Detection and Documentation of Forest Diseases in Jigme Dorji National Park in Bhutan for Forest Conservation

Progress Report

Submitted to the

Rufford Foundation

In fulfillment of the objectives of the project

Researcher

Phurpa

Master in Forestry

(Forest Pathology)

Under the Supervisor of

Dr. N.S.K.Harsh,

Scientist-G.

Forest Pathology Division

Co-supervisors

1 **Mr. Loady Phuntsho**

Deputy Chief Research Officer

RNR DCW, MoAF, Bhutan

2 Mr. Karma Thubten

Forest Officer

UWICE Bhutan

Table of contents

Acknowledgements

It is pleasant aspect and opportunity to express my gratitude and my heart will unaccomplished without acknowledging the overwhelming help and support I received during this endeavor.

With deep respect, I express my heartfelt gratitude to my advisor Dr. N. S. K. Harsh, Scientist-G, Forest Pathology Division, Forest Research Institute, (Indian Council of Forestry Research and Education) Dehradun, Mr. Loady Phuntsho, Deputy Chief Researcher, Ministry of Forest and Agriculture and Mr. Karma Thubten, Ugyen Wangchuck Institute for Conservation and Environment, Bhutan, for pragmatic suggestions, erudite guidance, warm appreciation and friendly cooperation and for maintaining diligence and interest for my project throughout my candidature.

I heartedly thank the Department of Forest and Park Service, Bhutan, Mr. Lhendrup Tharchen, Park Manager, Mr. Sangay Dorji, Research Officer, Ranges Officer and staff of JDNP for the kind cooperation, critical comments and lovely support during the course of data collection. I also thank the Head and staff of Forest Pathology Division, Forest Research Institute, Dehradun, India for kind support, comments and suggestions.

I am immensely thankful to Rufford Foundation for funding this project which is helpful in conservation for maintaining the biodiversity in Bhutan. I also thank all the friends and family which helped me in conducting this project in the best possible manner.

. *(Phurpa)*

List of figures

List of tables

List of annexures

Abbreviation

AOI : Area of Interest
CDB : Convention on Biological Diversity
CITES : Convention on International Trade in Endangered Species of Wild Flora and Fauna
D : Sipmson's index
DED : Dutch elm disease
DoFPS : Department of Forest and Park Services
DSI : Disease Severity Index
E : Pielou index
ERDAS : Earth Resources Data Analysis System
FAO : Food and Agriculture Organisation
GBH : Girth at Breast Height
GFRA : Global Forest Resources Assessment
GIS : Geographical Information System
GNH : Gross National Happiness
GPS : Global Positioning System
H’ : Shannon index
IPM : Integrated Pest Management
ISPM : International Standards for Phytosanitary Measures
IUCN : International Union on Conservation of Nature
IUFRO : International Union of Forest Research Organizations
JDNP : Jigme Dorji National Park
NEC : National Environment Commission
RGOB : Royal Government of Bhutan
SD : Standard Deviation
UNCCD : United National Convention to Combat Desertification
UNFCCC : United National Framework Convention on Climate Change

Abstract

Forest diseases are important limiting factors for structure and composition of healthy ecosystems, productive and protective equation, yet finding the references about forest disease in Bhutan is challenging. This study investigated 3,442 coniferous trees in JDNP belonging to 4 genera, 6 species and confirmed a total of 20 forest pathogens, 15 fungi belonging to 11 families, 3 mistletoes belonging to 2 families and 2 insect pests belonging to 2 families. Shinnon Wiener and Simpson diversity index (0.0001-2.4999, 0.000-0.8000) concluded that hosts have low to medium pathogens diversity with average of host to pathogens ratio 1:4. However, each host's pathogens are evenly distributed according to Pielou evenness index (0.7500-1.0000). Mean disease incidence percentage was 28.8 (SD=13.21) and disease severity index was 0.415 (SD=0.165) and shown that disease incidence and severity index have significant relationship (r=0.9, p=0.05). Further, disease incidence was directly proportional to increase in altitude (r=0.88) and GBH (r =0.88, multiple R=0.94, p<0.05). Study also showed significant variation of pathogens abundance in JDNP ranges ($\chi 2$=58, df=4, p=0.05) and confirmed 65% of diseases in pure forest and 35% in mixed forest ($\chi 2$=0.14, p=0.05, df=1). Most of fungal diseases were seen in northern aspect however, mistletoes and beetles were seen in southern aspect. Study concluded that there were different dominant predisposing factors for each hosts and only 19.16% of pathogens were sharing same host.

Key words*:* Forest disease, conifers, JDNP, pathogens, diversity, severity, incidence, index, predisposing factors.

INTRODUCTION

The contributions of forests to the well-being of humankind are extraordinarily vast and far-reaching. Forests play a fundamental role in combating rural poverty, ensuring food security and providing decent livelihoods (FAO, 2010). They provide critical refuge for terrestrial biodiversity and a central component of the earth's biogeochemical systems with potential to mitigate climate change by serving as net carbon sinks **(**Shvidenko *et al*., 2005; IPCC, 2007). They also offer promising opportunities and deliver vital long-term environmental services and Bhutan's 72% of forest is not an exception. It is estimated to sequester 6.3 million tons of CO_2, provide ecosystem services worth 15.5 billion US$ per/year, home for IUCN Red lists, livelihood for 69% of rural population and watershed which contribute 12.5 billion US$ annually by hydropower (DoFPS, 2011; Dorji, 2016).

Having magnificent dependence and correlations with forests, Government of Bhutan Constitution (2008) authorized, its citizens the fundamental right and trustees to conserve and protect the forests. It also has a mandate to conserve 60% of forest cover for perpetuity. Bhutan is also member of many forest related international parties and conventions such as; Convention on Biological diversity (CDB), United National Convention to Combat Desertification (UNCCD), the United National Framework Convention on Climate Change (UNFCCC), the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) and International Plant Protection Convention (IPCC) (Wangdi *et al*., 2014). Beside these, Forest Policy of Bhutan (1974, 2011), Forest and Nature Conservation Act (1995), Forest and Nature Conservation Rule (2006), Traditional Social Restriction System, Buddhist principles and Gross National Happiness (GNH) vision are the guardian and guiding principles for management, conservation and protection of Bhutanese pristine forests (Wangdi *et al*., 2014).

However, forest diseases constitute major biological deterrents of forest conservation, health and productivity. Because of insidious nature of pathogens and non-infectious diseases of forests, losses due to them are not fully realized unlike those from insects and fire where damage becomes evident (Bakshi, 1976; Agrios, 2006; Cooke and Jones, 2006). Hepting and Jemison (1957) have reported that losses from the forest diseases exceeded the combined losses from fire and insects. Boyce (1948) also supported above idea from the study of Indian forest (Agrios, 2006).

To add on issues, epidemic diseases and new virulent pathogens could be increased due to climate change. It would encourage warmth loving pathogens and climatic stress (dryness, water logging, soil acidification, etc.), which could reduce the resistance of trees to diseases (Porta *et al*., 2008; Sturrocka *et al*., 2011). The Fifth World Forestry Congress, held in Seattle (1960), agreed that a catastrophic loss of tree species to disease would occur (FAO, 1960). Further, FAO/IUFRO Symposium on Internationally Dangerous Forest Diseases and Insects (1964) and Second International Forest Disease Conference (1975) held in New Delhi, India urged in order to manage our forests wisely for current and future generations for the productive and protective functions, it is vital to have a clear understanding of forest diseases (FAO, 1965).

Forest diseases decline of *Abies densa* and outbreak of *Ips schmutzenhoferi* on *Pinus spinulosa* and *Pinus wallichiana* and *Ips longifolia* on *Pinus roxburghii* has been started from 1980s in Bhutan. These outbreaks have shown that diseases and insect pests can pose a great threat to forest management, conservation and biodiversity of Bhutan (Donaubauer 1986, 1987, 1993; Tshering and Chhetri 2000, Dorji 2007). However, Bhutan is unprepared in the forest pathology discipline and finding the references about forest diseases is not an easy task due to the lack of forest pathologist, forest disease research and documentation in Bhutan.

This present study has objectives to fill this major gap in the forestry pathology discipline by studying the forest diseases in JNDP. It will give the baseline data on diversity of pathogens, disease severity and incidence which will play vita role in conservation activities in Bhutan

Potential forest pathogens and diseases outbreak in international, regional and national which had significant impact in forestry history are given below.

Table 1 Important fungal diseases outbreak which has led to notable changes in the forest landscape in world (Heiniger, 2003)

Pathogen	Diseases name	Affected species	Country	Outbreak
Cryphonectria parasitica	Chestnut blight	*Castanea dentata*	USA, Europe	1990
Ophiostoma ulmi	Dutch elm	*Ulmus*	Europ	1910
Ophiostoma novo-ulmi	New Dutch elm	*Ulmus*	Europ	1975
Ceratocystis fimbriata	Plane tree wilt	*Platanus*	France	1960
Cronartium ribicola	White pine rust	*Pinus strobus*	USA	1906
Phytophthora ramorum	Sudden oak	*Quercus sp*	Switzerland	2004
Fusarium solani	Sissoo mortality	*Dalbergia* sissoo	India	2000

Table 2 Average area of forest annually affected by diseases by region and sub region (FAO, 2010)

Region / Sub-region	Number of Countries	% Of Forest Area	Area of Forest Affected by Diseases (Hectare)
Eastern and Southern Africa	4	4.7	n.s
Northern Africa	2	1.3	n.s
Western and Central Africa	4	5.3	4000
Total Africa	10	4.6	4000
East Asia	3	92.7	3,94,000
South and Southeast Asia	4	26.2	n.s
Western and Central Asia	12	42.6	4,1000
Total Asia	19	54.9	3,90,000
Europe Excl. Russian	33	71.8	17,86,000
Total Europe	34	94.6	29,18,000
Caribbean	6	48.9	n.s
Central America	1	18.9	n.s
North America	2	9.7	19,000
Total North and Central America	9	10.3	19,000
Total Oceania	4	4.7	3,20,000
Total south America	4	10.5	1,13,000
World	80	36.3	37,64,000

Table 3 Virulent forest pathogens reported in India (Bakshi, 1971; Ravarden and Johansen, 1980)

Forest Pathogens	Hosts (examples)
Phellinus badius	*Acacia catechu, Acacia nilotica, Albizzia* sp.
Phellinus durissimus	*Casuaria* sp, *Shorea robusta*
Phellinus caryophylli	*Mallotus philippinessis*
Phellinus gastropus	*Pinus wallichiana, Tsuga* sp.
Fomitopsis pinicola	*Pinus kesiya* and *Quercus* sp.
Fomes formentarius	*Quercus sp, Picea* sp, *Tsuga* sp, *Juglans* sp.
Phellinus pini	*Pinus wallichiana*
Phellinus sulphurascens	*Tsuga* sp, *Abies* sp.
Heterobasidion annosum	Conifers
Ganoderma appalatum	*Dalbergia Sisso, Tectona grandis, Acacia nilotica*
Ganoderma lucidum	Wide host range (Around 120 tree species)
Heterobasidion insularis	*Tsuga sp* and *Pinus wallichiana*
Armilaria mellea	Most conifers
Laetipors sulphereus	*Tsuga* sp, *Quercus* sp, *Pinus* sp.
Pleurotus ostreatus	*Acacia* sp, *Eucalyptus sp*, *Tsuga* sp
Rhizoctonia solani	*Acacia* sp, *Albizia* sp. and other legume trees
Fusurim oxysporum	*Albizia* sp
Pythium sp	*Abies procera,*
Phytophtbora sp	*Pinus sp. Eucyclaptus sp.*
Cronartium ribicola	*Pinus sp*
Rhizina undulate	Conifers on burnt sites, *Pinus sp, Tsuga sp,*
Glomerella cingulate	*A. mangium.*
Cylindrocladium quinqueseptatum	*A. mangium, A. auriculiformis*
Alternaria sp	*Malus domestica, Pyrus sp*
Verticillium sp	*Acer sp. Ulmus sp.*

