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DIEBACK DISEASE PREDICTIVE MODEL FOR SEXUALLY AND ASEXUALLY PROPAGATED *DALBERGIA SISSOO* (SHISHAM)

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Abstract

Dieback disease is a potential threat to *Dalbergia sissoo* **(**Shisham) which is a multipurpose tree of the Indian subcontinent. Different factors have been found associated with inciting shisham dieback. Fungal pathogens have been recognized as the major causal organism but changing climate is a main threat to forest dieback. Sexually (seedlings) and asexually (cuttings) propagated shisham were inoculated with the different fungi (*Fusarium solani, Botryodiplodia theobromae, Curvularia lunata* and *Ganoderma lucidum*). As environmental factors play critical role in the development of the disease, so the present study was designed to observe the impact of rainfall, temperature, relative humidity and wind velocity on the disease and for the management of disease predictive model was developed. A significant negative correlation was observed between disease and relative humidity both for seedlings ($r = -0.97$) and cuttings ($r = -0.487$), respectively while maximum temperature expressed significant positive correlation with seedlings and cuttings with coefficient of correlation $r = 0.734$ and $r = 0.629$, respectively. Path analysis expressed that with one unit increase in rainfall the disease would rise by 7.58 and 15.04 and for maximum temperature it was 2.47 and 5.27 units in seedlings and cuttings, respectively. Multiple regression analysis showed that coefficient of determination (R^2) value was 0.62 and 0.48 for cuttings and seedlings, respectively. Normed fit index (NFI) and comparative fit index (CFI) values indicate that model is quite a good fit. Similarly comparison of observed and predicted data also validated the model for forecasting the disease.

Key words: *Dalbergia sissoo*; Dieback; Seedlings and cuttings; Environmental variables; Path model.

Introduction

Shisham (*Dalbergia sissoo*) is a versatile tree of Indian subcontinent and native to Terai belt of Nepal (Khan, 2000). This multipurpose tree is also cultivated in tropical and subtropical areas of different continents of the world (Tewari, 1994; Afzal *et al.,* 2006). In Pakistan it is commonly found in the foothills of Himalayan mountains but can also grow in a variety of soil types (Champion *et al.,* 1965; Orwa *et al.,* 2009). Because of its nitrogen fixing properties and multiple uses it is considered as an important tree for agro-forestry practices (Idrees *et al.,* 2006; Kausar *et al.,* 2009; Lal & Singh, 2012).

Since the last few decades shisham is under severe threat of dieback disease in Pakistan (Ahmad *et al.,* 2013), India (Sharma *et al.,* 2000), Bangladesh (Tantau *et al.,* 2005) and has affected millions of trees in these countries (Vogel, 2011). Some common symptoms of diseased shisham trees are the desiccation of leaves and branches, then change in colour and wilting of crown which leads to the death of tree (Baksha & Basak., 2000; Tantau *et al.,* 2005). Symptoms of dieback disease of shisham almost similar like the dieback of mango in southern parts of Pakistan (Khan *et al.,* 2014). Because this disease is a complex, so both biotic and abiotic factors are considered as a cause of shisham dieback (Sharma *et al.,* 2000; Basak *et al.,* 2003). Different biotic and abiotic stresses including drought, heat, pathogens, insects etc, disturb the normal physiological functions of plants (Bukhari *et al.,* 2015; Naz *et al.,* 2015). Climate change along with imbalance nutrients and fungal pathogens have been assumed to be associated with the decline of forest trees around the globe (Simpson, 1993).

