

**STUDY OF BIODIVERSITY AND BIOACTIVITY OF ENDOPHYTIC FUNGI
OF SOME HIMALAYAN CONIFERS OF NEPAL**

A

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By

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RECOMMENDATION

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.....
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ABSTRACT

A study was conducted with the aim of studying the biodiversity and bioactivity of endophytic fungi from selected Himalayan conifers collected from different location viz. *Taxus wallichiana* ('Kavre', 2,930m), *Abies spectabilis* ('Kavre', 2,930m), *Podocarpus nerifolius* ('Kirtipur', 1,372m) and *Juniperus indica* ('Kirtipur', 1,372m)

Altogether 47 endophytes were isolated from the inner bark of the above mentioned Himalayan conifers. Only 29 fungal isolates could be identified. The most representative fungal isolates identified up to the genus level belonged to the genera *Pestalotiopsis*, *Alternaria*, *Trichoderma*, *Aspergillus*, *Paecilomyces*, *Nigrospora*, *Helicomyces*, *Drechslera*, *Helminthosporium* and an endomycorrhiza identified as *Glomus* spp.

The identified endophytic fungal isolates were subjected for screening purpose for their antifungal activities against the plant pathogenic fungi viz. *Fusarium oxysporum*, *Fusarium moniliforme*, *Sclerotinia* spp., *Helminthosporium* spp., *Alternaria* spp., *Geotrichum* spp., and *Gloesporium sporioides* by following the dual culture method. The observation revealed that most of the endophytic fungi showed antifungal activities against plant pathogenic fungi used in this study but *Pestalotiopsis* spp., *Alternaria* spp., *Trichoderma* spp. and *Helminthosporium* spp. were the ones among showing highest bioactivity against all the fungal plant pathogens used in the study. The bioactivity was due to the production of novel bioactive secondary metabolites by these endophytic fungi.

The different isolates of *Pestalotiopsis* spp., NT-801a, NP-601 and NJ-702 each from different plant source were chosen as the priority isolates, for fermentation and extraction of novel bioactive secondary metabolites. The dry weight of fungal extract recorded was 33 mg/500 ml for NT-801a isolate, 17mg/500 ml for NP-601 isolate and 32 mg/500 ml for NJ-702 isolate.

The presence of the bioactive secondary metabolite in the fungal extract was confirmed by the inhibitory activity against the test fungal plant pathogens. Among the fungal extract, the *Pestalotiopsis* spp. (NJ-702) isolated from *Juniperus indica*, showed the maximum inhibitory effect against most of the test fungal plant pathogens, compared to the other two fungal extracts of *Pestalotiopsis* spp. isolated from different plant sources.

The crude extract was further subjected to TLC analysis. The crude extract was made to run along with authentic taxol and its precursor compound (10-DAB III), as the reference compounds. The R_f value of the authentic taxol being 0.77 and that of the fungal analyte (NT-801a) being 0.78, (NP-601) being 0.79 and that of (NJ-702) being 0.78, suggests the possibility of presence of taxol in all the three fungal extracts.

Key words: bioactivity, biodiversity, endophytes, Himalayan conifer, taxol

TABLE OF CONTENTS

	Page No.
Title page	
Recommendation	
Certificate of approval	
Board of examiners	
Acknowledgement	
Abstract	
Table of contents	
List of abbreviations	
List of tables	
List of figures	
List of photographs	
List of appendices	
CHAPTER- I: INTRODUCTION	1
CHAPTER –II: OBJECTIVES	5
CHAPTER-III: LITERATURE REVIEW	6
3.1 Endophytes	6
3.1.1 History and Discovery	6
3.1.2 Distribution and Biodiversity	7
3.1.2.1 Distribution and biodiversity of endophytes of conifers	10
3.1.2.2 Bioactivity of Endophytes	17
3.2 Biological control of plant pathogens	25
3.2.1 Biocontrol of plant pathogens by use of endophytes	28
CHAPTER-IV: MATERIALS AND METHODS	33

