CHARACTERIZATION OF SHISHAM (*Dalbergia sissoo*) AGAINST DIEBACK DISEASE IN VARIOUS ECOLOGICAL ZONES OF PUNJAB

By

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M.Sc (Hons.) Forestry

A thesis submitted in partial fulfillment of requirements for the degree of DOCTOR OF PHILOSOPHY IN FORESTRY

DEPARTMENT OF FORESTRY, RANGE MANAGEMENT AND WILDLIFE

FACULTY OF AGRICULTURE, UNIVERSITY OF AGRICULTURE, FAISALABAD-Pakistan 2013
To
The Controller of Examination,
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Faisalabad.

“We, the Supervisory committee, certify that the contents and form of thesis submitted by Irfan Ahmad 2000-ag-1091 have been found satisfactory and recommend that it be processed for evaluation by the External Examiner (s) for the award of degree”.

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In The Name Of
Allah
The Most Beneficent,
The Merciful
DECLARATION

I hereby declare that the contents of the thesis “Characterization of Shisham (Dalbergia sissoo) against dieback disease in various ecological zones of Punjab” are the product of my own research and no part has been copied from any published source (except the references, equations, formulas/protocols etc). I further declare that this work has not been submitted for awards of any other diploma/degree. The University may take action if the information provided is found inaccurate at any stage. (In case of any default the scholar will be proceeded against as per HEC plagiarism policy).

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Irfan Ahmad
Regd. No. 2000-ag-1091
This humble effort is
Dedicated
To

HOLY PROPHET HAZRAT MUHAMMAD
(P.B.U.H)

The ocean of Knowledge and
The greatest reformer,

To

My Beloved

MOTHER AND THE SACRED SOUL OF MY FATHER

Who taught me the first word to speak and the first alphabet to write
And the first step to take
ACKNOWLEDGEMENTS

All praises and thanks are for ALMIGHTY ALLAH. The compassionate, the merciful, the only creator of the universe and the source of all knowledge and wisdom who blessed me with health, thoughts, talented teachers, cooperative friends and family members. It is the one of his infinite blessings that he bestowed me with potential and ability to complete the present research program and to make a material contribution to deep oceans of knowledge already existing.

With trembling lips, wet eyes, all love I have in my heart and soul; with respect, I have in mind; I praise for the personality who is the cause of creation of this universe, the Holy Prophet Hazrat Muhammad (Sallallah-o-Allaih-e-Wasallam), the beacon of enlightenment, the fountain of knowledge, the messenger of peace, biggest benefactor of the mankind ever had and the best among all of ever born on the universe.

I deem it my utmost pleasure to avail an opportunity to express my heartiest gratitude and deep sense of obligation to my honorable supervisor Professor Dr. Rashid Ahmad Khan, Department of Forestry, University of Agriculture, Faisalabad, for his kind behaviour, generous transfer of knowledge, moral support and enlightened supervision during the whole study period. His valuable words will always serve as a beacon of light throughout my life.

I express my deep sense of gratitude to my Supervisory Committee Prof. Dr. Muhammad Tahir Siddiqui, Chairman Department of Forestry and Dr. Riaz Ahmad, Professor in the Department of Agronomy for their kind, sincere and unprecedented guidance.

My sincere thanks are for a very hardworking and caring person Dr. Shahbaz Talib Sahi, Professor and Chairman of the Department of Plant Pathology for his time to time constructive criticism and valuable suggestions to improve the thesis. I am thankful to Dr. Muhammad Atiq, Assistant professor in the Department of Plant Pathology for his highly appreciable practical and productive guidance and discussion throughout my research work.

It is my pleasure to thank Dr. Abdul Hanan, Assistant Land Reclamation Officer and Mr. Muhammad Kashif, Lecturer, Department of Mathematics and Statistics, university of Agriculture, Faisalabad for help in data analysis and assistance during the write up of thesis.

I would like to show my gratitude to my friends Mr. Wasi-ud –Din, Lecturer, Department of Agronomy, Dr. Abdul Jabbar, Assistant Professor Department of Agronomy, Dr. Tariq Aziz Assistant Professor Institute of Soil and Environmental Sciences, Dr. Abdul Hanan, Assistant Professor, Department of Plant Pathology, Dr. Tahseen Azhar, Lecturer, Department of Plants Breeding and Genetics, Dr. Muhammad Farrakh Nawaz, Assistant Professor Department of Forestry, Mr. Muhammad Asif, Lecturer Department of Forestry and Dr. Muhammad Ayyoub Tanvir, Lecturer Department of Forestry.
No acknowledge could ever adequately express my obligation to my affectionate parents, whose hands always raised in prayers for me. Although my dear father Ahmad Din Wahla is not with me but I hope his sacred soul will be very happy with my achievement.

I also express my thankful feelings for my wife for her cooperation, my sisters and my cousin Adil Sher Mohar for their prayers and cooperation.

May ALLAH bless all these people with long, happiness & peaceful lives (Ameen)

(IRFAN AHMAD)
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<td>Mean percentage of healthy trees in different zones of Punjab according to age classes</td>
<td>90</td>
</tr>
<tr>
<td>5</td>
<td>Mean percentage of partially affected trees in different zones of Punjab according to age classes</td>
<td>91</td>
</tr>
<tr>
<td>6</td>
<td>Percentage of fully affected trees in different zones of Punjab according to age classes</td>
<td>91</td>
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**ABSTRACT**

Shisham (*Dalbergia sissoo*) is an important tree of Pakistan and is widely grown in different areas of the country mainly for furniture, timber and fuel. Dieback is a serious threat to this tree and has caused huge damage not only in Pakistan but also in India, Nepal and Bangladesh. A nursery experiment of sexually (seedlings) and asexually (cuttings) propagated *D. sissoo* was conducted during 2009 and 2010. Both seedlings and cuttings were inoculated with the most commonly found fungi in the dieback affected trees i.e *Fusarium solani*, *Botryodiplodia theobromae*, *Curvularia lunata* and *Ganoderma lucidum*. Overall highest disease incidence was observed in plants inoculated with *F. solani* (31.39%). In seedlings *F. solani* caused 46.18% disease while in cuttings it was only 16.61%. No disease was recorded in controlled conditions. A significant (*P*<0.05) correlation of seedlings and cuttings was observed with climatic variables. Good association of seedlings (*r* = 0.734) and cuttings (*r* = 0.629) was observed with maximum temperature. Disease predictive models of seedlings and cuttings were developed with climatic variables;

\[
Y = -58.3 +7.58x_1+0.0054x_2-1.14x_3+2.47x_4-1.09x_5 \quad \text{R}^2= 0.62 \quad \text{(2 years data)}
\]

Where *Y* = Disease in cuttings, *x_1* = Rainfall, *x_2* = Relative humidity, *x_3* = Minimum temperature, *x_4* = Maximum temperature and *x_5* = Wind velocity

\[
Y = -134 +15x_1+0.158 x_2-2.32 x_3+5.27 x_4-3.70x_5 \quad \text{R}^2=0.48 \quad \text{(2 years data)}
\]

Where *Y* = Disease in seedlings, *x_1* = Rainfall, *x_2* = Relative humidity, *x_3* = Minimum temperature, *x_4* = Maximum temperature and *x_5* = Wind velocity

A comprehensive survey of shisham was carried out in four agro-ecological zones (Sandy deserts, Northern irrigated plains, Barani areas and Sulaiman piedmont) of Punjab province. For survey eleven districts were selected being the most productive and shisham frequenting districts from the above said zones. Trees were divided into age classes and on the basis of disease severity into healthy, partially affected and fully affected trees. Age class one (1-20 years old trees) was observed as the healthiest while age class three (above 40 years) was found to be the most affected in all zones and districts. Maximum number of healthy trees was observed in age class one of Barani areas (92.16%) Maximum number of partially affected trees was seen in Sulaiman piedmont (22.89%) and less number was in Barani areas. Maximum number of dead or fully affected trees was found in class three of Northern irrigated palins (23.60%) and only 4.68% dead trees were recorded in age class one of Barani areas. In selected districts maximum number of healthy trees was in class one of Rawalpindi district (95%) and maximum partially affected trees were in age class three in Dera Ghazi Khan (22.89%). Maximum number of dead trees (33%) was found in age class three of Toba Tek Singh. Rawalpindi was the least affected district with only 5 % dead trees in class one. Water table between 15-20 feet of depth was considered as the most suitable depth where less number of dead trees were recorded in all age classes. Significant (*P*<0.05) correlation was observed between age class, relative humidity, minimum temperature, maximum temperature and wind velocity. Correlation was non significant with rainfall and water table. Good association was observed between tree age and dieback disease in Healthy (*r* =0.626), partially affected (*r* =0.539) and fully affected trees (*r* =0.613).

A disease predictive model based on two years disease survey data was developed:

\[
FA = 2.51 + 4.15 x_1 + 5.25 x_2 + 0.00866 x_3 - 0.113 x_4 - 0.0611 x_5 - 0.0115 x_6 - 0.0092 x_7 + 0.541x_8 \quad \text{R}^2= 0.89
\]

Where *FA* = Fully affected, *x_1* = Age, *x_2* = Year, *x_3* = Water table, *x_4* = Rainfall, *x_5* = Relative humidity, *x_6* = Minimum temperature, *x_7* = Maximum temperature, *x_8* = Wind velocity
Shisham (*Dalbergia sissoo* Roxb. Ex DC) is native to Nepal and also found in Afghanistan, Bangladesh, Bhutan, India, Nepal and Pakistan (Tewari, 1994; Afzal *et al.*, 2006; Bajwa and Irum, 2006; Idrees *et al.*, 2006). This species is in the family Fabaceae (sub-family Papillionaceae) and genus *Dalbergia* (Southon, 1994), which contains 100 species in Asia, America and Australia (Thothathn, 1987). Historically records indicate that this tree was deliberately cultivated from approximately 2500 years ago (White, 1994; Kumar *et al.*, 2002); it is an internationally important species of the rosewood genus, having importance for fuel, shade and shelter wood. It is known locally in Pakistan as “tally”. The species is cultivated in tropical and sub-tropical regions of Africa, Asia, Kenya, Mauritius, Nigeria, Palestine, South Africa, Sri Lanka and Zimbabwe (Tewari, 1994; Afzal *et al.*, 2006). Being a multipurpose tree, shisham has considerable importance in the socioeconomic development of Southern Asia (Nawashadul, 1994; Puri and Verma., 1996; Lowry & Seebeck, 1997; Khan and Khan, 2000; Bajwa and Irum, 2006; Idrees *et al.*, 2006; Kausar *et al.*, 2009; Edward *et al.*, 2011). It is a moderately fast growing tree with an average growth of approximately 5 m in 3 years, 11 m in 5 years and 15 m in 10 years under favourable environmental conditions. Rotation lengths vary from 30 to 60 years. Flower colour varies from pale white to dull yellow, and on average, pods contain 1-4 seeds, with seed numbers varying from 45000-55000/kg (Orwa *et al.*, 2009).

Shisham grows in a range of soil types, from pure sandy to fertile soils of riparian belts, but prefers well drained sandy loam soils with good moisture holding conditions. Growth is very poor in poorly aerated and hard clay soils, where the pH range is 5 to 7.7. The tree tolerates temperature ranges from -4 to 45°C, with maximum altitude of 1500 m and mean annual rainfall of 500-1500 mm. Shisham can survive drought conditions for six months. It is a light demanding species, requiring full light from seedling to maturity. It is one of most important species of riparian zones or the bela (Riverian) forests, where sunlight and water are available (Orwa *et al.*, 2009). As a deciduous tree, the foliage is shed in November-December; new leaves appear in January and February (Khan and Khan, 2000).

*D. sissoo* was introduced to Pakistan approximately 150 years ago, and is now an important timber tree (Bajwa and Irum, 2006; Afzal *et al.*, 2006). It is commonly found in the foothills of the Himalayan mountains. Although it is found extending from...
the Indus valley to the Attock district, it does not dominate in this area (Champion et al., 1965). In Pakistan, it is planted widely along canal banks, along agricultural field boundaries, as well as on road sides, in canopy gaps, in disturbed sites and in forest margins (Duke, 1983; Puri & Bangarwa, 1992; Puri et al., 1992; Zakaullah, 1999; Sharma et al., 2000; Gill et al., 2001; Langeland and Stocker, 2001; Rajput et al., 2008; Kausar et al., 2009).

The timber of *D. sissoo* is highly durable with excellent finishing colours and smoothness. It is used for veneer, furniture, cabinet, paneling, carving, small timber, plywood, musical instruments, and paper. Moreover, the tree is used in the treatment of variety of conditions and diseases (Singh, 1982; Sheikh, 1989; Lowry & Seebeck, 1997; Idrees et al., 2006), including skin and blood problems, syphilis, stomach problems, dysentery, nausea, eye and nose disorders, as an expectorant and as an aphrodisiac (Nadkarni, 1954; Duke, 1983; Hajare et al., 2000; Hajare et al., 2001; Sharma et al., 2001).

The tree is also used for fuel, shade, shelter, soil stabilization and for the control of erosion and is considered an important tree for agro-forestry practices, due to its nitrogen-fixing properties (Duke, 1983; NAS, 1983; Stewart & Flinn, 1984; Sheikh, 1989; Zabala, 1990; Gupta et al., 1995; Karpiscak et al., 1996; Sharma et al., 2000; Singh & Bhati, 2004; Sangha & Jolta, 2005; Kausar et al., 2009; Lal & Singh, 2012).

In the recent past, this valuable tree has been subject to two diseases: dieback and wilting (Khan, 1989; Baksha & Basak, 2000, Sharma et al., 2000, Joshi & Baral, 2000; Naz, 2002; Bajwa et al., 2003). In 1998, dieback appeared as an epidemic in the central irrigated tracts of Punjab, Pakistan (Naz, 2002). During this period approximately 70% of shisham trees were affected by dieback (Khan, 1999). This disease has been recorded in the different agro-ecological zones of Punjab with varying levels of severity (Bajwa and Irum, 2006).

Dieback disease has more specialized symptoms than wilt. Reductions in numbers of leaves lead to thinning of the crown. Shoots tips and twigs desiccate and the whole crown turns yellow (Bakshi, 1974). The infected trees develop sunken areas at the level of the root collar and the disease is characterized by a red-brown discoloration of the vascular cambium in this region, extending from below the root to 1.0 – 1.3 metres or more above ground level. Underneath the bark, dark lines occur, extending vertically up and down from the depressed tissues. The dark lines extend into the wood. In addition,
the feeder roots become discolored and unable to regenerate, leading ultimately to death of affected trees (Bakshi, 1974; Zakaullah, 1999; Bajwa and Javaid, 2007).

The true cause of shisham dieback remains uncertain and controversial (Javaid et al., 2003). Many biotic and abiotic factors have been suggested as responsible for the dieback of *D. sissoo* (Sharma et al., 2000). Climate change may have adversely affected physiology and growth of the trees. Extreme and rapid fluctuations of temperatures in summer and winter seasons have probably disturbed the physiological processes of the plants, with drought conditions further exacerbating the situation (Chaudhry, 2006). Entomological, pathological, silvicultural, soil and age related factors have each considered as important in the development and spread of dieback (Karki et al., 2000; Sah et al., 2003). According to Rehmatullah et al. (2006), there was no association between dieback disease and differing soil properties. It has also been confirmed that insects have no significant role in the development of the disease (Gul, 2004). Pathology, therefore, is considered the major factor in the development and spread of the disease, and many fungi, fungus-like organisms and bacteria, including *Botryodiplodia theobromae*, *Fusarium solani*, *Fusarium oxysporum*, *Fusarium dimarium*, *Fusarium semitectum*, *Pestalotia* spp., *Curvularia* spp., *Drechslera* spp., *Chaetomium* spp., *Cystospora* spp., *Colletotrichum gloeosporioides*, *Ganoderma* spp., *Phialophora* spp., *Phialocephala* spp., *Dothiorella* spp., *Phytophthora cinnamomi* (Oomycota), and *Xanthomonas* spp. (bacteria), have been isolated from tissues of diseased *D. sissoo* trees (Idrees et al., 2006). Pathogenecity tests with most of the commonly found fungi have been conducted on seedlings of *D. sissoo* (Idrees et al., 2006; Rajput et al., 2010). However, no such study has been carried out on asexually propagated plants. Controversy over the causal organism still prevails; therefore, intensive research efforts are required to investigate the problem and to help formulate precautionary and remedial measures to save this important timber species from severe damage and possible extinction.

