CHAPTER- VIII

CONCLUSIONS
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In plant pathology disease is understood as a host-pathogen, environmental interaction, and has been defined as a process with a sudden or well planned on set, following a short or prolonged course and ending in disability, death or recovery of host plants. It is thus not a steady state. The present study undertaken relates to Deuteromycetean pathogens from Melghat forest in which all the aspects i.e. status of host, pathogen characters, physiological behaviour and the prevailing environmental conditions were taken into consideration.

Melghat tract of Amravati District is mountainous terrain of Satpura ranges. The major part of the area is covered by a good forest. It floristically rich as more than 700 naturalized angiospermic plant species have been listed so far. They belongs to diverse families. The climatic features are such that certain floristic elements like Bryophytes, Pteridophytes, Orchids etc. are poorly represented. However, the flowering plant group affords a good diversity for pathogenic fungal form collection. The forest is of a Southern Dry Deciduous type with *Tectona grandis* as the dominant species. Different Teak forest associations show several tree species as the component. Some of the more common natural tree species as associates of tree are *Lannea Coromandeliana, Anogeissus latifolia,*
Emblica officinalis, Terminalia tomentosa, Adina cordifolia, Mitragyna parviflora, Lagerstroemia parviflora, Ougenia ooeinesis, Bauhinia racemosa, Caesearia elliptica, Schrebera switinooides, Zizyphus xylopyra, Bridelia retusa, Wrightia tinctoria, Butea monosperma, Cassia fistula and several others.

Common shrubby and liana elements in Melghat forest is represented by species like Celastrus paniculatus, Helicteris isora, Lantana camera, Nyctanthes arbor-tristis, Milletia auriculata, Colebrookia oppositifolia Carvia callosa, Mucuna prurita, Grewia sp. and several others. About 100 species of grasses and more than 350 species of annual herbs are the components of Melghat forest flora. Some of the shrubs, herbs and grasses show luxurient growth and contribute in a large measure to the biomass production of this forest. One of the Tiger Reserves is located in Melghat. Therefore, the forest is well protected and specific management plans are implemented by the Forest Department and the Melghat Tiger Reserve establishment. Thus the Melghat forest provides a rich variety of plant species for the study of pathogenic fungi.

Plant diseases are caused by a variety of agents. However, fungi as a group of pathogenic organisms is more important. From the symptoms it was observed that during the course of this study that a large number of flowering plant species are affected by fungal organisms. Disease symptoms such as spots, necrotic lesions, rust sori, wilting, blighting, gall formation, rotting, etc. were
observed on aerial parts of a very large number of plants. However, for the sake of uniformity and considering the facilities available only infections from leaves were collected.

Wild plants as well as cultivated plants of this region were continuously screened for more than four years in different seasons to observe the effect of climatic factors on disease development, and it was noticed that, the incidence of the disease is more from September onwards it is because the environmental factors influence the infection and invasion processes in disease by affecting the pathogens as well as the host. Factors such as moisture, temperature, light, pH affect germination of spores upon plant surfaces, penetration and colonization of plants. The optimum condition for any particular factor is different from each of the pathogenic activities involved in the disease process. The availability of optimum level of moisture is more significant in plant infection. Factors which prolong persistance of moisture usually bring about increased infection and thus increase the spread of many pathogens.

Melghat experiences dryness of climate from January onwards which is followed by hot dry summer from March onwards. There is south-west monsoon [rainy season] period from middle of June - September. The total annual rainfall in Melghat is quite high, some places receiving upto 2500 mm. in some very wet years. It mainly received during rainy season. During the same period the
temperature averages between maximum 30°C and minimum 20°C. Frequent rains, dense growth of vegetations accompanied by moderate temperatures results in the prevailing high relative humidity during the period of July to November. Factors which prolong persistance of moisture usually bring about increased infection and thus increase the spread of many pathogens. High humidity, mist, fog, dew and frequent rains are the conditions which can provide the moisture necessary for infection. Most diseases spread very fast under these conditions of moisture necessary for infection. Most diseases spread very fast under these conditions of moisture mostly because favourable condition for infection and persistance of disease prevails in the period July to December. And thus maximum infected hosts species, the individuals was observed during the period August to December.