Table 4 Common diseases of selected forest trees of Nepal (Forestry, 2010)

Tree	Pathogens	Disease nature
	Conifers diseases	
Cedrus deodara	*Heterobasidion annosum, Peniophora luna*	Butt & trunk rot
Abies pindrow	*Fomes fomentarius, Phellinus. robustus, P. pini*	Root rot , Heart rot
Pinus roxburghii	*Cronartium himalaynese, Phellinus pini, Fomitopsis pinicola, Ganoderma applanatum*	Needle rust, heart rot
Pinus wallichiana	*Phellinus pini*	Heart rot
	Hardwood disease	
Acacia catechu	*Ganoderma lucidum, Phellinus badius*	Root rot, heart rot
Dalbergia sissoo	*Fusarium solani, Ganoderma lucidum,*	Wilt disease, root rot.
Shorea robusta	*Aurificaria shoreae, Hymenochaete rubiginosa, Phellinus caryophulli, P. fastuosus*	Root rot disease, Heart rot
Tectona grandis	*Peniphora rhizomorphosulphurea, Corticium salmonicolour*	Root rots disease, Pink disease.
Santanum album	Mycoplasma	Spike disease
Eucalyptus hybrid	*Corticium salmonicolour, Ganoderma lucidum*	Pink disease, root rot

Table 5 Forest Diseases in Bhutan (Donaubauer 1986, 1987, 1993: Donald *et al*, *2003:* Chhetri and Tenzin 2012: Tshering and Chhetri 2000 :Konrad 2006 : Dorji 2007)

Name of diseases	Affected tree	Cause/ Pathogen	Reported
Mortality	*Pinus wallachina* and *Pinus spinulosa*	*Ips schmutzenhoferi*	1988, 2000, 2002
Dieback	*Pinus wallachina*	Drought	1994, 1999, 2001, 2003 and 2008.
Mortality	*Pinus roxbirghii*	*Ips longifolia*	1980's
Dieback	*Abies densa*	Prolonged drought	1993
Parasitism	*Pinus wallachina* and *Pinus roxburghii*	*Arceuthobium minutissimum* and *Taxillus kaempferi*	1986

3.1 Study area

Gazetted in 1974 as a wildlife sanctuary, Jigme Dorji National Park (JDNP) was officially upgraded to a National Park in 1993 and functionally operate with an area of 4,316 km^2 situated between 27.83'09.61"N and 89.77'55.33"E. JDNP is one of the oldest and second largest protected areas in Bhutan which covers the 5 districts of the Western Bhutan. JDNP is considered as conservation jewel, for housing many of worlds threatened flora and fauna (Tharchen,2013; Thinley *et al*., 2015).

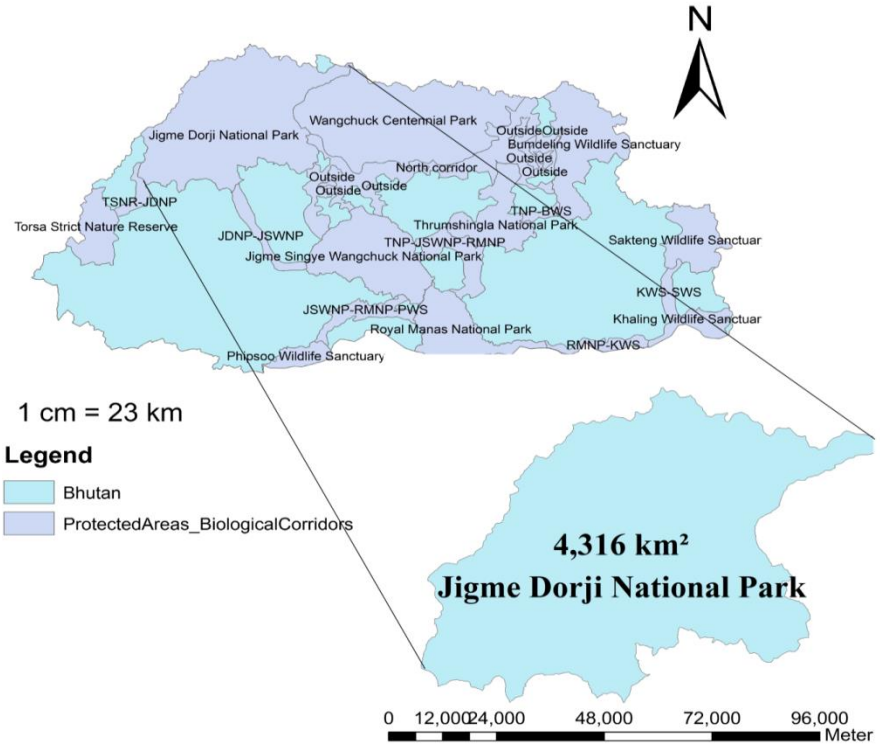


Figure 1 Study area (Jigme Dorji National Park, Bhutan)

JDNP alone contribute to 4% of the country' GDP. It provides Nu 81.54 million worth of resources to the Park residents on annual basis. At the regional level the Park provides ecosystem services worth of Nu 19.86 million and 1125.19 million at the national level considering that JDNP is conservation jewel (Thinley *et al*., 2015). Therefore, the conservation, management and protection of forests are crucial for the survival of rich flora and fauna for rich biodiversity and the economic contribution

3.2 Materials

Weighing balance (Anamed) was used for weighing chemicals and culture media. The pH of the solution and culture media were adjusted using HCL/NaOH and checked with digital pH

meter (Eutec Instrument). Glassware (Borosil) were sterilized in a hot air oven (Wieber) and culture media in an autoclave (NSM 227). Mircowave Oven (IFB-30SC2) was used to liquefy culture media and BOD incubator (NSW Caltan) was used for the culturing of fungi. Refrigerator (Zenith) was used for the storage of the cultures. All the isolation and culturing works were carried out under aseptic conditions in a Laminar air flow (Saveer Biotech). Binocular research microscope (Leica) was used for the microscopic studies of the cultures and slides of the samples. Paper bags were used to collect samples and GPS to take the coordinate and altitude of the sampling site. Hammer was also used to detect the heart rot of trees and measuring tape for measuring the DBH of the trees. Digital camera was used to take photograph of the samples and isolates.

3.3 Methodology

Research design such as Detection programmes and Purposive sampling techniques for forest diseases data were adopted.

3.3.1 Detection programmes:

3.3.1.1 Remote Sensing and GIS

Topographic maps of study area (scale 1:50,000) were scanned and transferred to ERDAS IMAGINE 8.7 for geo -referencing. Satellite image of study area was extracted from IRS-1D-LISS-III from USGS site and was rectified with topographic map of study area in ERDAS IMAGINE. After rectification subset of AOI (area of interest, JDNP) was classified into different land uses (agriculture, forest, settlement, snow and river, etc.) and different forest class (Fir forest, Conifer forest, mixed forest, Broadleaved forest and Scrub forest) as shown in Figure 2 using the non-supervise classification in ERDAS IMAGINE to use in area sampling.

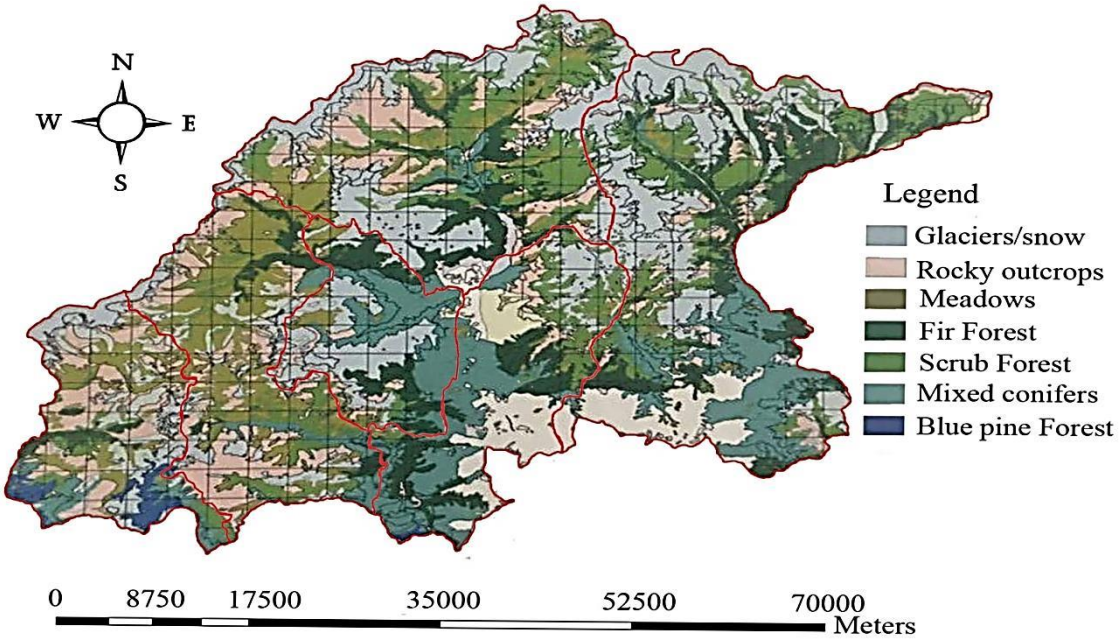


Figure 2 Land used classification of Jigme Dorji Nation Park, based on satellite image (LISS III)

3.3.1.2 Area Sampling.

JDNP was divided into six Ranges and was considered as separate area. In each range with use of Remote Sensing and GIS forest was classified into different forest class which was called as Clusters (smaller area). The ground truthing was performed with the help of Park officials. A total of 100 sampling units (50m *50m) were drawn randomly (20 in each range) and framed datasheets (annexure 2) were used to study diseases in clusters having tree species of interest.

Number of sampling units required

According to JDNP, Soe range, Lingzhi range, Gasa range, Ramina range and Laya range have average of 629 km^2 area. By using the given formula number of sampling units required was calculated and 20% sampling intensity was taken.

$$N1=C1/TA$$

Where, N1=Maximum number of sampling unit needed

C1= Size of the cluster (ha) or area of interest

TA= Size of sampling plot (ha)

Calculation

Average area of five range of JDNP= 629km^2~600km^2

Therefore, number of sampling unit required =600,000m^2/2500m^2=240

By taking 45% of sampling intensity=45/100*240=108 sampling units

But taking the consideration of other methods employed (field surveillance and purposive sampling), only 100 sampling units were taken.

3.3.1.3 Field surveillance

Forest disease detection depends upon the area the surveillance covers in forests and the intensity of surveillance. Since study area is protected area, field surveillance was done by foresters as regular assigned duties. Along with regular assigned forest surveillance, field surveillance for the study was done. Foresters were apprised about diseases symptoms to get exact information on types of diseases, species affected and location and magnitude as the site was known by the foresters.

In field surveillance, 5 meter area on both sides of the path was studied. Distance and area covered was marked with the help of GPS to see total area covered by surveillance. Framed datasheets was used to study diseases encountered in surveillance. Field surveillance covered a total area of 1.01km^2 (Total Length=102km: Breath=0.01km (5m on either side). Data were collected using the field datasheet (Annexure 2) and the samples were taken for laboratory analysis.

3.3.2 Purposive sampling

3.3.2.1 Questionnaire

Questionnaire was framed with the help of FAO website (Food and Agriculture Organisation) which is used for conducting research on forest diseases globally. Questionnaire was conducted from 5 District Chief Forest Officers (Thimphu, Paro, Haa, Gasa, Phunakha and Wangdue Phodrang) and Park Manager (Jigme Dorji National Park) which fall under the study area. Questionnaire was also done with the Forest Protection and Enforcement Division under Department of Forest and Park Service (Annexure 1).

3.4 Sample collections

Plant parts with recognizable signs and symptoms of diseases were collected. Special attention was paid to collect plant pathogens such as sporophores. Diseased portion of bark, stem and roots were also collected. Stem tissues from the leading edge of the lesion were removed with the help of sterile scalpel and collected. In case of root specimens, excess soil

particles were removed in running water. Roots were carefully teased using scalpel or forceps. Sporophores of the heart rot causing fungi were collected in a way so as to include all morphological features of the sporophore. Details of the diseases and hosts infected were recorded in datasheet (Annexure 2) and samples were taken for laboratory analysis.