The effective use of chemical agents against plant diseases requires knowledge of the epidemiology of disease. Understanding disease dynamics, including the pathogen life cycle, provides the basis for forecasting disease outbreaks. Because shisham decline massively reduces both the economic gains and the social uses of this tree, the aim of integrated disease management must be to reduce these losses. Approaches are required which aim to avoid frequent recurrence of shisham decline epidemics. Much research, therefore, remains to be done. Disease severity in relation to different age classes of shisham has not been assessed, and no correlation has been determined between disease
occurrence and the water table. Hence, the epidemiology of shisham dieback is poorly known. The impact of varying environmental conditions in relation to inoculum build up and spread of disease have not been studied quantitatively.

Shisham germplasm needs to be screened for relative susceptibility to dieback, and promising provenances and genotypes propagated through cuttings and seedlings to assess inoculum potential to obtain meaningful conclusions. In the absence of disease resistant varieties, tolerance may prove a useful phenomenon to use.

Based on these facts and observations, the present study was designed with the following objectives:

- to survey of shisham plantations to determine the severity and extent of damage in different agro-ecological zones of Punjab in Pakistan;
- to develop a technique for the identification of diseased trees of *Dalbergia sissoo* on the basis of different morphological characteristics of trees;
- to evaluate sexual and asexual propagated material of *Dalbergia sissoo* for relative susceptibility to die back disease by inoculating with different fungi isolated from mature trees suffering from the disease;
- to determine the environmental conditions conducive to the development of shisham dieback in the field; and
- using the data gathered in the objectives listed above, to develop a model for the prediction of disease.
2.1 Dieback and decline of forest trees

Dieback and decline are periodic events characterized by premature loss of tree health and stand vitality (Clatterbuck, 2006). The dieback and ultimate mortality of trees may be caused by a number of factors which are difficult to assess, but mostly are caused by human disturbances, especially to the environment (Lowman, 1991). Different terms such as dieback, stand level dieback and canopy level dieback have been used in the literature, depending on conditions. It is often very difficult to determine and diagnose the exact cause of dieback or decline, as compared to other diseases, because it is a complex issue and may differ from region to region (Manion, 1991; Platt & Statewide, 1999). The problem is caused by a number of interacting factors such as mechanical injury by wind, frost, drought, or attack by a destructive pathogen or insect, but without any particularly definable causal organism or factor. The damaging factor(s) ultimately disturbs the physiological functions of twigs and branches of trees sufficiently to initiate dieback, which may lead to death of entire plant (Ciesla & Donaubauer, 1994). Dieback has been considered a diverse disease of forests by many forest pathologists. In this situation, trees are affected by different environmental factors and finally tree tissues are invaded by pathogens, which lead to the death of tree (Housten, 1967, 1992; Manion, 1991).

Both winter and summer dieback has been observed. Winter dieback starts after a severe cold season with frost and then rapid rise in temperature, which causes wilting of branch tips. Symptoms progress from top to down, whereas in summer dieback, the problem begins after the end of warm season and at the start of cold season with symptoms progressing from bottom to top (South et al., 2002).

In dieback, the loss of trees often occurs in groups or whole stands, rather than as isolated individual trees. Death caused by forest fire or by flooding is not considered as dieback (Muller, 1986; Wylie et al., 1993).

2.2 History and Geographical distribution of forest dieback

No part of the world that has any tree cover is free from dieback as it is prevalent throughout the world, from the Sahara desert, the forests of Africa, Europe, Australia, to North, Central and South America have been subjected to different types of dieback (Lowman, 1991). Dieback is generally considered to be associated with geography, site quality and vegetation types (Jurskis, 2004a,b,c,d). Some reports suggested that, in recent decades, mortality rates have increased in the temperate and boreal forests of western
North America; in these varied regions, approximately 10 million hectares of different
forest types have been affected by dieback since 1997 (Breshears et al., 2005; Raffa et al.,
2008; Van Mantgem et al., 2009). In the early 20th century, stand level forest dieback was
reported in several parts of North America and lead to significantly increased mortality of
white pine (Pinus monticola), southern pines (P. echinata, P. taeda), birch (Betula spp.),
oak (Quercus spp.) and sugar maple (Acer saccharum) (Mueller-Dombois et al., 1983). In
the first half of the 20th century, few incidents of dieback in sugar maple were recorded
but later, dieback episodes became frequent (Marsden, 1950; Houston, 1998). Lowman
(1991) first reported dieback on chestnut (Castanea sativa) in America. Widespread
dieback of balsam fir (Abies balsamea) was reported in the mountain regions by Sprugeal
(1976).

At present, severe forest dieback events have been recorded in many of the forest
ecotypes of New Zealand, the smaller islands of Australasia, and the New England
tablelands in New South Wales, Australia (Ciesla, 1994). In early 1900, extensive dieback
was noted on several commercially important tree species like Eucalyptus and Acacia in
northeastern Australia due to severe drought (Fensham and Holman, 1999). Dieback of
jarrah (Eucalyptus marginata) in Western Australia was reported in the 1920s (Weste &
Marks, 1987). Three diverse types of diebacks were also reported from Tasmania (Felton,
Zealand (Grant, 1984; Hosking, 1989) and its widespread attack on Metrosideros spp. in
forest stands was considered a major threat to ecosystem sustainability (Batcheler, 1983).
Mangrove, Eucalyptus deglupta and Nothofagus spp. forests were also reported to be
showing symptoms of severe dieback in Papua New Guinea (Arentz, 1988; Ash, 1988).

The history of dieback attack in European countries dates back to the start of the
18th century (Ciesla, 1994); one of the first described declines is Tannesterban during
1810 (Ruzicka, 1937); a similar problem occurred in the 1970s and 1980s, which was
more severe than the previous (Kandler, 1993). Oak (Quercus spp.) are also affected in
many parts of Europe (Fuhrer, 1998) and Q. robur and Q. petraea species were the most
affected species; Marcu, 1966). Hartman and Blank (1992) suggested that oak dieback
episodes had occurred in Germany during 1739-1748, 1748-1911, 1929-1939 and 1939-
1944, prior to the 1970s/80s episode. Higher scale mortality of the different species of
beech (Fagus sylvatica), pine, spruce, fir and oaks in France caused great damage to
forest stands (Breda et al., 2006; Landmann et al., 2006; Vennetier et al., 2007). Fagus
sylvatica dieback was also reported by Ciesla (1994) in France, Germany, Switzerland
and Poland, and there are many reports of the problem on temperate conifers in Switzerland, Italy, Austria and Norway (Kienast et al., 1981; Minerbi, 1993; Cech & Tomiczek, 1996; Solberg, 2004). Deaths of temperate broadleaves of France (1980-1985), Poland (1979-1987) and Germany (2003-2006) were considered serious losses to forest stands in Europe (Negelelesien, 1994; Siwecki & Ufnalksi, 1998; Petercord, 2008).

Widespread dieback has also been reported in different African countries, including Benin, Botswana, Gambia, Sudan, South Africa, Tanzania, Uganda, Zambia and Zimbabwe were also reported by Ciesla (1994). Dieback of *Azadirachta indica* in Cameroon, Chad, Mali and Nigeria and *Acacia nilotica* in Sudan and adjoining countries was also recorded (Ei Atta, 1988; Boa, 1992), whereas Lawanga (2003) indicated similar occurrences in the tropical rain forests in Uganda.

Episodes of forest dieback along with its resultant decline have been reported in different parts of Asia (Allen, et al., 2010). Serious dieback cases in Asia included the mortality of *Cryptomeria japonica* in Japan during 1980s (Morikawa, et al., 1990), montane tropical rainforest of Sri Lanka from 1976-1980 (Werner, 1988), tropical rainforests of Malaysia, Indonesia (Woods, 1989; Kinnaird and O’Brien, 1998), tropical dry deciduous forests of the Indian Gujrat in 1987 (Khan, et al., 1994), subtropical coniferous and temperate coniferous plantations in China (Li, 2003; Wang et al., 2007), the boreal and temperate forests of eastern Russia during 2005-08 (Ermolenko, 2008) and the high death rate of temperate forests of South Korea during 2003-08 (Lim et al., 2008). Two decades ago, Ciesla (1994) also reported dieback in different parts of Pakistan.

### 2.3 Distribution of *Dalbergia sissoo* dieback

Dieback of forest trees is a major problem in the different countries of the Indian subcontinent (Shukla, 2002). *D. sissoo*, an important species of the subcontinent, suffers from dieback, wilt and many other pathological problems (Sah et al., 2003). In the last 20 or more years, dieback of *D. sissoo* has become a serious threat, affecting millions of trees in Southern Asia (Vogel, et al., 2011). The history of *D. sissoo* dieback in Pakistan goes back to the early 1900s, but regular research on this very important problem was first carried out in 1956 in an irrigated plantation in Khanewal (Khan, 1989). *D. sissoo* mortality has been observed frequently in the different regions of Pakistan, but unfortunately the causes are still not clear (Gill and Aziz, 2004). In India, *D. sissoo* dieback was first recorded in the forest stands of Utter Pardesh (Bakhshi, 1954). Half a century later, large scale mortality was recorded in 1998-2002 due to various pathogens and insects under different abiotic stresses in both natural stands and man-made
plantsations of the Bihar region (Chaturvedi et al., 2002). During the same period similar cases were reported in various other parts of Pakistan, which in 1998 was assumed to be an epidemic disease in Pakistan and other shisham growing countries (Afzal et al., 2006). The mortality rate raised from 5% to 25% from 1991 to 2000 (Gill et al., 2001). The disease also spread out in linear, compact and farmland shisham plantations in Punjab, Pakistan. At the same time, it became a threat for producers in the native range in Nepal and other neighbouring countries (Idrees et al., 2006) where it caused severe losses in natural forests and large private plantations (Baral, 1995 and Baral et al., 1997). Severe mortality of *D. sissoo* was also reported from other provinces of Pakistan including Sindh (Rajput et al., 2010), Punjab (Bajwa et al., 2003; Khan et al., 2004) and Khyber Pakhtunkhwa (Chaudhary and Ejaz, 2003). Muhlbach et al. (2009) reported that this tree was also under severe threat in Bangladesh, India, Nepal and Afghanistan.

Since 1993 mortality due to dieback in vast areas of Bangladesh was also observed and about two million shisham trees were found affected in the last seven years (Anon, 2002) whereas Basak et al., (2003) pointed out that 43% of the shisham trees died only due to dieback and even in some cases 100% death rate was also recorded (Mridha et al., 2003).

2.4 Symptomology

Heartwole and Lowman (1986) described the prominent symptoms of dieback. They stated that several stages are involved in a complete tree dieback which starts from a full healthy tree, then desiccation of foliage and twigs starts. Most of the smaller branches dry out, but some may remain healthy near ground, prior to ultimate death of the whole tree. A change in foliage colour and wilting of the crown are also symptoms of the disease (Tantau et al., 2005). Baksha and Basak (2000) and Sharma et al. (2000) added two further stages specific to *D. sissoo* dieback. Firstly, the foliage becomes yellow and dies; secondly, the foliage is shed, the trees wilt and finally the die within few months. These symptoms were confirmed by Kumar and Rai (2002) and Kumar et al. (2002). Khan (2000) and Muller-Dombois (1983, 1986) added further details, demonstrating that dieback started from the shoot tips, progressed downwards and in the final stages crowns were flat and stag headed; as the desiccation of twigs continued, the tree lost smaller branches.

In some trees, some branches remained green due to the presence of small amounts of live roots, leading to re-sprouting once the physiological stress passed. But the newly flushed foliage on these young branches were irregular, smaller in size and pale
in colour compared to foliage on unaffected trees. At the same time, the outer sapwood developed a pink to reddish stain, but there was no appearance of discolouration in heartwood (Zakauallah, 1999). In some trees, epicormic shoots developed from the main stem (Lowman, 1991).

2.5 Etiology

The precise cause of dieback on *D. sissoo* remains unclear and controversial (Bakhshi, 1974); many different factors both biotic and abiotic, have been associated with forest and shisham dieback (Sharma et al., 2000; Basak et al., 2003). Aslam (2004) stated that the intensity and causes of dieback varied region to region in Pakistan. Forestry experts and pathologists have suggested a number of possible causes of the high death rate of *D. sissoo*, including pathological, entomological, silvicultural, edaphic and age factors (Karki et al., 2000; Sah et al., 2003). Insects, bacteria and fungi are associated with trees developing dieback but the exact cause has not been identified to date. The lack of a clear cause is also responsible for the many contradictions regarding the etiology of the syndrome (Muhlbach et al., 2009). One major contributing factor in shisham dieback may be changes in land use patterns, resulting from different engineering practices, compaction of upper soil horizons, water logging, irregular irrigation, fertilizer and pesticide application. All these factors can contribute to attack by *Fusarium* and *Ganoderma* (Solanki, 2002). Afzal et al., (2006) identified the two major causes of *D. sissoo* dieback: according to them the primary cause was physiological drought, lopping and over maturity, whereas the second was attack by pathogens. Physiological problems and edaphic factors like soil and irrigation were considered many years ago as basic reasons for high scale mortality (Khan et al., 1965). Poor tree growth was associated with high water tables and shallow rooting of plants (Adams et al., 1972). But qualities such as soil texture, structure, pH, and organic matter did not directly cause dieback of *D. sissoo* (Webb et al., 2005) and there was no relationship between different soil physiological properties, plant nutrient status and dieback incidence (Rahmatullah et el., 2006).

Entomologists reported that different insects, such as pinhole larvae and beetles, caused the desiccation of the top portions of the trees (FORESC, 1997) but later, Gul (2004) found no role for insects in *D. sissoo* dieback.

A wide range of fungi have been reported from shisham trees showing symptoms of dieback, leading to the involvement of pathogens being considered the most likely cause of the problem (Bakshi, 1954). Fungi and fungus-like organisms isolated from dieback affected *D. sissoo* trees include *Botryodiplodia theobromae*, *Fusarium solani*, *F.*
oxysporum, F. dimarium, F. semitectum, Pestalotia spp., Curvularia spp., Drechserla spp., Chaetomium spp., Cystospora spp., Colletotrichum gloeosporioides, Ganoderma spp., Phialophora spp., Phialocephala spp., Dothiorella spp. and the Oomycota species Phytophthora cinnamomi (Dargan et al., 2002). Bacteria in the genus Xanthomonas spp. and nematodes have also been recovered from diseased tissue samples, but B. theobromae and F. solani appear to be common to most samples collected from all locations and from all parts of the trees (Idrees et al., 2006). B. theobromae, P. cinnamomi, F. solani, G. lucidum, Rhizoctonia solani, Alternaria alternata, Curvularia lunata, Aspergillus flavus, A. niger and Colletotrichum gloeosporioides were isolated from samples of affected D. sissoo collected from different areas of Punjab (Rehman et al., 2006). F. solani and G. lucidum were identified as the most common fungi in dieback-affected D. sissoo trees (Bakshi, 1954; Parajuli et al., 1999). Vigayan and Rehill (1990) reported Aspergillus flavus, Aspergillus niger, F. oxysporum and F. solani as causal organisms of D. sissoo dieback. Only F. solani, however, was identified as the major causal organism responsible for the dieback of D. sissoo (Bakshi, 1954; Shailendra et al., 2004; Bajwa and Irum, 2006; Rajput et al., 2008; Rajup et al., 2010).

### 2.6 Dieback and tree age

Age is considered an important factor in development of forest dieback (Manion, 1991). Nepstad et al. (2008) reported that as trees are perennial woody plants which grow slowly but the mortality rate of trees was very much higher, even within a period of few months as compare to its growth rate which is very interesting. Older trees were more susceptible to dieback compared with younger trees (Auclair et al., 2010). Sperry et al. (1991) suggested that in older trees, the membranes associated with vessels and tracheids rupture easily under ecological stresses. Baksha and Basak (2000) and Sharma et al. (2000) agreed with this conclusion, because no disease was recorded at nursery stage but mature trees were very much affected. Acharya and Subedi (2000) recorded mortality of over-mature dieback-affected D. sissoo trees in a short period of even few weeks. Along with tree age, certain species of fungi, such as F. solani and G. lucidum, killed over 20% of older trees of D. sissoo under abiotic stresses in Bihar (Chaturvedi, 2002). The same phenomenon has been reported in peach: low disease incidence was recorded in younger compared to older peach trees, with a significant linear regression between tree age and disease (Purcell et al., 1981).

