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TREES SURVIVAL EXPOSED TO DIEBACK DISEASE IMPLIES EVOLUTIONARY MODULATION RESISTANCE IN SHISHAM (*DALBERGIA SISSOO* ROXB .) IN VARIOUS AGRO ECOLOGICAL ZONES OF PUNJAB (PAKISTAN)

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A B S T R A C T

Plant pathogens cause mortality of species, effect on host population and composition of natural plant communities. Current study was conducted to evaluate ecological reason of possible survival of *Dalbergia sissoo* Roxb. (shisham) plantations after massive damage by dieback disease in various zones of Punjab, Pakistan. A broad base survey was conducted in 2005 and 2009 to assess morality percentage in five ecological zones of Punjab. Among selected zones, shisham dieback incidence was high in Barani (V) and Northern irrigated plains (IV) zones as compared to sandy deserts (IIIA & IIIB) and Sulaman piedmont (X) in 2005. The difference in dieback disease incidence revealed that variations in the physical environment have played a significant role in the development and spread of shisham dieback in agro ecological zones of the Punjab. It seemed that temperature and moisture influenced both pathogen virulence and host plant resistance responses. Current study concludes that thinning of shisham plantations is evident of high mortality in last decade; however the variation in shisham biotypes apparently is the major reason of shisham survival from high disease epidemic during 1998 to 2005. It seems those so called resistant biotypes have been evolved due to new genetic recombination and are the progeny of susceptible varieties that have almost vanished. At present stage, selection of improved gemplasm is crucial for success of plantation programme, in Punjab, Pakistan.

Keywords: Dieback, Shisham, resistance, *Dalbergia sissoo*, Punjab

INTRODUCTION

Dalbergia sissoo Roxburgi, (shisham) is an important and established forest species in Pakistan, Bangladesh, Nepal, Bhutan, India, and Afghanistan However, this precious tree is facing many devastating challenges from last six decades that dragging it towards disappearance from the subcontinent. Dieback and wilting are the two main diseases in Shisham plantations. last two decades, these two diseases has changed the forest situation in subcontinent altogether. Ecological study suggest that when plant pathogens cause mortality and reduce fertility of individual plants, drive host population dynamics, and affect the structure and composition of natural plant communities (Gilbert, 2002). However, there is a non-linear association between transmission and size of epidemics: small changes in the transmission potential may result in large differences in the dynamics of the epidemic. Changes in the disease transmission potential can be due to a wide variety of reasons such as changes in host ecology and environment, changes in host distribution, changes in host phenotype, changes in host genetics and changes in pathogen genetics (Woolhouse *et al*., 2005). The genetic variation within a host species is important for the survival of such a species in the long term. Up till now, almost nineteen varieties of Shisham have been identified on the basis of physical appearance of the plant (such as branching pattern, pod characters, leaf and leaflet size and shape, branching and leaf density and stem surface characteristics) and genetic (Javaid *et al.,* 2003 & 2004). A wide variation means that individuals in a population can adapt to new conditions, such as drought or a new pest or disease. However, pathogens are responsible for both numerical changes in host populations and evolutionary changes through selection for resistant genotypes. During a survey in 2009, a high percentage of healthy population of shisham trees was observed in

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plantations that encouraged exploring this phenomenon on the basis of pervious and current data. Keeping in view ecological and evolutionary dynamics in population, present study was conducted to compare the dieback morality trend in 2005 and 2009 to find out ecological reason of possible survival of shisham plantations against dieback disease in various zones of Punjab, Pakistan.

MATERIALS AND METHODS

Survey of different agro-ecological zones of Punjab

Site Selection: The survey sites were selected on the basis of shisham (*Dalbergia sissoo*) distribution in the various districts and zones of Punjab (Khan and Khan, 2000) as shown in Figure 1. To assess disease incidence in diverse shisham stands, site categories such as natural/managed stands, river-sides/canal sides, road sides and agricultural lands were extensively surveyed.

The detail of selected survey sites in various zones is as follows:

(a) Sandy Desert Zone (IIIA & IIIB): These two zones collectively cover a part from certain districts of Sindh and Punjab Province. Sandy desert-IIIA consists of Rahim Yar Khan (R. Y. Khan), Bahawalpur, Bahawalnagar districts and Cholistan desert, whereas sandy desert-IIIB comprises the areas of Muzaffargarh, Mianwali, Bhakkar, Khushab and Layyah. The climate is arid with very hot summer, mild winter and annual rain fall of 150-350 mm. The original tree vegetation consists of *Prosopis cineraria*, *Salvadora oleoides*, *Tamarix aphylla, T. dioca* and *Tecoma undulata*, whereas, *Calligonum polygonoides*, *Calotropis procera*, *Salsola foetida, Zizyphus nummularia* and *Haloxylon* spp., represent the shrubs. The major grass species include *Cymbopogon javarancusa,* Eleusine *compressa*, *Lasirus hirsutus*, *Saccharum benglense*, *Panicum antidotale* and *Pennisetum divisum,* used for grazing. However, the vegetation is sparse and lopped heavily for fuel, fodder and hutments.

(b) Northern Irrigated Plains Zone (IV): The districts of Multan, Sahiwal, Sargodha, Faisalabad, Lahore, Sheikhupura, Gujranwala, Sialkot, Kasur and Okara were selected as survey sites in this zone due to rich shisham plantations. The climate in this zone is semi-arid to arid with annual rainfall of 300 to 500 mm in the east and 200 to 300 mm in the southwest. The soils are sandy, clay-loam and loam. The canal-irrigated crops grown are wheat, rice, sugar cane, oilseed and millet in the north and wheat, cotton, sugar cane, maize, citrus and mangoes in the center and south.

