DECLARATION

I hereby declare that the work which is being presented in this thesis "**Diversity of *Suillus* species associated with conifer trees in North Western Himalayan region of India**" for the award of the degree of **Doctor of Philosophy** in the Department of Biotechnology, Thapar University, Patiala is true and original record of my own independent and original research work carried out under the supervision of Dr. M. Sudhakara Reddy, Professor, Department of Biotechnology, Thapar University, Patiala, Punjab, India. The matter embodied in this thesis has not been submitted in part or full to any other university or institute for the award of any degree in India or Abroad.

(Balwant Verma)

Dedicated to my Adored Parents .....

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(Balwant Verma)

List of publications

The following publications are outcome of the present research work:

1. **Balwant Verma** and M. Sudhakara Reddy. 2014. *Suillus triacicularis* sp. nov., a new species associated with *Pinus roxburghii* from northwestern Himalayas, India. *Phytotaxa*, 162 (3): 157–64.
2. **Balwant Verma** and M. Sudhakara Reddy. 2014. *Suillus himalayensis (Basidiomycota, Agaricomycetes,* Boletales), a new species associated with *Pinus wallichiana* from the northwestern Himalayas, India. *Nova Hedwigia*, 99 (3–4): 541–550.
3. **Balwant Verma** and M. Sudhakara Reddy. 2014. *Suillus indicus* sp. nov. (Boletales, Basidiomycota), a new boletoid fungus from northwestern Himalayas, India. *Mycology: An International Journal of Fungal Biology*, DOI: 10.1080/21501203.2014.988770.

Abstract

The diversity of the genus *Suillus* has not been explored thoroughly from the northwestern Himalayan region of India. By virtue of this, several basidiocarps of the genus *Suillus* were collected from different parts of this region and characterized morphologically as well as molecularly. The present research work described 7 species of *Suillus*. Among these, 3 taxa namely, *S. triacicularis* sp. nov., *S. indicus* sp. nov., and *S. himalayensis* sp. nov., were new to the science while *S. flavidus* and *S. placidus* were new records from India. Attempts were also made to isolate the cultures from each basidiocarps collected. In total, 8 *Suillus* isolates belonging to five different species (*S. triacicularis* sp. nov., *S. indicus* sp. nov., *S. himalayensis* sp. nov., *S. granulatus* and *S. sibiricus*) were obtained from basidiocarps. All these *Suillus* isolates exhibited inter-specific as well as intra-specific variations in axenic fungal growth, extracellular enzyme activities, and *in vitro* mycorrhizal capacities with *Pinus wallichiana* seedlings. On the basis of fungal growth, enzyme activities, mycorrhizal colonization, and the effects on seedlings growth, biomass and nutrients content; *S. sibiricus* SNW06 and *S. indicus* SNW02 were found to be the most effective and suitable *Suillus* isolates for growth promotion of *P. wallichiana* seedlings and therefore selected for mass inoculum production. Optimization of various parameters for mass inoculum production of both the *Suillus* isolates resulted in a significant increase in the radial growth and biomass yield of both the selected *Suillus* isolates. Further, the effects of both the selected *Suillus* isolates and biochar amendment on *P. wallichiana* growth and rhizosphere properties were evaluated. Based upon the present investigation, *S. sibiricus* SNW06 was found to be an efficient mycorrhizal strain as compared to *S. indicus* SNW02 and therefore, recommended for the production of mycorrhizal *P. wallichiana* seedlings in forestry practices. Also biochar application to the soil, especially along with mycorrhizal inoculations, was found to be advantageous for improvement of growth and nutrients content of *P. wallichiana* seedlings as well as the soil physico-chemical properties and enzyme activities.

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Abbreviations

A	Absorbance
ACPase	Acid phosphatase
AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of Variance
BC	Biochar
BI	Bayesian inference
BLAST	Basic local alignment search tool
bp	Base pair
BPP	Bayesian posterior probabilities
C	Carbon
°C	Degree celsius
Ca	Calcium
$CaCl_2$	Calcium chloride
Cd	Cadmium
CEC	Cation exchange capacity
cm	Centimeter(s)
cm^3	Cubic centimeter
C/N	Carbon nitrogen ratio
CTAB	Cetyl trimethylammonium bromide
Cu	Copper
DNA	Deoxyribonucleic acid
DNS	Dinitrosalicylic acid
dNTP	2'-deoxynucleotide-5'-triphosphate
ECM	Ectomycorrhizal
EDTA	Ethylenediamine-tetra acetic acid
$FeSO_4$	Ferrous sulphate
Fig.	Figure
g	Gram
h	Hour(s)
ha	Hectare
HNO_3	Nitric acid
H_2O_2	Hydrogen peroxide
IGS	Intergenic spacer
IPTG	Isopropyl β-D-1-thiogalactopyranoside
ITS	Internal transcribed spacer
K	Potassium
kg	Kilogram
KOH	Potassium hydroxide
l	Litre
LB	Luria-Bertani
L/W	Length/width
M	Molar
m	Metre(s)
ME	Malt extract
Mg	Magnesium

$MgCl_2$	Magnesium chloride
µg	Microgram
µl	Microlitre(s)
µM	Micromolar
µm	Micrometer(s)
mg	Milligram(s)
min	Minute(s)
ml	Millilitre(s)
mM	Millimolar(s)
mm	Millimetre(s)
MMN	Modified Melin-Norkans medium
mtDNA	Mitochodrial DNA
MUB	Modified universal buffer
N	Nitrogen
NaOH	Sodium hydroxide
NCBI	National Center for Biotechnology Information
ng	Nanogram
NH_3	Ammonia
NH_4^+	Ammonium ion
NH_4OH	Ammonium hydroxide
nLSU	Nuclear ribosomal large subunit
nm	Nanometer
NO_2^-	Nitrite ion
NO_3^-	Nitrate ion
nSSU	Nuclear ribosomal small subunit
OD	Optical density
OM	Optimized medium
P	Phosphorus
PCR	Polymerase chain reaction
PDA	Potato dextrose agar medium
PEG	Polyethylene glycol
%	Percent
pH	Potential of hydrogen
Pi	Inorganic phosphate
pNP	p-nitrophenol
ppm	Parts per million
PUE	Protease unit equivalents
Q	Quotient
RAPD	Random amplified polymorphic DNA
rDNA	Ribosomal DNA
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
rpm	Revolutions per minute
S	Sulphur
s	Second(s)
sp. nov.	Species novum
sq km	Square kilometer
TBE	Tris/Borate/EDTA

TE	Tris-EDTA
TOC	Total organic carbon
TPF	1, 3, 5-triphenyl tetrazolium formazan
Tris	Tris-(hydroxymethyl-) aminomethane
U	Unit
UV	Ultraviolet
V	Volts
v/v	Volume by volume
v/v/v	Volume by volume by volume
w/v	Weight by volume
X-Gal	5-Bromo-4-chloro-3-indolyl-β-D-galactoside
ybp	Years before present
Zn	Zinc