PATHOGENICITY TEST OF PATHOGENIC FUNGI ASSOCIATED WITH *Dalbergia sissoo* Roxb. DIE-BACK

By

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By

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CERTIFICATE

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Date: **June 2, 2005**

Photograph showing DIE BACK of *Dalbergia sisso*
In Changa Manga forest

AN ODE TO SHISHAM

SHISHAM THE GRAND OLD THREE OF PAKISTAN
PROSPERED FOR HUNDRENDS OF YEARS
A PARGON AMONGST ITS CLAN
FOR ITS FUTURE WE HEAD NO FEARS

BHOLD! IT WAS BEEN AFFLICATED
WITH A DISEASE OF WHICH NON IS SURE
ITS PLANTING WILL HAVE TO BE RESTRICTED
AS THERE IS NO IMMEDIATE CURE

THIS RICH AND THE POOR HAVE EQUALLY BEEN BLESSED
WITH ITS TIMBER, FODDER AND FUEL
SHALL IT NOW LEAVE US DISTRESSED
HOW A BENEFACTOR COULD BE SO CRUEL

WE SHALL NOT ALLOW IT TO DISAPPEAR
FROM THE LANDSCAPE IN AN INSTANT
BUT THE MESSAGE IS LOUD AND CLEAR
PLANT TREES WHICH ARE RESISTANT

RESEARCHERS SHOULD CONTINUE TO STRIVE
THERER IS NO CAUSE FOR DESPAIR
SHISHAM WILL ULTIMATELY SURVIVE
OUR SCIENTISTS POSSESS THAT FLAIR

M.I. SHEIKH

DEDICATION

TO MY BELOVED PARENTS WHO DREAMED ABOUT ME

TO THE LEVEL OF EXCELLENCE

WHERE I AM STANDING TODAY,

LOOKING FORWARD FOR THE MOST PROMISING

AND THE MOST GLOWING FUTURE AHEAD.

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1.	T	treatment
2.	R	Replication
3.	Spp	specie
4.	spps	species
5.	PDA	potato dextrose agar
6.	L	liter
7.	ml	mili liter
8.	g	gram
9.	C	centigrade
10.	*F.*	*Fusarium*
11.	*D*	Dalbergia
12.	°C	Centigrade

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Abstract

Shisham is an important forest tree in Pakistan has many uses such as furniture wood, building timber, and fuel wood etc. In recent few years this tree is facing severe attack of dieback and decline, which cause 5-95% loss of trees especially in linear plantation along road site, forest site and water channels. Symptoms of dieback in present studies were recorded on roots, collar portion, branches and leaves. Plants of all ages were facing this die-back problem and dying this problem is getting more severe day by day, however actual cause of this problem is still not known.

Pathogenicity of pathogenic fungi which were *Fusarium oxysporum, Fusarium solani, Macrophomina phaseolina, Botryodiplodia theobromae, Rhizoctonia solani,* done was carried by the mother cultures of Pathogenic fungi. These fungi were isolated and identified from the diseased specimens of Shisham (*Dalbergia sissoo* roxb.) including root and stem-bark. The samples were collected from different parts of Punjab at different locations like Forest site, Canal site, Road site and also site depending on Farmer's field according to severity scale.

For purification and reisolation of pathogenic fungi PDA media was used. On inoculation with *Fusarium solani, Fusarium Oxysporum, Macrophomina phaseolina, Botryodiplodia theobromae and Rhizoctonia solani* to the young plants of Shisham the maximum mortality was shown by the *Fusarium spps*. As percentage presence of *Fusarium* spps were maximum in the samples and Pathogenicity results also showed the maximum mortality rate on plants .*Fusarium solani* showed more mortality rate as compare to *Fusarium oxysporum* by showing yellowing of leaves and wilting. Shedding of leaves starts from the top and move downwards at the last stages the branches also dried *Botryodiplodia theobromae* showed the minimum mortality rate where as *Macrophomina phaseolina* and *Rhizoctonia solani* shows almost same mortality. Fusarium was isolated from the all four sites of sampling and all treatments done by *Fusarium* showed that may be the *Fusarium* is the causal agent of Die back. On the re-isolation of diseased plants maximums *Fusarium* spp were isolated besides others.

Results indicate that Fusarium *solani* and Fusarium *oxysporum* should be consider a causative and destructive agents of Shisham Dieback.

INTRODUCTION

INTRODUCTION

The forest statistics in Pakistan is not encouraging. Out of the total 87.98 million hectare area, only 4.28 million hectare (4.8%) is under forest. In Punjab, the forest crisis is further deepened. Only 2.72 percent of the total area of the province is under forests. Gap between demand and supply of timber and fuel wood is rising, while forest resources are declining. Forestry's share in the GDP and in agriculture is 0.15 and 0.62 percent respectively, which is not satisfactory and is a clear manifestation of forestry being neglected sector (Hasan, 2003). This news report shows that forest statistics of Pakistan is not satisfactory. It is important that we accelerate our efforts for establishment of forestry.

The genus *Dalbergia* is named after the Swedish Botanist Nichlas Dalberg. It belongs to the family leguminocae. The Species found in India, Nepal, Bhutan, Bangladesh, Malaysia, Pakistan and Afghanistan. William Roxburg, who named this species as *Dalbergia sissoo* early in 19th century, quoted that "upon the whole I scarcely know of any other tree deserving more attention, for its rapid rate of growth in almost every soil, its beauty, and uses, are taken into account, few trees can be compared of it", The Sissoo is widely recognized as an important multipurpose tree in Pakistan, India, Bhutan, Malaysia, Nepal, Afghanistan and Bangladesh. This tree had also been introduced into Java, Nigeria, Sri Lanka, Mauritius, Kenya, Northern Zimbabwe, Union of South Africa and USA (Sultan, 2002) Tewari (1994). Although it is a highly valuable timber tree but it also provides a range of other products such a fodder, fuel wood and medicine. Over 160 years scientists and recorders have amassed copious information on this tree species. Yet is a truism to note that it is still inadequately studied and a little progress has been made in improving the significant characteristics of either the tree form or wood qualities (Kevin, 1990). Generally it is considered that the deterioration of eco-edaphic conditions, debarking and heavy lopping fuel wood have induced stress conditions which eventually invites insect/pest attack as a natural sequence of events.

Shisham is an important timber tree that occupies more than 80% of forest area of Punjab (Anon. 2001). Besides Punjab it is also successfully growing in some parts of NWFP, Sindh and Azad Jammu & Kashmir. In Punjab Shisham is naturally growing along foot hills of Himalaya and extends up to Indus Valley till Attock. It has been planted in irrigated plantations, chak plantations, linear plantations and as shelter belt on farmlands through out the Punjab, since the mid of 19th century. It is preferred by farmers because of its deciduous habit and ability to increase soil fertility through the process of biological nitrogen fixation. Farmers are claming to grow this tree from generation to generation due to their elders' advice that this "magic" will always provide a relief during serve financial constraints, besides providing dowry for their daughters.