4.1 Materials	33
4.2 Methods	33
4.2.1 Collection of plant material	33
4.2.2 Isolation of endophytic fungi	33
4.2.3 Maintenance of fungal culture	34
4.2.3.1 Storage in sterile distilled water	34
4.2.3.2 Storage in PDA slants	34
4.2.4 Identification of the fungal isolates	34
4.2.4.1 Colony morphology on PDA	35
4.2.4.2 Lactophenol cotton blue mounting of the fungal specimen	35
4.2.4.3 Cover slip culture	35
4.2.4.4 Culture on water agar (WA) with sterilized carnation leaf	36
4.2.5 Screening of identified endophytic fungal isolates for antifungal activities	36
4.2.6 Extraction of secondary metabolites of a priority isolate of endophytic fungi	37
4.2.6.1 Fungal culture in liquid S-7 media	37
4.2.6.2 Fermentation	37
4.2.6.3 Extraction of secondary metabolite	38
4.2.6.4 Determination of dry weight of chloroform extract	38
4.2.7 Study of antifungal activity of the endophytic fungal extract extracted with chloroform	38

4.2.8 TLC analysis of the fungal extract	39
CHAPTER-V: RESULTS	40
5.1 Isolation and identification of endophytic fungi from the selected conifers	40
5.1.1 Isolation of endophytic fungi from the inner bark of selected conifers	40
5.1.2 Identification of the fungal isolates	40
5.1.3 Classwise and Generawise distribution of the identified isolates of endophytic fungi	48
5.2 Screening of the identified fungal isolates for their antifungal activities	50
5.3 Extraction of secondary metabolite of a priority isolates of endophytic fungi from different conifers	54
5.4 Study of antifungal activity of the endophytic fungal extract extracted with chloroform	55
5.5 TLC analysis of the endophytic fungal extracts	57
CHAPTER-VI: DISCUSSION AND CONCLUSION	59
6.1 Discussion	59
6.2 Conclusion	75
CHAPTER-VII:SUMMARY AND RECOMMENDATION	76
7.1 Summary	76
7.2 Recommendation	77
REFERENCES	80-94
APPENDICES: I-IV	I-XIV

LIST OF ABBREVIATIONS

CIEIA	Competitive Inhibition Enzyme Immuno Assay
cm	centimeter
° C	Degree Centigrade
10-DAB	Deacetyl Baccatin III
DMSO	Dimethyl sulfoxide
gm	gram
L	Liter
m	meter
M	molar
mg	milligram
ml	milliliter
mm	millimeter
µl	micro liter
ng	nanogram
NAST	Nepal Academy of Science and Technology
nm	nanometer
NPRL	Natural Product Research Laboratory
PDA	Potato Dextrose Agar
rDNA	ribosomal Deoxyribonucleic acid
R _f	Retardation factor
TLC	Thin Layer Chromatography
UV	Ultraviolet
v/v	volume/volume
WA	Water Agar
w/v	weight/volume
ZOI	Zone of Inhibition

LIST OF TABLES

- Table 1: Identification of endophytic fungal isolates of *Abies specabilis*
- Table 2: Identification of endophytic fungal isolates of *Taxus wallichiana*
- Table 3: Identification of endophytic fungal isolates of *Podocarpus nerifolius*
- Table 4: Identification of endophytic fungal isolates of *Juniperus indica*
- Table 5: Class wise and Genera wise distribution of the identified isolates of endophytic fungi from *Abies spectabilis*
- Table 6: Class wise and Genera wise distribution of the identified isolates of endophytic fungi from *Taxus wallichiana*
- Table 7: Class wise and Genera wise distribution of the identified isolates of endophytic fungi from *Podocarpus nerifolius*
- Table 8: Class wise and Genera wise distribution of the identified isolates of endophytic fungi from *Juniperus indica*
- Table 9: Screening of the identified isolates of endophytic fungi for their inhibitory activities against plant pathogenic fungi
- Table 10: Extraction of secondary metabolite of isolated endophytic fungi isolated from different conifers
- Table 11: Potency of the extract for the antifungal activities
- Table 12: R_f values of the spots obtained on TLC analysis