3.5 Isolation techniques

Disease specimens such as heart rots, roots, etc. were carefully examined to see the mycelium of the fungi. With the help of sterilized forceps about 5mm of mycelium along with plant tissue was cut and placed for surface sterilization. In case of roots samples, after washing in running water 5mm of roots were carefully teased using scalpel and forceps and placed for surface sterilization. From sporophores found on each host small piece from context was taken out with sterilized forceps and placed for surface sterilization.

3.6 Surface sterilization

The samples were surface sterilized by immersing in the mercuric chloride (0.1%) or ethanol (70%) for 1 minute. After that they were washed thoroughly in sterilized water for at least 3 times. With the help of sterilized forceps the sample were transferred to Petri plates containing PDA media (all media used have 0.2 or 0.5% antibacterial agent streptopenicillin or streptomycin).

3.7 Inoculation, incubation and purification

Multipoint inoculation was attempted usually by 5 bits of samples per Petri plate. For each sample two Petri plates were kept. Single point isolation was also attempted. Inoculated Petri plates were transferred to incubation room maintained at a temperature of 25±2^{o}C and humidity of more than 90%. In order to obtain adequate growth and sporulation of fungal colonies alternate 12h dark and light was provided for seven days. After seven days of inoculation fungal isolates from the Petri plates were purified by sub-culturing and pure culture was obtained.

3.8 Identification of fungal pathogens

Sections of the sporophores were cut and details of the context, hymenium, texture, colour, hyphae, etc., were recorded. Slides from sporophore were made for taxonomic identification.

The morphology of the culture was studied using both simple and compound binocular microscope. Details of fungal colony, growth rate, texture, smell and other important characteristics were recorded and matched with the cultural characters described by Stalpers (1978). They were also authenticated by matching with the cultures maintained in National

Type Culture Collection maintained by Forest Pathology Division, Forest Research Institute, India.

3.9 Data analysis

3.9.1 Biodiversity index of forest pathogens

Pathogen diversity in each host was tested using the Shannon-Wiener index (1949) and Simposon's index. These indexes incorporate both components of biodiversity (evenness and richness) and it provides a simple, synthetic summary in a single index

$$\textbf{Shannon index } (\mathbf{H}') = -\sum pi \ln pi$$

Where pi=S/N

S=Number of individual of one species
N=Total number of all individuals in the sample
Ln=logarithm to base e

$$\textbf{Simpson's diversity index } D = 1 - \sum p^2 i$$

Where pi= S/N

S=Number of individual of one species
N=Total number of all individuals in the sample

3.9.2 Pathogens evenness

Pielou's evenness (e) (1966) was used to calculate pathogens evenness index

$$\mathbf{e} = \boldsymbol{H'/(Ln\ S)}$$

H'=Shannon-Wiener diversity index

S=total number of species in the sample

3.9.3 Pathogen similarity

Statistical software, Biodiversity professional (version 2, 1997) was used to compute the Bray-Curtis index (1957) for pathogen similarity in each host species

3.9.4 Assessment of Disease Severity and Incidence

Diseases severity assessment was made in each disease encounter and it was rated on numerical scale (0-3). The average disease severity index (DSI) for each species was calculated from the sum of total number of trees of each disease severity rating (DSR) in all the plots multiplied separately by the disease index (1 - 3) and dividing it by the total number of trees assessed (N) as given in the following formula.

Disease severity (DSI) =n L x 1 + n M x 2 + n S x 3/N

Where: nL, nM, nS = Total number of trees with Low, Medium and Severe disease severity

1, 2, 3 = Low, Medium and Severe respectively

N = Total number of trees assessed in all the observation plots.

The per cent disease incidence was calculated from the total number of plants affected (nd) and total number of plants observed in all the plots (N):

Percent disease incidence = nd/N*100

Table 6 Disease scoring scale for assessing the disease severity (adopted from Sharma *et al*., 1985; Kumer, 2007)

DS	Foliage infection	Heart/root infection	Shoot infection	Parasites infection
Nil (0)	No disease	No disease	No disease	No disease
Low (1)	Up to 25%	Less than 5%	Dieback of branch (>25%)	Parasite 1-5%
Medium (2)	25-50% and >10% defoliation	Between 5-10%	Dieback of branch (>50%)	Parasite between 5-25%
High (3)	50-75% or >25% Defoliation	More than 10%	Extensive dieback and death of tree	More than 25%

3.9.5 Test and other tools

Non parametric chi-square test was performed to test the significant difference in proportion to pathogen in pure and mixed coniferous forests and to see the variation in pathogen abundance in JDNP range. Descriptive analysis, linear regression, Pearson correlation between the disease incidence and severity index was done with the SPSS and excel.

RESULTS AND DISCUSSIONS

Investigations were carried out in JDNP for detection of forest disease and results obtained in this study are presented below.

4.1 Potential forest pathogens in JDNP

Conifer forests form the most important natural vegetation in most parts of the Park area at elevations above 1800m amsl. They either form pure stands or stands of mixed species occasionally with hardwoods and play important role in rural constructions as well as for conservation. This study revealed, a total of 20 forest pathogens, 15 fungi belonging to 11 families, 3 flowering mistletoes belonging to two families and two insect pests (bark beetle) belonging to two families were recorded as shown in Tables 7, 8 and 9 which have potential to cause epidemic diseases.

Diseases caused by these pathogens broadly fall under root rot, heart rot, foliar damage, parasitism and mortality. Results also show that these pathogens are having detrimental effect on conifers. It also shows that 65% of pathogens were reported from pure coniferous stand and 30% from mixed coniferous forest.

Table 7 Fungal pathogens from Jigme Dorji National Park, their incidence percentage and abundance

Host	Fungal pathogen	Disease	Abundance	Fungal Incidence (%)
Juniperus recurva (N= 521)	*Heterobasidon annosum*	Root rot	73	14
Picea spinulosa (N=631)	*Fomitopsis pinicola*	Heart rot	27	04
	Chrysomyxa woroninii	Needle blight	08	01
Abies densa (N=876)	*Fomitopsis pinicola*	Heart rot	93	11
	Armillarea mellea	Root rot	39	04
	Armillarea ostoyoe	Root rot	53	06
	Herterobasidion annosum	Root rot	35	04
	Ganoderma appalatum	Root rot	46	05
	Lirula nervisequia	Needle cast	15	02
Tsuga dumosa (N=721)	*Fomitopsis pinicola*	Heart rot	154	21
	Heterobasidion annosum	Root rot	68	09
	Almillaria ostoyae	Root rot	52	07
Pinus wallichiana (N=397)	*Rhizina undulate*	Root rot	12	03
	Cronartium ribicola	Stem rust	26	07
	Meloderma desmazierii	Needle cast	09	02
	Dothistroma septosporum	Needle cast	07	02
Pinus roxburghii	*Phellinus pini*	Heart rot	11	04

(N=296)	*Coleosporium* sp.	needle	03	01
	Taxillus versicolor	Wood decay	08	03
	Lhophoderma sp.	Needle cast	05	02

N=Number of tree studied. Fungal incidence = Abundance /N

Table 8 Insect pests from Jigme Dorji National Park, their incidence percentage and abundance

Host	Insect pest	Symptoms	Abundance	Pest incidence (%)
Pinus roxburghii	*Ips longifolia*	Mortality	23	08
Pinus wallichiana	*Ips schimutzenhoferi*	Mortality	44	11
Picea spinulosa	*Ips schimutzenhoferi*	Mortality	26	04

Pest incidence = Abundance /N N= As given in table 7

Table 9 Phanerogamic Parasites from Jigme Dorji National Park, their incidence percentage and abundance

Host	Parasitic plant	Effect	Abundance	Parasites incidence (%)
Abies densa	*Taxillus kaempferii*	Parasitism	72	08
Pinus wallichiana	*Taxillus kaempferii*	Parasitism	78	20
Pinus roxburghii	*Taxillus kampferii*	Parasitism	27	09
	Arceuthobium minutissimum	Parasitism	26	09
Picea spinolosa	*Arceuthobium sichuanense*	Parasitism	56	09

Parasite incidence =Abundance/N N= As given in table 7

4.2 Biodiversity index of forest pathogens

Table 10 Pathogen diversity index JDNP host species

	Diversity index		
Host	Shannon index (H')	Sipmson's index (D)	Pielou index (e)
Juniperus recura	0	0	0
Picea spinulosa	1.205	0.67	0.871
Abies densa	1.832	0.83	0.941
Pinus roxburghii	1.720	0.81	0.884
Pinus wallichiana	1.453	0.72	0.811
Tsuga dumosa	0.985	0.59	0.896

Pathogen diversity was recorded highest in *Abies densa* (H'=1.832) followed by *Pinus roxburghii* and *Pinus wallichina* with H' 1.720 and H' 1.453, respectively. This proves that

Abies densa has more richness and evenness in terms of the forest pathogens diversity as compared to other host tree species. The least pathogens evenness and richness diversity was shown by *Juniperus recurva* (H'=0). Simpson's diversity index (D) also showed similar results, where highest value was found for *Abies densa* and *Pinus roxburghii* 0.82 and 0.81, respectively. However, according to Shannon diversity index score, *Juniperus recurva* and *Tsuga dumosa* were having very low pathogen diversity (0.0001-0.9999). *Picea spinulosa, Pinus wallichiana* and *Pinus roxburghii* were having low pathogen diversity (1.0000-1.7499). Only *Abies densa* was having medium pathogen diversity (1.7500-2.2999). Pathogens in each host were evenly distributed (0.7500-1.000) except in *Juniperus recurva* (0).

4.3 Disease incidence and severity index

Considering all the listed pathogens, the disease incidence was highest in *Pinus wallichiana* (44%) followed by *Abies densa* (40%), *Tsuga demusa* (38%), *Pinus roxburghii* (19%), *Picea spinulosa* (18%) and least in *Juniperus indica* (14%). Similarly the disease severity index was found maximum in *Pinus wallichiana, Abies densa, Tsuga dumosa, Pinus roxburghii, Picea spinulosa* and *Jumpierus indica* (58%, 53%, 49%, 46%, 25% and 18%, respectively) as showed by figure 3. Pathogens incidence rate was more in parasitic plants (n=259), followed by insect pests (n=93) and fungi (n=744). Descriptive analysis shows mean value of percent disease incidence as 28.8 (SD= 13.21) and mean disease severity index percentage as 41.5 (SD=16.5).