The species has grown for decades away from its natural ecological habitat as multipurpose trees. It is now almost a native species to different parts of Pakistan and in Nepal. *Dalbergia sissoo* has been widely planted outside its natural range. It has been established in irrigated plantations, along roadsides and canals, and around farms and orchards as windbreaks. The deterioration of eco-edaphic factors, debarking and heavy lopping for fuel wood has induced stress conditions in the species resultantly inviting insects/pest attack as a natural sequence of events. (Khan and Bokhari, 1970).

Dalbergia sissoo is considered to be native to Terai areas situated in the subtropical and dry temperate foot hills in Nepal growing at an altitude of 800 m to 1500 m with an annual rainfall range of 900 mm to 1500 mm. The popularity of sisso as a mountain tree on account of its multipurpose value and economic return with nitrogen fixing ability along with green maturing capacity and deciduous habit, rendering loose shade effect on agricultural crops, has made it the most preferred species for forestation in Teri. It is estimated to cover about 90% of the Mountain area in Terai. In India, it is extensively planted along the roadside, canal banks and on the private vacant and agricultural lands; especially in Bihar, Haryana, Punjab, Uttar Pradesh and many other parts of the country. In the sub-Himalayan tract it occurs along the rivers and streams, gregariously growing on the alluvial soil. It has been widely used for forestations, most parts of the country except in the very hot, cold and wet tracts (Sahim, 2001).

In Pakistan, it is found along the foothills of the Himalayan Mountains. As a species, it extends up the Indus valley of Attock, but does not dominate over any appreciable area (Champion et al., 1965) it was introduced in Changa Manga in 1866 to produce coal for the railways. Plantations exist at Kahanewal, Bhagat, Bhakkar and Mianwali. It causes white spongy rot in the sap wood. Lateral spread of the disease in Plantations is through root contact which result gaps in plantations. (Sahim, 2001)

Dalbergia sissoo is distributed throughout the canal system of Mardan, Charsadda, Bannu, and Peshawar. Since time immemorial, the species have been utilized for timber and medicines. It is a most valuable species and is preferred for plantations for its fast growth and multipurpose use in the Peshawar valley. It is grown in private land, community land and forest land, along the canals, Roadsides and railway lines. And even in the agriculture land as an agro-forestry crop. One of the most important benefits of this tree is that it can be grown on any well drained soil, even on pure sand. It is mainly grown for its heartwood, which is hard, heavy, strong, durable and elastic. It is among finest general use timbers of Pakistan and is commonly use for high class furniture, cabinet making and marine grade plywood. It is also suitable for all kinds of construction work and the wood is an excellent fuel (Siddiqui *et al.*, 1996). Its leaves are useful as livestock fodder, and being a nitrogen fixing tree, it enriches the soil (Hocking, 1993).

Shisham is one of the most important timbers of Pakistan. Its heartwood is excellent for high class furniture, paneling and other ornamental and constructional works. It is also used in the gun carriage parts and vehicle bodies. Shisham of this tract is gifted with the natural qualities of being find-grained with light brown colour and is, for these reasons, considered to be superior in quality to that found in the Punjab especially for high quality cabinet making. As matter of fact it is graded as of top most quality in the undivided sub-continent of Indo-Pak. The Pakistan Railways are using this timber in the construction of wagons especially the foot boards.

Shisham is one of the most useful multipurpose trees of South Asia. It is mainly grown for the timber which is among the finest for cabinets, furniture. The wood is excellent for fuel wood and charcoal. Leaves, young shoots and green pods are an important source of fodder. The leaves contain up to 24% crude protein. The fodder value is highest in April and May when other sources of green fodder are scarce. The species is nitrogen fixing and used in agro forestry systems with crops. Although Shisham trees can negatively effect crop production due to competition for nutrients, moisture and light. It provides shade and shelter. Its habit of developing root sucker and runners makes it useful for erosion control. It has some medicinal values as oil obtained from the seeds which is used to cure skin diseases. The powdered wood, applied externally as a paste, is reportedly used to treat leprosy and skin diseases. The roots contain tectoridin, which is used medicinally (Sheikh, 1988).

Dalbergia sissoo is a medium to large, deciduous, long-lived tree with a spreading crown and thick branches. It attains a height of up to 30 m and a girth of 2.4 m; the bole is often crooked. In the Rawalpindi district, Pakistan, it occurs in the form of a straggling bush at an altitude of 1500 m, clinging to crevices in the sides of sandstone cliffs. The bark is thick, rough and gray, and has shallow, broad. Longitudinal fissures exfoliating in irregular woody strips and scales (Luna, 1996). *D. sissoo* develop a long tap root from an early age has numerous lateral ramifying roots (Hocking. 1993).

The leaves of *Dalbergia sissoo* are compound, imparipinnate and alternate, with rachis 3.5-8 cm long, swollen at the base. There are 3-5 leaflets, each 3.5-9 x 3-7 cm; leaflets alternate, broadly ovate, conspicuously and abruptly cuspidate at the apex, rounded at the base, entire, pubescent when young and glabrous when mature. The terminal is larger than the others. There are 8-12 pairs of veins in the leaflets (Parker, 1956; Luna 1996).

The inflorescence of *Dalbergia sissoo* is an axillary panicle 3.5-7.5 cm long. The flowers are small, 7-9 mm long. White to yellowish –white a pervasive fragrance, sessile, papilionaceous and hermaphrodite. The standard petal is narrow at the base and forms a low claw; wing and keel petals are oblong. Pods are 4.5-10 x 0.7-1.5 cm, liner, oblong, indehiscent, stipulate, glabrous, apex acute, reticulate against the seeds, and usually 1-4 seeded.

Seeds are kidney – shaped, variable in size (8-10 x 4-5,5mm), and pale brown, brown to brownish-black, reniform, compressed, with papery testa (Parker, 1956; Singh, 1989; Luna, 1996). The fruit is a light brown indehiscent pod, 5-9cm long, 10-12 mm wide, thin and glabrous with conspicuous veins.

In Pakistan and India, rotations of 10-22 years are used for the production of fuel wood and small timber in affricated plantations. For production of larger timber, rotations of 40-60 years are used. In Pakistan, the cost benefit ratio of irrigated plantations is rather low. This may be due to a low crop density and growth rate as a result of indefinite irrigation systems (Haq, 1985).

In India, annual growth rates of 10-17 cubic meters per hectare have been recorded in plantations (Hocking, 1993; Gupta, 1993). A very high annual growth rate of 22.6 cubic meters per hectare was recently recorded in sample plots of the Pakistan Forest Institute, has computed yield and mean annual increment from 291 measurements of permanent and temporary sample plots in 17 major irrigated plantations in Pakistani Punjab.