Top dieback has been observed in mature and over-aged trees of Cryptomeria japonica in Japan (Morikawa et al., 1990). Pegg et al. (1980) also reported that in the
mangroves of Central coastal Queensland, large scale dieback occurred in larger trees of *Avicennia marina*. Similar findings were discussed by Jursiks and Turner (2002) who stated that in the declining forest stands of *Eucalyptus sieberi* in Australia, saplings were much healthier than mature trees. Older trees were killed by rising water tables, indicating that young trees were in a better position to adapt to the changing soil conditions when compared to older trees. The rapid growth rate of the roots of younger trees and greater ability to adapt to changing environmental conditions resulted in lower rates of mortality rate (Manion, 1991; Mueller-Dombois, 1993; Jurskis and Turner, 2002; Bi, 2004). Findings of Hosking and Hutcheson (1992) were also in line when they stated that dieback of *Cordyline australis* was associated with old age of trees and attack of pathogens.

Oak decline in central Europe was much more severe in forest blocks of greater than sixty years in age, whereas disease rates in stands below that age were not so alarming (Wulf and Kehr, 1996). Similarly, in the USA, oak and sugar maple decline was also in trees of over seventy years old (Oak, 1996; Houston, 1998). At the start of 21st century, Bentouati (2008) recorded dieback in all age classes of Cedar in Algeria, but he found that damage was due to drought. Most of scientists observed much more mortality in larger and older trees due to drought (Mueller et al., 2005; Nepstad et al., 2007; Floyd et al., 2009).

### 2.7 Climatic Change and tree dieback

Three basic theories have been presented for the causes of forest tree decline including climate change, fungal pathogens and nutrient deficiencies (Simpson, 1993). Climatic change has occurred since earth developed an atmosphere; these changes disturbed the existence and distribution of flora and fauna (IPCC, 1990).

Environmental stresses contribute to tree mortality and occurrence of forest diseases due to different pathogens will probably become more common and severe in the near future, as different biotic and abiotic factors are strongly influenced by climatic change (Sturrock et al., 2011). The rapidly changing environmental conditions influence the behaviour of the pathogens, the hosts and the host-pathogen interactions. Ultimately, therefore, changes will affect disease severity, biological activities of trees and pathogens which are marked due to fluctuations in temperature and humidity levels (Boland et al., 2004; Sturrock, 2007; Dukes et al., 2009; Tubby and Webber, 2010). Rising temperature is not considered a primary cause of forest dieback in hardwood forests (Auclair et al., 2005). However, short term climatic change in various parts of the world have caused
devastating forest diebacks, and disturbed the ecosystems. In the future, rapid climatic change is expected, which did not commonly occur in the past; such unprecedented changes will disturb and alter the geographic distribution of trees (Allen and Breshears 1998; IPCC, 2001). Dieback in northern hardwood forests due to low temperature was strongly linked with the global climatic change: the coefficient of determination value ($R^2 = 0.49$) in the regression analysis showed that climatic changes was responsible for 49% of the dieback occurring in northern hardwood forests (Auclair et al., 2005).

Seasonal droughts have intensified tree mortality rates in the rain forests of Costa Rica (Chazdon et al., 2005), Panama (Condit et al., 1995) and different regions of Brazil (Williamson et al., 2000; Rolim et al., 2005). Hot and severe drought spells in the Amazon basin and high surface temperatures have been linked with increasing tree mortality and loss of above ground biomass. In these conditions, Amazonian forests would become more vulnerable to moisture stress (Phillips et al., 2009). An increasing trend in the forest mortality and dieback due to drought was observed. In 1984 only 1% tree mortality was linked with drought, whereas by 2010 the figure was 4%; with an $R^2$ value of 0.61, the linear regression model suggested a very strong association between tree mortality and drought conditions due to low rainfall and high temperature (Phillips et al., 2009; Allen et al., 2010). The extent of forest dieback linked with global climate change would be greater than that observed in the past (National Research Council, 2001).

Maximum temperatures in the natural habitat of shisham range from 39 to 49 °C and minimum temperature from -3 to 6 °C. Rainfall in the natural range is from 750 to 5000 mm per annum. Based on analysis using the 1995-2000 meteorological data, unfavourable precipitation and sunshine hours in Amritsar and Bathinda were probably correlated with dieback and mortality of shisham, as monthly rainfall in Amritsar and Bathinda during that period ranged between 0-430 mm, far lower than the minimum requirements for the tree, leading to disturbances in plant growth (Kaushal et al., 2002). An increase in fog periods, a long dry spell, low soil temperatures and extremes of summer and winter seasons also increased the rate of dieback in the trees, which ultimately died over two years. Rapid changes in soil temperature especially if during winter, soil temperature was below freezing in the absence of moisture, these frosts retarded growth of shisham. Similarly, crown dieback was observed in the warm dry years (Negi, 2002; Sidhu et al., 2002; Chaudhry, 2006).
High levels of shisham mortality in India were linked with changing climatic conditions, such as increased drought, extremes of winter and summer, with severe foggy conditions which adversely affected photosynthesis and other physiological processes of the trees (Singh, 1980; Kaushal et al., 2002). For example, in Solan and Kunihar forest Divisions heavy frost was recorded in shisham affected areas (Kumar et al., 2002). Environmental, physiological, edaphic and water stresses resulted in large scale dieback of shisham. Moreover, young trees in farmlands were less affected than older trees in plantations, and similar conditions were observed in canal side plantations; the pathogen responsible for dieback in different agro-ecological zones appeared to be resistant to these fluctuating environmental and edaphic conditions (Jahan, S and Qureshi, 2006; Bajwa and Mukhtar, 2006).

These intensive and rapid changes in weather conditions were considered important in causing dieback of hardwoods (Auclair et al., 1990; 1992; Auclair, 1992, 1993 a, b; Pomerleau, 1991). Global warming is recognized as a serious threat to dominant forest trees in many regions. Drastic fluctuations in temperature can injure trees by disturbing the physiological processes. Freezing temperatures disturb water transport system of plants and kill roots. Similarly heat stresses and soil moisture deficiency due to low summer precipitation and high air temperature lead to drought; crown dieback of dominant forest trees is a common response to drought stresses with additional injuries resulting from low temperatures. Trees affected by these environmental extremes subsequently also had to face pathological and entomological problems (Greenidge, 1951; Soloman, 1986; Auclair, 1992; King and Nelson, 1992; Auclair et al. 2005).

2.8 Propagation methods and shisham dieback:

Dieback is considered the major factor in reducing production of shisham; the high mortality of seeds and seedlings has intensified the situation (Khan and Khan, 2000). The pathogens responsible for dieback and decline of shisham can colonize the embryo through the seed coat during storage, reducing viability of the seeds, and were considered responsible for the high scale mortality of shisham (Bhansli and Jindal, 1997).

Vegetative propagation is a method enabling rapid multiplication of desired genetic material, capturing the genetic characteristics of the mother plant (Chaperson et al., 1983). Asexual propagation of disease free plants of D. sissoo showed promising results; cuttings planted in spring and monsoon seasons produced roots but if taken in winter did not root at all (Puri and Verma, 1996). Singh (1982) stated that natural
regeneration of shisham had not been common and sexual propagation was the most commonly used method to obtain plants.

Despite being the most commonly used method, propagation of *D. sissoo* by seed can be unreliable due to poor germination and high mortality of young seedlings under natural conditions. It was considered essential, therefore, that superior clonal material be vegetatively multiplied to increase productivity (Afzal, 2004). Obtaining true to type and genetically superior plants within a short period of four months makes vegetative propagation advantageous compared to waiting for one year or more in raising plants from the seeds (Libby, 1974; Foster *et al.*, 1984; Puri and Shamet, 1988; Puri and Thompson, 1989; Afzal *et al.*, 2006). Moreover, the use of asexual or vegetative propagation from healthy *D. sissoo* could avoid dieback through the production of genetically resistant plants (Singh *et al.*, 2011). *F. solani* is frequently isolated from sexually propagated plants of *D. sissoo* (Manandhar and Shrestha, 2000). Jahan and Quraishi (2006) and Idrees *et al.* (2006) recommended that diseased plants found along canals and roads should be removed and replaced with vegetatively propagated, disease free saplings, taking advantage of the rapid growth rates and excellent survival of such plants.

PFRI (2004) reported the vegetative propagation of shisham using branch cuttings of dieback free plus trees. Saplings became ready to plant out within a period of only four months as compared to sexually propagated plants (seedlings), which can take over one year. Moreover, no dieback symptoms were observed on these plants after a period of five years growth at PFRI.

### 2.9 Surveys for shisham dieback disease

Shisham faces wilting and dieback problems in many areas of Punjab, which were not common before (Bajwa *et al.*, 2003). Road side surveys of 535 *Dalbergia sissoo* trees in Peshawar indicated that only 16% trees were healthy, crowns of over 20% of trees were dried, approximately 30% showed severe dieback, canopies of 20% of the trees were thinning and 14% were completely dead (Chaudhary and Ejaz, 2003). In a survey on losses of shisham to dieback in the irrigated plantations of Bhagat, Punjab, 31% financial losses were recorded. Total loss during last 20 years was more than 28% due solely to dieback (Khan and Bokhari, 1970). Later, Khan *et al.* (2004) estimated economic losses of 35 – 40% in Sahiwal and Gujranwala, 25-30% in Faisalabad, Sheikhupura, Kasur, T.T. Singh, Sargodha, Jhang and Norowal, 20.5% in Hafizabad and 24.7% in Vehari. Pathan *et al.* (2007) surveyed different areas in Sindh province and
found disease incidence up to 74% in Ghotki, 66.8% in Pano Akil, 54% in Mirpur Mathelo, 51.2% in Daharki, 38 – 43% in Hyderabad, 20% in Dadu, 32% in Tandojam and 24.80% in Hala. *Fusarium solani, Rhizoctonia solani* and *Curvularia lunata* were isolated from affected trees.

Trees affected by dieback in Gujranwala, Lahore, Jehlum, Rawalpindi, Khoshab, Bahawalpur, Multan, Sargodha and Sialkot (Pakistan) were surveyed and soil pH, water table and soil moisture determined. Maximum mortality (80%) was observed along the canals, reflecting high soil moisture as one a contributing factor. Moreover 30% affected trees occurred along roadsides and highways, whereas 10 % of trees in Agricultural and well managed lands were affected. Disease incidence was also high in dry areas. Furthermore, it was reported that older trees were more susceptible to disease, compared to younger ones (Bajwa and Javaid, 2007). In addition to these factors Dahiya *et al.* (2004) added that salinity, soluble calcium carbonate, and lime concentrations in soil, along with high water tables and insect pests were also responsible for dieback in shisham and kikar (*Acacia nilotica*) trees. Research in Nepal suggested that dieback affected all age classes of *Dalbergia sissoo* trees (Karki *et al.*, 2000). Stem and root borers and fungi (*Ganoderma lucidum*, *Fusarium solani* and *Fusarium* spp.) were claimed as possible cause of shisham decline (Lakhanpal *et al.*, 1998; Kumar *et al.*, 2002). In contrast to other work, Webb and Hossain (2005) in survey work in Bangladesh, suggested that mortality due to dieback was not correlated with tree age, but recorded a positive and strong correlation with In planting density. Further complications arise from the report of Chturevdi *et al.* (2002) that trees of lower age and diameters were more susceptible to dieback, and that mortality in younger trees was higher (30%) than in older ones (5%).

### 2.10 Pathogenicity:

A range of fungi has been associated with wilt, dieback and death of *D. sissoo*, including *Fusarium solani, F. moniliforme, F. equiseti, F. oxysporum, F. semitectum, Rhizoctonia solani, Alternaria alternata, Curvularia lunata*, *Aspergillus niger* and *Penicillium* spp. (Pathan *et al.*, 2007; Rajpu *et al.*, 2008) Amongst these species, *F. solani* appears to be the most damaging fungus, followed by *R. solani* and *C. lunata. F. solani* was mainly isolated from stem tissues (34.6-85.50%) collected from all locations where the tree is affected by dieback, with an infection frequency of 75% in stem colonization. Some examples of this association include (percentages of infected trees are in brackets) in Ghotki (85.5%), Pano Akil (70.0%), Mirpur Mathelo(65.5%), Daharki (56.5%) and Hala (34.6%). *F. moniliforme* isolations from infected trees ranged from 3-18.5%. *F.*
solani infested soil reduced seed germination up to 50% and the seedling mortality rate was over 93%, followed by soil infested with R. solani (60.0%) with a mortality rate (66.7%) and C. lunata (70.00%), mortality rate 42.9% (Rajput et al., 2010).

Rajput et al. (2008) studied the virulence of F. solani, R. solani and C. lunata isolated from dieback affected shisham trees by inoculating each species alone and in the combination R. solani and C. lunata with F. solani. Plants inoculated with F. solani alone became diseased, whereas plants inoculated with R. solani or C. lunata showed very few or no symptoms. F. solani alone and in combined inoculations with R. solani and C. lunata, resulted in a brown discoloration of stem and root tissues, along with chlorosis of the leaves; in control plants there was no change in leaf colour. Mortality rates of the seedlings was also higher in plants inoculated with F. solani alone (92.3%), followed by the combination of F. solani and R. solani (78.6%). Plants were inoculated by injecting a spore suspension of the fungus into stem by a soil drench, or by spraying a spore suspension onto the plants. Growth rates were lower and reductions in root and shoot weights greater in plants inoculated using the injection method.

Pathan et al. (2007) also confirmed that F. solani was the major fungus responsible for shisham dieback disease, as the pathogen caused more mortality, compared to R. solani and C. lunata, which were also associated with the trees affected by dieback. Likewise, Reductions in germination of D. sissoo seeds are also reported following infection by Aspergillus flavus, A. niger, F. oxysporum and F. solani (Vigayan and Rehill, 1990). Similar results were obtained by Shailendra et al. (2004), who found high rates of mortality and wilting in D. sissoo trees when seed were infested with F. solani.

Shukla (2008) found mortality of D. sissoo trees due to dieback over vast areas of India and neighbouring countries. The disease was found almost in all age classes of the tree, leading to increased problems in state forest departments and for farmers. F. solani was isolated from the roots of the dying shisham trees. Young plants of D. sissoo were raised from seeds collected from 107 mature healthy trees, from 21 heavily infected localities in the country and disease resistance testing carried out on a limited number of provenances. Seedlings were inoculated with F. solani using soil drenching and root-dip methods. After the symptoms appeared survival was recorded enabling the classification of provenances in terms of relative susceptibility. Plants from Amritsar (Punjab) showed maximum survival and were considered very resistant whereas seedlings from Dehra...
(Kangra), Himachal Pradesh, were the most susceptible. The most virulent isolate of *F. solani* was that from Nihal Gate (Haldwani), Uttarakhand, as compared to other fungi (Shukla, 2008). Shakya and Lakhey (2007) collected samples of pods, roots and twigs of trees growing in forests, propagating orchards and other areas of Nepal, which were affected by dieback. Using their testing methods, only *F. solani* was found on roots, pods and green branches of affected trees.

Shakya and Lakhey (2007) inoculated the saplings of *D. sissoo* with *F. solani* by making a 1 cm long cut at a right angle on the stem surface at approximately 5 cm above soil level, using a sterilized blade so that it injured the xylem to some extent. Inoculum was inserted into the cut and the wound bound with grafting tape. Within ten to fifteen days saplings inoculated with the pathogen showed the characteristic dieback symptoms, and *F. solani* was re-isolated from the symptomatic plants, confirming pathogenicity and establishing that this fungus was the major cause of *D. sissoo* dieback in Nepal.

Shakir *et al.* (1999) confirmed that, compared to *Aspergillus, Cladosporium, Verticillium* and nematodes, *F. solani* was the most likely causal organism for shisham decline. Similar results were reported by Chaturvedi *et al.* (2002), in collections of samples from affected trees in north Bihar (India): maximum mortality (approximately 10-22%) was due to *F. solani* infection, followed by *Rhizoctonia* sp. (8-10%) and 2-3% due to *Ganoderma lucidum*. In the nursery stage of production, *F. solani* caused 5-30% mortality.