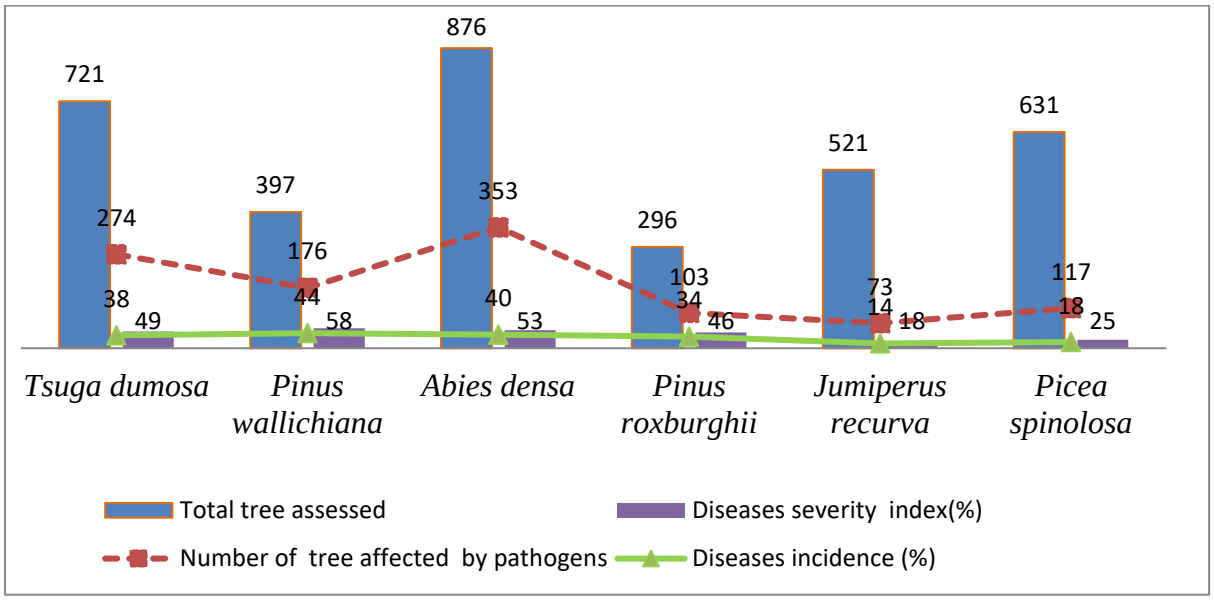


Figure 3 Diseases incidence and severity index percentage

It was also found that from total diseased trees (N=1096), 74% (n=807) fall in low, 19% (n=211) medium and 7% (n=74) in high categories of disease severity index. Study further concluded that root rot, heart rot and parasite disease lead with 34.4%, 29.9%, 23.6% respectively and insect and foliar disease least with 8.4% and 4.2% in JDNP. Further, Pearson correlation between disease incidence and severity index show as a significant relationship (r=0.998, p=0.05)

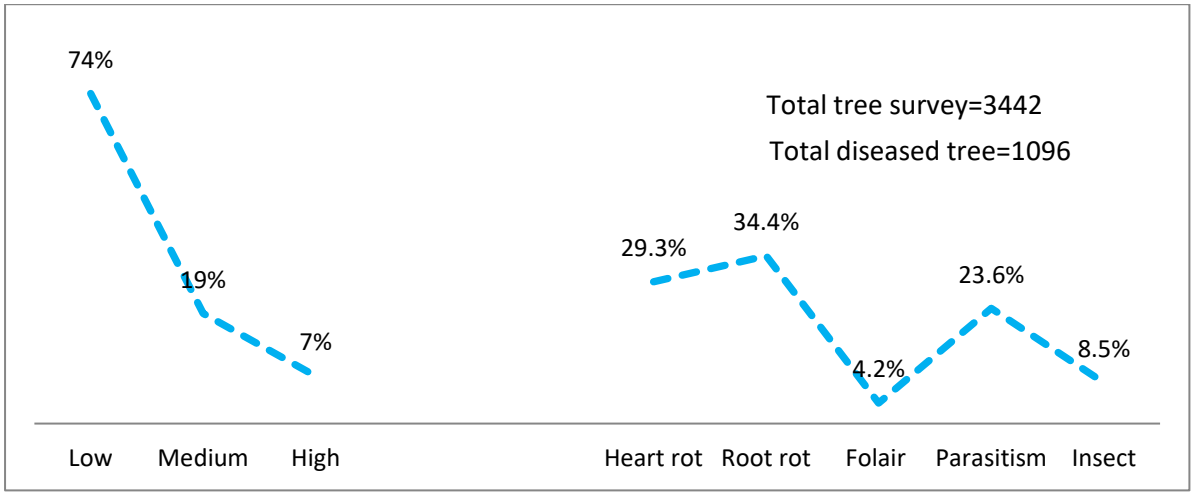


Figure 4 Disease severity and type percentage

4.4 Disease rate in JDNP ranges

Study site analysis showed that Linzhi range has more disease (28%), followed by Soe range (22%) and Laya range (19%). Least disease was noticed in Gasa and Ramina range with 16% and 15%, respectively as shown in Table 11.

Table 11 Disease rate in JDNP ranges

	Disease occurrence					Disease %
JDNP Range	**Heart rot**	**Root rot**	**Foliar**	**Parasite**	**Insect**	
Ramina	52	37	8	39	27	15
Gasa	105	57	2	11	6	16
Laya	92	72	2	33	9	19
Soe	52	82	12	58	32	22
Lingzhi	18	130	23	118	19	28
Total	319	378	47	359	93	1096/100%

Similarly, chi square test ($\chi 2=58.18$, $p=0.05$, $df=4$) also showed significant abundance variation in JDNP range thereby rejecting null hypothesis of having equal pathogen abundance in each JDNP range.

Table 12 Chi- square test for pathogen abundance variation in JDNP ranges

Range	Observe value (O)	Expected value (E)	Deviation (O-E)	(O-E)/E	$\chi 2$
Ramina	163	219.2	-56.2	3158	14.4
Gasa	181	219.2	-38.2	1459	06.6
Laya	209	219.2	-10.2	104	0.47
Soe	236	219.2	16.3	265	1.21
Lingzhi	308	219.2	88.3	7796	35.5
Total	1096	1096	00.0	12782	**58.18**

P=0.05, df =4, $\chi 2$ tabulated=9.49

4.5 Host to pathogen ratio

Hosts to pathogens ration was worked out on the basis of number of fungi, mistletoes and the insect pests identified from each host. It was confirmed that host to pathogen was lowest in *Juniperus indica* with 1:1 and highest in *Abies densa* and *Pinus roxburghii* with 1:7 each. Other hosts like *Picea spinulosa*, *Tusuga dumosa* and *Pinus wallichina* were having host to pathogen ratio of 1:4, 1:3 and 1:6, respectively. It showed that average of host to pathogen ratio is 1:4.

4.6 Pathogens similarity in host

A Bray-Curtis dendrogram based on abundance of individual pathogen in each host (Figure 5) showed that the host preferences by pathogens were less similar (19.16%). *Abies densa* and *Tsuga dumosa* have the most similar (53.26%) pathogens followed by *Juniperus recurva* and *Tsuga dumosa* (39.19%). *Juniper recurva*, *Pinus spinulosa* and *Pinus roxburghii* have no similar pathogens (Table 12). *Pinus roxburghii* cluster apart from other hosts suggested that the pathogens occurrences are most distinctive.

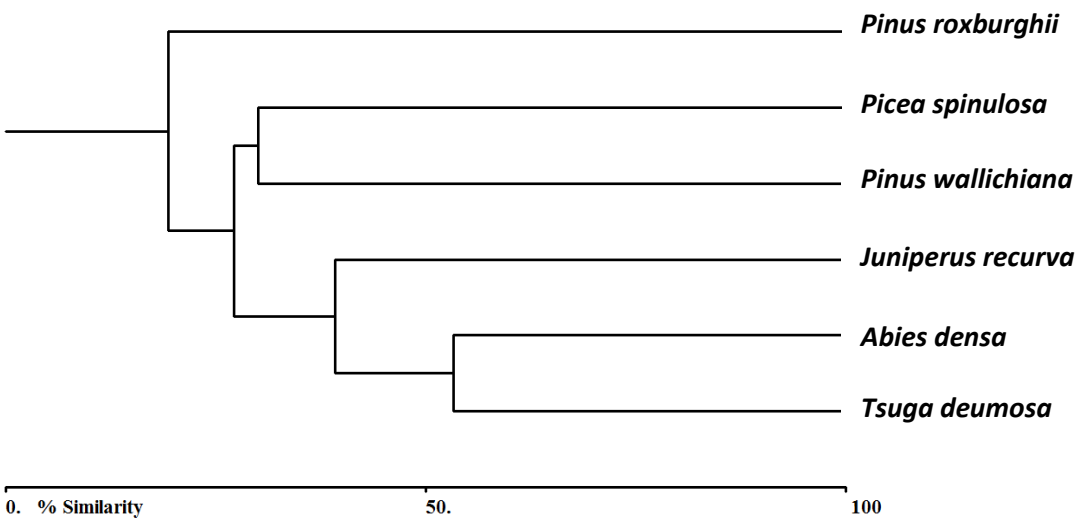


Figure 5 Bray-Curtis linked cluster dendrogram for the hosts, calculated using total number of pathogens per host

Table 13 Pathogens percentage similarity matrix

Host species	*Tsuga Dumosa*	*Pinus Wallichiana*	*Abies densa*	*Pinus roxburghii*	*Juniperus Recurva*	*Picea spinulosa*
Tsuga Dumosa	*	0.00	53.26	0.00	39.19	13.81
Pinus Wallichiana	*	*	27.22	19.35	0.00	30.03
Abies densa	*	*	*	11.84	16.43	11.48
Pinus roxburghii	*	*	*	*	0.00	0.00
Juniperus recurva	*	*	*	*	*	0.00
Picea spinulosa	*	*	*	*	*	*

4.7 Analysis of parameters

4.7.1 Altitude

Lowest altitude of diseases occurrence was in Ramina range (1629m asl) and highest was in Lingzhi range (3870 asl). 30% of diseases occurrences were in altitude between 1000m asl to 3000 m asl were *Pinus roxburghii* and *Pinus wallichina* formed dominants vegetation. 70% between 3000m asl to 4000m were *Abies densa*, *Tsuga dumosa, Picea spinulosa* form pure strand or mixed vegetation. In lower range, most of the pathogens were mistletoes and the beetles (*Ips*) and few fungi but in higher altitude most of the diseases were related to fungi. It's concluded that with increases in evaluation abundance of pathogens also increase (r=0.88).

4.7.2 Aspect

The disease is most prevalent on north-facing slopes, while south facing slopes are normally unaffected. Trees on north-facing slopes produce poor height growth and those showing the poorest growth eventually succumb to the diseases. A characteristic feature of the diseased slopes is the absence of sunlight in winter. Dieback is most prevalent on north-facing slopes and normally affected young trees as they were about to form canopy. About 58% of diseases due to fungi were seen in northern aspect with only 18% prevailing in west, 15% east and 9% in south but for the mistletoes they were prevailing with 45%, 29%, 14 %, and 12% for east, south, west and north, respectively. Similarly *Ips* attack was prevalent with 50%, 35%, 10% and 5%, for south, east, and west and north respectively.

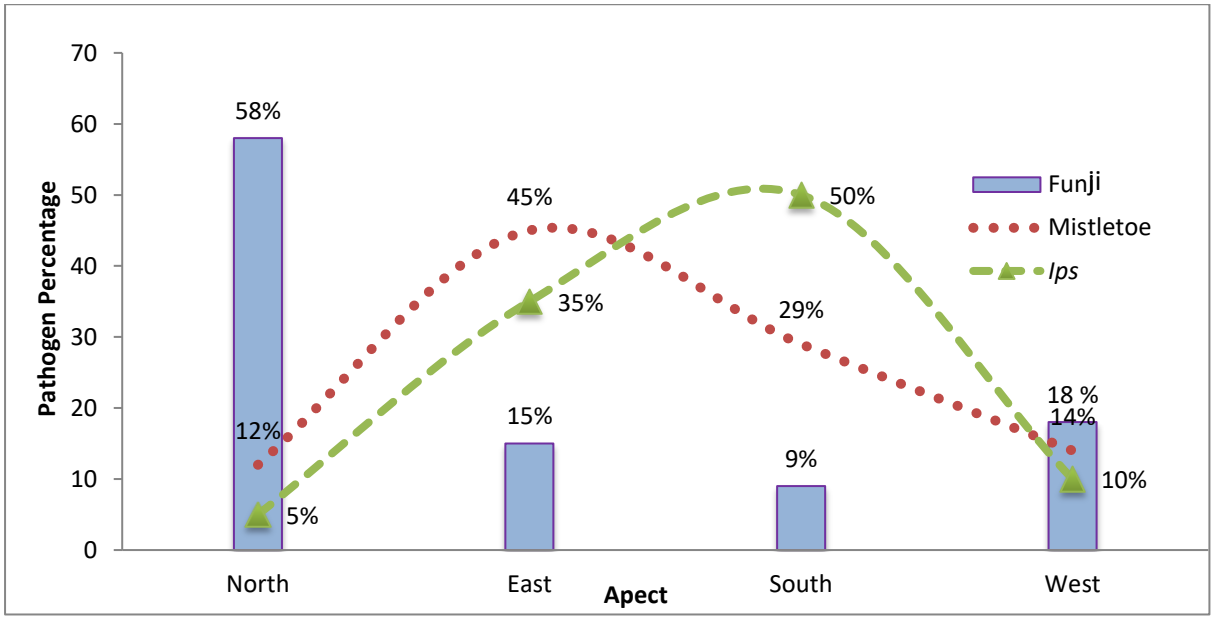


Figure 6 Pathogen or insect pest percentages with respect to aspect

4.7.3 Composition (mixed and pure forest)

In lower altitude (1000-2000m amsl) 80% of sampling unit were pure stand of *Pinus roxburghii* and *Pinus wallichiana* however, mixed forest intercepted by broad leaves and other conifers was also seen (20%) in few sampling units. At higher altitude between 300 to 4000 m amsl both pure and mixed conifers interception by *Rhodendron, Acer campelli, Larix griffithii* and different species of bamboo with 60% and 40% of sampling units were found respectively . Chi square test ($\chi 2=0.14$, $p=0.05$, $df=1$) proved about 65% of diseases occurred in pure forest and 35% in mixed forest.