In fact that microorganisms are mostly heterotrophic in their nutrition makes them dependant on suitable substratum in their environment. Hosts serve this purpose for the pathogenic microorganisms. There is infection court i.e. the host surface where the pathogen makes contact with it. This area on host surface accommodates several other microorganisms other than pathogen. Within the infection court are found the infection sites at which the pathogen is successful to penetrate the host tissue and cause infection. It here again that the pathogen builds in inoculum for infection. The environmental
conditions in Melghat forest are most suitable during August to October for development of inoculum potential. However, the external manifestation of the disease in case of the infection collected was maximum during the period October to December.

The survey of plant diseases of Melghat forest showed that, mainly the Deuteromycetean fungi were associated with leaf spot diseases of most of the plants. A total of 19 genera viz. Curvularia, Drechslera, Bipolaris, Exserohilum, Volutella, Periconia, Dictyocarphium, Alternaria, Fusarium, Cladosporium, Verticillium, Cercospora from order Moniliales, Pestalotia and Colletotrichum from order Melanconiales, and Phoma, Macrophomina, Coleophoma, Phyllosticta and Botryodiplodia from order Sphaeropsidales were isolated from leaf spots of different plants. For all practical purposes identification of plant disease is essential. Since, the diseases in plant are caused by some infection agents and environmental factors, the first step in their identification would be to ascertain the cause of the disease correctly. Development of symptom as characteristic of disease reveals the sickness of the plant, besides the nature of the causal agent. Symptoms produced by the pathogens collected were carefully noted and recorded. Besides the characters of the culture and reproductive structures, the symptom characters were found to be very useful.

Diseased portion found on Wrightia tinctoria, Casearia elliptica, Butea monosperma, Ficus arnottiana, Grewia scabrophyllea,
Adina cordifolia, Milletia auriculata Lagerstroemia parviflora, Mytragyna parviflora and many other plants indicated that with a particular type of symptom there could be an association of more than one organism. Presence of same fungus on different hosts showed the capacity of Deuteromycetean fungi to obtain food from different plants and therefore, such fungi have better chances for survival and infecting new hosts, as they do not seem to be very rigid regarding their nutritional requirements. Species of Curvularia, Bipolaris Drechslera, Fusarium, Phoma and Botryodiplodia which were of wide distribution could be grouped in the above category. During the present investigation the plant parts were collected from geological and ecological conditions, out of which majority of the hosts were new host records for India, giving indication that Deuteromycetean fungi can survive in any type of climatic conditions.

Host range could not be directly co-related with systematics of angiosperms as fungi were isolated from all the families of angiosperms. Similarly no specification in taxa was observed. This shows that fungal organisms infect those plants which are available in ample quantity in nature. Even taxonomic relation of fungi could not be drawn on the basis of angiosperm taxonomy. One genus showed its existance on majority of hosts which were from different taxons; and one host showed different organisms belonging not only to different genera but of different orders.
The fungi collected from the lesions of *Butea monosperma* [Lam.] Taub. [Papilionaceae] were of three different orders i.e. Moniliales Melanconiales and Sphaeropsidales, while those collected from *Emblica officinalis* Gaertn. [Euphorbiaceae], *Butea superba* Roxb. [Papilionaceae] *Uaria rufescens* [DC] Schinol. [Papilionaceae] *Syzygium cumini* [L.] Skeels were of two different orders, it may be Moniliales and Melanconiales or Moniliales and Sphaeropsidales, while those collected from lesions of *Butea monosperma* [Lam.] Taub. [Fabaceae], *Lagerstroemia parviflora* Roxb. [Lythraceae], *Wrightia tinctoria* R. Br. [Apocynaceae], *Impatiens balsamina* L. [Balsaminaceae], *Acalypha indica* L. [Amaranthaceae], *Brideia retusa* [L.] Spr. [Amaranthaceae], *Mangifera indica* L. [Anacardiaceae]., *Ougenia ooejensis* [Roxb.] Hochreut. [Fabaceae], *Cassia fistula* L. [Fabaceae], *Bauhinia vahlii* Wt and Arn. *Mitragyna parviflora* [Roxb] Korth. [Rubiaceae]. *Brachiaria setigera* [Retz.] C.E. Hubb [Poaceae] showed two or more than two genera of same order viz. Moniliales and Sphaeropsidales. Two species of one genus from the same lesion was not observed in any plant.
Though fungi from all the three order of Deuteromycetes i.e. Moniliales, Melanconiales and Sphaeropsidales were observed on different hosts, but out of them Moniliales showed its dominance over Sphaeropsidales and Melanconiales. Fungi belonging to Moniliales were reported Moniliales were reported from Ninety five hosts, out of which many hosts were new records for India; Sphaeropsidalean fungi were reported from farly thirty two hosts, while fungi belonging to Melanconiales showed their existance on twelve hosts.