Fungal pathogens including *Fusarium solani, Fusarium oxysporum, Phytohpthora cinnamomi, Curvularia lunata, Ganoderma lucidum, Rhizoctonia solani* have been recognized as the possible causal organisms responsible for the sudden death and decline of shisham in the Indian subcontinent (Bajwa *et al*., 2003; Pathan *et al*., 2007; Ahmad *et al*., 2013). The fluctuating environmental conditions (temperature and humidity levels) influence the behavior and biological activity of trees and pathogens (Boland *et al.,* 2004; Dukes *et al.,* 2009; Tubby & Webber, 2010). Rising temperature is not considered as the only cause of forest dieback (Auclair *et al.,* 2005). Short term climatic changes throughout the world have disturbed the ecosystems and wrecked the forest dieback (Anon., 2001). Seasonal droughts have intensified the tree mortality in tropical forests (Rolim *et al.,* 2005) and a very strong association has been observed between tree mortality and low rainfall along with fluctuating temperatures (Allen *et al*., 2010). High mortality of shisham in Amritsar and Bathinda regions of India between1995-2000 was strongly correlated with low precipitation and rising temperatures (Kaushal *et al.,* 2002).

Increasing mortality of forest trees has disturbed the forest professionals and policy markers through the globe. Under the changing climate scenario researchers and policy makers has recommended four categories for the successful management of forest diseases which are: monitoring, predicting, proper planning, and make use of mitigating approaches. The features of these strategies have been practiced in the USA for the proper management of declines of aspen and cedar.

Shisham dieback was linked with changing climatic conditions including extremes of summer and winter, drought stresses with severe foggy conditions which adversely affected the physiological processes of the trees (Singh, 1980; Kaushal *et al.,* 2002). Previously different studies have been carried out to study the affect of different fungal pathogens on the growth of shisham at nursery stage but no study has examined the affect of environmental factors on the appearance of disease on sexually and asexually propagated shisham plants. The following study was designed to check the effect of environmental variables (rainfall, relative humidity, minimum and maximum temperature, wind velocity) on the sexually and asexually propagated plants of *Dalbergia sissoo* artificially inoculated with different fungi (*Botryodiplodia theobromae, F. solani, C. lunata,* and *G. lucidum*).

Materials and Methods

The study was carried out in the nursery of the Department of Forestry and Range Management, University of Agriculture, Faisalabad for two years 2009 and 2010. Planting material for the study was collected from the Irrigated plantation of the Punjab Forest Department and raised in nursery for the experiment during 2008 and 2009. For sexual propagation seedlings of *Dalbergia sissoo* were grown from the healthy seeds collected from the disease free and plus trees. Similarly for asexual propagation cuttings of 23 cm length and 1-1.5 cm diameter were used. To create the homogeneous conditions during the study polythene tubes were used to grow experimental material. Plants grown during 2008 were used for study during 2009 and similarly grown during 2009 were under trial in 2010. Plants of same height both from sexually and asexually propagated plants were chosen for the inoculation trials of different fungi (*G. lucidum, C. lunata*, *B. theobromae* and *F. solani*) most commonly isolated from the diseased trees of *D. sissoo*.

Inoculation was carried out by making one cm long cut at about 6 cm above the ground level with the help of sterilized knife to injure the bark to some extent. Preparation of spore suspension for all fungi was carried out separately from 1 to 2 week old culture on the potato dextrose agar medium and was adjusted at 2.6×10^6 spores/ml by counting with haemocytometer. 2 ml spore suspension was used for injection into the wounds of both sexually and asexually propagated plants (Shakya & Lakhey 2007; Rajput *et al.*, 2008). Disease development data after the fungal inoculation were recorded for a period of 2 months during 2009 and 2010. Randomized complete block design was used for trials (RCBD), with three blocks, ten plants receiving each treatment and fifty plants in each block of seedlings and cuttings.

Statistical analysis

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 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 $p<0.05$). The influence of each variable on dieback disease was determined by correlation and regression analysis (Chatterjee & Hadi, 2006). A multiple regression model $Y= \beta_0+\beta_1x_1+\beta_2x_2+\cdots+\beta_i x_i+$□ and path analysis were performed to identify the magnitude of each environmental variable towards disease development (Steel *et al*., 1997). R– square and mean square error (MSE) were the criteria used to select the best models (Myers, 1990).