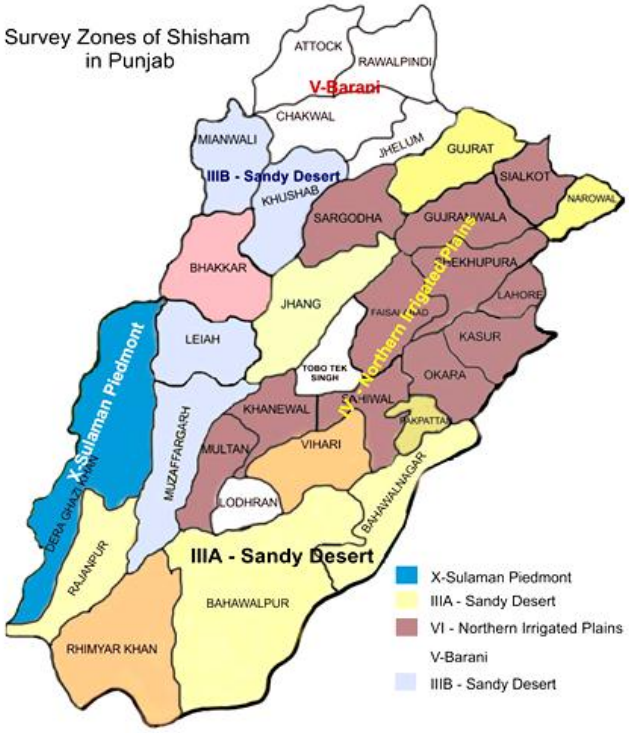


Figure 1. Map of Punjab province showing selected agroecological zones to assess the Shisham dieback disease distribution trend during 2005& 2009.

(c) Barani (rainfed) Lands Zone (V): The main shisham growing districts viz., Jehlim, Chakwal, Rawalpindi, Attock were selected for the dieback assessment in this zone. This covers the Salt Range, Potohar Plateau and Himalayan piedmont plains. In the North mean monthly rainfall is 200 mm in summer and 35 to 50 mm in winter. The climate in the southern part is semi-arid and hot. The main crop cultivation includes wheat, millet, oilseed and pulses.

(d) Sulaman Piedmont Zone (X): Plains of the Sulaman range with arid and hot climate, mean monthly rainfall is less than 15 mm. Irrigation relies on floods of the hill torrents. Wheat, millet and gram are the main crops. Dara Ghazi Khan (D.G Khan) was only district selected for disease assessment in this region.

Survey: A survey of 21 districts in five agro ecological zones was carried out during 2005 and 2009. Shisham irrigated plantations (government / private), linear plantations (canal banks and roadsides) and agricultural lands were the survey sites in above-mentioned zones. For the visual observation tree stands were selected randomly at each site to assess disease prevalence, incidence, severity, and disease index. Each stand consisted of maximum ten trees. Prevalence was calculated with the help of following formula:

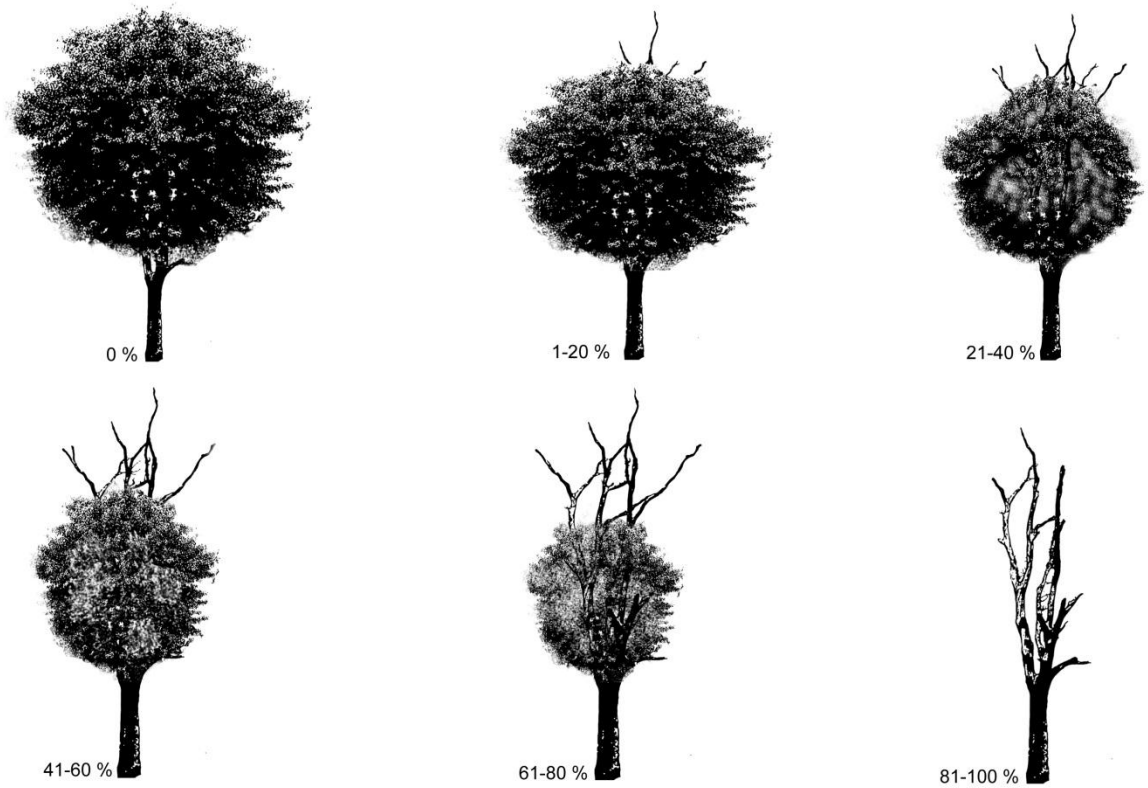
$$\text{Prevalance \%} = \frac{\text{Standars showing dieback symptoms}}{\text{Total number of locations}} \times 100$$

Disease incidence was calculated with the help of following formula:

$$\text{Disease Incidence} = \frac{\text{Total number of trees}}{\text{Total number of examined trees}} \times 100$$

Disease severity was based on visual rating scales i.e. in case of stagheadness, mortality status of the trees in strand were categorized as (i) healthy, (ii) low mortality (less then 20%), (iii) medium mortality (21-40%), (iv) high mortality (41-60%), (v) sever mortality (61-80%) and (vi) dead tress (81-100%). Disease index was calculated with the help of following given below. Rhizospheric soil were also collected and sealed in sterilized air tight plastic bags for further soil pH and texture studies. Soil samples were collected randomly from 0-30 inches depth in the vicinity of healthy and diseased shisham trees.

$$\text{Diseease Index} = \frac{1(\text{Tree in class 1}) + 2(\text{Tree in class 2}) + 3(\text{Tree in class 3}) \ldots\ldots + n(\text{Trees in class n})}{\text{Total number of trees in a location}} \text{ x } \frac{100}{n}$$



Sample Preparation and pH Measurement of Soils: The rhizospheric soil pH of both infected and healthy trees was determined by standard electrode method. A 10gm of soil was added in 50 mL of distil sterile water and continuously stirred for 5 min. The soil suspension was kept undisturbed for about 1 hr to allow most of the suspended clay to settle down from the suspension for pH measurement. Electrodes were adjusted in the clamps of the electrode holder so that upon lowering the electrodes into the beaker, the glass electrode would be immersed just deep enough into the clear supernatant to establish a good electrical contact through the ground-glass joint or the fiber-capillary hole. Data for soil texture was taken from record of Soil Survey of Pakistan Lahore.