A number of bio- mass production studies carried out in India and Pakistan gave the results of 7.7-17.4 tons/ha per year (Sharma *et al.*, 1988; Swaminath, 1988). In a 24-year – old plantation, the most bio-mass was accumulated in the bole and the least in the foliage, with 55% and 2.6% respectively. (Luna, 1996).

The term die-back used to describe the stress conditions is characterized by the reduced growth, shortened internodes, root necrosis, premature fall coloring, sprouting from adventitious buds and increased prevalence and Pathogenicity of root decay fungi, nematodes, insects etc. (William and Edwin, 1994) Generally it is considered that these abnormalities resulted form deterioration of eco-edaphic conditions, debarking and heavy lopping, intensive cultivation of agricultural crops and extensive use of chemicals and changed pattern of agricultural practices which eventually inviting insect/pest attack as a natural sequence of events (Anon, 2001.) This disease came to limelight since mid 1998, after which different institutions like Ayub Agricultural Research institute (AARI), University of Agriculture Faisalabad (UAF) Nuclear Institute of Biological and Genetic Engineering (NIBGE) Pakistan Forest Institute (PFI) Peshawar are under-taking diagnostic studies to work out the remedial measures. This study is designed to evaluate the extant of die-back in state owned plantations and on farmlands of district Jhang. This valuable timber is subjected to various diseases causing colossal damage to the plantation crops and individual trees growing on water channels and on the border of agriculture fields.

It is well established fact that Shisham is facing severe threat of die back, yet unidentified causes. However, there are some reports available in literature that indicate, die back is perhaps due to collar rot and root decay. In this situation the top leaves of the infected plants turn light yellow and withering of individual branches takes place from top to down. Bark at the collar region turns brown and gives rot like appearance, often it splits away, separated from cambium which can be easily pealed off. There are holes in the bark and galleries in the cambium. This disease attacks the plant regardless of its age. (Zentmyer, 1980).

In spite of this disease other diseases like powdery mildew, leaf rust, leaf blight and *Ganoderma,* root rot, leaf rot, leaf wilt have been reported (Khan and Bokhari 1979).

Nowadays Shisham trees in Pakistan are found to be attacked with two types of diseases; wilting and dieback, the latter being more prevalent than the former (Bajwa *et al.,* 2004). In case of dieback, during 1990-91 disease incidence recorded was 5 % which has raised to 25 % in 19 districts of the Punjab during 1999-2000, especially in the linear plantations along the canal bank, road side and water channels. Since then no control measure has been adopted, therefore the disease is on the increase (Gill *et al.*, 2001).

Hasan (2002) emphasized on ecological value of this tree and highlighted the need of effective remedy to the deadly quick Shisham decline. According to him the officials of Punjab Forestry Department link the cause of disease to consistent drought conditions in the country as a result; growth of Shisham has been adversely affected because of diminished water availability. More over, the pathogen is not only confirmed to Sissoo tree but other plants e.g. Mango, Guava and Citrus orchards are also attacked.

Sahim (2001) reviewed the literature of dieback and drew the conclusion that adverse climate, biotic pressure, high susceptibility and decline in management practices are the main causes of this menace. He also suggested working out the proper site, planting material, mixed cropping, avoiding debarking, use of Bordeaux mixture and certain fungicides to control the Shisham dieback.

Champion *et al.* (1965) assigned the natural forests of this specie to tropical dry mixed deciduous and dry deciduous scrub forest types, occurring in open and low forest formations. These are composed entirely of deciduous trees and some trees of the thorn forest type, with a predominantly deciduous trees and some trees of the thorn forest type with a predominantly deciduous shrub layer and are limited to Himalayan foothills. *Dalbergia sissoo* colonized newly formed land, preceded by Saccharum munja, S. spontaneum and Tamarix dioca when stream and rivers alter their courses or add fresh deposits of sand and shingles (Troup, 1921).

Dalbergia sissoo dieback is a common problem in countries like Nepal, India, Bhutan, and Bangladesh. *Dalbergia sissoo* dieback is now acknowledged as a threat of national significance to the biodiversity and ecological sustainability causing irreversible loss to natural vegetation.

The disease is a slow dieback and yellowing disease of *Dalbergia* sisso whose first report was published in 1999 (Shakoor *et al.,* 1999). Declining *Dalbergia sisso* trees show visible symptoms leading to death of plant. Pathogen tends to kill the tree from the top to down. A tree's ability to produce seeds is not eliminated by the pathogen until it dies completely. The disease manifest itself by yellowing of leaves development of sunken areas at collar level and characterized by red-brown (cinnamon) discoloration of the cambial region which extends from below the root collar to 3-4 inch or above the ground. Underneath the bark, dark lines are formed extending vertically up and down from the depression. The infected feeder roots turn black and brittle as they die. Lastly lack of root regeneration leads to decline and death of completely girdled trees. In finally diseased trees the leaves sometimes turn reddish brown and remain sticking to the branches. Regardless of how rapidly crown dies, their root system are always in an advance stage of deterioration by the time crown symptoms appear. Death of the smallest root is often the indication that a tree is beginning to die of decline, followed by larger roots that die and then the death of inner bark in vertical streaks along the tree bole occur. Once a tree dies, it generally remains standing long after death (Zakaullah, 2003). This disease is reported in trees of all ages (Gill *et al.,* 2001).

Decline is the general term that describes deterioration in a tree's crown or an overall reduction of tree vigor. Trees of all ages may be affected but decline or dieback usually occurs when trees reach to a significant size and maturity (Hiemann *et al.*, 1999).

Concerning to the examination producer Hiemann *et al;* (1999) explained the causes that may be involved in shade tree disorder, decline or dieback. These workers stressed physical injury, construction injury, soil compactation, weather conditions, water logging, salinity and use of herbicides as major factors for tree dieback. Further they established an elaborated procedure for accurate diagnosis of the entire diseased plant which involves field examination and laboratory analysis.

Forests being naturally dynamic are always undergoing change even in an area largely unaffected by humans. This dynamic status of forests play key role in their decline all over the world. Therefore, we are not alone in our efforts to try and resolve the puzzle of forest decline. There are several dozen tree's declines in different forests scattered all over the world.

Hennon (1993) worked on Yellow Cedar decline in Alaska and explained it as a naturally occurring phenomenon. According to him it was the slow passing of one specie independent from human activity from an ancient family of trees. The study showed that primary cause appeared to be some environmental stress possibly soil toxins and freezing damage that first affected the fine root system of these valuable trees which then became attacked by several fungi, bark beetles and nematodes resulting in ultimate death of victim.

Grant (1998) used ecologic study approach to understand correlation between acid deposition and ozone dose for Oak decline and excess mortality of Oaks and Hickories, in which ozone doze was found to be very highly correlated with Oak decline in the south eastern U.S. In this study the excess mortality was thought to be because of acid deposition and ozone exposure. Mortality data showed that Red Oak were more sensitive to ozone than White which were highly affected by acid deposition.