Dhingra *et al.* (2003) reported that seed borne fungal pathogens also reduced seedling emergence and the quality of *Dalbergia nigra* seedlings in Brazil. *Cladosporium cladosporioides, Colletotrichum crassipes, F. semitectum, Phomopsis dalbergiae* and *Pestalotiopsis* spp. were the only fungi consistently isolated from seed samples of a number of trees. Colonization of both pericarp and seed by these fungi started soon after pod formation and continued to increase until pod maturity. Except *C. cladosporioides*, all the fungi were reported highly pathogenic to seeds and seedlings, causing seed decay, root rot and reduced seedling height and survival. Leaf spots caused by *P. dalbergiae* infection extended into the hypocotyl causing dieback, whereas those caused by *C. crassipes* remained restricted to the foliage. The seed borne fungi also latently colonized the hypocotyl. Foliar inoculation of seedlings with *Pestalotiopsis* sp. induced severe chlorosis and defoliation. The findings of this study revealed the heavy losses suffered by nurserymen due to low quality seedlings. From the seed samples of *Bauhinea variegata,*
Dalbergia latifolia, Gmelina arborea, and Hardwickia bipinnata, many different fungi such as Aspergillus niger, A. flavus, A. flavusoryzae, A. columnaris, A. ochraceus, Penicillium spp., Fusarium moniliforme, F. solani and F. oxysporum were isolated. Seedlings of Gmelina arborea and Bauhinea variegata raised in nursery beds showed high incidence of leaf blight. Powdery mildew in Bauhinea variegata and Fusarium wilt in Hardwickia bipinnata were also observed at high incidence. Dead Dalbergia latifolia seedlings showed high incidence of Aspergillus flavus-oryzae (Mahendra et al., 2005).

Negi (2002) pointed out that attack of D. sissoo by F. solani and G. lucidum was directly related to high water tables; when the water table was high and the roots of trees were touching the water, wilted and death occurred.

Shukla (1992) raised seedlings of Azadirachta indica (Neem) from F. solani infected seeds; resulting plants wilted and died. Kapoor et al. (2004) reported wilting of Acacia nilotica seedlings caused by F. oxysporum: wilted plants became desiccated and in some cases, died. Disease severity was higher in seedlings raised in polythene tubes (37.7%) than those raised in pots (16.9%) and the source of infection was soil.

F. oxysporum, F. solani and F. moniliforme are also known to cause damping off in seedlings of Psyllium (Elwakil and Ghoneem, 1999) and F. lateritium is known to cause wilting and death of Acacia nilotica seedlings (Uniyal et al., 2004). These problems are not restricted to sub-tropical and tropical regions. Martin-Pinto et al. (2008) found that Fusarium spp. were responsible for damping off of forest tree seedlings in nurseries in Spain. F. verticillioides was frequently isolated from diseased plants and Fusarium spp. inoculated on seedlings of trees caused maximum mortality as compared to other fungi.
MATERIALS AND METHODS

Chapter 3

The present work was carried out over a period of three years, from 2009-2011. All field experiments related to nursery stage plants were carried out in the nursery area of the Sub-Campus, University of Agriculture Faisalabad at Depalpur, Okara. Field data on dieback affected shisham trees was collected from cities in different ecological zones of Punjab, Pakistan.

3.1 Survey of *D. sissoo* dieback in different agro-ecological zones of Punjab:

A large area of shisham plantations was surveyed throughout the agro-ecological zones of Punjab to assess the extent of damage caused by dieback disease. The work was carried out in five zones based on the classification of the Pakistan Agricultural Research Council (PARC) (Khan and Khan, 2000):

i. IIIa Sandy deserts

ii. IV-a-Northern irrigated plains

iii. V-Barani areas

iv. X- Sulaiman Piedmont

These agro-ecological zones were selected because of different climatic conditions (Bajwa and Mukhtar, 2006), cropping patterns and diversity in the perennial vegetation cover.

Shisham (*Dalbergia sissoo*) trees are present in high numbers in these areas, being the most favoured species of the local people. Sandy deserts have arid to semi-arid climatic conditions with low rainfall and high temperatures, whereas the Northern irrigated plains have moderate climatic conditions with fertile soils. Barani areas have no artificial irrigation systems and crop cultivation depends entirely upon rainfall. The Sulaman piedmont is also an area which is quite different from other zones. The eleven districts Faisalabad, Toba Tek Singh, Lahore, Sargodha, Okara, Sahiwal, Rawalpindi, Multan, Bahawalpur, Dera Ghazi Khan and Rahim Yar Khan were selected as the most productive districts in which shisham is frequent, from the ecological zones listed above.

Disease severity was recorded on the basis of physical appearance of trees which was divided into three categories:

i. Healthy (vigorous green trees with no signs of dieback and disease)

ii. Partially affected trees (Trees affected by dieback, but not nearing death)

iii. Fully affected (All parts affected, trees almost dead)
Plate 1 Map of the Agro-ecological zones of Punjab (Pakistan).

I. III-a-Sandy deserts
II. IV-a-Northern irrigated plain
III. V-Barani areas
IV. X- Sulaman Piedmont
Plate 2 Partially affected Dalbergia sissoo in Lal Suhanra National Park, Bahawalpur
Plate 3. Fully affected trees of *Dalbergia sissoo* in Toba Tek Singh district.

Plate 4. Healthy and fully affected trees of *Dalbergia sissoo* at the same location in Lal Suhanra National Park Bahawalpur.
As severity of disease is related to different age classes of shisham, it was decided that the most susceptible age of tree to dieback disease should be determined. Trees were divided into three age classes:

i. Age class I (1-20 years in age)
ii. Age class II (21-40 years)
iii. Age class III (over 40 years)

Healthy, partially affected and fully affected trees were observed in each age class. Trees of different ages were selected in each district at random and data recorded weekly for two years (six months in each year). The first data set was completed between November 2009 to April 2010 and the second from November 2010 to April 2011. Weekly observations were recorded to determine the speed of disease development and spread and the age of the trees in which the problem is most severe. In both years, the same experimental units were used for data collection. Number of trees in each district and age class was differed depending on availability at the selected sites.

3.2 Propagation of Dalbergia sissoo

Both sexually and asexually propagated D. sissoo plants were inoculated with fungi to study disease development with time.

3.2.1 Vegetative propagation of D. sissoo

Branch cuttings of D. sissoo were prepared from disease-free plus trees from the Piplee Pahar Irrigated plantation, Depalpur, Okara and were planted in spring 2008 in a nursery on the Sub-campus, University of Agriculture Faisalabad at Depalpur. Cuttings were 23 cm in length and 1-1.5 cm in diameter. Each cutting include 4-5 axillary buds. Cuttings were struck in polythene tubes of 10×15 cm placed in 1.2 x 4.8 meter beds in the nursery. Tubes were filled with the mixture of sand and silt in 1:1 ratio. Cuttings were watered immediately after planting and 24 hour intervals or according to the requirement of the plants. All experimental plants remained in the nursery until February 2009. Vigorous plants of approximately 60 cm in height were selected for inoculation tests. The same procedures were repeated in 2010 in the same experimental area and nursery, using materials from trees in the Piplee Pahar irrigated plantation.

3.2.2 Sexual propagation of D. sissoo

In order to compare the performance of sexually and asexually propagated plants in inoculations, shisham plants were also raised from seeds collected from the same healthy plus trees used for vegetative propagation.
During 2008, seed were sown in 10 x 15 cm polythene tubes containing a mixture of sand and silt in 1:1 ratio, creating the same conditions as those used for cuttings. During February 2009, vigorous seedlings of about 60 cm in height were selected for inoculation. The same procedure was repeated in the following year.

### 3.2.3 Screening of Shisham for dieback resistance in the nursery

Isolates of the fungi most commonly obtained from affected *D. sissoo* trees were used to inoculate was both sexually and asexually produced plants in the nursery.

The following five treatments were used for inoculation

- T₁ = Control
- T₂ = *Ganoderma lucidum*
- T₃ = *Curvularia lunata*
- T₄ = *Botryodiplodia theobromae*
- T₅ = *Fusarium solani*

The inoculation methods used by Khan *et al.* (2004) and Rajput *et al.* (2008) were followed. A cut was made in the stem near the root collar using a sterilized knife. Spore suspensions of the fungi were prepared from 8-10 days old cultures growing on PDA. Sterilized water was poured in fully grown culture plates and then plates were vigorously shaken to prepare spore suspension. The spore densities were determined by repeated haemocytometer and suspensions adjusted to 2.6×10⁶ spore/ml. A 2 ml volume of spore suspension was injected in the wound on the stem on plants raised from both seeds and cuttings. Control plants were treated with 2 ml sterile distilled water. After inoculation, wounds were wrapped in Parafilm; plants were watered.

Data on disease development was recorded on a weekly basis for two months (March and April, 2009 and 2010). Observations were recorded in percentages, Plants showing symptoms of wilt were considered diseased.
Disease incidence was calculated using:

\[
\text{Disease incidence (\%) } = \frac{\text{No. of infected plants}}{\text{Total No. of plants examined}} \times 100
\]

The experiment was repeated in 2010. Experiments were designed as randomized complete block designs (RCBD), with three blocks, ten plants receiving each treatment and fifty plants in each block of seedlings and cuttings.

3.3 Characterization of environmental factors conducive to *D. sissoo* dieback at the nursery stage and development of disease predictive models

Tree dieback and wilting is a complex phenomenon and there is a possibility that different biotic and abiotic factors are involved, which may contribute to disease incidence and ultimately plant mortality. At the nursery stage the plants are more subjected to local weather conditions so the current work was designed to correlate the climatic conditions with disease.

Weekly data for various climatic factors, including minimum and maximum temperature, rainfall, relative humidity and wind speed, were obtained from the Regional Meteorological station, Lahore, for March and April 2009 and 2010. The influence of each variable on dieback disease was determined by correlation and regression analysis.

3.4 Development of dieback disease predictive model for sexually and asexually propagated *D. sissoo* plants
3.4.1 Regression analysis

Regression analysis was used to determine any links between environmental/climatic factors and disease incidence/development (Chatterjee and Hadi, 2006). There are two types of models i.e simple and multiple regression models. For developing simple linear regression models, the response variable (Y), in this case disease, was a function of the single predictor or explanatory variable (X), here an environmental/climatic variable. The equation for simple linear regression is:

\[ Y = \beta_0 + \beta_1X \]

Where \( \beta_0 \) is the intercept and \( \beta_1 \) is the slope. In multiple linear regression, however, there are more explanatory or predictor variables when compared with simple linear regression. The relationship is described by:

\[ Y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \cdots + \beta_ix_i + \epsilon \]

Where \( x \) represents the collection of \( i \) predictors \( x_1,x_2,\ldots,x_i \) in the model, and \( \beta_1,\beta_2,\ldots,\beta_i \) are the corresponding regression coefficients and \( \epsilon \) is the random error or disturbance.

The environmental data (maximum and minimum temperature, rainfall, relative humidity and wind velocity) and dieback disease incidence data at the nursery stage of both sexually and asexually propagated plants collected during March-April 2009 and 2010 were subjected to analysis of variance and differences in climatic factors and disease incidence were determined by least significant difference test (LSD at \( P<0.05 \)). A multiple regression model was developed based on these data.

3.5. Characterization of environmental factors conducive for shisham dieback in field conditions (Survey)

Data collected (November 2009-April 2010 and November 2010-April 2011) for the incidence of shisham dieback from the different ecological zones of Punjab were subjected to correlation analysis with different climatic variables. The influence of each environmental variable (maximum and minimum air temperature, rainfall, relative humidity wind velocity, age and water table) on the severity of shisham dieback occurrence was determined by correlation.

3.6. Development of disease predictive model for the field study (Survey)

A predictive model for the field data on disease incidence and severity was also developed. Weekly environmental data comprising of maximum and minimum air temperatures, relative humidity, average rainfall and wind speed were collected from the
Regional Meteorological Station, Lahore. All the collected environmental data, tree age, underground water table data and dieback disease incidence data in the different districts of Punjab (Faisalabad, Lahore, Sargodha, Sahiwal, Okara, Rawalpindi, Multan, Bahawalpur, Rahim yar khan and Dera Ghazi Khan) from November 2009 to April 2010 and the same months during 2010-11, were subjected to analysis of variance to evaluate the effect of abiotic factors on disease incidence by using the Least Significant Difference Test (LSD at P<0.05).

The influence of these environmental conditions on disease severity was determined by correlation analyses (Steel and Torrie, 1997). R², Mallows Cp and mean square error (MSE) were the criteria used to select the best models (Khan and Illayas, 1999).
RESULTS AND DISCUSSION

4.1 Screening of sexually and asexually propagated shisham (*Dalbergia sissoo*) at nursery stage for susceptibility to fungi isolated from trees with dieback

A pathogenecity test was carried out using the fungi most commonly isolated from shisham trees affected by dieback using inoculations of sexually and asexually propagated shisham plants in the nursery. The experiments were conducted twice to confirm results.

4.1.1 Disease incidence with different fungi

Inoculation treatments gave significantly different (p=0.003) disease incidences (Table 4.1; Figure 4.1). The highest disease incidence followed inoculation with *F. solani* (31.4%; treatment T5) followed by *B. theobromae* (19.0%; T4) and *C. lunata* (12.22%; T3). No disease was detected following inoculation with *G. lucidum* or with mock inoculation (Fig. 4.1). Analysis of variance (Table 4.1) showed significant (p=0.006) differences among all treatments in both years. On yearly basis, the highest incidence was found with T5 (32.99%) during 2010 and (29.80%) during 2009. Similar trends were observed with T4 (19.87%) during 2010 and (18.20%) during 2009 and with T3 (13.27%) during 2010 and (11.16%) during 2009 (Fig. 4.2).

4.1.2 Comparison of disease incidence in sexually and asexually propagated *D. sissoo*

Disease incidence differed significantly between sexually propagated (seedlings) and asexually propagated plants (cuttings) (Fig. 4.3) with mean disease incidence of 17.24% in seedlings, compared to 7.83% in cuttings for combined treatments.

4.1.3 Interaction of different fungal pathogens with different planting materials

Interactions between shisham seedlings and cuttings inoculated with different fungal pathogens are shown in Fig. 4.4. Maximum disease incidence occurred in seedlings (46.18%) inoculated with *F. solani* (treatment T5), which was significantly different (p=0.004) than 16.61% maximum disease incidence in cuttings as presented in Table 4.1. *B. theobromae* caused 24.23% disease incidence appeared in seedlings compared with 13.86% in cuttings, and *C. lunata* 15.76% in seedlings and 8.67% in cuttings. No disease symptoms were observed in seedlings or cuttings inoculated with *G. lucidum*, or mock-inoculated. Disease incidence in seedlings was significantly higher (p=0.009) in both years, compared with disease incidence in cuttings during the same years (Fig 4.3).
Fig. 4.1 Disease incidence rate in shisham (*D. sissoo*) after inoculation with different fungal pathogens (see text for full names of fungi).

Fig. 4.2 Disease incidence rate in shisham (*D. sissoo*) after inoculation with different fungal pathogens in two consecutive years (2009 and 2010).
4.1.4 Changes in disease severity with time

Disease progress, in terms of incidence, over 8 weeks from inoculation with the different fungi is presented in Fig 4.5. Increasing numbers of plants showed symptoms in treatments 3, 4 and 5 over the 8 week assessment period, with 54% of plants showing wilting in treatment T5. However, the highest rate of increase in disease incidence in T5 (nearly 14.5%) was observed between weeks 4 (24.50%) and 5 (39%). After 5 weeks, incidence continued to increase at a lower rate. Treatments T4 and T3 exhibited high rates of disease incidence, i.e 30.5% and 20.5% respectively, after 8 weeks, but no disease was found in treatments T1 (control) and T2 (G. lucidum).

Increases in disease incidence over time separately for seedlings and cuttings are shown in Fig 4.6. Maximum disease incidence (77%) was observed in seedlings inoculated with F. solani (T5) at 8 weeks post-inoculation, but the highest increase in disease incidence (nearly 20%) occurred between 4 (38%) and 5 weeks (58%). In cuttings, the highest disease incidence (31%) was also observed at 8 weeks post-inoculation with T5, significantly lower than that observed in seedlings (P<??%). At 8 weeks post-inoculation, treatment T4 (B. theobromae) lead to 37% disease incidence in seedlings, compared to 24% in cuttings. Treatment T3 (C. lunata) caused 25% disease incidence in seedlings and 16% in cuttings at 8 weeks post-inoculation. No disease symptoms were recorded in treatments T1 (control) and T2 (G. lucidum).

![Fig. 4.3 Average disease incidence in cuttings and seedlings of shisham (D. sissoo) in the two consecutive years 2009 and 2010. The average disease incidence in these two years combined is also shown.](image-url)
Fig. 4.4 Disease incidence in shisham (*D. sissoo*) seedlings and cuttings inoculated with different fungal pathogens.