Statistical Analysis: Graphical presentation of data was carried out using Microsoft Excel program (Microsoft Corporation, Los Angeles, CA, USA). All the data were expressed as mean value of three replicates. For comparison of treatment means, standard errors were also computed using Microsoft Excel program. All graphical presentations of parameters measured contained individual standard errors of means. The data were also subjected to statistical analysis using Statistical Package (Costat-2003, Co-Hort, v 6.1.). Levels of significance ($P \leq 0.05$) were determined (ANOVA) using 1, 2 or 4-factors as applicable, factorial CRD by following the principles outlined by Steel *et al.* (1997). The differences among the means were compared by Duncan's Multiple Range (DMRt) test (Duncan, 1955).

RESULTS

Survey of Agro Ecological Zones: Survey revealed that Shisham (*Dalbergia sissoo* Roxb.) dieback disease was prevalent in five selected agro ecological zones of Punjab both during 2005 and 2009. A total of 128 Shisham growing areas [Irrigated plantations (government and private plantations); linear plantations (road sides and canal sides) and farm lands] were surveyed to assess the extent of disease incidence (Table 1).

General Observations during Survey: Various categories of factors were evaluated for Shisham dieback on the basis of ocular observations. Most common observations in various agroecological zones were recorded as;

- Shisham dieback is not a nursery disease.
- Mature trees of 25years of age or above were susceptible.
- Variation in disease distribution in different zones is some how related to zonal environment and biotypes diversity in Shisham

Sandy Desert (IIIA): A total of 13 different locations including road sides, canal sides, farm lands and four major Shisham plantations had been surveyed in two main areas viz., Bahawalpur and RahemYar Khan (Table 1). In general, the disease incidence ranged between 13.3 to 20%. The lowest disease incidence of 13% with severity (0.43), index (8.67), rang (0-3) and prevalence (66.7%) was recorded in Bahawalpur in 2005. The lowest disease incidence was noted at farm lands as compared to canal sides and plantation sites in both these districts during 2005. Shisham dieback was completely disappeared during 2009 at farm lands in this zone (Fig. 2 -4). Total disease incidence was lower in Sandy desert IIIB (13%) followed by IIIA (16.7 %) as compared to other selected zones in 2005.

According to survey data in 2009, disease was recorded up to 5.8-6.6% in this zone. While, the disease reduction from 2005 to 2009 was slightly higher in RaheimYar Khan (R. Y. Khan) as compared to Bahawalpur region (Table 2). The lowest disease incidence (5.8%), severity (1.3), index (2.2), rang (0-5) and prevalence (42%) was recorded in Bahawalpur in 2009. Disease incidence was slightly higher (5.0%) from 4.6% of Northern irrigated plains (IV) and 3.6% sandy desert IIIB zone in 2009 (Table 2). Data indicates that Shisham dieback completely disappeared during 2009 at farm lands (Fig. 2 -4) in sandy desert (IIIA) however, the highest disease reduction of 50% was noted at plantation sites in Bahawalpur and 78% was recorded at canal sides in R. Y. Khan. Total disease reduction of 61.7% was observed from 2005 to 2009 in sandy desert IIIA (Fig. 5).

Sandy Desert (IIIB): The districts Khushab, Mianwali, Muzaffargarh and Layyah were extensively surveyed at 21 different plantations sites for Shisham dieback assessment in IIIB Sandy desert zone during 2005 and 2009 (Table. 1). The disease incidence range was 25-6.6% however, the highest disease incidence (11%), severity (0.9 %) and index (18%) was recorded in Mianwali whereas Khushab was least affected area during 2005 (Table 2). The highest disease incidence was observed on road sides (4%-19%) and Shisham plantations (4%-18%) as compare canal sides (10%-2%) and farmland areas (7%-2%) during 2005 and 2009 (Fig. 4). The disease incidence was lowest in Sandy desert III-B (13%) as compared to other selected agro ecological zones in 2005, however disease prevalence was slightly higher then V- Barani zone (2.6%) in 2009 (Table 2).

Table 1. Survey locations and dieback incidence in various agro ecological zones of the Punjab, during 2005 and 2009.