Kendall (2004) studied the different abiotic and biotic factors involved in decline of White bark Pine in Montana U.S. and reported that an exotic fungus white pine blister rust had killed many White bark Pine trees.

Shisham die-back is very common in sub-continent especially in Pakistan, Nepal, India and Bangladesh (Sahim, 2001). Anyone who travels on any road, along farms and canal banks is shocked to see the dry Shisham tree with no branches. It is believed that the primary causes of dieback are variety of physical and soil based physiological stresses resulting from intensive cultivation of agricultural crops, extensive use of chemicals and changed pattern of agricultural practices on the farm lands (Anon, 2001).

The terms like dieback, decline, stand level die-back and canopy level die-back are used to describe the stress conditions resulting into the death of the trees. Die-back is characterized by the presence of symptoms such as reduced growth, shortened internodes, root necrosis, Pathogenicity of root decay fungi, nematodes, insects etc. Another feature of decline is its progressive nature and differences in the progression of symptoms between trees in the same stand. Some trees may have slight symptoms while others are in intermediate conditions and still others are dead (William and Edwin, 1994).

Although Shisham has been suffering from dieback for the last 8-10 years but it has started out bursting from the last 5-6 years. It is becoming more and more serious. In Pakistan quick wilting, drying, decline and die-back came to lime light in 1998.

Many fungi are recorded on Shisham which attack and branches girdle and kill them. Frequently these fungi attack trees, which are otherwise weakened, an in some cases damage may be noticeable. Dying of portions of branches or of entire branches may be an indication of canker disease. Affected trees should be relived of suppression and affected portions removed and burnt. The fungi which have been recorded on Shisham are: *Cryptovalsa rabenhorstii, C. mori. Eutypa subtecta, Eutyella russodes, Nodulosporium gregariumn.*Otherwise bad compartment, the healthy Shisham trees show good root extension where the soil is not compact. Under conditions of the plantations, lateral growth is more marked in the 3rd year buy major part of the root system is shallow due to faulty irrigation practices.

The moisture determinations have shown that wherever the soil moisture percentage is lower than the moisture equivalent, there the plantation suffered in all cases and where an adequate moisture level is maintained in the soil, a healthy growth is noticed. In irrigated forests, therefore, proper up-keep of the soil moisture is very essential for normal and healthy growth of plants. When the portion of soil profile available for root expansion below the plants is shallow, frequent and low depth watering are called for, to keep soil moisture level suitable to meet the requirement of plants Shallow rooted plants thrive on such a profile (Sahim 2001)

The physical and nutritional factors are more important in contributing to the disease. The soil and water temperatures lag behind the air temperatures, sometimes by as many as 24 F or –4 C. The lower soil temperatures reduce the absorption of water by the roots. The high temperatures during the day increase losses of water by evaporating from the soil due to direct isolation, which may be excessive in a thinly stocked area and thus prove injurious by rapidly depleting the water reservoir in the root absorption zone. This defect however, can be overcome by creating optimum percolation conditions in the soil and ensuring proper crop density, which will also considerably influence the soil temperature.

Shisham has proved to be a failure in common with Melberry and Bakain in different areas. The spirited for the compact soil should, therefore, be such that they can either penetrate the hard compact layer or break through this soil in such a way as to pave the way for the percolation of water along the root which would provide better reservoir for moisture after irrigation. Once the water reaches there, it will be better reservoir for moisture after irrigation. Once the water reaches there, it will be less subject to atmospheric changes. There fore, we must look to the species growing successfully in similar lactones and still possess Julie flora come in handy under such conditions.

The fungus primarily causes sap rot but can also attack heartwood, growing both on living trees and dead wood. The rot produced is a characteristic and constant feature (Bagchee, 1952). In early stage bleached pockets and formed. The fungus causes a typical white rot surrounded by a dark brown band, the wood becoming light cream in color. The rotten wood splits tangentially along the annual rings and radically along the wood rays. The cracks may sometimes be filled with pale brown hyphae. Finally the wood is reduced to a spongy mass. Zone lines are also formed. It causes severe heart rot within the living host and it is responsible for destruction of large quantities of wood annually. Although it attacks living trees and has been shown to attack living cells, it mainly functions as a saprophytes growing on dead tree stumps and slash. It probably would never attack living tissues of healthy trees and is frequently found on living hosts injured by lightning, fire, frost, or mechanical means, which expose the sapwood. By attacking the heartwood it it unfit renders for use. Like *Ganoderma lucidum*, in collar infections, the fungus works in association with *Fusarium*, but as a butt rot, it is associated with other canker organisms, mostly a socomycetes.

The Sporophores of *P. gilvus* are always associated with dead and dying trees. It is a widely distributed fungus in the Indo-Pakistan, in the hills as well as plains. The association of the fungus with cankers on affected trees is one of the chief symptoms of the diseases. The Sporophores are found growing in imbricate fashion. Gummosis occurs from the cankers. Foliage is yellow, reduced in size, forming clustered growth, giving the trees somewhat bushy appearance. Finally the tree becomes 'stag headed' (Some of the symptoms found in Punjabi and NWFP).

One finds *Ganoderma lucidum* on the top of the list of pathogens of Shisham followed by *Poria ambigua*. *Polyporus gilvus* causes die back and caner but are not serious in Pakistan as in rainy wet areas of India. Besides these there are Powdery mildew and Rust, which are of minor importance. Wilt due to *Fusarium oxysporum* is quite serious in some localities subject to excessive moisture and water logging.

Khan and Bokhari (1970) carried out a complete survey in one of the typical irrigated Shisham plantations, the Bhagat reservoir plantation, with a past history of fungus diseases. The existing forest becomes all clear because more tress had been removed during the previous operations had required in normal feeling to eliminate diseased ones. Out of a total eight percent of diseased Shisham trees, the most common pathogens are: *Ganoderma lucidum (Fomes lucidus) 7.85%) and Poria ambigua*

*(0.46%).*It is perhaps apparent that poor soil water conditions predispose Shisham to fungal attack.

Ganoderma lucidum (root and but-rot) the disease is common both in natural forests and plantations. Trees of advanced age are normally attacked by the pathogens. The disease occurs in light as well as in heavy textured soils. In light soils the pathogen spreads rapidly on roots and trees are killed in assort period. In stiff soils, however, the fungus spreads slowly on roots. The Disease spread through root contact and, therefore, in pure plantation, the spread is rapid resulting in large gaps in the forest. The affected trees exhibit a stag headed appearance in which condition they may continue for a few Years before being killed. The semi-circular to kidney shaped fruiting bodies (perennials) appears at the base of the diseased trees. The pathogen causes white sponge rot generally confined to the root and butt portion of the trees. In a monoculture susceptible crop, the disease is known to occur in an epiphytotic from and the forest managers are forced to adopt a pathological rotation.