Table 14 chi-square test for significant difference in proportion of pathogens in pure and mixed forest

	Pure forest	Mixed forest	Total
Disease observed value (O)	718	378	1096
Disease expected Value (E)	712 (65%)	384 (35%)	1096
Deviation= (O-E)	06	-06	00
(O-E)2	36	36	72
(O-E)/E	0.05	0.09	$\chi 2$ =0.14

P=0.05, df=1, Tabulated value=3.85

4.7.4 Predisposing factors

For each species there were different dominant predisposing factors (Figure 7) such as fire, human impact (pruning, injuries, logging, poor silviculture system, etc.), soil (pH, moisture, etc.), snow/frost and others. Percentage of predisposing factors were, 37%, 24%, 12%, 8% and 19% of fire, human impact, soil (pH, moisture, drought), frost / snow and others, respectively.

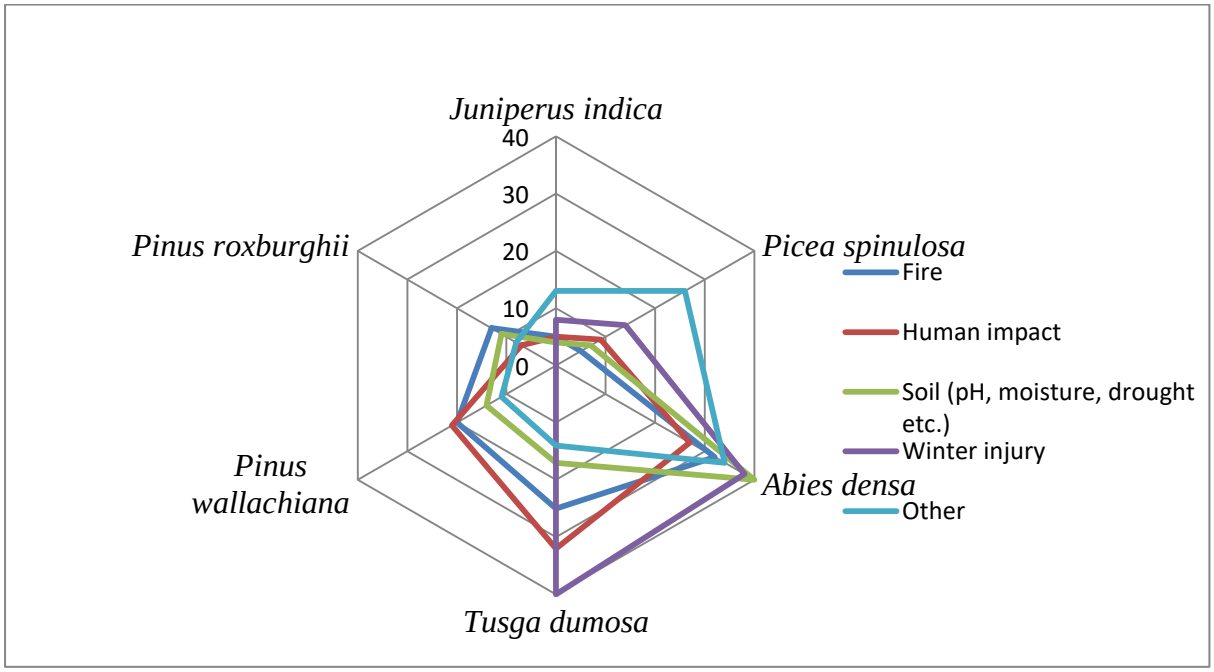


Figure 7 Predisposing factors of forest disease (%)

4.7.5 Girth class (GBH) relation with pathogens

Conifers were found susceptible to diseases from young age to maturity. Descriptive analysis in each GBH class showed that the occurrence of diseases was highest in 160-180 cm girth class (mean=56.8, SD=58.5) followed by girth class 140-160 cm (mean 40.4, SD=36.6). Least disease was appeared in girth class 20-40 cm (mean=4.6, SD=5) (Table 15 and Fig 8).

Table 15 Descriptive analysis of pathogens in each GBH class

GBH class	Pathogen abundance						Descriptive analysis	
cm	Heart	Root	Foliar	Parasite	Insects	Total	Mean	SD
010-020	0	3	33	0	0	36	7.2	14.5
020-040	2	9	11	1	0	23	4.6	5.0
040-060	12	11	0	59	3	85	17	24.0
060-080	5	7	1	29	7	49	9.8	11.0
080-100	17	15	0	70	4	106	21.2	28.2
100-120	43	44	0	43	11	141	28.2	21.1
120-140	57	72	0	11	21	161	32.2	30.9
140-160	72	86	2	27	15	202	40.4	36.6
160-180	111	131	0	19	32	293	56.8	58.5

Regression analysis also generated statistical relationship between the GBH class (predictor variables) and the pathogens abundance (response variable) with R^2 =0.88 and multiple R=0.94 (p value=<0.05). Regression further concluded that predictor variable was associated with changes in the response variable.

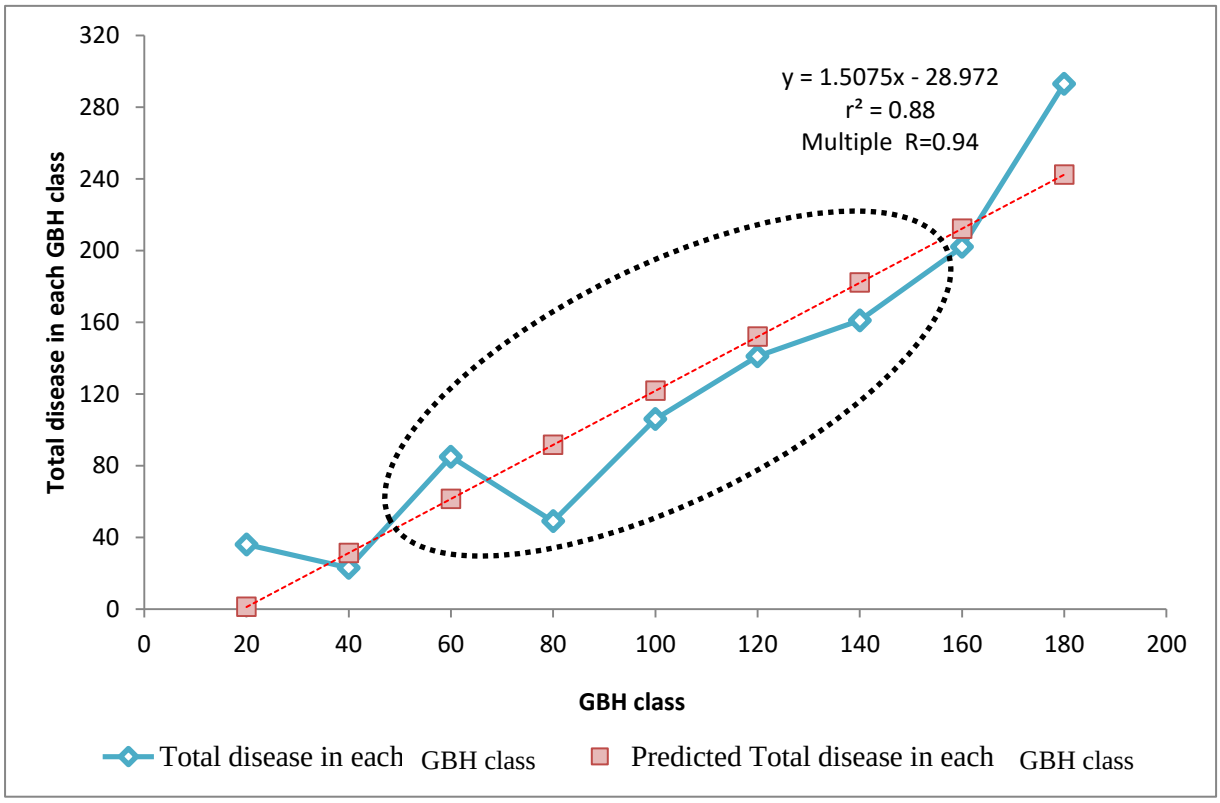


Figure 8 GBH class line fit plot

CONCLUSION

In Bhutan, forest resource is vast and will provide wood and ecological functions for a multitude of purposes virtually indefinitely, if managed wisely and used sustainably. Though, native insect pests and diseases are an integral part of forest ecosystems that kills the weakened and senescent trees to make way for new, vigorous forests they also help to recycle forests by decomposing trees to replenish the soil and supply vital nutrients necessary for forest growth. Process of forest regeneration, growth and renewal has been repeated for millennia and is essential to stable, healthy forest ecosystems (Farjon and Page, 1999).

However, human need for lumber and other wood products often conflicts with the natural loss of these products through diseases. Disease outbreaks also affect other resources valued by society such as aesthetics, recreation, water and wildlife. If conservation and productivity are to be sustained, forests have to be protected against fire, indiscriminate cutting, encroachments and equally important against insect pests and diseases. Whilst the protection of forests against the human hazards are partly be achieved through law enforcement and extension services. But, protection against diseases requires, in addition a much more integrated approach incorporating specialized knowledge on types and nature of diseases. Utilization of such knowledge is not being achieved due to insufficient knowledge about forest pathology in Bhutan at present. As reported in the Second National Communication (2011), Bhutan is highly vulnerable to adverse impacts of climate change due to the fragile mountainous ecosystem and economic structure. The most vulnerable sectors are water resources, agriculture, forests, biodiversity, and hydropower sectors.

Therefore, a comprehensive inventory of diseases in the JDNP was undertaken and 20 species of pathogens affecting different species were recorded which are known to cause large forests damage worldwide. Therefore, to fulfil the Constitution mandates, GHN principles, International Conventions, Pledges and domestic Policy and law, following measures are need to be considered:

Bhutan has large forest cover and fall under higher climate change risk zone. 69% of rural population also depend on forest and it is key for economic, ecological and biodiversity. Yet, Bhutan lack forest pathologist and entomologist who play vital role for forest protection and management. Scientific knowledge on forest pathology and resource persons is lacking which create huge gab in forest pathology. Therefore, it’s imperative to enrich and create human capacity in forest pathology science with respect to climate change in Bhutan.

Forest protection, conservation and management activities are concentrated only to wildfire, social restriction system, Buddhist principles and Gross National Happiness (GNH) vision. Diversification of forest management to forest diseases and pests inventory, incorporation of forest pathology and entomology discipline are paramount importance for 72% forest for current and future ecological and protective equations.

Finding the reference about forest diseases and research are challenging in this Himalayan County. It receives a little concerned despite destroying a large area of forest since first outbreak from 1980s. Low budget allocated in forest department (eg.4.1 billion in 2013-2014) also hampered the research work in forestry. Therefore, incorporation of pathological science and traditional knowledge in forest protection, conservation and management programmes with proper budget allocation for pathology research are necessary.