Zones	Districts	Number of Survey sites	Locations	Dieback (%) in each district 2005	Dieback (%) in each district 2009	Reduction (%) in dieback between 2005-2009
IIIA-Sandy Desert	Bahawalpur	8	Lal Suhanra National Park (LSNP), Bahawalpur plantion, cannal sides, road sides, Public parks in city, farm lands	13.3	5.8	56.39
	RaheimYar Khan	5	Khanpur plantation, Abbasis Bed Ny. Cpt No. 2 plantation, road sides, farm lands	20	6.6	67.0
V- Barani	Jhelum	5	Railway track sides, road sides, Suleman paris plantation, farm lands, riversides	43.3	3.3	92.40
	Chakwal	6	Chaua Chowk Nursery, road sides, Kallar Kahar plantation, farm lands	16	2.2	83.00
	Rawalpindi	8	G. T road sides, farm lands, parks	7.7	2.2	71.42
	Attock	7	Fam lands, road sides, Fathh Jang area	50	3.3	93.00
IIIB-Sandy Desert	Khushab	5	Private strands, raily way track sides, farm lands	6.6	4.4	33.33
	Mianwali	7	Hernoli Plantation, Mianwali Khusab road, farm lands	25	2.2	91.20
	Muzaffargarh	5	Khanpur plantation Cpt. 542. road sides, farm lands, cannnal sides	9.13	3.3	64.00
	layyah	4	Inayat Cpt. 168. Plantation, road sides, farm lands	11.1	4.4	60.36
IV-Northern Irrigated Plains	Multan	6	Forte lake Nursery, Cannal sides, farm lands, Khanewal Multan road	27	3.3	88.00
	Sahiwal	6	Chichawatni planttation, Headquater Nursery, Road sides, farm lands, cannal banks	15	4.0	73.33
	Faisalabad	5	Gatwala Forest park, , Bhalak dust, road sides, canal sides, farm lands	23	4.12	82.10
	Sargohda	5	Bhalwal Chak No. 7/S Nursery, Cannal sides, Kari More Nursery, Road sides, farm lands,	25	3.3	87.00
	Lahore	7	Jallo park Nursery, Lahore Jaranwala road, farm lands, cannal side, University of punjab,	28	7.4	74.00
	Sheikhupura	5	Sheikhupura by Pass Nursery, farm lands, Farooqadad Nursery, G.T. road sides	33	8.3	75.00
	Gujranwala	4	Kamoki G.T. road, Gujranwala G.T. road, farm lands	28	7.4	74.00
	Sialkot	7	Sialkot pasrur Rord, farm lands, Head Marala nursery, cannal sides	40	2.5	94.00
	Kasur	7	Changa Manga Plantation, Khudian Plantation, Cannal sides , fam lands	12.2	2.2	82.00
	Okara	10	Piplipahar plantation Cpt. 96, Upper Sohag plantation, Depalpur road, farm lands, cannal sides	14.2	3.3	77.00
X-Sulaman Piedmont	D.G .Khan	6	Bhatti Metal, road sides, farm lands	25	5	80.00
Total No. of locations		128		23	4.44	82.00

Table 3.2. Comparison of Incidence, severity, index, rang and prevalence of Shisham dieback in various agro ecological zones of the Punjab, during 2005 and 2009.

Zones	Districts	Year 2005					Year 2009				
		Incidence (%)	Severity	Disease Index (%)	Rang	Prevalence (%)	Incidence (%)	Severity	Disease Index %	Rang	Prevalence (%)
IIIA-Sandy Desert	Bahawalpur	13.3	0.43	8.67	0-3	67	5.8	1.3	2.2	0-5	42
	R.Y.K.	20	0.57	11.33	0-5	100	6.6	0.13	2.2	0-4	33
V- Barani	Jhelum	43.3	1.30	26.4	0-5	100	3.3	0.09	1.4	0-3	25
	Chakwal	16.0	0.40	8.5	0-4	50	2.2	0.04	0.73	0-3	23
	Rawalpindi	7.7	0.10	2.1	0-4	50	2.2	0.05	0.9	0-3	34
IIIB-Sandy Desert	Attock	50	1.40	25	0-4	50	3.3	0.11	1.8	0-5	33
	Khushab	6.6	0.13	2.66	0-2	67	4.4	0.16	2.7	0-3	45
	Mianwali	25	0.9	18	0-4	67	2.2	0.05	0.9	0-2	26
	Muzaffargarh	9.13	0.27	7.6	0-5	83.3	3.3	0.08	1.35	0-4	33
	Layyah	11.1	0.77	4.41	0-4	78	4.4	0.13	2.13	0-3	45
VI-Northern Irrigated Plains	Multan	27	0.9	18	0-4	100	3.3	0.05	0.9	0-3	33
	Sahiwal	15	0.36	3.3	0-3	60	4.0	0.11	1.8	0-4	42
	Faisalabad	23	0.67	13.3	0-4	67	4.12	0.11	1.9	0-4	33
	Sargohda	25	1.00	21	0-4	67	3.3	0.35	1.65	0-4	45
	Lahore	28	0.90	23	0-4	100	7.4	0.18	4	0-4	58
	Sheikhupura	33	1.15	23	0.5	100	8.3	0.21	3.5	0-5	66
	Gujranwala	28	0.83	16.66	0-4	100	7.4	0.03	4.3	0-5	50
	Sialkot	40	1.20	25.3	0-4	67	2.5	0.10	3.6	0-5	33
	Kasur	12.2	0.33	4.4	0-4	100	2.2	0.08	1.2	0-4	20
	Okara	14.2	0.36	6.0	0-5	88.8	3.3	0.11	1.6	0-3	33
X-Sulaman Piedmont	D.G .Khan	25	0.75	15	0-4	50	5	0.32	2.8	0-4	33
Over all situation		21.80	0.668	12.812	0-5	70.932	4.64	0.273	2.09	0-5	43.148

The data revealed disease range of 2.2-4.4% was observed in this zone during 2009. The disease reduction from 2005 to 2009 was high in Mianwali (91.20%) followed by Muzaffargarh (64%). The lowest disease incidence (2.2 %), severity (0.05 %) and index (0.9 %) was recorded in Mianwali with dieback reduction of up to 91% in plantations and road sides, while it was 100% in farmlands both in Mianwali and Muzaffargarh district in 2009 (Fig. 2& 3). However, the total dieback disease reduction of 62.2 % was observed from 2005 to 2009 in sandy desert III- B (Fig. 5).

Barani (V): In Barani area, disease assessment surveys were carried out in four districts viz., Attock, Chakwal, Jhelum and Rawalpind during 2005 and 2009. These areas comprise of four major Shisham plantations along with linear and farm land Shisham stands. A total of 26 Shisham plantation sites were extensively surveyed in Barani (V) zone. The highest dieback incidence range of 43% to50% was recorded both in Jhelum and Attock respectively in 2005 (Table 2). The disease incidence at road sides (25%), main Shisham plantations (29.15%) and farm land stands (21%) was almost similar in V- Barani zone during 2005.