Khan (1961) reported the fungus *Ganoderma sp.* on wide variety of host such as *Querers, Acacia and Dalbergia* etc. It was observed that in warmer climates, *Ganoderma* naturally grows well on *Dalbergia sissoo*. Khan and Bokhari (1970) carried out a complete survey in one of the typical irrigated Shisham plantations, the Bhagat reservoir plantation, with a past history of fungus diseases. The results of these studies focused attention on the serious nature and vast extent of such losses caused by fungal diseases. It has been shown that the number of trees after the third thinning became nearly half as compared to the normal. At least one tree per acre was found diseased out of 74 standing trees. The existing forest became healthier because more trees had been removed during the previous operations, required in normal felling to eliminate disease ones.

Fusarium solani (wilt disease) The Disease is prevalent in the Punjab in plantations Raised on unsuitable sites, i.e., on stiff and clayey soils, Where aeration is poor, roots do not develop healthy and may also die from asphyxiation. The pathogen enters through Dead and weakened roots from where it progresses into healthy tissues. Wilt, like many vascular wilts, is favored by high soil moisture in sites with poor drainage. Wilt disease is systemic and manifests it self during rains between July and September. Symptoms include yellowing and death of leave in acropetal succession up the advanced age trees. Eventually, the leaves drop-off rendering the branches increasingly bare. Affected trees die with in a few months. The pathogen is mostly restricted to roots and in some cases may extend to stem basses. The outer sapwood exhibits a characteristic pink to reddish pink stain. In diseased roots, vessels are plugged with hyphae and infiltrated with jelly – like substances which hinder sap flow to the crown resulting in wilt symptoms. The toxins like fasare acid are produced and that may be responsible for causing wilt.

Fusarium oxysporum which is a common disease in nursery as welt as of larger plants of shisham, Babul (*Acacia arabica*) Khair (*A catechu*), Albizzia, *Teak* and *Sal* (*Shorea robusta*). Shisham wilt is of common occurrence in Indian taungyas, Sind and Punjab plantations. In wilt, as the term signifies, there is the sudden withering or wilting of plants, as if under drought but actually such conditions being absent. In seeding, it is manifest when the entire top shows simultaneous withering. Amongst trees the entire up may wilt or individual branches may be affected. The disease may be seen during or after the rains, sometimes after the flush.

Pinkish brown discoloration is seen in bark and peripheral sapwood several feet up from the base (Fusarium Wilt). Production of fermenting liquid and plugging of the conducting vessels by hyphae occurs. Verticillium wilt of maple shows greenish areas in the vessels, which is absent in Fusarium wilt. The disease may be growing in soil with sufficient moisture to support their life. Wilt is generally the result of *Fusarium* attack at the roots or even lower portions of stem where is growth interferes with the conduction of water and excretes toxins of the nature of conjugated phenols (Davis et al. 1953). It is quite destructive and a vast variety of conifers and hardwoods such as *Pinces, Oaks, Elders, Chestnuts, Maples, Linden, Shisham, Muberry, Guava, Wainut, Siris,* are susceptible to it (Bakshi, 1954; Beg and Jamal, 1974).

Shisham wilt is common amongst trees growing in marshy or water-logged tracts, canal side plantations, in depressions along the roadside, in orchards and tea gardens where the land is ploughed or hoed. In India it has received active attention of Bsagchee and his associates (1945). Although the wilt of Shisham, Babul, Khair in the sapling stage is a persistent menace in the taugyas, yet the trees do not suffer from damping off in the seedling stage. The occurrence of wilt, therefore, is a separate phenomenon from damping off which takes place under different soil conditions. A number of other fungi also cause wilting of plants. Amongst the Deuteromycetes, beside *Fusarium* and *Verticillium*, the *Cephalosporium* is common. The Pycomycetes include some of the most active pathogens namely *Phytophthora* and *Pythium* which cause, beside wilt, a number of other diseases in a large variety of plants.

Fusarium wilt occurs mostly in young Shisham trees and is a menace in the taungyas of U.P (India) and plantations Punjab and Sind (Pakistan) (Bagchee 1945, Beg and Jamal 1974). The *Fusarium oxysporum* is an active parasite and enters the host through wounds by air borne spores, besides direct mycelial and spore contamination from the soil. The same fungus is named by Bagchee as *F. solani*. It is also recorded on Teak (as *Nectria haemotococca*). It becomes most active during or after the monsoons when the temperatures are high, combined with excessive moisture in the soil and air. The disease decreases when both these condition come to normal. The fungus enters through wounds or cracks at the junction where lateral roots arise, and spreads through veeds which are plugged by hyphae and gummy substances are produced during the attack. Once the disease is active it is impossible to save the plant. In Shisham a soft rot of pinkish brown colour sets in cambium and sapwood. The bank peels off and pinkish

Phytophthora wilt sometimes occurs overnight as the fungus under favorable conditions completes its life cycle in nine hours. It also attack leaves branches and steam of various plants. When branches and stem are attacked, wilting of the whole plant or a part of it may result. This fungus besides wilt causes bud rot, fruit rot, damping off, canker and black thread disease of a large number of plants .

Rhizoctonia solani (Corticium solan) has been reported from India, causing collar rot of Shisham seedling of one to two age under moist conditions. The fungus causes yellowing or Browning of the hypocotyls. The discolored area collapses and the seedling dies. *Rhizoctonia bataicola* (*Macrophomina phaseolina*) has also been reported to kill 6-9 moths old seedling of Shisham in forest nurseries.

Damage done by soil fungi (*Rhizoctonia solani* and *Fusarium oxysporum*) on Shisham has been estimated at 6% in Bhagat Shorkot plantations. They block the conducting systems besides toxin production. Patristic plants reported to cause considerable damage to sissoo include *Loranthus longiflours* and *Tapinenthus dononeiflius*; in alluvial forests, climber like *Dregea volobilis, cryptoleps volobilis, cryptolepis buchanina and Acacia pennata* cause the same damage. Leaf diseases include the powdery mildew *funus, Cercospora sissoo, collectotrichum sissoo*, and *fusarium solani dalbergiae*. Wood pathogens recorded include *Daedalea flavida* and *fomes durissimus* (stump rot fungus).

A number of authors have worked on the fungal diseases, besides hanerogamic parasites and physiological disorders of *Dalbergia sissoo* in depth studied. In contrary with conditions prevailing in Indo-pak Sub- continent have been carried out , notably by sultan (1956), pakshi *et .al.*(1961).

Bakshi (1954) identified the *Fusarium solani* as wilt causing agent in mature Shisham. He also studied behavior of *Fusarium solani* which revealed that it has quick and wide power of saprophytic colonization of dead roots. Bakshi (1957) during a survey of diseases in Sissoo forests, that the wilt and other root diseases are absent in the River beds, where Sisso grow naturally. He also found *Fusarium solani, Ganoderma lucidum* and *Phillinus gilvus* in varying degree on sites away from River bed.