Deuteromycetean fungi recorded in the present studies produced various types of symptoms on the host lamina. Initiation of Infection in most of the cases was observed in the laterpart of rainy season [August and September] but the expression of symptoms was more pronounced in October to December. Generally the initiation of infection started from periphery or middle part of lamina in the form of brown or yellowish brown spots which at advanced stages increased in dimensions and often coalesed to form irregular patches. Detachment of the infected parts as well as defoliation were also observed at the severity of infection. Monilian fungi exhibited infection from margin or apex of leaf lamina which proceeds towards midrib having regular or irregular spots which may or may not be surrounded by boundary. The pustules were not prominant but drying or cracking of lamina takes place at severity of infection. Sphaeropsidalean fungi showed brown to olivaceous brown regular to irregular lesions which
develops in intra or interlaminar region, but with prominent boundary and black pin-hole like bodies resulting in shot holes or etiolation of leaf lamina at the severity of infection, while lesions of Melanconilian fungi were not definite. Diseased portions which contain more than one on fungi of two different orders exhibited mixed symptoms.

On the basis of present investigation of collections made from plant of Melghat forest, it was observed that, plants grown in different ecosystems and environments were susceptible to diseases. It was also noticed that plants growing in hilly areas were found to be more disease resistant as compared to plants growing in plains. In most of the cases; it was noticed that, same host grown in hilly and plain area did not show similar efficiency for infection at the same time. This indicates that, climatic conditions govern the resistance of plants against diseases. As the literature about mycological surveys of Amravati district, it concludes that the geological and climatic factors governs the disease development. During investigation, it was also noticed that, fungal flora could not be co-related with angiospermic flora.

It was revealed from the available literature that, Curvularia, Drechslera, Bipolaris, Helminthosporium and Exserohilum of order Moniliales showed most of the similarities in morphological characters. Some of the species of Helminthosporium, Bipolaris and Curvularia were converted to Drechslera and Exserohilum by certain mycologists
only on the basis of their morphological differences. Hence author decided to study in detail morphological and taxonomical studies of the species collected so far, of these genera i.e. *Curvularia, Drechslera, Bipolaris* and *Exserohilum* to know their characteristic features. This separation of few species and shifting to genus *Exserohilum* was carried out by Leonard [1974]. The new genus *Exserohilum* was established by Leonard for the species formerly included in *Bipolaris* [graminicolar *Helminthosporium* species] in which the conidal hilum is distinctly protuberant. Shoemaker [1959] recognised the separation of *Bipolaris* from *Helminthosporium*, as *Bipolaris* was distinguished for having fusiform conidia and bipolar germination. Most of the species of *Helminthosporium* viz. *H. flagelloideum, H. maydis, H. flumantaceum, H. nodulosum, H. oryzae, H. tetramera, H. turricum* and *H. sativum, H. ravenalii, H. sacchari*, and *H. bicolor* which are included in “Fungi of India” by Mundkar [1938] have been named by Shoemaker [1959] as *Bipolaris flagelloides, B. maydii, B. monoceras, B. sacchari, B. sorokiana, B. ravenelii, B. nodulosa, B. tetramera, B. turcica* and *B. bicolor* respectively.