Dorji (2010), reported about 247 species of exotic plants in Bhutan, which accounts for about 5.6% of the total recorded plant species in the country; hence it is likely that forest diseases are also introduced in country. Increasing in the international trade, might also introduce forest pathogens as most of virulent pathogens are reported from neighbouring countries such as Nepal and India. This called necessary measurements and programmes in plant quarantine sectors.

The high levels of incidence and infection severity caused by mistletoes suggests past and present forest management practice. This is because it is common practice to preferentially cut uninfected trees with good wood quality and to leave infested residual trees. Incorporating principles of disease management, particularly pruning and sanitation in a silviculture system will reduce the mistletoes infection. High heart rot incidence also suggests effect of past primary focus on the protection of the forests. Therefore, now it called for balance harvests of mature stand following conservation with sustainable management which is economical and scientific sound.

Hence, this study fulfilled the objectives to document the diversity of forest diseases and provide information about disease incidence and severity index along with key measures and recommendations. Therefore, this study will also act as base line information for forest pathology and will be valuable data for future references in JDNP, Bhutan.

SELECTED BIBLIOGRAPHY

Agrios, G. (2006). *Plant Pathology.* New Delhi: Elsevier, Division of Reed Elsevier Indian Private Limited.

Bakshi, B. K. (1971). *Indian Polyporaceae (On trees and Timber).* New Delhi: Indain Council of Forest Reserch and Education.

Bakshi, B. K. (1976). *Forest Pathology; Principal and practic in Foresty.* Dehardun: Controller of Publication.

Chhetri, P. B. and Tenzin, K. (2012). *State of Forest Genetic Resources of Bhutan. Report.* Department of Forests and Park Services,Royal Government of Bhutan.

Cooke, B. M. and Jones, D. G. (2006). *The Epidemology of Plant Diseases.* New York: Springer publication.

DoFS. (2011). *Forestry development in Bhutan. Policies, Programmes and institutions*: Celebrating international year of forest. MoAF, Thimphu , Bhutan.

Donald, P.A., Stamps, W, T. and Linit, M. J. (2003). Pine wilt disease. *The Plant Health Instructor*. DOI:10.1094/ PHI-I-2003-0130-01.

Donaubauer, E. (1986). Technical advisory services for forest development, Bhutan, Forest Pathology. Department of Forests, *Field Document, 11*:37.

Donaubauer, E. (1987). Technical advisory services for forest development, Bhutan, Forest Pathology. Department of Forests, *Field Document, 12*:14.

Donaubauer, E. (1993). On the decline of fir (*Abies densa* Griff.) in Bhutan. *Field Document, 12*:14.

Dorji, S. (2007). Himalayan dwarf mistletoe (*Arceuthobium minutissimum*) and the leafy mistletoe *Taxillus kaempferi* on Blue pine: A case study in Western Bhutan. *Diploma thesis,* 127.

Dorji, T. (2016). Hydropoer Sale. *Kuensel*:5

Dorji, Y. (2010). *A red data list for the flowering plants of Bhutan*. WWF, Bhutan.

FAO. (1960). An international review of forestry and forest products. *Unasylva, Vol. 14*(4).

FAO. (1965). An international review of forestry and forest products.*Unasylva, 19*(3).

FAO. (2010). Global Forest Resources Assestment. *Foresty Paper* :169.

Farjon, A. and Page, C. N. (1999). Conifers. Status Survey and Conservation Action Plan. IUCN/SSC Conifer Specialist Group. IUCN, Gland, Switzerland and Cambridge,UK.:121

Forestry, N. (2010). *Common Diseases of Selected Forest Trees.*

Heiniger, U. (2003). Das Risiko eingeschleppter Krankheiten für die Waldbäume. *10*: 410-414.

Hepting, G. (1963). Climate and forest diseases. *Annual Review of Phytopathology 1*, 31-50.

Hosagoudar, V. B., Sabeena, A. and Mathew, S. P. (2013). Additions to asterinaceous fungi (Ascomycetes) in India. *Journal of Threatened Taxa* 5(2): 36703672 doi:10.11609/JoTT.o3228.3670-72.

IPCC. (2007). *Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, UK: Cambridge University Press, 1–18.

Kirisitis, T., Dorji, S., Gyenthen, S. and Chhetri, D. B. (2007). Commom Shoot, Stems, leaves and Branch Diseases of Conifer in Bhutan. *Acta, Silva, Ling, Hung.*, 241-247.

Konrad, H. (2006). *Molecular ecology of forest pathogens causing Dutch elm disease, blue-stain and Sirococcus shoot blight*. University of Natural Resources and Applied Life Sciences, Vienna (BOKU), Austria. *Dissertation*. 57.

Porta, N. L., Capretti, P., Thomsen, I. M., Kasanen, R., Hietala, A. M. and Weissenberg, K. V. (2008). Forest pathogens with higher damage potential due to climate change in Europe. *Plant Pathol., 30*, 177-195.

Ravarden, L. and Johansen, I. (1980). *A primary polypore flora of East Africa.* Oslo, Norway.

Sturrocka, R. N., Frankel, S. J., Brown, A. V., Hennond, P. E., Kliejunasb, J. T. and Lewis, K. J. (2011). Climate change and forest diseases. *Plant Pathology, 60*, 133-149.

Tharchen, L. (2013). *Protected Area and Biodiversity of Bhutan.* Kolkota, India: CDB Printer Pvt.Ltd.

Thinley, P., Tharchen, L. and Dorji, R. (2015). Conseravtion Management Plan of Jigme Dorji National Park for period january 2015-2019. *Biodiversity in Persuit of Gross National Happiness*.

Tshering, G. and Chhetri, D. B. (2000): *Important forest insect pests and diseases of Bhutan withcontrol measures*. Renewable Natural Resources Research Centre, Yusipang, Bhutan, *Field Guide, 57.*

Wangdi, S., Dorji, T., Wangchuck, S. and Thinley, K. (2014). *Social Restriction in Traditioal Forest Managent System and its impication in Biodiversity Conservation in Bhutan*. 112-122.

	ANNEXURE 1- Questionnaire on types of diseases and *their outbreak (if)* for purposive sampling											
					Outbreak period (If outbreak was reported in your area)							
	Disease	Tree affected	Pathogen	Units (Ha/year or m3 or No of trees)	1980-1990	1991-2000	2001-2010	1996-2000	2001-2005	2006-2010	2011-2015	2016-
Si No	Name	Name	Name									
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
12												
13												
14												
15												
					(Specify in brackets if the figure is O=Official, E=Estimate, A =Average or U =Unknown)							
	TOTAL AREA AFFECTED OR											
	TOTAL NUMBER OF TREES AFFECTED OR											
	TOTAL WOOD VOLUME AFFECTED											

ANNEXURE 2 TREE DISEASES CHARACTERISATION IN FIELD

NAME OF DISEASES:						
DISEASES NO:			**SAMPLING UNIT NO;**			
GPS coordinate;		Aspect;	Altitude;		Wind direction;	
1.PRIMARY CAUSAL AGENT						
a. Scientific name		b. Common name		c. Host tree		
2 EXACT LOCATION AND TIMING OF DISEASE						
a District		b. Block		c. Season		
3. FOREST INFORMATION (Tick one)						
A. Forest types	a. Fir forest	e. Broad leaved mixed with conifer forest		B. Age class of affected trees (Tick one) and DBH		
	b. Mixed conifer forest	f. Broadleaved hardwood forest		a. Seedling	a. DBH	
	c. Blue fine forest	g. Forest scrub forest		b. Young		
	d. Chir pine forest			c. Mature		
4. CAUSES AND EFFECTS OF DAMAGE						
A. Predisposing cause(s) of damage (Tick one or two)				B. Visible effects (Tick one)		
	a. Fire	f. Pollution		a. Mortality		
	b. Storm	g. Human impact		b. Dieback		
	c. Drought	h. Others		c. Defoliation		
	e. Frost			d. Wood decay		
5. SOIL CONDITION						
A. Soil Ph			B. Moisture contents			
	a. Acidic	c. Alkaline	a. High	c. Low		
	a. Neutral		b. Medium.			

Annexure 3 Fruting body of fungal pathogens

Figure 2 *Phellinus pini* on *Pinus roxburghii*

Figure 3 *Ganoderma appalatum*

Figure 11 *Heterobasidion annosum*

Figure 12 *Rhizina undulata*

Figure 13 *Armillaria solidipes* (formerly *Armillaria ostoyae)*

Figure 14 *Armillaria mellea*

Annexure 4 Pure culture of pathogens

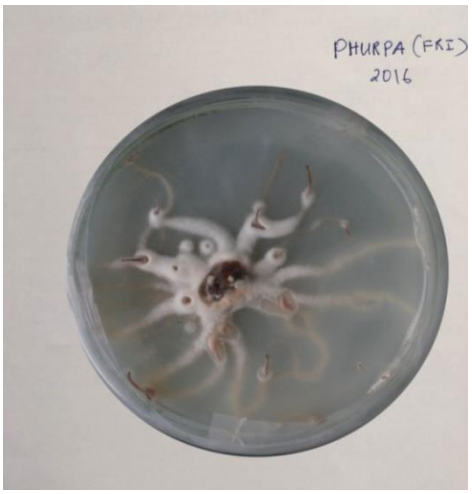


Figure 15 *Armillaria solidipes* (*A. ostoyae*)