Fig. 4.5 Effects of different fungal pathogens on disease incidence in inoculated shisham (*D. sissoo*) plants over 8-week, post-inoculation period.
Fig. 4.6 Increases in disease incidence in inoculated seedlings and cuttings of shisham (D. sissoo) over an eight week period. Treatments: T<sub>1</sub> = Control (mock inoculation); T<sub>2</sub> = Ganoderma lucidum; T<sub>3</sub> = Curvularia lunata; T<sub>4</sub> = Botryodiplodia theobromae; and T<sub>5</sub> = Fusarium solani
Table. 4.1 Results of an ANOVA performed on data from the screening of sexually and asexually propagated *D. sissoo* inoculated with different fungi

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>4</td>
<td>738057</td>
<td>184514</td>
<td>62912.7</td>
<td>0.003</td>
</tr>
<tr>
<td>Type</td>
<td>1</td>
<td>109061</td>
<td>109061</td>
<td>37185.8</td>
<td>0.000</td>
</tr>
<tr>
<td>Year</td>
<td>1</td>
<td>2187</td>
<td>2187</td>
<td>745.60</td>
<td>0.000</td>
</tr>
<tr>
<td>Week</td>
<td>7</td>
<td>176324</td>
<td>25189</td>
<td>8588.59</td>
<td>0.000</td>
</tr>
<tr>
<td>Treatment*Type</td>
<td>4</td>
<td>150855</td>
<td>37714</td>
<td>12859.1</td>
<td>0.004</td>
</tr>
<tr>
<td>Treatment*Year</td>
<td>4</td>
<td>1649</td>
<td>412</td>
<td>140.52</td>
<td>0.006</td>
</tr>
<tr>
<td>Treatment*Week</td>
<td>28</td>
<td>175890</td>
<td>6282</td>
<td>2141.86</td>
<td>0.000</td>
</tr>
<tr>
<td>Type*Year</td>
<td>1</td>
<td>457</td>
<td>457</td>
<td>155.97</td>
<td>0.009</td>
</tr>
<tr>
<td>Type*Week</td>
<td>7</td>
<td>13739</td>
<td>1963</td>
<td>669.20</td>
<td>0.000</td>
</tr>
<tr>
<td>Year*Week</td>
<td>7</td>
<td>2729</td>
<td>390</td>
<td>132.91</td>
<td>0.000</td>
</tr>
<tr>
<td>Treatment<em>Type</em>Year</td>
<td>4</td>
<td>874</td>
<td>218</td>
<td>74.46</td>
<td>0.030</td>
</tr>
<tr>
<td>Treatment<em>Type</em>Week</td>
<td>28</td>
<td>31231</td>
<td>1115</td>
<td>380.31</td>
<td>0.000</td>
</tr>
<tr>
<td>Treatment<em>Year</em>Week</td>
<td>28</td>
<td>3423</td>
<td>122</td>
<td>41.68</td>
<td>0.021</td>
</tr>
<tr>
<td>Type<em>Year</em>Week</td>
<td>7</td>
<td>192</td>
<td>27</td>
<td>9.34</td>
<td>0.000</td>
</tr>
<tr>
<td>Treatment<em>Type</em>Year*Week</td>
<td>28</td>
<td>1168</td>
<td>42</td>
<td>14.23</td>
<td>0.030</td>
</tr>
<tr>
<td>Error</td>
<td>4760</td>
<td>13960</td>
<td>3</td>
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</tr>
<tr>
<td>Total</td>
<td>4919</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.2 Correlation of environmental variables with dieback disease of *D. sissoo* at nursery stage:

The correlation between the various different environmental factors (rainfall, relative humidity, minimum and maximum temperature and wind velocity) with dieback of both seedlings and cuttings was significant (*P*<0.05). It is clear from the data given in Table 4.2 that the effects of relative humidity and maximum temperature were the most important factors. A negative correlation was observed between relative humidity and disease incidence in both seedlings and cuttings. The coefficient of correlation (*r* = - 0.97) in cuttings suggested a very strong negative association between disease and relative humidity. A moderate positive correlation was observed between dieback disease and maximum temperature in both cuttings (*r* = 0.734) and seedlings (*r* = 0.629). A weak
association between disease in both seedlings and cuttings occurred with rainfall, minimum temperature and with wind velocity (Table 4.2).

For further confirmation of the relationship between disease and environmental variables, the data were analysed using regression analysis taking each factor separately as an independent variable. Figures 4.7 and 4.8 represent the scatter plots of dieback disease in seedlings and cuttings in relation to environmental variables. With cuttings, 53.9% variation in dieback disease was explained by maximum temperature independently, followed by relative humidity 36.2%, minimum temperature 10.8% and wind speed 1.4% (Table 4.3). Rather similar results were observed in the regression equations in case of seedlings with the environmental variables (Table 4.4) where 39.8% variability correlated with maximum temperature followed by relative humidity 25.3%, minimum temperature 12.8%, rainfall 8.4% and wind speed 0.3%.

Table 4.2 Correlation between weekly environmental data and dieback disease incidence in seedlings and cuttings of *Dalbergia sissoo* inoculated with fungi associated with dieback at the nursery stage.

<table>
<thead>
<tr>
<th>Environmental parameters</th>
<th>Disease incidence in cuttings</th>
<th>Disease incidence in seedlings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainfall</td>
<td>-0.327* 0.000</td>
<td>-0.294* 0.000</td>
</tr>
<tr>
<td>Relative humidity</td>
<td>-0.97* 0.000</td>
<td>-0.487* 0.000</td>
</tr>
<tr>
<td>Minimum temperature</td>
<td>0.434* 0.000</td>
<td>0.350* 0.000</td>
</tr>
<tr>
<td>Maximum temperature</td>
<td>0.734* 0.000</td>
<td>0.629* 0.000</td>
</tr>
<tr>
<td>Wind velocity</td>
<td>0.115* 0.000</td>
<td>0.044* 0.028</td>
</tr>
</tbody>
</table>

Upper values indicate Pearson’s correlation coefficient
Lower values indicate level of probability at p=0.05
Fig. 4.7 Relationships between environmental variables and disease incidence in cuttings of *D. sissoo*.

Table 4.3 Regression equations showing the relationships between environmental variables and disease incidence in cuttings of *D. sissoo*.

<table>
<thead>
<tr>
<th>Regression equation</th>
<th>$R^2$</th>
<th>S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cuttings= 9.52-6.18 Rainfall</td>
<td>10.8%</td>
<td>8.70</td>
</tr>
<tr>
<td>Cuttings= 40.2-0.473 R.H</td>
<td>36.2%</td>
<td>7.36</td>
</tr>
<tr>
<td>Cuttings= -9.37+0.954 Min. Tem</td>
<td>19.1%</td>
<td>8.28</td>
</tr>
<tr>
<td>Cuttings= -38.7+1.34 Max. tem</td>
<td>53.9%</td>
<td>6.25</td>
</tr>
<tr>
<td>Cuttings= 6.54+1.52 wind speed</td>
<td>1.4%</td>
<td>9.17</td>
</tr>
</tbody>
</table>
Fig. 4.8 Relationships between environmental variables and disease incidence in seedlings of *D. sissoo*

Table 4.4 Regression equation showing the relationship between environmental variables and disease in seedlings of *D. sissoo*

<table>
<thead>
<tr>
<th>Regression equation</th>
<th>R²</th>
<th>S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seedlings = 20.7 - 12.4 Rainfall</td>
<td>8.4%</td>
<td>20.11</td>
</tr>
<tr>
<td>Seedlings = 79.1 - 0.904 R.H</td>
<td>25.3%</td>
<td>18.15</td>
</tr>
<tr>
<td>Seedlings = -14.8 - 1.78 Min. Tem</td>
<td>12.8%</td>
<td>19.62</td>
</tr>
<tr>
<td>Seedlings = -74.0 + 2.63 Max. Tem</td>
<td>39.8%</td>
<td>16.30</td>
</tr>
<tr>
<td>Seedlings = 16.0 + 1.63 Wind speed</td>
<td>0.3%</td>
<td>20.98</td>
</tr>
</tbody>
</table>
4.3 Predictive model of Dieback disease on *D. sissoo* cuttings based on two years data

In the present study, a multiple regression model was developed by combining the environmental conditions and disease incidence data from inoculated cuttings in two years. The significant model ($R^2 = 0.62$) at $P<0.05$ was used to predict the probable attack of dieback at the nursery stage under a given set of environmental variables:

$$Y = -58.3 + 7.58x_1 + 0.0054x_2 - 1.14x_3 + 2.47x_4 - 1.09x_5$$

Where $Y =$ Disease in cuttings, $x_1 =$ Rainfall, $x_2 =$ Relative humidity, $x_3 =$ Minimum temperature, $x_4 =$ Maximum temperature and $x_5 =$ Wind velocity

The predictive model clearly indicated that the main environmental factors associated with incidence of dieback disease were rainfall, temperature and wind speed. For example, with a one unit change in rainfall the model predicted a 7.58 unit increase in disease. The (negative) change would be -1.14 units with a one unit change in minimum temperature. With a one unit increase in maximum temperature, disease will increase by 2.47 units, whereas higher wind speeds will result in less disease.

4.3.1 Model Validation:

The model was validated using the procedures described by the Chatterjee and Hadi (2006):

1. Comparison of the dependent variable (dieback disease) and regression coefficients with physical theory,
2. Comparison of observed and predicted data.

| Table 4.5 Statistics for regression of predictive model applied to shisham dieback in cuttings based on two years data (2009-2010). |
|---|---|
| Regression statistics | |
| R-Square | 0.62 |
| Adjusted R-Square | 0.62 |
| Standard Error | 5.67 |
| Observations | 2460 |
Table 4.6 Results of analysis of variance for predictive model for dieback in shisham cuttings based on two years data (2009-2010)

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>5</td>
<td>130125</td>
<td>26025</td>
<td>809.50</td>
<td>0.000</td>
</tr>
<tr>
<td>Error</td>
<td>2454</td>
<td>78895</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2459</td>
<td>209020</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.7 Coefficient of variables, standard error, t statistics and P-values

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Coefficients</th>
<th>Standard error</th>
<th>T stat</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-58.27</td>
<td>2.84</td>
<td>-20.52</td>
<td>0.000</td>
</tr>
<tr>
<td>Rainfall</td>
<td>7.58</td>
<td>0.40</td>
<td>18.92</td>
<td>0.000</td>
</tr>
<tr>
<td>Relative humidity</td>
<td>0.005</td>
<td>0.019</td>
<td>0.27</td>
<td>0.784</td>
</tr>
<tr>
<td>Minimum temperature</td>
<td>-1.14</td>
<td>0.059</td>
<td>-19.15</td>
<td>0.000</td>
</tr>
<tr>
<td>Maximum temperature</td>
<td>2.47</td>
<td>0.62</td>
<td>39.71</td>
<td>0.000</td>
</tr>
<tr>
<td>Wind velocity</td>
<td>-1.09</td>
<td>0.178</td>
<td>-6.13</td>
<td>0.000</td>
</tr>
</tbody>
</table>

4.3.2 Comparison of the dependent variable (dieback disease incidence) and regression coefficients with physical theory

The coefficient of determination ($R^2$), an important consideration in the reliability of the model, was 0.62 (62%) (Table 4.5). The regression model was significant ($P<0.05$; Table 4.6). Rainfall, minimum temperature, maximum temperature and wind velocity were significantly correlated with disease incidence ($P<0.05$) (Table 4.7). The coefficients of determination, standard error, t-statistics and $P$ values are given in Table 4.7.

4.3.3 Evaluation of model by comparing observed and predicted data

The second stage of model assessment included comparison of observed and predicted data. Most predictions were between the 95% predictive interval (PI; Fig. 4.9). A good relationship was observed between predicted and observed data points.
A multiple regression model for disease incidence in seedlings was also developed by combining the environmental conditions and disease incidence data recorded over two years. The model justified ($R^2 = 0.48$; at $P<0.05$) was used to predict the likely occurrence of dieback at the nursery stage under a given set of environmental variables:

$$Y = -134 + 15x_1 + 0.158x_2 - 2.32x_3 + 5.27x_4 - 3.70x_5$$

Where $Y =$ Disease in seedlings, $x_1 =$ Rainfall, $x_2 =$ Relative humidity, $x_3 =$ Minimum temperature, $x_4 =$ Maximum temperature and $x_5 =$ Wind velocity

The regression equation suggested that the main environmental factors responsible for the disease were rainfall, temperature and wind velocity. The equation indicated that a one unit change in rainfall there would be a 15 units increase in disease. For maximum temperature with one unit change, the increase in disease would be 5.27 units. A negative relation was observed between disease incidence and wind velocity (-3.70) and minimum temperature (-2.30).
4.4.1 Seedlings model assessment

Validation of the regression models is very important because the objective of model development is to recognize the best probable set of variables for disease development in a particular system. Models are used for forecasting the diseases in future, that’s why the reliability of the model is very important. Assessment of the model was carried out using the methods of (Snee 1977; Chatterjee and Hadi, 2006).

4.4.2 Comparison of the dependent variable (dieback disease incidence) and regression coefficients with physical theory:

In the model, coefficient of determination (R²) was 0.48 (48%) (Table 4.8). Standard error was 15.23 and the regression model was significant at P<0.05 (Table 4.9). Rainfall, relative humidity, minimum temperature, maximum temperature and wind velocity were significantly correlated with incidence of dieback (P<0.05; Table 4.10).

Table 4.8 Regression statistics of predictive model for Shisham dieback in seedlings based on two years data (2009-2010)

<table>
<thead>
<tr>
<th>Regression statistics</th>
<th></th>
</tr>
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<tbody>
<tr>
<td>R-Square</td>
<td>0.48</td>
</tr>
<tr>
<td>Adjusted R-Square</td>
<td>0.48</td>
</tr>
<tr>
<td>Standard Error</td>
<td>15.23</td>
</tr>
<tr>
<td>Observations</td>
<td>2460</td>
</tr>
</tbody>
</table>

Table 4.9 Analysis of variance of predictive model for Shisham dieback in seedlings based on two years data (2009-2010)

<table>
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<td>Regression</td>
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<tr>
<td>Error</td>
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</tr>
<tr>
<td>Total</td>
<td>2459</td>
</tr>
</tbody>
</table>

Table 4.10 Coefficient of variables, standard error, t stat and P-value

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Coefficients</th>
<th>Standard error</th>
<th>T stat</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-134.06</td>
<td>7.63</td>
<td>-17.57</td>
<td>0.000</td>
</tr>
<tr>
<td>Rainfall</td>
<td>15.04</td>
<td>1.08</td>
<td>13.97</td>
<td>0.000</td>
</tr>
<tr>
<td>Relative humidity</td>
<td>0.16</td>
<td>0.053</td>
<td>2.99</td>
<td>0.003</td>
</tr>
<tr>
<td>Minimum temperature</td>
<td>-2.32</td>
<td>0.16</td>
<td>-14.48</td>
<td>0.000</td>
</tr>
<tr>
<td>Maximum temperature</td>
<td>5.27</td>
<td>0.17</td>
<td>31.54</td>
<td>0.000</td>
</tr>
<tr>
<td>Wind velocity</td>
<td>-3.69</td>
<td>0.48</td>
<td>-7.72</td>
<td>0.000</td>
</tr>
</tbody>
</table>
4.4.3 Evaluation of model by comparing observed and predicted data

The second stage of model assessment was carried out by comparing the observed and predicted data. It is clear from the results shown in Figure 4.10 that despite an $R^2 = 0.48$, most of the predictions laid within the 95% predictive intervals (PI). A good relationship was observed between predicted and observed results. Based on these parameters, therefore, the model can be used for forecasting the disease.

![Fitted line plot for dieback disease in seedlings with predicted and observed data points at 95% confidence and predictive interval](image)

Fig 4.10. Fitted line plot for dieback disease in seedlings with predicted and observed data points at 95% confidence and predictive interval
4.5 Survey of Shisham growing areas of the Punjab to determine severity and extent of damage due to dieback

An extensive survey in eleven major shisham growing districts of four agro-ecological zones in Punjab province was carried out to calculate the severity and extent of damage caused by dieback disease. Trees were divided into three major age classes to determine the most damaged age class; in each age class the trees were further classified into healthy, partially affected and fully affected or almost dead trees, to find out the ratio of healthy, diseased and dead trees in the study area.

4.5.1. Healthy trees in different ecological zones of Punjab within different age groups

The number of healthy trees differed significantly between all age groups in the ecological zones of Punjab (Fig 4.11). The highest number of disease free trees was found in Barani. In all ecological zones, age class I (1-20 years) was the least affected by dieback. In Barani 92.16% of trees were healthy in age class I, 83.41% in age class II (20-40 years) and 77.65% in age class III (above 40 years). The lowest number of healthy trees was found in the Suleman piedmont zone with 69.27% in age class I, 64.86% in age class II and 55.99% in age class III. Similar trends were observed in the northern irrigated plains and sandy deserts with 77.19% and 79.20% in age class I and 68.84%, 67.78% in age class II and 59.43 and 62.24% in age class III, respectively (Appendix 4).