Subramaniam and Jain rejected the separation of these species of *Helminthosporium* into *Bipolaris* and *Drechslera*, because they did not regard the distinction upon which the separation was based to be of sufficient magnitude to justify it. They also felt that for a few species, the characters used by Shoemaker were too
inconsistent to be reliable in determining which of the new genera the species belong to. But Leonard [1974] objected to the views of Subramanian and Jain that Bipolaris and Drechslera were based on differences of insufficient magnitude, and suggested that Drechslera, Bipolaris and Helminthosporium should be considered as three distinct genera, where phylogenetic relationships are not recognised taxonomically from present investigation we could not isolate Helminthosporium.

Fungi causing leaf spot diseases come in contact with diverse types of carbon and nitrogen sources in nature. In the present investigation, effect of various carbon, nitrogen sources and pH on growth, sporulation of five genera of Deuteromycetes viz., Curvularia lunata, C. senegalensis, C. prasadii, Drechslera rostrata, Bipolaris hawaiiensis, Fusarium equiseti, F. scirpi, F. oxysporum, Alternaria alternata was examined. Carbohydrates induced diverse degree of vegetative and reproductive growth on all the genera under study. They exhibited different degree of growth on various carbon sources. None of the same extent in all the genera under investigation. D-glucose favoured the excellent to good growth of species of Curvularia, Drechslera, Bipolaris, Alternaria and Fusarium. Carbohydrates induced diverse degree of vegetative and reproductive growth of all the genera under study. They exhibited different degrees of growth on various carbon sources. D-glucose favoured the excellent growth of Curvularia lunata and Drechslera rostrata; good to moderate in Bipolaris
hawaiiensis, Alternaria alternata, Curvularia senegalensis, C. prasadii, Fusarium equisetii, F. scirpi and F. oxysporum. It was also reported as an excellent source for the growth of Curvularia lunata [Pachkhede, 1988]; good source for the growth of many fungi like Curvularia siddiqueii, Fusarium oxysporum and Botryodiplodia theobromae by Panwar [1972], Chary [1982] and Shreemali [1971] respectively.

Curvularia lunata, Bipolaris hawaiiensis showed moderate growth on D-glucose, while it acted as good carbon source for Drechslera rostrata, Curvularia senegalensis, C. prasadii, Alternaria alternata, Fusarium equiseti and excellent for Fusarium scirpi and F. oxysporum D-galactose supported good growth of Curvularia lunata, Drechslera rostrata, Fusarium equiseti, Alternaria alternata but poor growth of Bipolaris hawaiiensis. In Curvularia senegalensis and C. prasadii it favoured mycelial growth while in case of Fusarium scirpi and F. oxysporum it showed excellent growth. D-xylose and D-galactose were reported as moderate to poor carbon sources by Bilgrami [1962] for the fungi studied by him.

Curvularia lunata exhibited excellent growth on D-fructose while Drechslera rostrata C. senegalensis, C. prasadii, Alternaria alternata, Fusarium scirpi, F. oxysporum showed good growth. Moderate growth was observed for Bipolaris hawaiiensis. D-fructose was also reported as a good source for the growth of Curvularia pallescence [Bais et al., 1970] and Alternaria alternata [Shivkumar and Tandon, 1978].
Sucrose induced excellent mycelial growth of *Curvularia lunata* and good growth of *Drechslera rostrata* and *Fusarium equiseti*, while poor in *Bipolaris hawaiensis* and *Alternaria alternata*. *Curvularia senegalensis* and *C. prasadii* showed good mycelial growth while *Fusarium scirpi* and *F. oxysporum* [Chary, 1982] showed excellent growth on sucrose. Cellobiose and lactose were observed as moderate to poor carbon sources from the majority of the species of *Curvularia, Drechslera, Bipolaris, Alternaria* and *Fusarium* [Chary, 1982].