LISTS OF FIGURES

- Figure 1: Schematic diagram for the isolation of endophytic fungi
- Figure 2: Scheme of identification of the endophytic fungal isolates and their screening for their antifungal activities by Dual culture method
- Figure 3: Schematic diagram for the study of antifungal activity of the extract of the endophytic fungal isolate
- Figure 4: Class wise distributions of the isolated endophytic fungi
- Figure 5: Genera wise distribution of identified isolates of endophytic fungi from *Abies spectabilis*
- Figure 6: Genera wise distribution of identified isolates of endophytic fungi from *Taxus wallichiana*
- Figure 7: Genera wise distribution of identified isolates of endophytic fungi from *Podocarpus nerifolius*
- Figure 8: Genera wise distribution of identified isolates of endophytic fungi from *Juniperus indica*
- Figure 9: Extent of inhibition of *Fusarium oxysporum* by the identified isolates of endophytic fungi
- Figure 10: Extent of inhibition of *Fusarium moniliforme* by the identified isolates of endophytic fungi
- Figure 11: Extent of inhibition of *Sclerotinia* spp. by the identified isolates of endophytic fungi
- Figure 12: Extent of inhibition of *Helminthosporium* spp. by the identified isolates of endophytic fungi
- Figure 13: Extent of inhibition of *Alternaria* spp. by the identified isolates of endophytic fungi
- Figure 14: Extent of inhibition of *Geotrichum* spp. by the identified isolates of endophytic fungi
- Figure 15: Extent of inhibition of *Gloeosporium sporioides* by the identified isolates of endophytic fungi

- Figure 16: Yield of the Chloroform extract of *Pestalotiopsis* spp. (NT-801a, NP-601 and NJ-702)
- Figure 17: Zones of inhibition shown by the fungal extract (NT-801a, *Pestalotiopsis* spp.) for the fungal plant pathogens
- Figure 18: Zones of inhibition shown by the fungal extract (NP-601, *Pestalotiopsis* spp.) for the fungal plant pathogens
- Figure 19: Zones of inhibition shown by the fungal extract (NJ-702, *Pestalotiopsis* spp.) for the fungal plant pathogens

LIST OF PHOTOGRAPHS

- Photograph 1: Indigenous High altitude Himalayan Conifers of Nepal
A: *Abies spectabilis* D.Don; B: *Juniperus indica* Bertol.
- Photograph 2: Indigenous High altitude Himalayan Conifers of Nepal
C: *Taxus wallichiana* Zucc.; D: *Podocarpus nerifolius* D. Don
- Photograph 3: Twelve days old colony of *Pestalotiopsis* spp. (NT-801a) on PDA plate
- Photograph 4: Photomicrograph of fusiform transversely septate conidia of *Pestalotiopsis* spp. (NT-801a) (X 100)
- Photograph 5: Fourteen days old colony of *Nigrospora* spp. (NT-805) on PDA plate
- Photograph 6: Photomicrograph of inflated dark conidia of *Nigrospora* spp. (NT-805) (X 100)
- Photograph 7: Ten days old colony of *Alternaria* spp. (NJ-705) on PDA plate
- Photograph 8: Photomicrograph of chain of conidia of *Alternaria* spp. (NJ-705) (X 100)
- Photograph 9: Ten days old colony of *Drechslera* spp. (NA-911) on PDA plate
- Photograph 10: Photomicrograph of *Drechslera* spp. (NA-911) (X 100)
- Photograph 11: Seven days old colony of *Trichoderma* spp. (NP-603) (X 100) on PDA plate
- Photograph 12: Photomicrograph of *Trichoderma* spp. (NP-603) (X 100)
- Photograph 13: Ten days old colony of endophytic fungal isolate, NA-903 (unidentified) on PDA plate.
- Photograph 14: Photomicrograph of endophytic fungal isolate, NA-903. (X 100)
- Photograph 15: Inhibition of *Geotrichum* spp. by *Pestalotiopsis* spp. (NJ-702) on PDA plate
- Photograph 16: Inhibition of *Fusarium moniliforme* by *Pestalotiopsis* spp. (NJ-702) on PDA plate
- Photograph 17: Rotatory Vacuum Evaporator

Photograph 18: Zone of inhibition produced by the filtrate extract of *Pestalotiopsis* spp. (NP-601) against *Sclerotinia* spp.

Photograph 19: Zone of inhibition produced by the filtrate extract of *Pestalotiopsis* spp. (NJ-702) against *Fusarium moniliforme*

Photograph 20: TLC analysis (1: authentic taxol, 2: 10-DAB (10-Deacetyl Baccatin III), 3: Fungal extract (NT-801a), 4: Fungal extract (NJ-702), 5: Fungal extract (NP-601), 6: control extract)

Photograph 21: Investigator performing the laboratory work

LIST OF APPENDICES

APPENDIX-I: Lists of materials

APPENDIX-II: Composition and preparation of different culture
Media

APPENDIX-III: Characteristics of the coniferous plant used in this study

APPENDIX-IV: Characteristics of fungal plant pathogens used in this
study