Beg and Jamal (1974).Nepal & Pak PFI. Bakshi (1974) described the damage caused by *Ganoderma lucidum* to young plantations of Khair, Sissoo, and eucalyptus. He suggested the removal of residual roots, planting of mixed species and digging of isolation trenches as control measure.

Bagchee (1952) reported the research investigations on different disease of Shisham for the first time in the sub continent. He also found that *Phillinus gilvus* is the primarily casual agent of sap rot, it is susceptible to root-rot due to *Ganoderma lucidum* either pure or in mixture and vascular wilt due to *Fusarium spp.*

Harsh *et al.* (1992) reported a new seedling wilt disease of Shisham (*Dalbergia sissoo*) and the pathogen was identified as *Fusarium oxysporum*. After one year it was observed that 25% of the 8000 saplings began to wilt and 5% of them have completely been died, after on year. The root samples of affected saplings were collected and used for isolation of fungi. A fungus *Fusarium solani* was identified as common soil born pathogen. It was also proposed to exercise two successive soil drenching with 1% commercial formalize at 10 days interval along with the reduction in water logged conditions could control the spread of wilt in Shisham. Tewari (1994) PFI (Nepal & Pakistan die - back)

Wester and Marks (1987) estimated that 14% of forest area of *Eucalyptus margimata* in Australia was affected with die back disease. The disease was also recorded on 59 indigenous plant species present in these forest areas of *Eucalyptus.*

West and Marks (1987) estimated that 14% of forest area of *Eucalptus margimate* in Australia was affected with die-back disease. The disease was also recorded on 59 indigenous plant species present in these forest areas of eucalyptus. Shearer and Tippet (1989) stated that the *Eucalyptus margimata* (Jarrah) die-back caused by *Phytophthora cinnamomi* is a major factor affecting the ecology and management of the Jarrah forest. Mark and smith (1991) reported that the *Phytophthora cinnamomi* is a primary cause of die-back in coastal forest of Victoria.

Harsh *et al.* (1992) reported a new seedling wilt disease of Shisham and the pathogen was identified as *Fusarium oxysporum.*

Tabassum (1996) investigated fungi associated with Shisham pods and seeds and found it to be affected by seed storage condition and diseases of seedling in Shisham nurseries. For this he studied 200 Shisham pods and seeds each and revealed nine fungi and one bacterium.

Khan *et al.* (1999) identified *Phytophthora, Fusarium, Phoma and Botrytis* from diseased parts of Dalbergia sissoo and also identified a root rot fungus *Ganoderma lucidurm.*

Shakier *et al.* (1999) gave first report on Shisham dieback in Pakistan and after early investigation reported Fusarium solani, being isolated from infected roots along with *Aspergillus, Verticillium, Cladosporium* and nematodes.

Tewari (1994) worked on heart wood borers of *D.sissoo* and found two important species *Crossotarsus saunders* and *Aristobia horridula*. The beetles of *C. saunders* bore into dying or newly felled trees where as the larvae of *A. horridula* feed into the meristematic tissues and then penetrate into the sapwood and heart wood, making irregular galleries.

Manandhar *et al.* (2000) examined roots and one seed sample of *Dalbergia sissoo* and conducted Pathogenicity test of *D.sissoo* seedling. The seeds were examined and *Aspergillus spp.* and *Alternaria spp.* were found associated with them.

Joshi and Barel (2000) conducted a study in 1998-99 in Nepal and recorded several fungal pathogens, insect pest and bacteria as the major factors of Sissoo mortality. Culture made from root and stem of the trees revealed that Sissoo was seriously attacked by fungus *Ganoderma lucidum, Fusarium solani* and a bacteria. They recommended conducting a general survey of Sissoo die-back, use of appropriate fungicide, proper silvicultural operations, and selection of resistant varieties

Niaz (2001) held various meetings with different scientists working on the Sissoo mortality and recommended to remove all dead and dying trees in all localities, use of certified seeds collected from plus trees, prevention of heavy lopping Shisham, avoiding over irrigation of Shisham and introduction mixture of indigenous tree species instead of monoculture.

Zakaullah (1999) reported that a new destructive fungal pathogen (*Phytophthora spp.*) occur in an epiphytotic from in the irrigated areas of Punjab causing collar rot, die-back and complete death of infected Shisham trees growing generally of farm land and canal-side plantations.

Khan (2000) compiled a report on Shisham die back in Pakistan and its remedial measures in which he concluded that Sissoo die back could be due to the attack of root and stem rotting parasites. He suggested that disease can be prevented by combining different practices like Ban on debarking, application of fungicides, proper sit selection, deep plating, sanitary precautions, mixed cropping, removal of over aged trees, avoiding injuries. Furthermore, he also proposed a comprehensive survey in different ecological zones to evaluate the damage and causal agent in the natural and man made plantations of the country.

Negi *et al.* (1999) conducted a survey in Haryana, UP and Behar to assess the mortality of sissoo and analyzed the soil and plant parts of the affected plants. This survey indicated that the northern districts were badly affected where the soil pH was ranged between 7.5-9.7 as compared to healthy locatives where pH neutral. Besides, chemical analysis of leaves showed lower concentration of Potassium, Phosphorus as compared to healthy sides which could have resulted in poor uptake of nutrients.

Sharma *et al.*, (2000) compiled a report on *Dalbergia sissoo* in India, where in it was mentioned that recent surveys to different status of India i.e. Bihar, Hayyana, Dehli, Punjab, Himachal Pradesh and U.P indicated that Sissoo mortality was prominent either in isolated trees or /in trees growing on agricultural bunds, roads and canal side. In the exposed roots of stressed trees, the feed ling roots were blackish in colour and partially decomposed. An ocular estimation of area revealed that mortality percentage was in the range of 20-30%. They have also mentioned the occurrence of *Fusarium solani, Ganoderma lucidum, phellimus spp.* Root, and butt rot and root-knot nematode in Shisham. In 19 different localities of India Joshi and Barel (2000) conducted a study in 1998-99 in Nepal and recorded several fungal pathogens, insect pest and bacteria as the major factors of Sissoo mortality. Culture made from root and stem of the trees revealed that Sissoo was seriously attacked by fungus *Ganoderma lucidum, Fusarium solani* and a bacteria. They recommended conducting general survey of sissoo die-back, use of appropriate fungicide/insecticide, proper silvicultural operations, selection of resistant varieties and proper selection of planting material.

Shukla (2002) reported that different surveys conducted to assess the mortality of sissoo in India revealed that the adverse climatic factors, construction of roads, canals and railway lines are the major causes of mortality with particular reference of *Fusarium solani* which is a parasite on the dead roots and collaborative organism for the wilting in Shisham. He also suggested choosing proper site selection, sanitation, tree improvement and introduction of antagonistic fungi against pathogens.