Amongst polysaccharides, starch favoured good to excellent growth of species of *Curvularia, Drechslera, Fusarium* [Chary 1982] and *Alternaria* and poor growth of *Bipolaris hawaiensis*. Singh and Tandon [1967, 1971], Hasija [1970], Singh and Prasad [1973] reported good growth of fungi studied by them on starch. Raffinose induced good to moderate growth for species of *Curvularia, Drechslera, Bipolaris, Alternaria* and *Fusarium*. Raffinose is reported to be poor carbon source for lower fungi [Hasija 1970] but for pathogenic Deuteromycetes it is good carbon source [Kapoor and Tandon, 1969C].

In general *D*-glucose, *D*-fructose, Sucrose and Starch were observed as good carbon sources; *D*-xylose, raffinose and *D*-galactose as moderate and cellobiose and lactose as poor carbon sources for various species of *Curvularia, Drechslera, Bipolaris,*
*Alternaria* and *Fusarium* under study. Species of *Curvularia* exhibited excellent mycelial growth on these sources followed by species of *Drechslera, Alternaria, Fusarium* and *Bipolaris*. Nitrogen sources also influence the growth of present fungi. Amongst nitrate nitrogen, potassium nitrate supported excellent growth of *Curvularia*, while ammonium nitrate and sodium nitrate favour good to excellent growth of the species of *Drechslera, Alternaria* and *Fusarium*. In general nitrates have been reported to be excellent sources of growth of imperfect fungi by Lilly and Barnett [1951], Thind and Randhawa [1957a], Agarwal and Ganguli [1960], Bilgrami [1964] and Shreemali [1969]. While working on *Curvularia lunata, Alternaria tenuis* and *Helminthosporium rostratum* Mathur et al. [1960], Singh and Khanna [1966] and Agarwal and Shinkhede [1959], respectively observed that ammonium nitrate induced moderate to poor mycelial growth of these fungi.

Ammonium sulphate induced moderate growth of *Curvularia prasadii, C. senegalensis, Fusarium scirpi, F. oxysporum, Alternaria alternata* and poor growth in *Curvularia lunata, Drechslera rostrata, Bipolaris hawaiiensis, Fusarium equiseti*. Similar results were obtained by Mathur et al. [1960] and Agarwal and Shinkhede [1959] while working with *Curvularia lunata* and *Helminthosporium rostratum* respectively.
Amongst organic nitrogen sources urea L-aspargin and L-tyrosine favoured excellent to good; glycine, DL-leucine and L-cystine moderate and DL-valine poor mycelial growth of majority of fungi under study. There was no relative constancy about role of organic nitrogen sources on growth. Glycine was reported as excellent nitrogen source for the growth of Fusarium oxysporum and F. scirpi [Chary, 1982] and Alternaria alternata [Mathur 1978]. L-tyrosine was reported as a good source for the growth of Curvularia pallescensce [Bais et al., 1970].

In general, potassium nitrate, sodium nitrate, ammonium nitrate and L-aspargin were observed as good nitrogen sources while moderate to poor sources were ammonium sulphate, Urea, DL-alanine, glycine, D-L-leucine, L-cystine, DL-valine, Arginine and L-tyrosine.

It was observed that effect of carbon and nitrogen sources on vegetative as well as on degree of sporulation was more or less same. The carbon and nitrogen sources which induced excellent mycelial growth showed excellent sporulation. In general, amongst hexoses, D-glucose and D-fructose favoured excellent sporulation as compared to D-xylene and D-galactose. Amongst diasmaccharides, sucrose was observed as an excellent source than lactose and celllobiose, while raffinose favoured sporulation of majority of species as compared to starch polysaccharides.
Amongst nitrogenous compounds potassium and sodium nitrate favoured excellent sporulation while ammonium nitrate reacted as a moderate source and ammonium sulphate relatively moderate to poor source for maximum of the fungi. Amongst organic nitrogenous compounds, DL-alanine, L-asparagine, Urea, glycine, L-arginine, and DL-valine supported good sporulation; L-tyrosine induced poor sporulation.