Results

Relationship of environmental variables with dieback disease of *D. sissoo* in cuttings and seedlings: The correlation between environmental factors (rainfall, relative humidity, minimum and maximum temperature and wind velocity) with dieback in cuttings was significant ($p<0.05$). The effect of relative humidity and maximum temperature were most prominent. A significant negative correlation was observed between disease and relative humidity both for seedlings (r = -0.97) and cuttings (r = -0.487), respectively while maximum temperature expressed significant positive correlation with seedlings and cuttings with coefficient of correlation r = 0.734 and r = 0.629, respectively. A week association was observed b/w minimum temperature, rainfall, wind speed with disease incidence both in case of seedlings (r = 0.350, 0.294, 0.044) and cuttings (r = 0.434, 0.327 and 0.115), respectively.

The data was further analyzed using regression analysis taking each factor separately as an independent variable. With cuttings, 53.9% while with seedling 39.8% variation in dieback disease was explained by maximum temperature independently while relative humidity explained 36.2 and 25.3% variability in disease incidence with cuttings and seedlings respectively. Similarly minimum temperature expressed 10.8, 12.8% and wind speed 1.4 and 0.3% dieback disease variability with cuttings and seedlings (Table 1 and Fig. 1).

Table 1. Regression equations showing the relationships between environmental variables and disease incidence in cuttings and seedlings of *D. sissoo*.

Regression equations	R^2	r	SE
Cuttings = 9.52-6.18 Rainfall (mm)	10.8	-0.327*	8.70
Cuttings = 40.2-0.473 R.H (%)	36.2	-0.97*	7.36
Cuttings = -9.37+0.954 Min. Temp. (°C)	19.1	0.434*	8.28
Cuttings = -38.7+1.34 Max. Temp. (°C)	53.9	0.734*	6.25
Cuttings = 6.54+1.52 wind speed (Km/h)	1.4	0.115*	9.17
Seedlings = 20.7-12.4 Rainfall (mm)	8.4	-0.294*	20.11
Seedlings = 79.1-0.904 R.H (%)	25.3	-0.487*	18.15
Seedlings = -14.8-1.78 Min .Temp. (°C)	12.8	0.350*	19.62
Seedlings = -74.0+2.63 Max. Temp. (°C)	39.8	0.629*	16.30
Seedlings = 16.0+1.63 Wind speed (Km/h)	0.3	0.044*	20.98