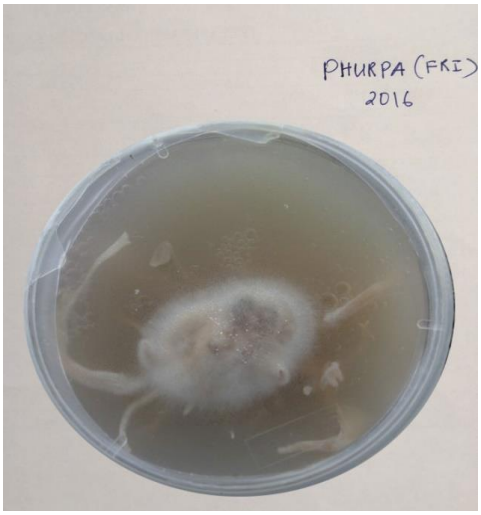


Figure 16 *Armillaria mellea*

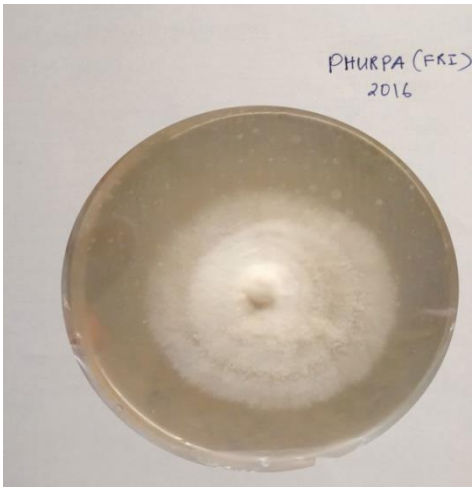


Figure 17 *Fomitopsis pinicola*

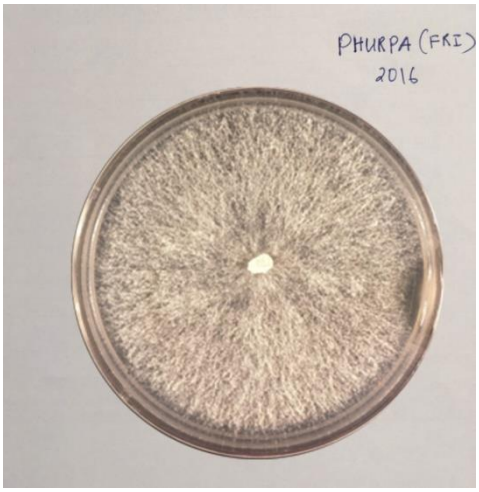


Figure 18 *Heterobasidion annosum*

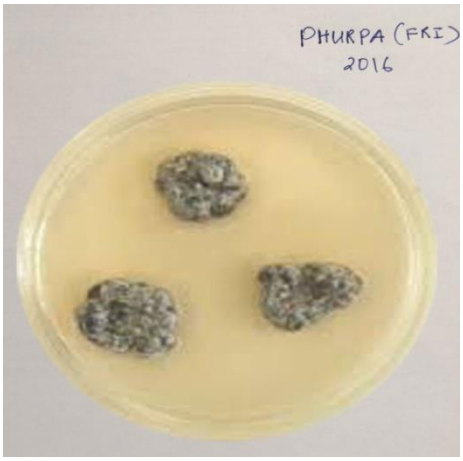


Figure 19 *Dothistroma septosporum*

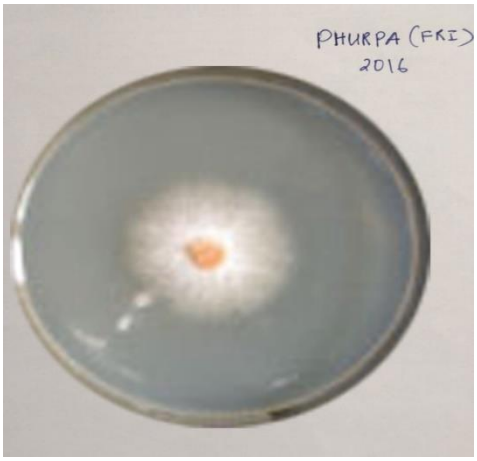


Figure 20 *Ihophoderma* sp

Annexure 5 Forest pest (*Ips longifolia*) and damage

Figure 21 Beetles (*Ips* affect) on *Pinus roxburghii*

Figure 22 *Pinus roxburghhii* Mortality by *Ips longifolia*

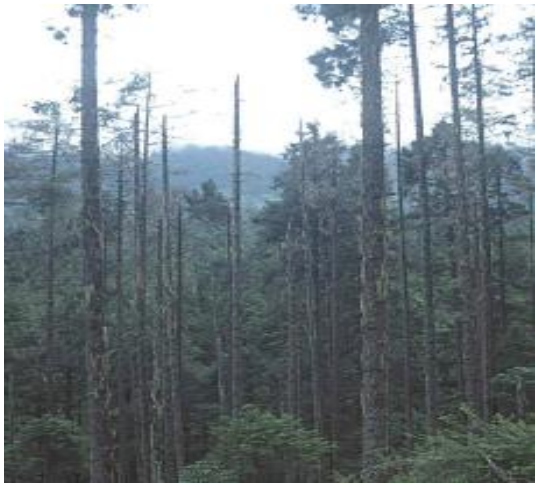


Figure 23 *Picea spinulosa* mortality by *Ips*

Figure 24 *Ips Longfolia* on *Pinus roburghii*

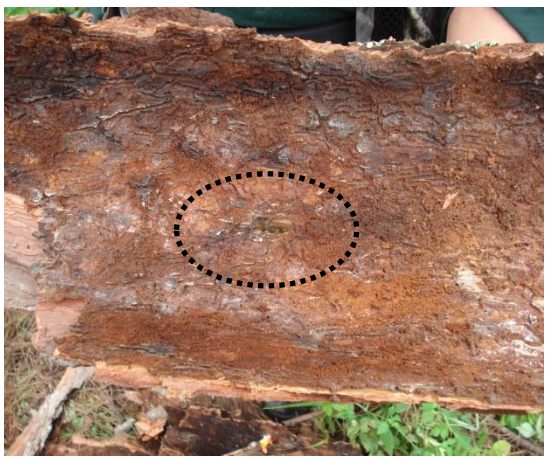


Figure 25 *Ips schmutzhonferi* on *Pinus wallichiana*

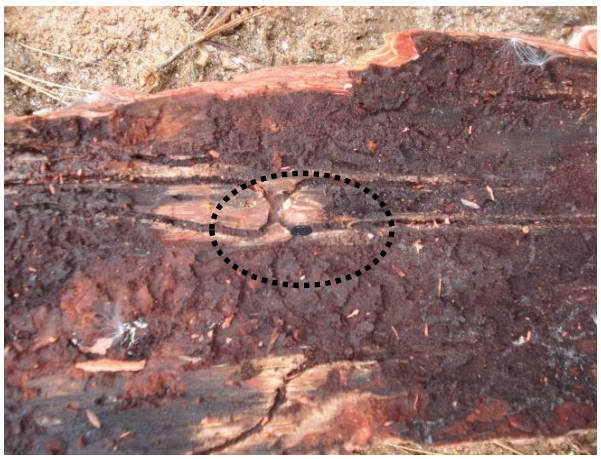


Figure 26 *Ips Schimutzhonferii* on *Picea Spinulosa*

Annexure 6 Fungal pathogens and damange

Figure 27 Hear rot of *Abies densa*

Figure 28 Hear rot of *Juniperus recurva*

Figure 28 Root and But rot of T*usga dumosa*

Figure 30 White rot of *Abies densa*

Figure 31 White rot of *Tsuga dumosa*

Figure 32 Heart rot of *Picea spinulosa*

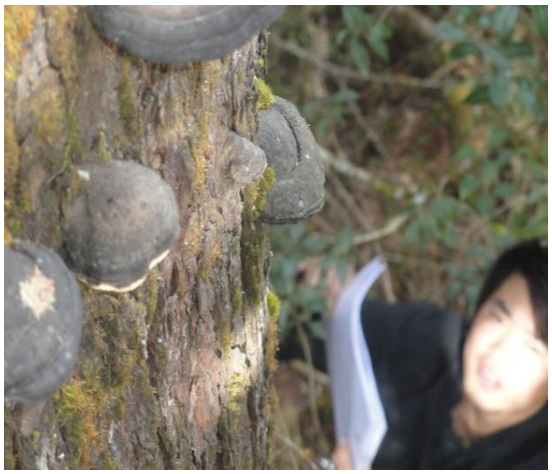


Figure 33 Assessing of fruiting body

Figure 34 *Fomitops pinicola* on *Abies densa*

Figure 35 Human impacts on *Juniperus recurva*

Figure 36 Pathogens inoculation

Figure 37 Identification process

Annexure 7 Flowering Mistletoes

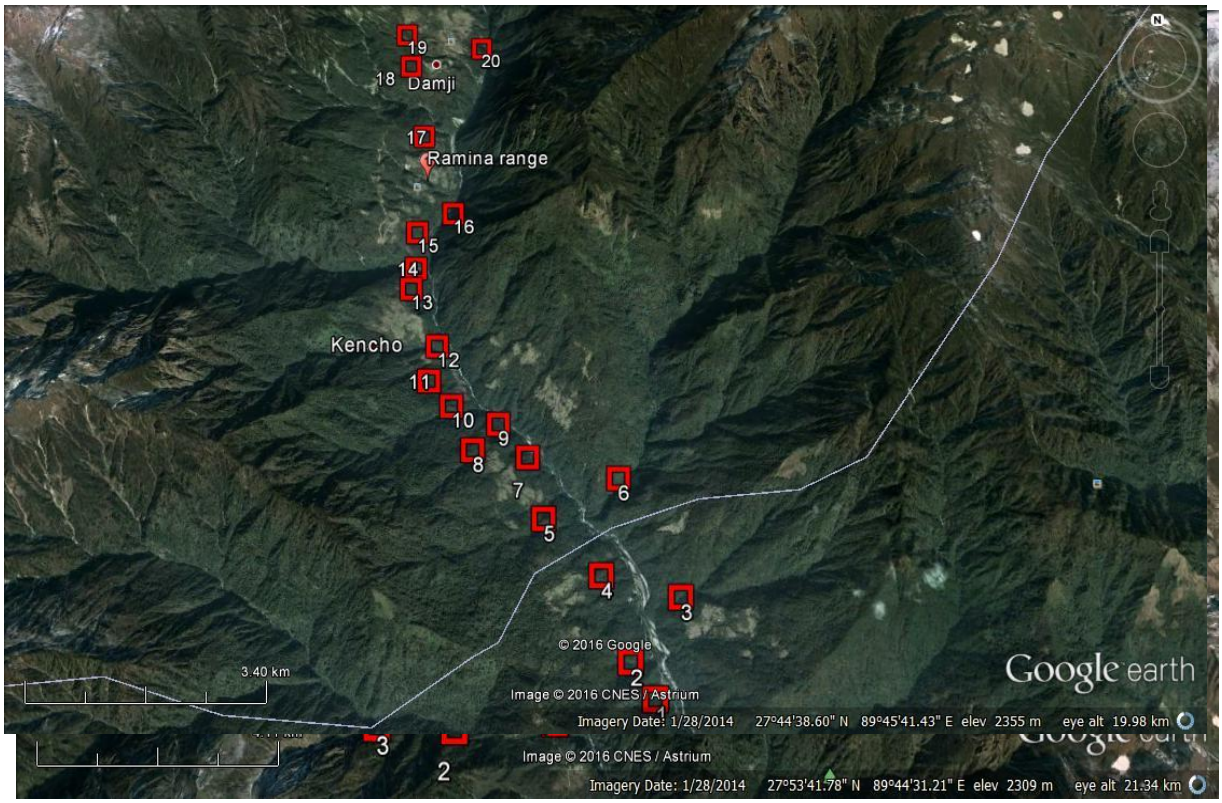
Figure 38 Leafy mistletoe *T. kaempferi* on *P. wallichiana* (Kirisitis)

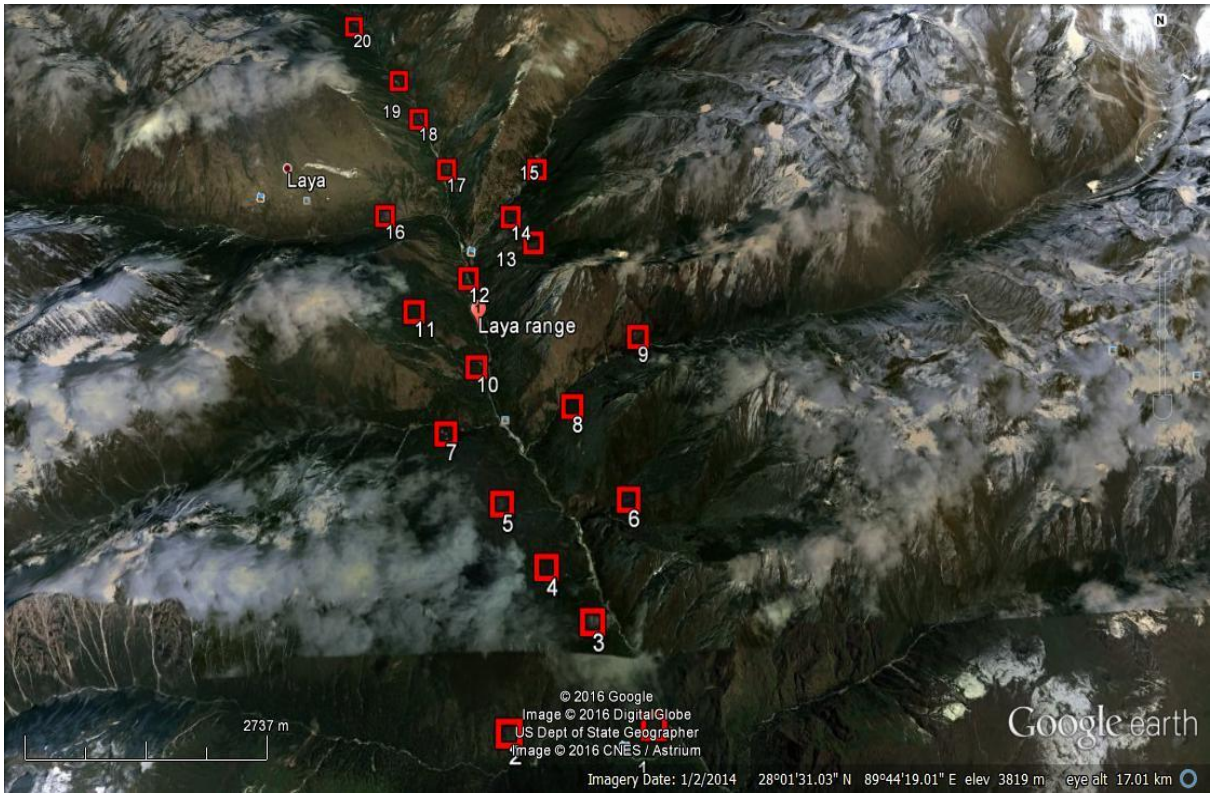
Figure 39 *A. minutissimum* on P. wallichiana: (A) Stem swelling as symptom of dwarf mistletoe infection and shoots of A. minutissimum, (B) Pistillate (Female) plants of A. minutissimum, (C) Female A. minutissimum at plants (Kirisitis)