Anon (2003) analyzed 135 soil samples collected from different localities of Punjab and concluded that soil texture, soil pH, soil electric conductivity and percentage of soil organic matter were not responsible for Shisham die back. It is also found that the nutrients are available from satisfactory to adequate level except zinc. They suggested to under taking an investigation o soil physical aspects and pathogenic aspects under field conditions.

Wheeler (1969) stated that the fungi of genus *Colletotrichum* were much less common as cause of damping-off and seedling blight. However, they cause damage to many tree species. The mycelium of these fungi is seed-borne and persists for relatively long periods in crop residues. The fungi are mainly seed borne either as spores on the seed coat or as mycelium within the outer layers.

Pavgi and Singh (1971) reported that *Blomerella cingulata* (Stonem) Spaulding and Shrink and *Septothyrella dabergiae* (Pavgi and Singh) were recorded on pods of Shisham from Varanasi (U.P). A species of *phoma* was common on pods of Shisham forest in western Uttar Paradesh.

Hiremath (1991) studied the seed-borne nature of causal agent of Shisham blight. *Colletotrichum gloeosporioides* was isolated from all heavily infected pods of Shisham, even after surface sterilization. Isolation from discolored and shriveled seeds was 84.62% and from apparently healthy seeds 58.88%. surface sterilization of seed with 0.1% mercuric chloride reduced the percentage isolation depending on the length of treatment but even after 300 seconds, 2.5% of the apparently healthy seeds yielded the fungus.

Harsh *et al.*, (1992) studied Fusarium wilt of *Dalbergia sissoo*. The results are reported of a study of seedling wilt disease of *D.sissoo* found in nurseries and young plantations in Madhya Paradesh. The pathogen was identified as *Fusarium oxysporum*. The disease was severe during summer and high temperature and moisture flavored its development. The results of laboratory and field control tests indicated that a soil drench with 0.2% Bavistin or seed dressing with 0.2% Topsin-M were the effective methods. Other fungicides tested were captan. Fytolan and Pentachlornitrobenzene.

D. sissoo is attacked by 125 insect pests, out of which ten are considered important (Luna, 1996). *Plecoptera reflexa* causes defoliation by heavy and repeated attacks, resulting in reduced growth and mortality in plantations (Qadri, 1952; Chaudhry, 1954; Chaudhry and Gul, 1984). For effective control of *plecoptera reflexa*, proper selection of insecticides and timely applications are important; this pest can also now be controlled by botanical pesticides (Chaudhry and Bajwa, 1992).

The biggest threats to *D.sissoo* are heartwood borers. Two important species are *Crosstarsust saunders:* bore into dying or newly felled trees and construct tunnels running radically towards the heartwood and then turn parallel to the circumference. Vertical tunnels run upwards and downward from the main tunnel. The larvae of *Aristobia horriduala* feed into the meristematic tissues and then penetrate into the sapwood and heartwood, making irregular galleries (Tewari, 1994).

Agrilus dalbergiae is a bark borer and the grub feeds on the bark of *D. sissoo* causing weakening and mortality of trees (Gul and Chaudhry, 1983).

Bruchus pisorum infests seeds of *D. sissoo*. Infestation is normally initiated in the filed but breeding can continue during storage (Rehman, 1993).

The origin of *Dalbergia* dieback is still unknown but it is suspected of being caused by fungi (Gill et al., 2001). Guava, Mango and Citrus orchards show similar symptoms which suggests that the disease may not be confirmed to Dalbergia (Hasan, 2003). *Dalbergia* decline had so far been observed mainly in sites that have undergone significant disturbance, such as farmland, road side and canal banks.

The objective of the study was to do Pathogenicity test of fungi as it is not cleared that which fungi is most pathogenic so that be able to confirm that whether these pathogenic fungi reported by different pathologist are cause of this die back or there are other factors associated with the die-back of *Dalbergia sisso.*

MATERIAL AND METHODS

Materials and Methods

Pathogenicity of pathogenic fungi was carried by taking the mother cultures of Pathogenic fungi .These fungi were isolated and identified from the diseased specimens of Shisham (*Dalbergia sissoo* roxb.) including root and stem-bark. They were collected from different parts of Punjab at different locations which are Forest site, Canal site, Road site and Farmer's field. (Ishaq, 2005)

Preparation of pure cultures:-

Pure cultures of fungi were achieved from the mother cultures by single spore technique under Laminar flow hood with stereomicroscope. It was preserved on PDA slants with the help of cork borer and kept in refrigerator for the further use.

Pathogenicity test:

Five different pathogenic fungi were selected for the Pathogenicity test on the basis of their severity and possibility of occurrence on infected samples, these are

1. *Fusarium solani*
2. *Fusarium oxysporum*
3. *Macrophomina phaseolina.*
4. *Botryodiplodia theobromae.*
5. *Rhizoctonia solani.*

Different methods were used to test their Pathogenicity according to the mode of infection and nature of pathogenic fungi. Each experiment was run in three replications along one set of control. In each treatment one plant is set as a standard or control. Pathogenicity of pathogenic fungi was conducted on *Dalbergia sissoo* plants of 2-3 feet carried out in earthen pots of 12" containing autoclaved soil. Present tests are carried out in four treatments having three replications.

Treatments:-

- **T1** = Treatment 1= Inoculation at the junction of stem
- **T2** = Treatment 2= Inoculation at the junction of 2 branches
- **T3** = Treatment 3= Tooth pick technique
- **T4** = Treatment 4= Soil inoculation through spraying

Inoculation at junction, stem and 2 branches:-

In first two treatments the Roots and stems of separate plants were inoculated by making a cut in the stem or at the junction of stem and root, using a sterilized knife. A 1×2-cm inoculum block from 5-7 days old pure culture was placed in the gap and the inoculated portion was wrapped with Para film. The wrapping material was removed from the stems after 2 weeks of inoculation. The observations were carried out after every 3 days, up to 45 days. (Nene *et al.*, 1981)All were conducted in triplicate.

Tooth pick technique:-

Tooth pick packets were collected, made up of bamboos and pointed from both sides; these were put in a jar containing potato broth. The volume of the nutrient solution was enough to dip the tooth picks. Autoclaved the bottles along the tooth picks dipped in potato broth with loose lid for 25 mints at 180 °C, than when jars was cooled up. They were inoculated with 2 mm agar blocks containg pure culture of different pathogenic fungi. The bottles were put at 25°C -30°C for 8-15 days depending upon the growth of pathogenic fungi. The observations were done with out opening the bottles which had tooth picks completely colonized by the fungal growth. After colonization of fungi colonies on tooth picks these tooth picks were inserted completely into stem of healthy plants and the cut surface was sealed with the petroleum (Vaseline) to prevent desiccation. Symptoms were observed after 20 -25 days of inoculation (Calvert and Wyllie, 1967). All were conducted in triplicate.