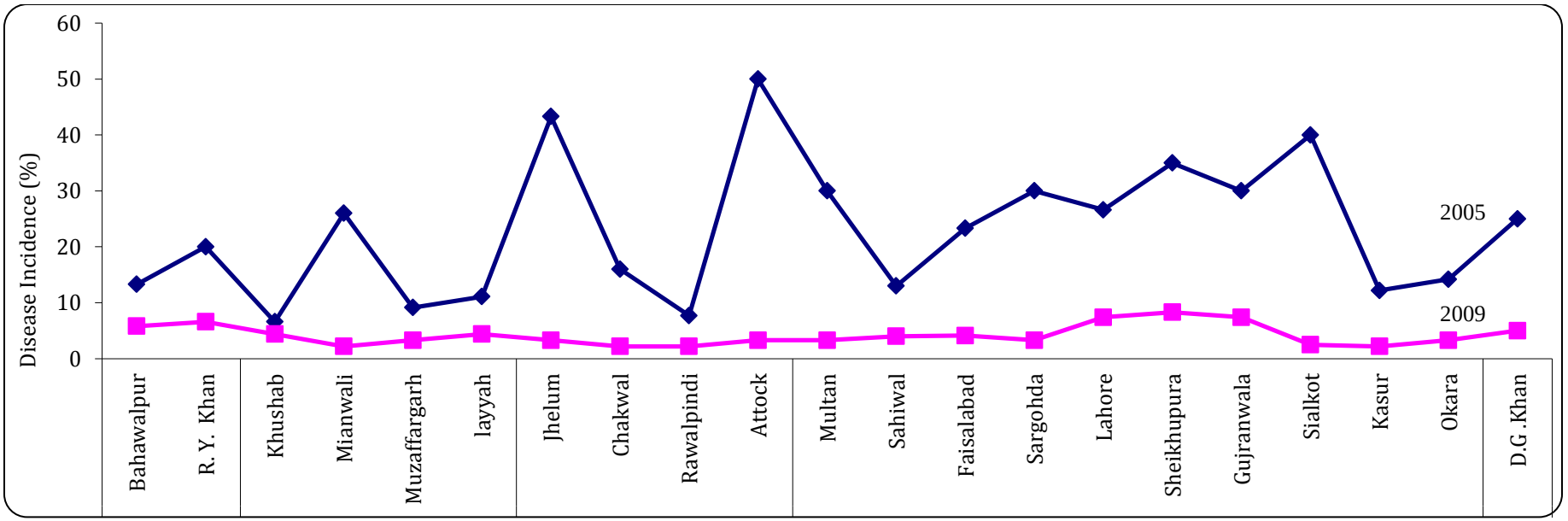


Figure. 3.2. Status of Shisham dieback in different agro ecological zones and areas of Punjab in 2005 and 2009.

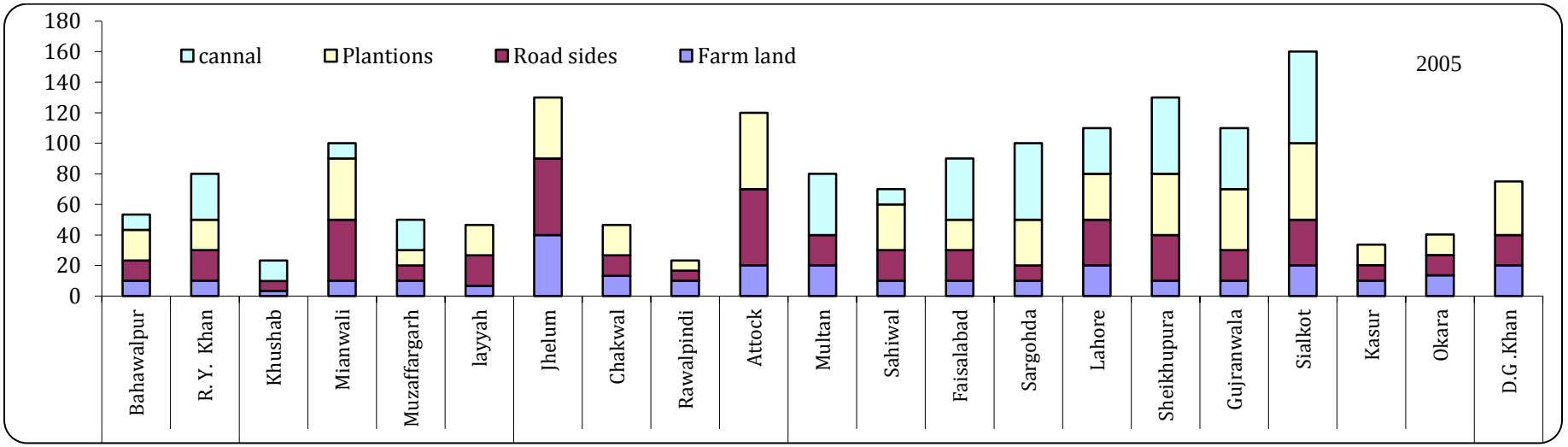


Figure. 3.3: Dieback (%) distribution in 2005 in farm lands, road sides, plantations in five agroecological zones of Punjab.

Table 3.3. Extant of Shisham die back influenced by pH, average temperature, average rainfall and soil texture.

Zones	Districts	Soil pH healthy tree	Soil pH healthy tree	Soil pH diseased tree	Soil pH diseased tree	Average temperature	Average rainfall	Soil texture
		Range	Average	Range	Average	°C	mm	
Sandy Desert (IIIA)	Bahawalpur	7.42-7.57	7.42±0.09	7.52-7.60	7.56±0.32	50	165	Sandy clay
	R.Y. Khan	7.56-7.71	7.63±0.004	7.52-7.62	7.58±0.06	48	200	Clay/ Silty clay
Barani (V)	Jhelum	7.23-7.53	7.38±0.09	7.30-7.52	7.44±0.01	42	880	Silt loam/ sand loam
	Chakwal	7.56-7.61	7.58±0.02	7.54-7.58	7.55±0.003	42	880	Silty clay loam/ Silty clays
	Rawalpindi	7.58-7.60	7.59±0.01	7.56-7.59	7.57±0.003	46	1550	Silt loam
	Attock	7.48-7.57	7.52±0.03	7.39-7.56	7.44±0.26	40	783	Silt clay loam/silty loam
Sandy Desert (IIIB)	Khushab	7.65-7.70	7.67±0.02	7.70-775	7.71±0.01	49	300	Sandy loam
	Mianwali	7.62-7.75	7.62±0.02	7.60-7.70	7.65±0.02	51	250	Sandy loam
	Muzaffargarh	7.53-7.67	7.61±0.03	7.54-7.63	7.56±0.04	47	125	Silt clay loam
	Layyah	7.44-7.63	7.54±0.05	7.47-7.69	7.54±0.07	50	270	Sandy loam
Northern Irrigated Plains (IV)	Multan	7.52-7.63	7.51±0.07	7.38-7.56	7.53±0.11	49	140	Clay/ Silty clay
	Sahiwal	7.43-7.59	7.52±0.05	7.42-7.52	7.48±0.04	47	177	Silt loam
	Faisalabad	7.58-7.63	7.62±0.04	7.49-7.61	7.55±0.03	48	400	Silt loam
	Sargohda	7.78-7.62	7.70±0.05	7.75-7.65	7.70±.014	49	240	Sandy loam/ Silty clay loam
	Lahore	7.56-7.64	7.63±0.02	7.52-7.66	7.54±.01	46	590	Silt loam
	Sheikhupura	7.48-7.67	7.54±0.012	7.51-7.62	7.57±.01	47	635	Silt loam
	Gujranwala	7.56-7.76	7.61±0.07	7.55-7.59	7.56±0.003	45	888	Silty clay loam/Silty loam
	Sialkot	7.48-7.68	7.56±0.035	7.55-7.62	7.56±0.06	48	1000	Silt loam
	Kasur	7.47-7.72	7.61±0.07	7.45-7.69	5.64±0.08	46	250	Sandy loam
	Okara	7.40-7.61	7.52±0.07	7.42-7.65	7.55±0.08	44	220	Silty clay loam
Sulaman Piedmont (X)	D.G .Khan	7.34-7.60	7.47±0.043	7.40-7.53	7.46±0.04	48	350	Loam/silty clays