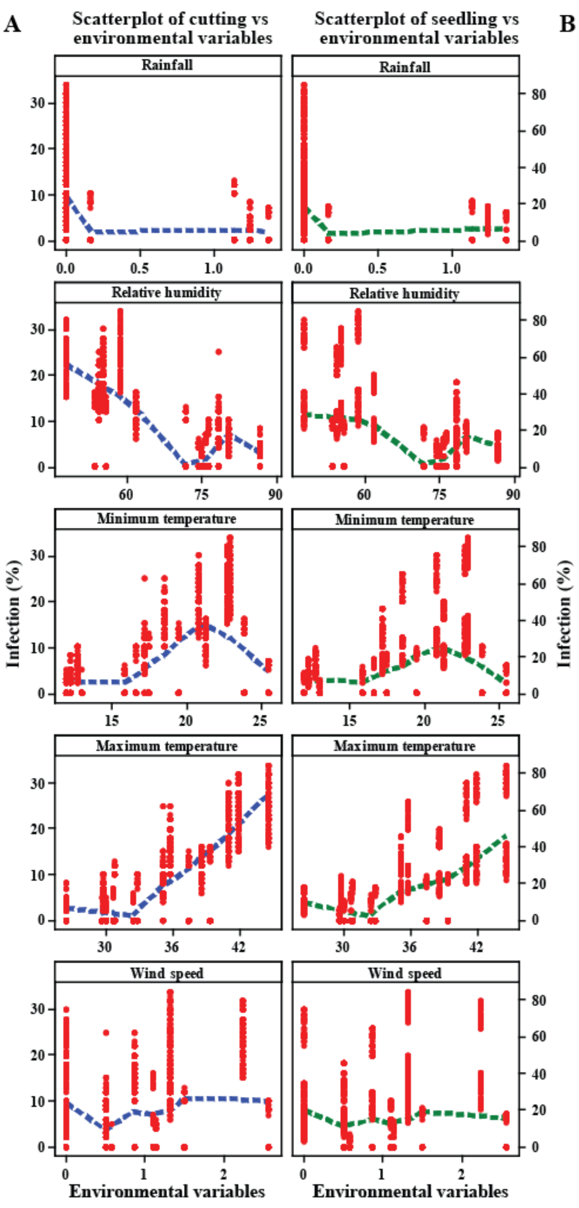

Fig. 1. Relationships between environmental variables and disease incidence in cuttings (A) and seedlings (B) of *D. sissoo.*

Path model for seedlings and cuttings: Path analysis was performed to identify the magnitude of each environmental variable towards disease incidence. Where Y= Disease in cuttings, x_1= Relative humidity, x_2= Rainfall, x_3= Minimum temperature, x_4= Maximum temperature and x_5= Wind velocity. The statistically justified model (Squared Multiple Correlations = 0.62 for seedlings and SMC for cuttings = 0.481 at p□0.05) was used to predict the probable attack of dieback at the nursery stage under a given set of environmental variables. Results of the path analysis clearly indicated that the main environmental factors associated with incidence of dieback disease were rainfall, temperature and wind speed (Table 2). Path model expressed that with one unit increase in rainfall, the probability of developing the disease would rise by 7.58 units in case of seedling for cuttings it was 15.04. The decrease in disease incidence would be -1.14 units with a one unit change in minimum temperature for seedlings and for cuttings -2.32 units. Similarly with a one part increase in maximum temperature, disease will increase by 2.47 parts for seedlings and for cuttings the increase in disease would be 5.27 units, whereas higher wind speeds will result in less disease both for seedlings and disease, respectively. The associated variances and covariance have also been shown in the path model (Fig. 2) with error magnitude of 32.07 231.42 for cuttings and seedlings, respectively.

Model validation: The model was validated using the procedures described by the Chatterjee & Hadi (2006).

1. Evaluation of model fit summary with physical theory,
2. Comparison of observed and predicted data.

The path model (Table. 3) was significant at $p<0.05$ and root mean square residual (RMR) of zero and goodness-of-fit index (GFI) of one indicates a perfect fit (Hu & Bentler, 1999) NFI (normed fit index) indicates the proportion of improvement of the overall fit of the model relative to the independence model. The CFI (comparative fit index) is also a descriptive fit index and is interpreted in the same way as the NFI, but may be less affected by sample size. For these indices, values close to 1 are generally considered to indicate a good fit, so our values for NFI (1) and CFI (1) suggest that our model is quite a good fit.