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>3</td>
<td>141.3</td>
<td>47.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>2</td>
<td>35462.4</td>
<td>17731.2</td>
<td>358.90</td>
<td>0.0000</td>
</tr>
<tr>
<td>Climate</td>
<td>3</td>
<td>38327.8</td>
<td>12775.9</td>
<td>258.60</td>
<td>0.0000</td>
</tr>
<tr>
<td>Age* Climate</td>
<td>6</td>
<td>1455.5</td>
<td>242.6</td>
<td>4.91</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>1569</td>
<td>77515.5</td>
<td>49.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1583</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Unhealthy and trees showing substantial dieback were designated as partially affected trees. Numbers of trees in this category varied considerably in all zones of Punjab; proportions of these trees also differed greatly in all age classes (Table 4.12; Fig 4.12). Most partially affected trees were observed in the Suleman piedmont zone. Age class III was considered as the most affected class in all zones of Punjab province examined. In Suleman piedmont 22.89% of partially affected trees were found in age class III followed by 18.81% and 18.31% in age classes II and I, respectively. Fewer diseased trees were found in the Barani area with 11.08% in age class III followed by 7.87% and 3.17% in classes II and III, respectively. Similar trends were observed in all age classes of Northern irrigated and Sandy desert zones (Fig 4.12; Appendix 5).
Fig. 4.12: Partially affected trees in different agro-ecological zones of Punjab with different age classes

Table 4.12: ANOVA of partially affected trees in different ecological zones with different age Classes

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>3</td>
<td>22.4</td>
<td>7.47</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>Age</td>
<td>2</td>
<td>6829.1</td>
<td>3414.56</td>
<td>279.92</td>
<td>0.000</td>
</tr>
<tr>
<td>Climate</td>
<td>3</td>
<td>11655.2</td>
<td>3885.06</td>
<td>318.49</td>
<td>0.0000</td>
</tr>
<tr>
<td>Age* Climate</td>
<td>6</td>
<td>739.4</td>
<td>123.3</td>
<td>10.10</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>1569</td>
<td>19139.3</td>
<td>12.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1583</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.5.3. Fully affected trees in different ecological zones of Punjab with different age groups

The numbers of dead trees within different age groups in the different ecological zones were significantly different (Table 4.13). High disease incidence was recorded in Northern irrigated plains, with 23.60% of trees in age class III, 17.97% in class II and 12.78% in class I (Fig 4.13). In Suleman piedmont, the highest mortality in age class III
was 21.14%, compared with 20.36% in the sandy desert. Little difference, however, was recorded in the other age classes in these two zones. The Barani area was the least affected zone, with 11.17% mortality of trees in age class III, 8.85% in class II and 4.68% in class I (Appendix.6).

![Fig. 4.13: Fully affected trees in different agro-ecological zones of Punjab with different age classes](image)

![Table 4.13: ANOVA of fully affected trees in different ecological zones with different age classes](table)

<table>
<thead>
<tr>
<th>Source</th>
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<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>3</td>
<td>46.0</td>
<td>15.34</td>
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<td></td>
</tr>
<tr>
<td>Age</td>
<td>2</td>
<td>11152.5</td>
<td>5576.27</td>
<td>292.71</td>
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<tr>
<td>Climate</td>
<td>3</td>
<td>12473.0</td>
<td>4157.67</td>
<td>218.25</td>
<td>0.0000</td>
</tr>
<tr>
<td>Age* Climate</td>
<td>6</td>
<td>544.9</td>
<td>90.82</td>
<td>4.77</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>1569</td>
<td>29889.9</td>
<td>19.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1583</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.5.4. Healthy trees with different age groups in different districts of Punjab

Numbers of healthy trees within different age groups differed significantly in different districts of Punjab (P<0.05; Fig. 4.14, Table 4.14). The greatest numbers of disease free trees were recorded in Rawalpindi district, with, in age class I, 95% trees, age class II 84% and age class III 78% healthy, respectively. Fewer healthy trees were observed in the Toba Tek Singh district, with 75, 60 and 35 % healthy in age classes I, II, III, respectively. Sargodha district had 83% healthy trees in age class I, compared with 81% in Sahiwal. In all other areas including Bahawalpur, Dera Ghazi Khan, Faisalabad, Lahore, Multan, Okara and Rahim Yar Khan, age class I had high proportions of healthy trees as compared to other age classes (Appendix.1).

Fig. 4.14: Comparison of healthy trees with different ages in different areas of Punjab

Table 4.14: ANOVA of healthy trees in different districts with different age classes

<table>
<thead>
<tr>
<th>Source</th>
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<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
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<td>47.1</td>
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<tr>
<td>Age</td>
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<td>75745</td>
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<td>1103.42</td>
<td>0.000</td>
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<td>Climate</td>
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<td>5846.8</td>
<td>170.35</td>
<td>0.000</td>
</tr>
<tr>
<td>Age* Climate</td>
<td>20</td>
<td>58468</td>
<td>285</td>
<td>8.30</td>
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</tr>
<tr>
<td>Error</td>
<td>1548</td>
<td>53132</td>
<td>34.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1583</td>
<td>193185</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.5.5. Partially affected trees within different age groups in different districts of Punjab

The numbers of partially affected trees were significantly different (P<0.05) in all surveyed districts of Punjab. In all age classes in the Rawalpindi district the lowest numbers of affected trees were recorded: 3.16% in age class I, in age class II 7.87% and in age class III 11.08% diseased trees. In Sargodha, the disease rate was similar, with 3.16% in class I, 9.43% in age class II and 13.12% in age class III. The highest numbers of partially affected trees were observed in Dera Ghazi Khan with 22.89% in age class III, 18.81% in class II and 18.31% in class I. In all the surveyed areas, disease incidence was higher in age class III: in Faisalabad it was 18.93% followed by Lahore 18.56% and Multan 18.29%. Similar disease trends were observed in all other districts (Table 4.15; Fig 4.15; Appendix 2).

![Comparison of partially affected trees with different ages in different areas of Punjab](image)

**Fig. 4.15:** Comparison of partially affected trees with different ages in different areas of Punjab
Table 4.15: ANOVA of Partially affected trees in different districts with different age classes

<table>
<thead>
<tr>
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<th>MS</th>
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<th>P</th>
</tr>
</thead>
<tbody>
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<td>7.47</td>
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</tr>
<tr>
<td>Age</td>
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<td>12979.4</td>
<td>6489.71</td>
<td>801.52</td>
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<tr>
<td>District</td>
<td>10</td>
<td>16475.4</td>
<td>1647.54</td>
<td>203.48</td>
<td>0.000</td>
</tr>
<tr>
<td>Age* District</td>
<td>20</td>
<td>2522.9</td>
<td>126.14</td>
<td>15.58</td>
<td>0.000</td>
</tr>
<tr>
<td>Error</td>
<td>1551</td>
<td>12535.6</td>
<td>8.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1583</td>
<td>44535.6</td>
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<td></td>
</tr>
</tbody>
</table>

4.5.6. Mortality of shisham in different districts in relation to age

The number of fully affected trees was always higher in the most mature age class of trees, compared to the younger age classes (Appendix 3) (Fig 4.16). Disease incidence was significantly different (P<0.05) in all age classes and districts (Table 4.16).

Rawalpindi district was the least affected area, in which only 5% mortality was observed in age class I, followed by 8% in class II and 11% in age class III. Toba Tek Singh was the most affected district with a mortality rate of 33% in age class III, 25 % in class II and 18% in class I. Faisalabad was the second- most affected area with 25.33% dead/dying trees in age class III, followed by Okara (23.29%), Lahore (22.89%), Rahim Yar Khan (22.64%), Sargodha (22.31%), Dera Ghazi Khan (21.16%) and Multan (20.22%).
Fig. 4.16: Comparison of fully affected (dead) trees within different ages in different areas of Punjab

Table 4.16: ANOVA of fully affected trees in different districts within different age classes

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>3</td>
<td>46.0</td>
<td>15.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>2</td>
<td>25872.7</td>
<td>12963.3</td>
<td>1109.20</td>
<td>0.000</td>
</tr>
<tr>
<td>District</td>
<td>10</td>
<td>23237.8</td>
<td>2323.8</td>
<td>199.25</td>
<td>0.000</td>
</tr>
<tr>
<td>Age* District</td>
<td>20</td>
<td>1627.2</td>
<td>81.4</td>
<td>6.98</td>
<td>0.000</td>
</tr>
<tr>
<td>Error</td>
<td>1548</td>
<td>18042.9</td>
<td>11.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1583</td>
<td>68826.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.5.7. Effect of different water tables on shisham mortality in different age classes

The relationship between numbers of dead shisham trees in different age classes in relation to depths of the underground water tables was examined. There was great
variation in death rate with different depths of water tables (Fig 4.17). When the water table was between 15-20 feet fewer numbers of dead trees were recorded in all age classes. High shisham mortality rates were recorded when the water tables were below 15 feet and above 20 feet. When the water table was below 10 feet 13% mortality was 13, 17.4 and 22.3% in age classes I, II and III, respectively; a similar trend was observed at the 10-15 feet depth. Between 20-25 feet depth, mortality was higher, with 13.4% in class I, 18.7% in class II and 24.2% in class III, but after 25 feet mortality decreased again. At water table depth of 35 feet, mortality was 9% in class I, 13% in class II and 17.4% in class III.

Fig. 4.17: Relationship between water table depths on mortality of Shisham in different age classes
Table 4.17: ANOVA of fully affected trees in different age classes in relation to water table depth.

<table>
<thead>
<tr>
<th>Source</th>
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<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
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<td>15.3</td>
<td>1374.68</td>
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</tr>
<tr>
<td>Age</td>
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<td>22413.0</td>
<td>11206.5</td>
<td>272.76</td>
<td>0.0000</td>
</tr>
<tr>
<td>Water table</td>
<td>13</td>
<td>28906.1</td>
<td>2223.5</td>
<td>6.87</td>
<td>0.0000</td>
</tr>
<tr>
<td>Age* Water table</td>
<td>26</td>
<td>1455.6</td>
<td>56.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>1539</td>
<td>12546.1</td>
<td>8.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1583</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.6 Correlation of age and environmental variables with dieback disease of *D. sissoo* in different zones of Punjab:

There was a significant correlation between tree age, underground water table and environmental factors (rainfall, relative humidity, minimum temperature, maximum temperature and wind velocity) with dieback disease in healthy, fully affected and partially affected trees of shisham in different areas of Punjab was found significant (*P*<0.05). Water table and rainfall were not significantly correlated with tree mortality (Table 4.18). A moderate association was observed between tree age and dieback disease in healthy (*r*=0.626), partially affected (*r*=0.539) and fully affected trees (*r*=0.613). A weak or negative association was found between maximum temperature and disease in healthy (*r*=−0.190), positive in partially affected (*r*=0.176) and fully affected trees (*r*=0.175) followed by wind velocity in healthy (*r*=−0.166), partially affected (*r*=0.184) and fully affected trees (0.129). A similarly weak association was found for minimum temperature (healthy, *r*= -0.105; partially affected, *r*=0.150; fully affected, *r*=0.138) and for relative humidity in healthy (*r*=0.106), partially affected (*r*=−0.125) and fully affected (*r*= -0.076) trees.

Regression analyses of environmental variables against dieback disease were carried out independently to observe the effects of each environmental variable separately on the disease development in the major shisham grown areas of the Punjab province. Fig.4. 18 represents the scatter plots and Table. 4. 19 shows the regression equations and *R*^2^ of the correlations of healthy trees with the environmental variables. *R*^2^ value shows that 39.2% of variation in dieback disease was explained by the age classes, followed by time (20.5%), maximum temperature (3.6%), wind speed (2.7%), minimum temperature (2.4%), relative humidity (1.1%), rainfall (0.3%) and water table (0.2%).

For partially affected trees, age class was responsible for 29.1% of variation, followed by time (15.7%), wind speed (3.4%), maximum temperature (3.0%), minimum temperature (2.2%), relative humidity (1.5%), water table (0.2%); rainfall did not explain any variation (Table 4.20; Fig. 4.19). A similar trend was detected in fully affected trees, (Table 4. 21; Fig 4. 20) where 37.6% variation was explained by age class, followed by time span (18.6%), maximum temperature (3.1%), minimum temperature (1.9%), wind speed (1.7%), relative humidity (0.6%), rainfall (0.5%) and water table (0.2%).
Table 4.18 Correlations between incidence of shisham dieback disease and environmental factors under field conditions

<table>
<thead>
<tr>
<th>Factors</th>
<th>Healthy</th>
<th>Partial Affected</th>
<th>Fully Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.626*</td>
<td>0.539*</td>
<td>0.613*</td>
</tr>
<tr>
<td></td>
<td>0.000</td>
<td>0.006</td>
<td>0.000</td>
</tr>
<tr>
<td>Water table</td>
<td>0.044</td>
<td>-0.046</td>
<td>-0.037</td>
</tr>
<tr>
<td></td>
<td>0.080</td>
<td>0.070</td>
<td>0.142</td>
</tr>
<tr>
<td>Rainfall</td>
<td>0.053</td>
<td>-0.021</td>
<td>-0.072*</td>
</tr>
<tr>
<td></td>
<td>0.035</td>
<td>0.414</td>
<td>0.004</td>
</tr>
<tr>
<td>Relative humidity</td>
<td>0.106*</td>
<td>-0.125*</td>
<td>-0.076*</td>
</tr>
<tr>
<td></td>
<td>0.000</td>
<td>0.000</td>
<td>0.002</td>
</tr>
<tr>
<td>Temperature (Min)</td>
<td>-0.105*</td>
<td>0.150*</td>
<td>0.138*</td>
</tr>
<tr>
<td></td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Temperature (Max)</td>
<td>-0.190*</td>
<td>0.176*</td>
<td>0.175*</td>
</tr>
<tr>
<td></td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Wind velocity</td>
<td>-0.166*</td>
<td>0.184*</td>
<td>0.129*</td>
</tr>
<tr>
<td></td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Upper values indicate Pearson’s correlation coefficient
Lower values indicate level of probability at p=0.05

![Diagram](image)

Fig 4.18 Effect of different environmental variables on proportions of healthy *D. sissoo* trees in Punjab
Table 4.19 Regression equations of healthy trees with different variables

<table>
<thead>
<tr>
<th>Regression equation</th>
<th>R²</th>
<th>S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy = 86.6 - 8.47 Age class</td>
<td>39.2</td>
<td>8.61</td>
</tr>
<tr>
<td>Healthy = 84.7 - 10.0 Time (Year)</td>
<td>20.5</td>
<td>9.85</td>
</tr>
<tr>
<td>Healthy = 68.7 + 0.0416 Water table</td>
<td>0.2</td>
<td>11.03</td>
</tr>
<tr>
<td>Healthy = 69.5 + 0.491 Rainfall</td>
<td>0.3</td>
<td>11.03</td>
</tr>
<tr>
<td>Healthy = 63.7 + 0.078 R.H</td>
<td>1.1</td>
<td>10.98</td>
</tr>
<tr>
<td>Healthy = 72.8 - 0.262 Min. Tem</td>
<td>2.4</td>
<td>10.91</td>
</tr>
<tr>
<td>Healthy = 77.1 + 0.273 Max. Tem</td>
<td>3.6</td>
<td>10.84</td>
</tr>
<tr>
<td>Healthy = 71.1 - 1.50 Wind speed</td>
<td>2.7</td>
<td>10.89</td>
</tr>
</tbody>
</table>

Fig 4.19 Effect of different environmental variables on incidence of dieback disease in the partially affected class in Punjab
Table 4.20 Regression equations of partially affected trees with different variables

<table>
<thead>
<tr>
<th>Regression equation</th>
<th>R²</th>
<th>S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.A=6.59 + 3.50 Age class</td>
<td>29.1</td>
<td>4.46</td>
</tr>
<tr>
<td>P.A=7.30 + 4.20 Time (Year)</td>
<td>15.7</td>
<td>4.87</td>
</tr>
<tr>
<td>P.A=14.1-0.0221 Water table</td>
<td>0.2</td>
<td>5.29</td>
</tr>
<tr>
<td>P.A=13.6-0.086 Rainfall</td>
<td>0.0</td>
<td>5.30</td>
</tr>
<tr>
<td>P.A=16.9-0.0432 R.H</td>
<td>1.5</td>
<td>5.26</td>
</tr>
<tr>
<td>P.A=12.2+0.120 Min. Tem</td>
<td>2.2</td>
<td>5.24</td>
</tr>
<tr>
<td>P.A=10.4+0.120 Max. Tem</td>
<td>3.0</td>
<td>5.23</td>
</tr>
<tr>
<td>P.A=12.9+0.797 Wind speed</td>
<td>3.4</td>
<td>5.21</td>
</tr>
</tbody>
</table>

Fig 4.20 Effect of different environmental variables on the mortality rate of *D. sissoo* trees due to dieback disease in Punjab
Table 4.21 Regression equations of fully affected trees with different variables

<table>
<thead>
<tr>
<th>Regression equation</th>
<th>$R^2$</th>
<th>S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F.A= 6.80+4.95 \text{ Age class}$</td>
<td>37.6</td>
<td>5.21</td>
</tr>
<tr>
<td>$F.A= 8.15 + 5.70 \text{ Time (Year)}$</td>
<td>18.6</td>
<td>5.94</td>
</tr>
<tr>
<td>$F.A= 17.2-0.022 \text{ Water table}$</td>
<td>0.2</td>
<td>6.59</td>
</tr>
<tr>
<td>$F.A= 16.9-0.410 \text{ Rainfall}$</td>
<td>0.5</td>
<td>6.58</td>
</tr>
<tr>
<td>$F.A= 19.3 - 0.0331 \text{ R.H}$</td>
<td>0.6</td>
<td>6.57</td>
</tr>
<tr>
<td>$F.A= 15.1+0.139 \text{ Min.Tem}$</td>
<td>1.9</td>
<td>6.53</td>
</tr>
<tr>
<td>$F.A= 12.6+0.150 \text{ Max.Tem}$</td>
<td>3.1</td>
<td>6.49</td>
</tr>
<tr>
<td>$F.A= 16.0+0706 \text{ Wind speed}$</td>
<td>1.7</td>
<td>6.53</td>
</tr>
</tbody>
</table>

4.7 *Dalbergia sissoo* dieback disease predictive model based on two years field survey data (2009-10 and 2010-11)

Data of dead shisham trees due to dieback disease about three different age classes were collected from different shisham grown areas of Punjab (Pakistan) through a comprehensive survey. The effect of age classes, environmental variables (Minimum and maximum temperature, rainfall, relative humidity and wind velocity), years and underground water table on dieback disease development was determined by correlation and regression analysis.