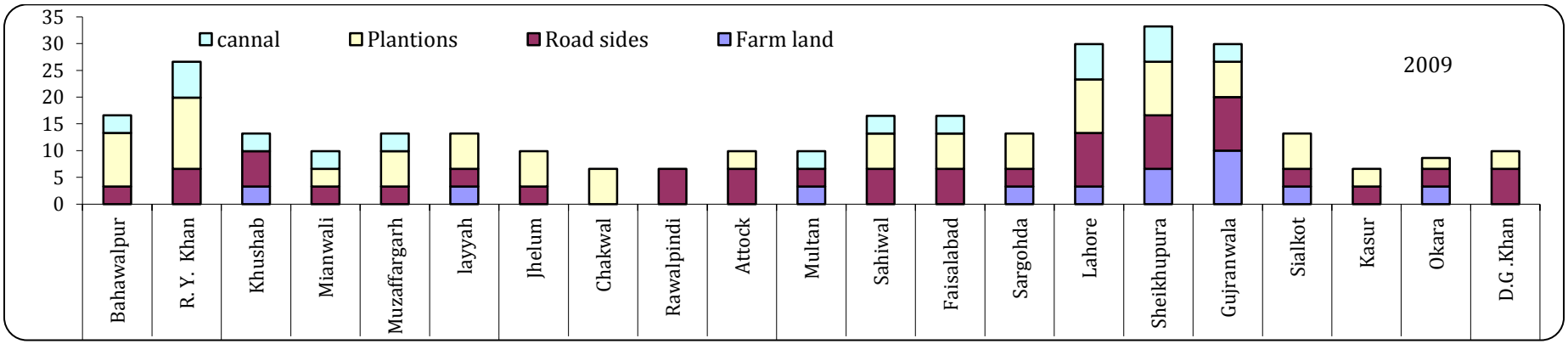


Figure. 3.4. Dieback (%) distribution in 2009 in farm lands, road sides, plantations in five agro ecological zones of Punjab.

Disease reduction percentage was quite high in Jhelum (92.4%) and Attock (93%) in 2009 (Table 1). The highest disease reduction of 93.4% was also recorded at road sides in Jhelum, during 2009. On the other hand, in Attock, Chakwal, Jhelum and Rawalpind, the Shisham dieback was found totally vanished in farmlands in 2009 (Fig. 2). The total disease reduction of 85% was observed from 2005 to 2009 in Barani region (Fig. 5).

Northern Irrigated Plains (IV): Ten selected districts viz., Multan Sahiwal, Faisalabad, Sargohda, Lahore, Sheikhupura, Gujranwala, Sialkot, Kasur and Okara, were surveyed at 62 different Shisham sites including 14 main Shisham plantations during 2005 and 2009 for dieback assessment (Fig. 1). Highest disease incidence (40%), severity (1.20%) and index (25.3%) were recorded in Sialkot followed by Sheikhupura and Lahore district in 2005 (Table 2). In general, highest disease incidence was observed on canal sides (32%) and Shisham plantations (26.4%) as compared to road sides (20.4%) and farm land areas (13.6%) during 2005 (Fig. 4). According to 2009 surveys, disease incidence was high in Sheikhupura (8.3%), Lahore (7.4%) and Gujranwala (7.4%) districts, as compared to other areas in this zone. On the basis of 2005 and 2009 survey analysis, highest disease reduction was observed in Sialkot (94%), Multan (88%) and Sargohda (87%) followed by Faisalabad (82%) and Kasur (82%). According to survey, the highest dieback reduction of 92% was observed on canal sides followed by Shisham plantations (78%), farmlands (76%) and road sides (71%) in 2009. However a tremendous disease reduction of 100% was observed in farm lands of Faisalabad, Sahiwal and Kasur district followed by Sialkot region during 2009 (Fig. 3& 4). Dieback incidence was 25% in 2005 which reduced up to 81% in 2009 that is more or less similar as in V-Barani zone (Fig. 5).