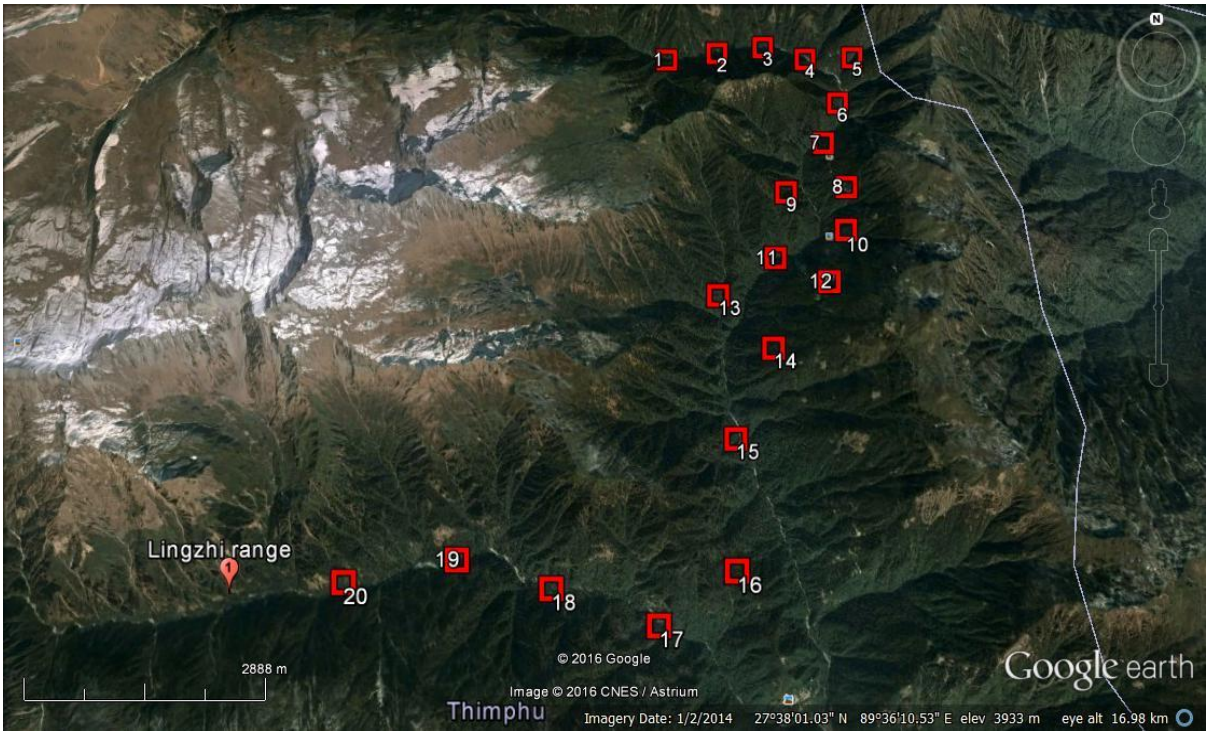
Figure 40 *A. sichuanense*

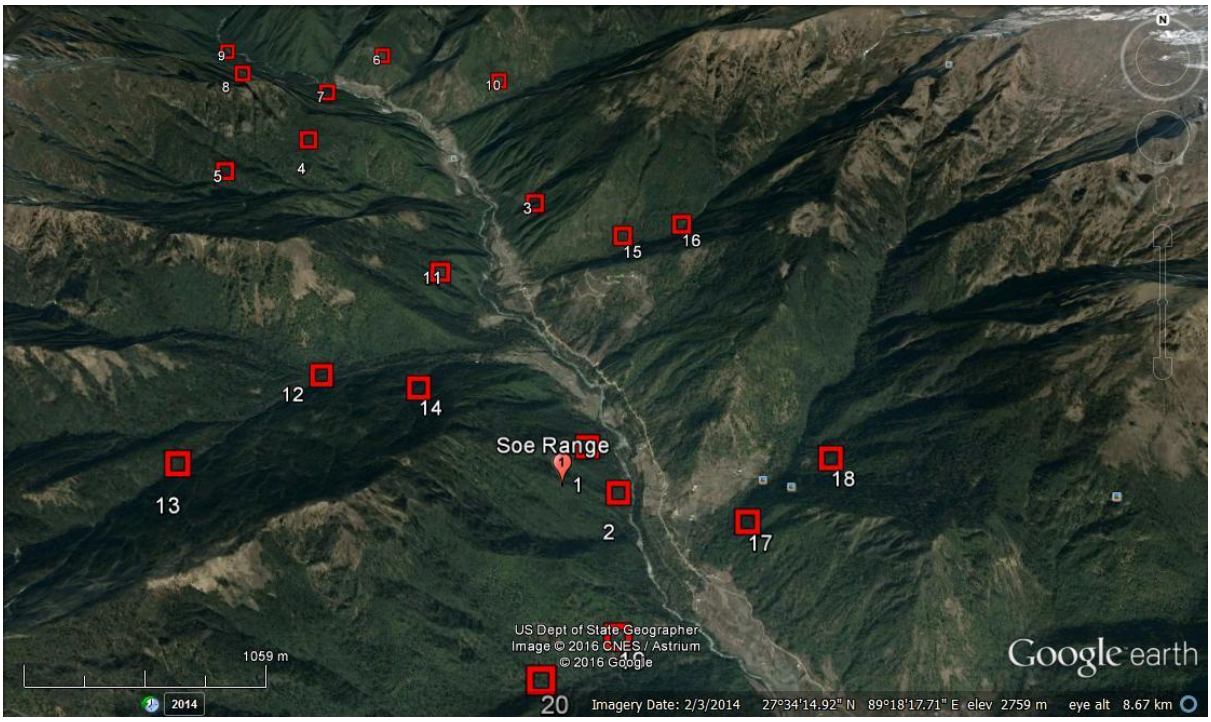
Figure 41 Fire as predisposing factor

Annexure 8 Sampling units in JDNP ranges






1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
Lingzhi range
2888 m
© 2016 Google
Image © 2016 CNES / Astrium
Thimphu
Imagery Date: 1/2/2014 27°38'01.03" N 89°36'10.53" E elev 3933 m eye alt 16.98 km
Google earth


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
20
Soe Range
1059 m
2014
US Dept of State Geographer
Image © 2016 CNES / Astrium
© 2016 Google
Imagery Date: 2/3/2014 27°34'14.92" N 89°18'17.71" E elev 2759 m eye alt 8.67 km
Google earth

Annexure 9 Disease abundance in each sampling unit

	Sampling units																			
Range	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	Total
Ramina	12	9	17	07	11	08	10	08	13	08	05	12	09	03	09	08	05	07	02	163
Gasa	07	12	02	09	12	03	22	23	02	12	10	09	09	12	09	09	08	09	02	181
Laya	08	11	01	02	05	15	12	09	12	08	09	11	12	09	05	21	23	23	12	208
Soe	12	23	07	12	10	13	07	12	05	12	02	11	06	12	12	34	12	12	22	236
Lingzhi	12	23	10	23	22	12	23	11	13	15	12	08	22	12	32	12	23	08	15	308
Total disease tree																				1096

Annexure 10 Pathogen Diversity calculation

1. ***Picea spinulosa***

Species	**Abundance**	**pi=n/N**	**pi2**	**ln p_i**	**p_i (ln p_i)**
Fomitopsis pinicula	27	0.230	0.052	-1.469	-0.337
Chrysomyxa woroninii	8	0.068	0.004	-2.688	-0.182
Ips schimutzenhoferi	56	0.478	0.228	-0.738	-0.352
Arceuthobium sichuanense	26	0.222	0.049	-1.505	-0.334
	117	D	0.83	H'=	1.205

Species Evenness: (H'/ln(S)): 0.871

2. ***Abies densa***

Species	Abundance	pi	pi2	ln p_i	p_i (ln p_i)
Formitopsis pinicula	93	0.263	0.069	-1.334	-0.351
Armillarea mella	39	0.110	0.012	-2.203	-0.243
Armillarea ostoyoe	53	0.150	0.022	-1.896	-0.285
Heterisodion annosum	35	0.099	0.009	-2.311	-0.229
ganoderma appalatum	46	0.130	0.016	-2.038	-0.266
Lirula nervisequia	15	0.042	0.001	-3.158	-0.134
Taxillus kaemferii	72	0.204	0.041	-1.590	-0.324
		D=	0.83	H'=	1.832

Species Evenness: (H'/ln(S)):0.941

3. ***Pinus roxburghii***

Species	Abundance	pi	pi2	ln p_i	p_i (ln p_i)
Phellinus pini	11	0.118	0.013	-2.135	-0.252
Coleosporium sp	3	0.032	0.001	-3.434	-0.111
Taxillus versicolor	8	0.086	0.007	-2.453	-0.211
Lhophoderma sp	5	0.054	0.002	-2.923	-0.157
Ips longifolia	23	0.247	0.061	-1.397	-0.346
Taxillus kampferii	27	0.290	0.084	-0.237	-0.359
Arcethobium minitissimum	16	0.172	0.029	-1.76	-0.303
		D	0.81	H'	1.720

Species Evenness: (H'/ln(S)):0.884

4. ***Pinus wallichiana***

Species	**Abundance**	**pi**	**pi2**	**ln p_i**	**p_i (ln p_i)**
R.undulata	12	0.068	0.004	-2.686	-0.183
cronarcium ribucola	26	0.148	0.021	-1.686	-0.283
D. septosporum	7	0.04	0.001	-3.225	-0.128
m. mesmazer	9	0.051	0.002	-2.973	-0.152
Ips schimutzenhoferi	44	0.25	0.062	-1.386	-0.347
Taxillus Kampferii	78	0.443	0.196	-0.914	-0.361
		D	0.72	H'	1.453

Species Evenness: (H'/ln(S)):0.811

1. ***Tsuga dumosa***

Species	**Abundance**	**pi**	**pi2**	**ln p_i**	**p_i (ln p_i)**
Formitopsis pinicula	154	0.562	0.315	-0.576	-0.324
Heterisodion annosum	68	0.248	0.061	-1.394	-0.346
Armillrea mella	52	0.19	0.036	-1.662	-0.315
		D	0.59	H’	0.985

Species Evenness: (H'/ln(S)):0.896

Annexure 11 Forest Disease severiy calculation

1		Total diseased *Tsuga dumosa* =274			
	N=721	Low	211	1*211	
		Medium	42	2*42	
		High	21	3*21	
			274		
			DSI=	358/721	0.496533

2		Total diseased *Pinus wallichiana* =176			
	Low		132	1*132	
N=397	Medium		32	2*32	
	High		12	3*12	
			176		
			DSI	232/397	0.584383

3		Total diseased *Abies densa* =353			
	N=876	Low	256	1*256	
		Medium	77	2*77	
		High	20	3*20	
			353		
			DSI	470/876	0.53653

4		**Total diseased *Pinus roxburghii* =103**		
N=296	Low	74	1*74	
	Medium	22	2*22	
	High	7	3*7	
		103		
		DSI	139/296	0.469595

5		**Total diseased *Jumiperus indica* =73**		
N=521	Low	52	1*52	
	Medium	12	2*12	
	High	6	3*6	
		70		
		DSI	94/521	0.180422

6		**Total diseased *Picea spinolosa*=117**		
N=631	Low	82	1*82	
	Medium	23	2*23	
	High	12	3*12	
		117		
		DSI	164/631	0.259905