A multiple regression model was developed to determine the inter-relationships between the development of dieback disease and all environmental and edaphic variables assessed. With an $R^2$ value of 0.89, the model was statistically justified at $P<0.05$ and, therefore, can be used to predict the likelihood of dieback occurring under a given set of environmental and other variables.

**Full predictive model for field data**

\[
FA = 2.51 + 4.15 x_1 + 5.25 x_2 + 0.00866 x_3 - 0.113 x_4 - 0.0611 x_5 - 0.0115 x_6 - 0.0092 x_7 + 0.514 x_8
\]

$R^2 = 0.89$
Where FA=fully affected trees, x1= age, x2= year, x3= water table, x4= rainfall, x5= relative humidity, x6= minimum temperature, x7= maximum temperature, x8= wind velocity

The model indicated that most predictor variables had a small role in disease development. Age class and time span, however, had an important role. The model equation suggests that with one unit increase in age, dieback incidence will increase by 4.15 units in shisham plantations; with a single unit increase in time span (years), the change in dieback would be 5.25. For rainfall, relative humidity, minimum and maximum temperature, the negative values indicate that an increase in these variables will reduce disease incidence. In the principle component analysis, using the forward selection method, an optimum model was developed. The summary of the forward selection process (Table. 4.25) shows that the partial R² value after entering the age variable was 0.5253 and for year, relative humidity and wind velocity it was 0.2943, 0.0552 and 0.0154, respectively. Beyond these variables, there was negligible increase in the R² value. The R² value for both the full model and optimum models was the same (0.89).

Table 4.22 Regression statistics of the full predictive model for the fully affected trees of Shisham due to dieback based on two years data (2009-10 and 2010-11)

<table>
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</thead>
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</tr>
<tr>
<td>Adjusted R-square</td>
<td>0.89</td>
</tr>
<tr>
<td>Standard error</td>
<td>1.543</td>
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<tr>
<td>Observations</td>
<td>1583</td>
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</tbody>
</table>

Table 4.23 Analysis of variance of full predictive model for the fully affected trees of Shisham due to dieback based on two years data (2009-10 and 2010-11)

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<th></th>
</tr>
</thead>
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</tr>
<tr>
<td>Error</td>
<td>1575</td>
</tr>
<tr>
<td>Total</td>
<td>1583</td>
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</tbody>
</table>
Table 4.24 Coefficient of variables, standard error, t stat and P-value

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Coefficients</th>
<th>Standard error</th>
<th>T stat</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
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<tr>
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<td>0.000</td>
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<tr>
<td>Year</td>
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<td>66.25</td>
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</tr>
<tr>
<td>Water table</td>
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<td>0.003</td>
<td>2.54</td>
<td>0.000</td>
</tr>
<tr>
<td>Rainfall</td>
<td>-0.113</td>
<td>0.034</td>
<td>-3.31</td>
<td>0.011</td>
</tr>
<tr>
<td>Relative humidity</td>
<td>-0.061</td>
<td>0.004</td>
<td>-13.36</td>
<td>0.000</td>
</tr>
<tr>
<td>Min. Temperature</td>
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<td>0.016</td>
<td>-0.69</td>
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<tr>
<td>Max. Temperature</td>
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<td>0.014</td>
<td>-0.66</td>
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<tr>
<td>Wind velocity</td>
<td>0.540</td>
<td>0.038</td>
<td>14.36</td>
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</tbody>
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Table 4.25 Summary of forward selection

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<th>Steps</th>
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<th>Model R-square</th>
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<td>1</td>
<td>Age class</td>
<td>0.5253</td>
<td>0.5253</td>
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</tr>
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<td>Time span (Year)</td>
<td>0.2943</td>
<td>0.8196</td>
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<td>0.8748</td>
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<td>4</td>
<td>Wind velocity</td>
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<td>5</td>
<td>Rainfall</td>
<td>0.0009</td>
<td>0.8911</td>
<td>&lt;0.0002</td>
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<tr>
<td>6</td>
<td>Water table</td>
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<td>0.8915</td>
<td>&lt;0.0235</td>
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<td>7</td>
<td>Min Temperature</td>
<td>0.0003</td>
<td>0.8919</td>
<td>&lt;0.0405</td>
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</tbody>
</table>

4.7.1 Model Validation:

Model was evaluated according to the procedures described by the Chattefue and Hadi, (2006). They used the following options to assess the model,

1. Comparison of the dependent variable (Dieback disease) and regression coefficients with physical theory,

2. Comparison of observed and predicted data

4.7.2 Comparison of the dependent variable (Dieback disease) and regression coefficients with physical theory:

Coefficient of determination ($R^2$) was an important parameter derived from the present work (Table. 4.22 and 4.26). Standard error was good (1.5532). The regression model was significant at P<0.05 (Table. 4.23). Minimum and maximum temperatures were non significant in the full predictive model (Table. 4.24). Role of water table and rainfall were also not much significant and summary of forward selection represents the same (Table. 4.25) while all other parameters were significant (Table. 4.28). In the optimum model age, year, relative humidity and wind velocity were the major contributors in the disease development. The coefficients, standard error, t stat and P
values of full and optimum models are given in Tables 4.24 and 4.28 respectively. It has been concluded from the above results that the model is very good for prediction.

**Optimum predictive model for field data**

\[ FA = 1.65 + 4.15 \text{ Age} + 5.20 \text{ Year} - 0.0523 \text{ RH} + 0.544 \text{ WV} \]

\[ R^2 = 0.89 \]

**Table 4.26 Regression statistics of optimum predictive model for the fully affected trees of Shisham due to dieback based on two years data (2009-10 and 2010-11)**

<table>
<thead>
<tr>
<th>Regression statistics</th>
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<tbody>
<tr>
<td>R-square</td>
<td>0.89</td>
</tr>
<tr>
<td>Adjusted R-square</td>
<td>0.89</td>
</tr>
<tr>
<td>Standard error</td>
<td>1.5532</td>
</tr>
<tr>
<td>Observations</td>
<td>1583</td>
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</tbody>
</table>

**Table 4.27 ANOVA for optimum predictive model for the fully affected trees of Shisham due to dieback based on two years data (2009-10 and 2010-11)**

<table>
<thead>
<tr>
<th>ANOVA</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Source</td>
<td>Df</td>
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<td>Regression</td>
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</tr>
<tr>
<td>Error</td>
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</tr>
<tr>
<td>Total</td>
<td>1583</td>
</tr>
</tbody>
</table>

**Table 4.28 Coefficient of variables, standard error, t stat and P-value**

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Coefficients</th>
<th>Standard error</th>
<th>T stat</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>1.65</td>
<td>0.29</td>
<td>5.57</td>
<td>0.000</td>
</tr>
<tr>
<td>Age</td>
<td>4.15</td>
<td>0.047</td>
<td>86.91</td>
<td>0.000</td>
</tr>
<tr>
<td>Year</td>
<td>5.20</td>
<td>0.078</td>
<td>66.44</td>
<td>0.000</td>
</tr>
<tr>
<td>Relative humidity</td>
<td>-0.052</td>
<td>0.0029</td>
<td>-17.62</td>
<td>0.000</td>
</tr>
<tr>
<td>Wind velocity</td>
<td>0.54</td>
<td>0.036</td>
<td>14.87</td>
<td>0.000</td>
</tr>
</tbody>
</table>
4.7.3 Validation of model by comparing observed and predicted data

The model was also assessed by comparing the observed and predicted data. Fig. 21 shows that majority of the predictions fell between the 95% confidence intervals and 95% predictive intervals, demonstrating a very good relationship between predicted and observed data. Based on the $R^2$ (89%), the CI and PI model can be used for forecasting the dieback disease of shisham in various climatic conditions in the future.

![Fitted line plot between observed and predicted disease incidence % (Fully affected)](image)

**Fig 4.21** Fitted line plot for dieback disease in fully affected trees of Shisham with predicted and observed data points at 95% confidence and predictive interval
4.8 Discussion

Provision of disease free and healthy nursery stock is key to success in obtaining a healthy forest plantation crop. In the work reported here, shisham plants were raised in a nursery using sexual (seedlings) and asexual (cuttings) propagation to compare the susceptibility of plants arising from each method to dieback disease. Past studies were focused solely on disease incidence in seedlings of shisham (Idrees et al., 2006; Rajput et al., 2010) and little work has been reported on the evaluation of the responses of seedlings versus cuttings to different fungal pathogens.

Many authors have postulated that a number of factors – silvicultural, pathological, entomological, edaphic and ontogenic – can contribute to dieback disease of shisham. Amongst these factors, pathogen related mortality has been considered the most important and is commonly reported (Karki et al., 2000; Sah et al., 2003). Many pathogens have been found associated with shisham, but fungi are the group most consistently isolated from diseased trees (Rajput et al., 2008).

In this work four fungi commonly isolated from shisham, *Fusarium solani*, *Botryodiplodia theobromae*, *Curvularia lunata* and *Ganoderma lucidum*, were inoculated onto seedlings and cuttings of shisham. Disease was caused by *F. solani*, *B. theobromae* and *C. lunata*, of which, the highest incidence resulted from inoculations with *F. solani*. In contrast, shisham plants inoculated with *G. lucidum* failed to develop symptoms of disease. The high increase in disease incidence at 4 to 5 weeks post-inoculation probably resulted from favourable climatic conditions, when the temperature was in the range of 20-25 °C, which was quite suitable for the growth of *F. solani* and *B. theobromae*. Kausar et al. (2009) found that 25 °C is the optimum temperature for attack by *F. solani*. Similar findings were reported by Rehman et al. (2006). These results also corroborated with the findings of Rajput et al. (2008) who found maximum disease incidence in shisham seedlings appeared 30 to 40 days after inoculation with *F. solani* and other fungi. Higher disease incidence caused by *F. solani* compared with the other tested fungi in this study also supported previous reports that *F. solani* reduced the vigor of forest tree seedlings including shisham species (Vijayan and Rehill, 1990). Some other reports revealed that seed-borne fungi, including *F. solani*, increased susceptibility of seeds and seedlings to other soil-borne pathogens (Mamatha et al., 2000).

Shukla (2008) isolated *F. solani* from the roots and stems of diseased shisham trees, and confirmed the pathogenic role of *F. solani* in inoculation tests on seedlings. Rajput et al. (2010) also isolated 10 fungi from shisham trees and found *F. solani* most virulent,
causing a 50% reduction in seed germination. In another study Rajput et al. (2008) inoculated seedlings with *F. solani*, *Rhizoctonia solani*, and *C. lunata* and showed that *F. solani* produced the greatest disease symptoms.

PFRI (2004) produced plants from branch cuttings of shisham: in those plants no symptoms of dieback or wilting appeared even after five years of planting. In the present study, sexually propagated plants (seedlings) of shisham were compared with asexually propagated plants (cuttings), and significantly more disease incidence was observed in seedlings. In seedlings, *F. solani* produced a maximum disease incidence of 46.08% followed by *B. theobromae*, 24.22%. In cuttings, however, *F. solani* caused a maximum disease incidence of 16.61% and *B. theobromae* 13.85%. These results showed that disease incidence in seedlings was significantly different from that in cuttings; the results were almost unchanged in 2 years after planting. Previously, Singh et al. (2011) expressed the opinion that seed-derived shisham plants were more susceptible to dieback disease as compared to vegetatively propagated plants. Some other (Puri and Sharma, 1996; Rafiqul Hoque, 2008) demonstrated that vegetative propagation produced healthier trees than the trees propagated from seeds.

In present study mortality of *D. sissoo* seedlings was higher than in cuttings following inoculation. It is known that, during storage fungal pathogens can enter the seed coat and embryo of many plants, which reduces germination and ultimately survival rate of seedlings while in vegetative propagation, fresh and disease free branches were planted resulting in healthy and vigorous plants (Bhansli and Jindal, 1997).

Furthermore, the controlled inoculations in this study indicated the that *F. solani* is a major contributor to shisham dieback disease.

Studies related to the prediction of dieback disease of *D. sissoo* are scarce in the literature, and little work has been conducted on this serious problem. According to the findings of Sturrock et al. (2011) different environmental variables may have profound impacts on tree health, and can lead to dieback and mortality of forest stands. Climatic changes have been associated with dieback and death of forest trees and have also disturbed the health of other flora and of fauna (IPCC, 1990; Simpson, 1993).

According to the findings of nursery experiments, the incidence of disease after inoculation of sexually and asexually propagated *D. sissoo* plants with different fungal pathogens was correlated with different climatic factors, especially with maximum temperature and relative humidity. There is a general consensus that rapid changes in temperature and humidity disturb physiological processes which leads to disease.
development and even mortality of trees (Boland et al., 2004; Sturrock, 2007; Dukes et al., 2009; Tubby and Webber, 2010). Disease prediction models for seedlings and cuttings at the nursery level were developed to determine the role of different environmental variables in the disease development. Almost all climatic factors, including rainfall, relative humidity, minimum and maximum temperatures and wind velocity, played a role, although rainfall and maximum temperature were most important. According to Singh (1980) and Kaushal et al. (2002), mortality of shisham is strongly linked with extreme, summer and winter temperatures, drought periods and foggy conditions.

Dieback of *D. sissoo* is a major threat to this important tree species and has caused huge damage in all areas where the species is cultivated. In Pakistan dieback of shisham has been recorded in different regions but severity differs with region (Gill and Aziz, 2004). Part of the present work included a detailed survey of *D. sissoo* in four different agro-ecological zones of Punjab, Pakistan and considerable variation was observed in the incidence of dieback disease in the different zones. Approximately ten years ago, Bajwa et al. (2003) also surveyed the incidence of dieback in shisham plantations in all regions of Punjab.

In the present survey trees were classified into healthy, partially affected and fully affected, and disease incidence in three different age classes was also recorded. The survey showed that the Barani area was the least affected region, with 92.16% healthy trees in Rawalpindi district. This area, in the foothills of the Himalaya Mountains, is considered a zone where shisham is natural in Pakistan. Bajwa and Javaid (2007) also recorded a low incidence of dieback in the Barani area compared to other regions of the Punjab. In the present studies, the Suleman Piedmont zone was the most affected, with the highest proportions of dead and partially affected trees. A similar trend was reported by Bajwa and Mukhtar (2006) with high disease rate in Suleman piedmont and a lower number of affected shisham in Northern irrigated plains.