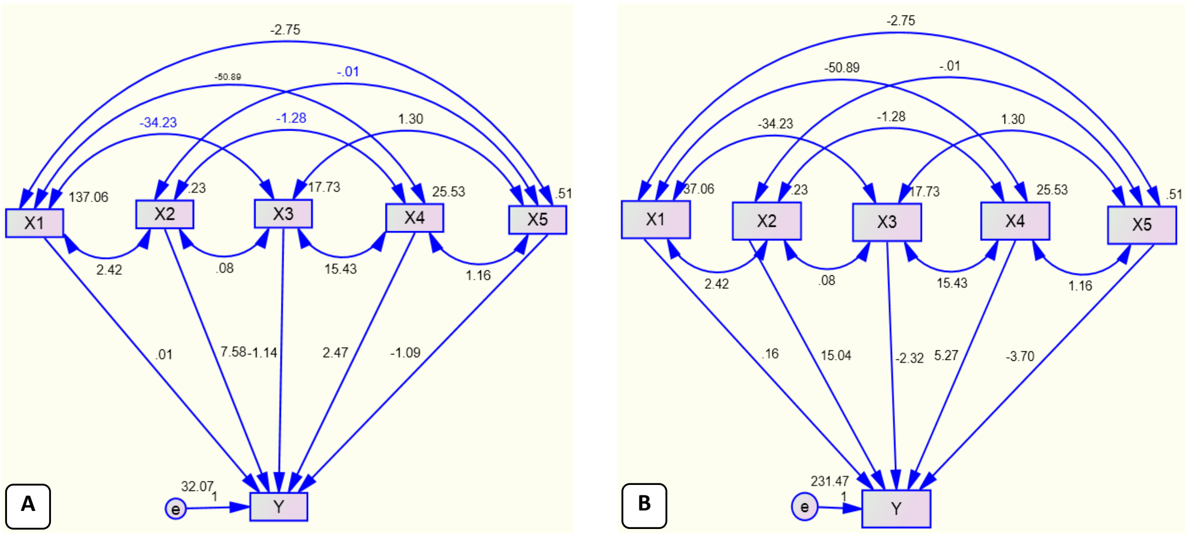

Fig. 2. Path model for A) cuttings B) seedlings with environmental variables.

Table 2. Regression estimates of path model for seedlings / cuttings.

Dependent variable	Independent variable	Seedlings/ cutting	Estimate	S.E.	C.R	P
Y = Disease incidence	x_1=Relative humidity	Seedlings	0.158	0.053	2.99	***
		Cuttings	0.005	0.02	0.274	0.784
Y = Disease incidence	X =Rainfall	Seedlings	15.043	1.076	13.986	***
		Cuttings	7.583	0.4	18.941	***
Y = Disease incidence	x_3=Mini. temperature	Seedlings	-2.325	0.16	-14.496	***
		Cuttings	-1.144	0.06	-19.165	***
Y = Disease incidence	x_4= temperature	Seedlings	5.272	0.167	31.568	***
		Cuttings	2.471	0.062	39.75	***
Y = Disease incidence	x_5= Wind velocity	Seedlings	-3.697	0.478	-7.727	***
		Cuttings	-1.092	0.178	-6.132	***

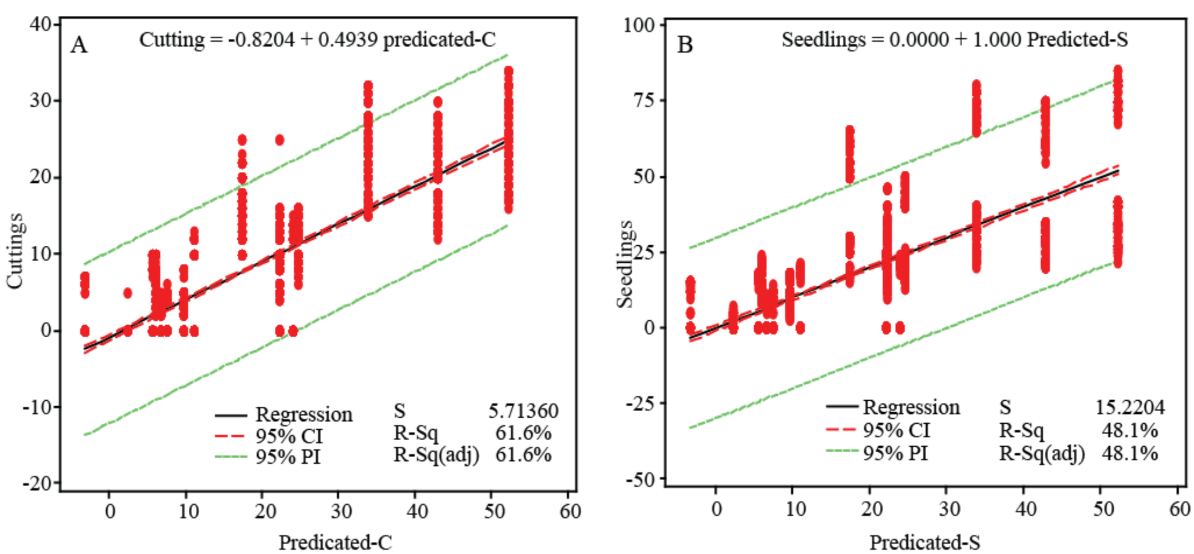

Fig. 3. Fitted line plot for dieback disease in cuttings (A) and seedlings (B) based on predicted and observed data points at 95% confidence and predictive interval.