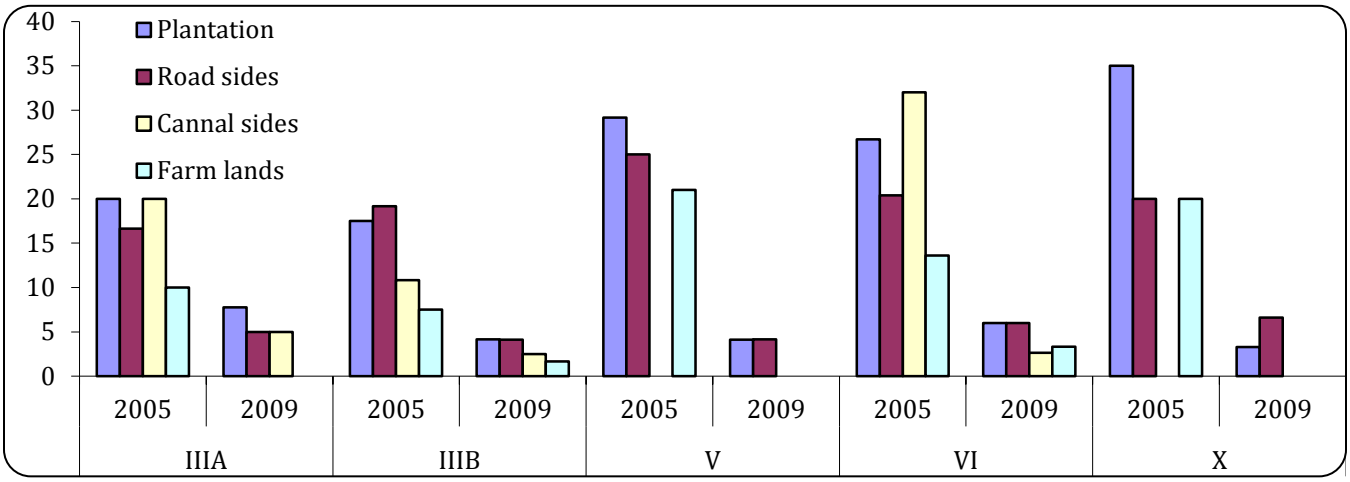


Figure. 3.5. Comparison of dieback (%) distribution in 2005 and 2009 in five Agro ecological zones of Punjab.

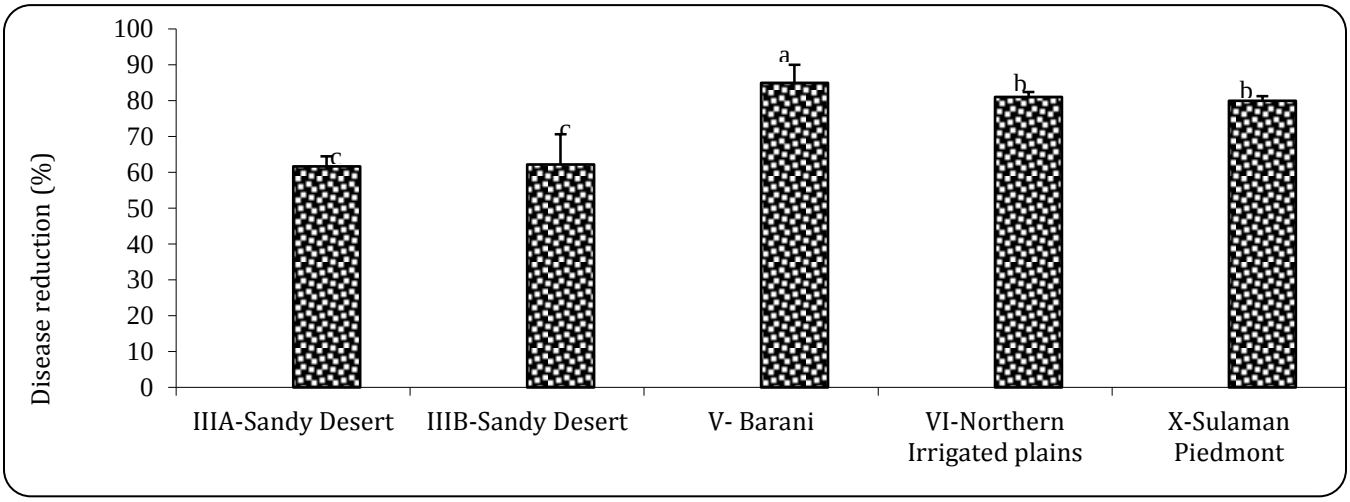


Figure 3.6. Comparison of dieback reduction (%) between 2005 to 2009 in various Agro ecological zones of Punjab.

Sulaman Piedmont (X): This area comprises of one major Shisham plantation along with road sides and farm land stands. Surveys were carried out in 2005 and 2009 at six different locations in Dara Ghazi Khan (D. G. Khan) district for Shisham dieback (Table 1). Survey observations in 2005, indicated that disease incidence was 25% with 0.75% disease severity at 0-4 range and 50% prevalence in D.G. Khan. Dieback incidence was comparable at road sides (20%) and farmlands (20%) during 2005.

Disease incidence of 1.6% was recorded in 2009 with total reduction of 94% (Table 2) in D. G. Khan district. However, dieback disease totally vanished from farmlands during 2009 (Fig. 4). From 2005 to 2009, total disease reduction was 91% on plantation sites (Fig. 4). The dieback incidence was 25% in 2005 which reduced up to 86% in 2009, similar as in Northern Irrigated plains (IV) (Fig. 5).

Relationship of Physical Characteristics of Soil in Selected Zones: Soil texture and pH is summarized in the Table 3. This table shows that soil pH under diseased trees in general was alkaline. However the degree of alkalinity varied from slight to highly alkaline. Slightly alkaline pH (7.44-7.48) was recorded at the sites of Jehlum, Attock, Sahiwal and D. G. Kahn, while highly alkaline (7.65-7.72) in districts of Khushab, Mianwali and Sargodha. All sites with high mortality like Jhelum, Attock, Sheikhupura and Sialkot districts indicated slight to moderate alkaline pH range in 2005.

The trees in the high mortality districts (Attock, Jhelum, Sialkot and Sheikhupura) were found to grow both on the loosely textured and the heavily-textured soils. The districts with low mortality (Kushab, Muzaffargarh, Bahawalpur and Chakwal) have sandy soil with no water logging however slight top dying was observed in these areas. During sites inspection, it was also noted that healthy Shisham stands as well as stands with low to moderate mortality, have good water drainage conditions in a varied range of the soil texture, both silty loam to sandy clay. On the basis of location, sandy desert of both A&B zones of Punjab had low percentage of Shisham dieback as compared to IV- Northern irrigated plain and Barani-V zone during 2005. However, the variations in disease incidence with reference to soil structure, was not pronounced in 2009.