Effect of pH of growth and sporulation of species Curvularia, Drechslera, Bipolaris, Alternaria and Fusarium were experimented and it was observed that, growth of all species of Curvularia and Drechslera was minimum at 4.0 to 5.5 and highest on the pH 5.5 or 6.0 [Pachkhede, 1988] Bipolaris hawaiensis exhibited maximum growth at 5.0. [Vyawahare, 1988]. For Fusarium oxysporum and F. scirpi pH 5.5 and 6.0 are best for maximum growth [Chary, 1982] while 5.5 to 6.5 pH are best for maximum growth pH 5.5 and 6.0 favoured good sporulation for species of Curvularia and Drechslera. Hasija [1970] observed a wide pH range and relatively lower pH optima for growth of Curvularia pallescens. Alternaria alternata favoured 4.5 to 5.5 pH for growth of sporulation. Bipolaris hawaiensis exhibited maximum sporulation at pH 5 [Vyawahare, 1988]. Fusarium oxysporum exhibited maximum growth of sporulation on pH 5.5 and 6.0 Fusarium equiseti exhibited excellent sporulation at pH 5 and 6.0 while F. scirpi favoured 5.5 and 6.0 pH for excellent sporulation.
The spore germination of various genera under investigation, was significantly influenced by different nutritive substances. Nutrients play an important role in germination of fungal spores. D-glucose and potassium nitrate favoured the rate of germination of species of Curvularia, Drechslera, Bipolaris, Alternaria and Fusarium. While the conidial germination of the present fungi was poor in L-glutamic acid. Shreemali [1969] reported poor germination of Botryodiplodia theobromae on L-glutamic acid.

Germination studies also revealed that species of Curvularia, Drechslera, Bipolaris, Alternaria and Fusarium varied in the mode of spore germination. Curvularia lunata and Fusarium equiseti showed unipolar germination. Drechslera rostrata showed unipolar or bipolar germination from terminal as well as from intermediate cell of conidium, constant bipolar germination from both the terminal cells was observed in Bipolaris hawaiensis. The spores of Alternaria alternata exhibited germination both from terminal as well as other cells.

There are several reports showing variations in amino acid contents of closely related fungal species. In present investigation such variations were also observed amongst different species of Curvularia lunata, Drechslera rostrata, Bipolaris hawaiensis Alternaria alternata and Fusarium equiseti.

Chromatographic analysis of the free and bound amino
acid constituents of species of *Curvularia*, *Drechslera*, *Bipolaris*, *Alternaria* and *Fusarium* in free as well as in bound state.

In all, 18 amino acids were detected in free state in the culture filtrates of these fungi, but none of them universally represented. Amongst these, DL-alanine and glycine were detected in three species DL-amino-n-butyric acid, L-glutamic acid, L-leucine, L-lysine HCl, DL-serine, L-tyrosine and DL. Valine in two species, while L-arginine HCl, L-cystine, D-L-Dopa, L-hydroxyproline, DL-B-phenyl alanine, L-proline, DL-methionine, DL-tryptophan and L-cystine HCl could be traced in a single species.

Eighteen different bound amino acids were detected. Chromatographically during the investigation. Among these DL-alanine alone was detected in all the fungal species. L-glutamic acid and L-leucine were detected in four species, DL-aspartic acid, L-cystine and L-tyrosine in three species whereas DL-amino-n-butyric acid L-arginine HCl, L-cystine HCl, L-hydroxy proline, L-lysine HCl, L-ornithine, DL-threonine and DL-tryptophane could be found in a single species.

Following are contradictory views regarding the presence of DL-amino-n-butyric acid in the bound form. Steward and Thompson [1954] reported that it was a decarboxylation product of L-glutamic acid was not a constituent of protein. On the contrary Venkatram [1957], Natrajam [1958] detected as a constituent of proteins.
Forests are valuable in our daily lives, crucial to our nation's economy, integral to our long term health of the environment. Maximum photosynthesis resulting in increased biomass production is most essential so far as the optimum production from a forest is concerned. Along with other damages to leaf lamina like insect attack, the fungal infections and formation of lesions reduce the photosynthetic surface which results in biomass productivity. This one of the several damages caused to the plant species by fungal pathogens and is more relevant in case of the hosts recorded during the course of this study.