Table 3. Model fit parameters for seedlings and cuttings

Model fit parameters	Estimates	
	Seedlings	Cuttings
Root mean square residual	0.00	0.00
Goodness-of-fit index	1.00	1.00
Normed fit index	1.00	1.00
Comparative fit index	1.00	1.00
Squared multiple correlations	0.481	0.623

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 3 that despite an R^2 = 0.61 0.48 (for cuttings/seedlings), 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.

Discussion

Forests are very important for ecological functions as well as for monetary, physical and spiritual health of human beings. Climate has always played an important role in shaping the Earth's forests, but over the past 2-3 centuries, human interference due to the exploding population has remarkably changed the world's climate. The interaction of the climate change with the forest disturbances including pathogens, insects and fire has mainly affected the sustainability, growth and geographic distribution of the world's forests. Indigenous and introduced forest pathogens are considered to be responsible for epidemic of certain forest diseases, which become more disastrous and severe in dry spells and other stresses. Under the climate change circumstance with unpredictable future temperatures, it is predicted that different forest diseases like dieback, decline caused by pathogens were exaggerated by climatic disturbances (Van Mantgem *et al*., 2009).

Management of forest plant diseases like shisham decline is greatly influenced by environmental factors. Because not much information is available about the affect of climate change on forest trees at nursery stages, therefore it was difficult to predict the effect of environmental factors on disease development. That is why present study was planned to observe the affect of climate changes on the occurrence of decline disease in *D. sissoo* which are grown from seeds and from cuttings. Correlation, regression and path models revealed that maximum temperature, relative humidity and rainfall have critical role in the disease development. Disease predictive models both for sexually and asexually propagated *D. sissoo* plants were developed which will be very helpful in formulating forest plant disease management strategies. The results of present studies are in line with Chakraborty & Pangga, (2004) who observed that temperature, light relative humidity and moisture, wind and the presence of inoculums played a significant role in the occurrence, development and spread of forest plant diseases while Ghini *et al.* (2008) reported that plant growth and success depends upon the presence of several abiotic, or non-living, environmental factors. The effect of environmental factors such as temperature, relative humidity, wind speed and rainfall are important to the disease progress as such factors play a significant role in disease development.

Climate indirectly affects the pathogens and these pathogens infect the hosts that are stressed by fluctuating weather conditions. These pathogens infect the healthy host and remain dormant until the host is stressed. Temperature and moisture affect the ability of pathogens to sporulate, spread and infect new hosts. For example, an increased incidence of summer drought will enhance the chance that trees will be infected by pathogens whose activity is facilitated by host stresses such as root pathogens, wound colonizers and latent colonizers of sapwood (Brasier & Scott, 1994; Lonsdale & Gibbs, 2002; Desprez-Loustau *et al*., 2006). The environment can affect host plant growth and vulnerability; pathogen reproduction, dispersal, survival and activity; as well as host-pathogen interaction. Rapid changes in the maximum temperature, wind and humidity and other climatic factors disturb the physiological processes which leads to disease development and ultimately the death of trees (Boland *et al.,* 2004; Dukes *et al.,* 2009; Tubby & Webber, 2010; Allen *et al*., 2010). These findings are confirmed in this present study. Warm temperature and consequent moisture deficiency were considered the cause of increase of mortality among trees of all sizes. It was concluded by recent assay on drought and heat induced mortality that some of the world's forested ecosystems may already be affected by climate change, and further increase in mortality should be expected even in water-limited environments.

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