Furthermore, in each ecological zone, the main shisham grown districts were selected to determine the extent of variation in dieback between different districts. The greatest number of dead trees was recorded in the Toba Tek Singh and Faisalabad districts, whereas the Rawalpindi district was less affected, having high proportion of healthy trees. These results confirm the report of Khan et al. (2004) which showed a high disease rate in Toba Tek Singh and surrounding areas.

Tree age is considered an important factor in the development of forest dieback (Manion, 1991). In the present study, trees were divided in to three age classes. Class one
was up to 20 years, class two from 20 to 40 years and class three was above 40 years of age. In all ecological zones surveyed, a major difference was obvious regarding the tree mortality, where age class three was the most affected, followed by trees in age class two. Indian scientists (Baksha and Basak, 2000; Sharma et al., 2000) also reported that older and over mature trees were more susceptible to dieback disease. Sperry et al. (1991) suggested that in mature trees, membranes in the tracheids and vessels rupture easily under different environmental stresses including climatic, edaphic and biotic factors, which can lead to death of forest stands; this phenomenon seems true in shisham in the present study.

The data obtained in the present work also showed that younger trees of age class one were least affected by dieback, with high numbers of healthy and lower numbers of partially and fully affected trees, supporting the previous conclusions of Auclair et al. (2010). Fast growth rates of the roots of younger trees and adaptability to changing environmental conditions are considered to lower mortality rates of younger trees (Manion, 1991; Mueller-Dombois, 1993; Jurskis and Turner, 2002; Bi, 2004).

Position of the water table plays an important role in tree growth, because high and low water tables disturb growth processes. A high water table creates anaerobic conditions and deep percolation of water leads to drought conditions; consequently both hinder normal growth and metabolism (Adams et al., 1972; Floyd et al., 2009). In the present study dieback affected trees in different age classes were correlated with the variation in underground water tables and negligible disease symptoms were observed when the water table was at a depth of between 15 to 20 feet. Water tables below and above that range increased the disease severity. Dahiya et al. (2004) suggested that a high water table was responsible for dieback of both *D. sissoo* and *Acacia nilotica*. Present findings regarding the water table are also in line with the results of Solanki (2002) and Afzal et al. (2006).

The current work showed that tree age, and climatic factors including relative humidity, minimum and maximum temperature and wind velocity, were significantly correlated (*P*<0.05) with incidence of dieback disease in the different agro-ecological zones. Tree age showed a prominent and strong association with dieback disease in all the examined districts and zones. Amongst the climatic factors, maximum temperature and wind velocity were more important compared to other variables. Baksha and Basak (2000) and Sharma et al. (2000) correlated mortality of *D. sissoo* with different ages of trees, and found low disease incidence in the early stages of growth, but greater
susceptibility in older and larger trees leading to a high mortality rate compared to young plants. These results also support the conclusions of (Boland et al., 2004; Sturrock, 2007; Dukes et al., 2009; Tubby and Webber, 2010). The disease predictive model developed through regression analysis of the field survey data in this work utilized age class, environmental variables (minimum and maximum temperature, rainfall, relative humidity and wind velocity), time span (years) and underground water table to generate the model. Age class and time span played very important roles in the development of dieback under the field conditions. Hosking and Hutcheson (1992) showed that dieback of *Cordyline australis* was associated with old trees and attack of pathogens. Acharya and Subedi (2000) reported mortality of over mature dieback affected *D. sissoo* trees in a short period of even few weeks. Phillips et al. (2009) and Allen et al. (2010) observed an increasing trend in the forest dieback and mortality due to drought on the basis of literature available from 1984 to 2010. In 1984 only 1% tree mortality was linked with drought, whereas in 2010 the figure was 4%; the value of $R^2=0.61$ in the linear regression model showed a very strong association between tree mortality and drought conditions due to low rainfall and high temperature. These results support the findings reported here, that with the passage of time environmental stresses increased forest mortality. Different researchers (Negi, 2002; Sidhu et al., 2002; Chaudhry, 2006) observed that the reoccurrence of climatic changes in *D. sissoo* plantations for more than two years may seriously disturb plant growth and reduce resistance of plants to different pathogens which ultimately causes the death of the trees. These findings are confirmed in this present study.
Dieback of shisham (*Dalbergia sissoo*) is a serious threat to this commercially important tree species. This disease has caused huge losses and shisham timber is getting rare in the market. Demand, however, remains high due to the excellent quality wood. Fungi are the major causal organisms of dieback disease.

*D. sissoo* plants were raised in the nursery from both sexual and asexual propagation, using seeds and cuttings, respectively, and inoculated with fungi commonly isolated from dieback-affected shisham. *F. solani* caused the greatest amount of disease (31.39%) followed by *B. theobromae, C. lunata* and *G. lucidum*. Control plants remained healthy. Results were consistent over a two year period, 2009 and 2010. Seedlings were more susceptible than cuttings to disease development, with mean maximum disease symptoms of 17.24% as compared to 7.83% in cuttings. Disease incidence in seedlings inoculated with *F. solani* was 46.18%, whereas in cuttings it was 16.61%. Inoculation of other fungi produced greatest disease symptoms in seedlings as compared to cuttings. No disease appeared in plants inoculated with *G. lucidum* or in control plants. It was concluded, therefore, that *F. solani* was the fungus responsible for dieback disease. The highest disease incidence was recorded in nursery plants 8 weeks after inoculation with fungi. After 8 weeks in *F. solani* inoculated seedlings, maximum (77%) disease was recorded while in cuttings, it was 31%. In both seedlings and cuttings, the main increase in disease incidence occurred between 4 to 5 weeks after inoculation.

Disease incidence in seedlings and cuttings was correlated with different climatic variables i.e rainfall, relative humidity, minimum/maximum temperature and wind velocity. Correlations were significant, except for wind velocity with seedlings. Strong and positive associations were observed between maximum temperature and disease in both propagation methods, whilst strong negative associations were observed between relative humidity and disease in cuttings. Regression analysis confirmed the correlation analyses, where maximum temperature independently caused the greatest variation in disease with 53.9% in cuttings and 39.8% in seedlings.

A predictive model for disease in cuttings was developed using the environmental factors and disease data collected over two years (2009-2010):

\[ Y = -58.3 + 7.58x_1 + 0.0054x_2 - 1.14x_3 + 2.47x_4 - 1.09x_5 \]

\[ R^2 = 0.62 \]
where $Y=$ disease in cuttings, $x_1=$ rainfall, $x_2=$ relative humidity, $x_3=$ minimum temperature, $x_4=$ maximum temperature and $x_5=$ wind velocity.

The model showed that rainfall and maximum temperature were the most important environmental factors influencing disease incidence.

A similar predictive model was developed for disease in seedlings, using disease incidence and environmental variable data (2009-2010):

$$Y = -134 + 15x_1 + 0.158x_2 - 2.32x_3 + 5.27x_4 - 3.70x_5$$

$R^2 = 0.48$

where $Y=$ disease in seedlings, $x_1=$ rainfall, $x_2=$ relative humidity, $x_3=$ minimum temperature, $x_4=$ maximum temperature and $x_5=$ wind velocity

All environmental factors played a role in disease incidence in seedlings, but the main influencing factors were rainfall and temperature.

Both models were validated by comparing the observed and predicted data and secondly by comparing the dieback disease and regression coefficients with physical theory.

A comprehensive survey of four major agro-ecological zones of Punjab (Barani, Northern irrigated plains, Sandy deserts, Suleman Piedmont) was conducted to determine the severity and extent of damage to shisham in different tree age classes. Trees were classified into healthy, partially affected and fully affected, in three age classes, I (within 1-20 years old), II (21-40 years) and III (over 40 years). In this survey major shisham grown districts were chosen to include in the study.

In the selected zones, the greatest number of healthy trees was recorded in the Barani area, where 92.16% of trees in age class I were healthy, whereas in the Suleman piedmont zone 55.99% of trees in age class III were healthy. The percentage of partially affected trees was higher (22.89%) in age class III the Suleman piedmont, but fewer numbers of trees affected in this way were found in the Barani areas. For fully affected or dead trees, little difference occurred between the northern irrigated plains, sandy deserts and Suleman piedmont in age class I and II, but in age class III the highest occurrence was in the northern irrigated plains where a 23.60% mortality rate was recorded. As compared to other zones, a low mortality rate was recorded in all age classes in the Barani area (4.68% in class I, 8.85% in II and 11.17% in III).

In selected districts of the Punjab, the highest numbers of healthy trees were found in Rawalpindi (95% in age class I) and the fewest healthy trees were observed in Toba Tek Singh (35% healthy trees in age class III). The highest numbers of partially affected trees were recorded in age class III (22.89%) trees in Dera ghazi khan, whereas only
3.16% of such trees were recorded in age class I in the Rawalpindi district. Data on fully affected or dead trees showed that Toba Tek Singh was the most affected district with 33% dead trees in age class III, followed by Faisalabad with a 25% mortality rate in class III. The Rawalpindi district was the least affected with fewer dead trees in all age classes.

Data from the different zones and districts showed that Barani zone was the least affected and the Northern irrigated zone was most affected. In all zones studied, older trees in age class III were the most badly affected. A similar trend was observed in all districts, where age class II was the most severely damaged. The highest mortality rate was observed in Toba Tek Singh and the least in Rawalpindi.

An effect of water table depth on shisham mortality in different age classes was observed. A water table depth of 15-20 feet was the most suitable for shisham growth, resulting in low rates of mortality in all age classes, whereas higher death rates were recorded where water tables were below or above this depth.

Correlations of dieback disease incidence in different ecological zones of Punjab with tree age, relative humidity, minimum/maximum temperature and relative humidity were significant, whereas rainfall and water table were not significant. Regression analysis confirmed the results of the correlations, in which each factor was studied independently.

A disease predictive model based on two years disease incidence and other variables data was developed:

\[ FA = 2.51 + 4.15 x_1 + 5.25 x_2 + 0.00866 x_3 - 0.113 x_4 - 0.0611 x_5 - 0.0115 x_6 - 0.0092 x_7 + 0.541 x_8 \]

\[ R^2 = 0.89 \]

where \( FA \) = fully affected, \( x_1 \) = age, \( x_2 \) = year, \( x_3 \) = water table, \( x_4 \) = rainfall, \( x_5 \) = relative humidity, \( x_6 \) = minimum temperature, \( x_7 \) = maximum temperature, \( x_8 \) = wind velocity.
Conclusions and Recommendations

Based on the data obtained in this work, and the above discussion, the author suggests the following tentative conclusions:

- *Fusarium solani* caused the greatest dieback disease in the shisham nursery compared to the other fungi tested. *F. solani* can be considered as the major fungus responsible for the dieback of *Dalbergia sissoo*. Suitable fungicides should be used for the control of the fungus.

- Greatest disease incidence occurred in inoculated seedlings whereas in cuttings, disease incidence was lower. In the future the planting of cuttings for the propagation of shisham should be promoted.

- Optimum temperature and humidity should be maintained during the nursery stages, to discourage the growth of fungus

- Older and mature trees were mostly affected from dieback disease. Mature trees should be harvested as soon as possible and replaced with younger ones derived from cuttings.

- Disease incidence was lower where the water table was at a depth of between 15-20 feet. It is recommended, therefore, that trees be planted where the water table is at this depth.

- Less disease occurred in the natural zone of shisham, compared to other zones and districts. It would be useful, therefore, to cultivate more trees in that area and in other areas with similar environmental conditions. New irrigated plantations of shisham should be developed in such areas.
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## APPENDICES

**Appendix 1: Mean percentage of healthy trees in different districts of Punjab according to age classes**

<table>
<thead>
<tr>
<th>Districts</th>
<th>Healthy Age Classes</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±STDEV 1</td>
<td>Mean±STDEV 2</td>
<td>Mean±STDEV 3</td>
<td></td>
</tr>
<tr>
<td>Bahawalpur</td>
<td>77.62±3.27</td>
<td>67.22±3.96</td>
<td>63.96±5.01</td>
<td></td>
</tr>
<tr>
<td>DG_Khan</td>
<td>69.27±4.94</td>
<td>64.86±7.72</td>
<td>55.99±5.45</td>
<td></td>
</tr>
<tr>
<td>Faisalabad</td>
<td>75.68±5.66</td>
<td>67.87±6.45</td>
<td>55.71±7.24</td>
<td></td>
</tr>
<tr>
<td>Lahore</td>
<td>78.69±3.89</td>
<td>70.42±6.30</td>
<td>58.54±6.28</td>
<td></td>
</tr>
<tr>
<td>Multan</td>
<td>73.26±4.35</td>
<td>67.90±6.71</td>
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<td></td>
</tr>
<tr>
<td>Okara</td>
<td>77.83±4.92</td>
<td>72.48±5.61</td>
<td>59.44±5.90</td>
<td></td>
</tr>
<tr>
<td>RahimYar_Khan</td>
<td>80.79±6.21</td>
<td>68.33±7.89</td>
<td>60.53±5.90</td>
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<tr>
<td>Rawalpindi</td>
<td>92.16±4.84</td>
<td>83.41±4.80</td>
<td>77.65±5.81</td>
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<tr>
<td>Sahiwal</td>
<td>80.48±2.18</td>
<td>70.23±4.73</td>
<td>68.10±3.56</td>
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</tr>
<tr>
<td>Sargodha</td>
<td>83.48±3.95</td>
<td>73.68±4.03</td>
<td>64.63±3.48</td>
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</tr>
<tr>
<td>TTSingh</td>
<td>70.93±7.19</td>
<td>59.28±9.25</td>
<td>48.12±8.86</td>
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**Appendix 2: Mean percentage of partially affected trees in different districts of Punjab according to age classes**

<table>
<thead>
<tr>
<th>Districts</th>
<th>Partially affected Age Classes</th>
<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Mean±STDEV 1</td>
<td>Mean±STDEV 2</td>
<td>Mean±STDEV 3</td>
<td></td>
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<tr>
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<tr>
<td>DG_Khan</td>
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<td>18.83±4.33</td>
<td>22.87±2.60</td>
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<tr>
<td>Faisalabad</td>
<td>10.40±2.62</td>
<td>12.37±3.23</td>
<td>18.90±3.64</td>
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<tr>
<td>Lahore</td>
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<td>12.39±3.17</td>
<td>18.57±3.08</td>
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</tr>
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<td>Multan</td>
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<td>16.17±3.75</td>
<td>18.30±4.45</td>
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<tr>
<td>Okara</td>
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<td>11.18±2.98</td>
<td>17.28±4.51</td>
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</tr>
<tr>
<td>RahimYar_Khan</td>
<td>8.49±2.42</td>
<td>15.01±2.18</td>
<td>16.90±2.81</td>
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<tr>
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<td>7.74±2.14</td>
<td>11.18±3.09</td>
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<tr>
<td>Sahiwal</td>
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<td>15.15±2.52</td>
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<tr>
<td>Sargodha</td>
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<td>TTSingh</td>
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### Appendix 3: Mean percentage of fully affected trees in different districts of Punjab according to age classes

<table>
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<th>Districts</th>
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<tr>
<td>DG Khan</td>
<td>12.46±3.64</td>
</tr>
<tr>
<td>Faisalabad</td>
<td>13.91±3.27</td>
</tr>
<tr>
<td>Lahore</td>
<td>11.88±2.97</td>
</tr>
<tr>
<td>Multan</td>
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<tr>
<td>Okara</td>
<td>11.61±2.80</td>
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<tr>
<td>RahimYar Khan</td>
<td>10.72±3.95</td>
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<tr>
<td>Rawalpindi</td>
<td>4.68±3.54</td>
</tr>
<tr>
<td>Sahiwal</td>
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### Appendix 4: Mean percentage of healthy trees in different zones of Punjab according to age classes

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<td>Barani_area</td>
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<tr>
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<tr>
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<tr>
<td>Suleman piedmont</td>
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<tr>
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Appendix.5: Mean percentage of partially affected trees in different zones of Punjab according to age classes

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<td>Barani_area</td>
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<tr>
<td>Sandy desert</td>
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<td>18.31±4.65</td>
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Appendix.6: Percentage of fully affected trees in different zones of Punjab according to age classes

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<tr>
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<tr>
<td>Barani_area</td>
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<td>4.68±3.54</td>
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<tr>
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<td>12.46±3.64</td>
<td>16.31±5.05</td>
<td>21.14±3.17</td>
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<tr>
<td>Total</td>
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