DISCUSSION

The present investigations have revealed very promising results regarding shisham dieback distribution pattern and trend of dieback disease in five agroecological zones of the Punjab. Among selected zones, shisham dieback incidence was high in Barani (V) and Northern irrigated plains (IV) zones as compared to sandy deserts (IIIA & IIIB) and Sulaman piedmont (X). During present study, no relation between the soil texture and the intensity of dieback has been found. Shisham dieback was evidenced only in mature trees (above 25 year old trees) indicating that it is not a nursery disease. The results indicated that total disease incidence was lower in Sandy deserts (A-III and III-B), whereas higher in Barani (V) and Northern irrigated plains (IV) zones in 2005. The difference in dieback disease incidence revealed that variations in the physical environment have played a significant role in the development and spread of shisham dieback in agro ecological zones of the Punjab. The data also specified that degree of disease severity is highly dependent on rainfall. Apparently, the significant amount of rainfall during monsoon season makes the Northern irrigated plain (IV) and Barani (V) an ideal location for dieback. In parallel investigations (Khan and Khan, 2000; Bajwa and Mukhtar, 2006, Mukhtar, 2012), Northern irrigated plain (IV) and Barani (V) zones, shisham plantations have also been found severely infected by dieback disease. The variability in dieback incidence in these regions is perhaps due to climate impact and moisture level.

The classic disease triangle theory as well as previous investigations supports that environmental variables such as temperature and moisture can influence both pathogen virulence and host plant resistance responses (Coakley, 1979; Scherm and Yang, 1995; Garrett *et al.*, 2006; Zhu *et al*., 2010). In another study, Aslam (2004**)** has also reported that the number of causes for tree disease may vary in intensity from region to region in Punjab (Pakistan). However, several workers have suggested that mixed cropping plantations might be more suitable for survival of shisham (sissoo) due to an 'immune environment' created by non-sissoo species (Negi *et al.,* 1999; Khan, 2000).

In recent past, phenotypically different varieties of Shisham have been grouped under resistance, susceptible and unsusceptible varieties on the basis of their relative resistance to dieback disease (Javaid *et al.,* 2003 & 2004). During 2009 survey, thinning of shisham stands has been observed as a consequence of heavy loss of tree cover and slow plantation strategies. Biotypes variation in shisham can also be accounted for as an important factor in spread of shisham dieback in

agroecological zones of Punjab. The results of present study revealed that disease reduction (%) trend was similar in all selected regions during 2009. It may be due to disappearance of certain biotypes of shisham during dieback epidemic leading to thinning of forest during 1998 to 2005. Difference in resistance among populations, whether environmental or genetic in origin, may influence the dynamics of an epidemic across geographic areas (Burdon, 1987). Many studies support that plant populations and communities can be dramatically altered by disease epidemics (Burdon *et al*., 2006; Gilbert, 2002**)**. In other investigations, populations of forest trees in the cases of white pine blister rust (*Cronartium ribicola*) (Kinloch, 1992) and Jarrah dieback (*Phytophthora cinnamomi*) (Stukely and Crane, 1994; Stukely *et al*., 2007), some degree of resistance to invasive pathogens have been found due to variation in biotypes (Sniezko, 2006). In recent past, Dhakal *et al.* (2005) also studied genetic variation in mortality by comparing survival of Shisham progenies, originating from different parts of Nepal and found a significant difference in mortality percentage between Shisham biotypes. However in pervious surveys (Khan and Khan, 2000; Bajwa and Mukhtar, 2006) in Punjab, biotypes variations of shisham and cover thinning has not been taken in to account to link the distribution pattern and severity of shisham dieback in various zones. However, recently, various shisham biotypes have been evaluated for resistant against dieback pathogen (Mukhtar, 2012) that directly support hypothesis of genetic evolution in shisham against this disease in Punjab. Result also supports a linkage between resistant biotypes in shisham plantation and low incidence of dieback disease in Punjab during 2009.

Rhizospheric soil pH analysis of dieback infected trees, showed variation from slightly to highly alkaline range according to area of origin in five selected zones. All high mortality sites like Jhelum, Attock and Sheikhupura and Sialkot districts have slight to modrate alkaline soil pH. Similar pH range (even high pH value up to 8.5) has been reported from healthy shisham forest in India and Nepal (Sharma *et al.,* 2000; Sah *et al.,* 2003). In recent past, Sah *et al.* (2002**)** has reported that the water logging is the major factor responsible for the decline of shisham stands. According to this conclusion, it can be assumed that only the loosely textured soils with good drainage are suitable for proper growth of shisham tree. However presently, there is no such relation between the soil texture and the intensity of shisham dieback has been found. The trees in the high mortality districts (Attock, Jhelum, Sialkot and Sheikhupura) were found to grow both on the loosely textured (sandy loam/ Silty clay loam) as well as on heavily-textured soils (Silt loam/ Silty clay loam) with dominantly water logged soils in some areas in these districts. In other districts with low to high shisham mortality due to dieback, no relation has been observed between disease incidence and waterlogged soil and or well drained soil. Majority of both healthy and infected shisham stands were found under good water drainage conditions however on a varied range of the soil texture. Sandy desert IIIA& IIIB zones of Punjab has low shisham dieback disease incidence as compared to northern irrigated plain (VI) and Barani (V) zone.

The present study in general, has not indicated any correlation of soil texture and water table level with the spread and severity of shisham dieback. These findings are in conformity with earlier investigations (Shah *et al.,* 2002; Bajwa and Mukhtar, 2006). The present investigations provided an insight into patron and trend of shisham dieback disease. The disease incidence is clearly related to biotype and zonal environmental factors. However, due to high genetic variability among the pathogenic isolates of *Fusarium solani*, this fungal species is the major cause of epidemic and persistence of shisham dieback (Mukhtar, 2012). Therefore, dieback pathogen is uncontrollable due to high genetic variability of pathogen, however resistant biotypes is the only solution to eradicate this menace from shisham plantations. Recently, resistance of some biotypes against dieback pathogen has been evaluated and proved that some biotypes have resistance against various pathogenic isolates.

CONCLUSIONS

Current study concludes that thinning of shisham plantations is evident of high mortality in last decade; however the variation in shisham biotypes apparently is the major reason of shisham survival from high disease epidemic during 1998 to 2005. It seems those so called resistant biotypes have been evolved due to new genetic recombination and are the progeny of susceptible varieties that have almost vanished. At present stage, selection of improved gemplasm is crucial for success of plantation programme, in Punjab, Pakistan.

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