological screening revealed
    - Broad spectrum activity
    - Bioactive against *Aspergillus fumigatus* involved in poultry feed contamination



# Conclusions

- Metabolically diverse compounds
  - Potent against *Aspergillus fumigatus*
  
- Further exploration of these strains
  - Large scale study
  - Further bioactivity screening
  - HPLC-MS and NMR
  - Antiviral activity against Covid-19



# Acknowledgment

Our sincere thanks to **Prof. Dr. Tahir Yaqub**, Director IOM, UVAS Lahore, for his support by making available the facilities in the institute



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