Potato broth:-

Peeled diced potato	250g
Dextrose	20g
Distilled Water	1 Liter (1000ml)

The Ingredients of broth were weighed, taken in conical flask and were autoclaved. These were placed in Laminar flow hood where cooled at40°C-45°C before antibacterial was added. Finally the broth was poured in jar containg tooth picks.

Spraying of inoculum:-

In fourth treatment the plants were sprayed with the aqueous suspension of culture mixed @ one Petri plate per litre of autoclaved distl. Water in a blender and 250 ml of culture suspension was applied in each pot. The inoculation pressure will be applied continuously on soil by adding the aqueous suspension of culture mixed @ one Petri plate per litre of autoclaved distl.water. The same procedure will carry on until 40 to 45 days besides the observations. The whole procedure was run in three replications. Plants were placed in the growth room by maintaing the temperature 25±30°C for the development of disease symptoms (Gangopadhyay, 1984). The whole experiments were done on randomized block design.

Confirmation of Pathogenicity test:-

The Confirmation of Pathogenicity test is done by taking roots and stems samples of treated plant. For re-isolation of fungi from the root and stem-bark samples that were separated and washed thoroughly in running tap water for 10 to 15 min which was cut in 3 mm/5 mm long segments. First of all these roots and stem-bark segments were surface sterilized by dipping them in 1% Clorox for 3 min and then three times rinsed with autoclave distilled water. These samples were placed on sterilized filter paper in Petri plates (90mm) for drying. After the placement of these sterilized samples in the Petri plates enough autoclaved sterilized distilled water was added to moisten the filter paper however there should be no excess free water in the Petri plates. These filter paper plates were placed for 24 hours at 25 °C under photo period and then 24 hours at 18 °C in dark period after continuous light and dark period the presence of organisms on sections ware recorded under Stereomicroscope (De wolf *et al.* 1998). While on PDA plates the sections were transferred to medium in 90mm Petri plates to isolate the fungi. These Petri plates were incubated at 20°C - 25°C for 5-7 days. Fungi were identified under stereomicroscope to prove the *Koch's postulates*, based on characteristics of fungal morphology in accordance to literature available (Booth, 1970; Barnett, 1960; Padwick, 1940 and Ellis, 1976).

RESLUTS

RESULTS

Different pathogenic fungi were isolated from the infected samples collected from the different locations of Punjab province (table-3.1). Pure cultures of pathogenic fungi were made under Laminar flow hood with the help of stereomicroscope which is very important to get the pure Pathogenicity test's result .Different four treatments were done on the plants those were Inoculated at the junction of stem, and at the junction of 2 branches, Tooth pick technique, soil inoculation through spraying(Fig-3.7) (table-3.2) .Five fungi which were *Fusarium solani(Fig-3.2), Fusarium oxysporum(Fig-3.3),Macrophomina phaseolina(Fig-3.3), Botryodiplodia theobromae(3.4)* and *Rhizoctonia solani(Fig-3.5)* were selected to find out their pathogenic nature.

Two species of *Fusarium* i.e. *Fusarium solani, Fusarium oxysporum* were inoculated on plants because their percentage of presence on infected samples were maximum as compare to other as showed in previous studies. *Fusarium solani* showed the typical symptoms (table-3.3) i.e. yellowing of leaves, wilting, shedding of leaves which started from the top and than moved downward (Fig- 3.17). On inoculating *F. solani* at the junction of stem showed symptoms earlier as compare to inoculation at the junction of 2 branches (Fig-3.16). Wilting was very strong in by *F.solani* (Fig-3.17) .When plants were inoculated by tooth picks they did not show strong symptoms .The strong symptoms were observed when plants were sprayed with suspensions and the suspension pressure were maintained(Fig-3.16). Continuous pressure gave symptoms earlier as than other treatment's symptoms.

Similarly *Fusarium oxysporum* also showed typical symptoms (table-3.4) when inoculated at the junction of stem (Fig-3.20). *F.oxysporum* showed wilting and drying of branches at the later stages (Fig-3.19). The most interesting thing was that the symptom shown by inoculation of soil through spraying is not that much strong as compare to the *F. solani*.yellowing and shedding leaves were observed after 30 days of inculcation. It is strongly recommended that the symptom of die-back is the yellowing and shedding of leaves and wilting of leaves in the case of Fusarium species (Fig-3.17, 3.19). After shedding off the roots, the blacking at the tap roots caused by F species was seen; as the plants were small that's why it was not very prominent on the secondary roots.

Botryodiplodia theobromae showed the typical symptoms of disease but not as strong as showed by the *Fusarium* species (table-3.5) (Fig-3.21). The maximum symptoms were shown by inoculating soil through spraying. Gummosis also formed at the junction in only on plant which was inoculated at the junction of stem. Decline and death of branches and leaves also seen on plants inoculated with *B. theobromae*.

Macrophomina phaseolina showed collar rot. The maximum symptoms showed by the inoculation at the stem junction and tooth pick technique (Fig-3.24) (table-3.6). The interesting symptoms showed by the *M. phaseolina* was the black and red-brown discoloration extended up and down in vascular tissue for up to many centimeters or in continuous and discontinuous streaks (Fig-3.15). The minimum symptoms showed by the treatments on branches.

Rhizoctonia solani showed its symptoms after the 15 days of inoculation than the change in symptoms were not very fast. The symptoms shown by *R. solani* were not very strong and typical (Fig-3.26) (table-3.7). It caused the blacking of roots and reddish-brown in vascular bundles (Fig-3.28). Very less symptoms were seen on plants which were inoculated with *Rhizoctonia solani.*

On the re-isolation the maximum fungi which were isolated were the *Fusarium spps* where as *Rhizoctonia solani* also isolated from all 4 samples but the symptoms it showed were not very typical and strong as compare to the *Fusarium spps.Botrydiplodia theobromae* and *Macrophomina phaseolina* also re-isolated but with minor fluctuations that is they were isolated from treatment 2 and 3 respectively (table-3.8).

Mortality rate (table-3.9) was maximum in the *Fusarium solani (Fig-3.16)* and minimum in the *Rhizoctonia solani (Fig-3.26)* where as *Rhizoctonia solani* were re-isolated from the all four treated samples. Here we can say that *Rhizoctonia solani* is the not the destructive agent like *Fusarium spps.* All the four treatments showed the maximum mortality rate in the *Fusarium solani* and *Fusarium oxysporum* other than the treatment 3 which was the tooth pick technique and this result make *Fusarium solani* more destructive than the *Fusarium oxysporum.* Over all mortality rates were higher in the plants which were inoculated by soil trough spraying .and minimum in the tooth pick technique.

It is concluded on the bases of results which obtained (table-3.9) that *Fusarium solani* and *Fusarium oxysporum* is the causative and destructive agent of *Dalbergia sisso* Die-Back.

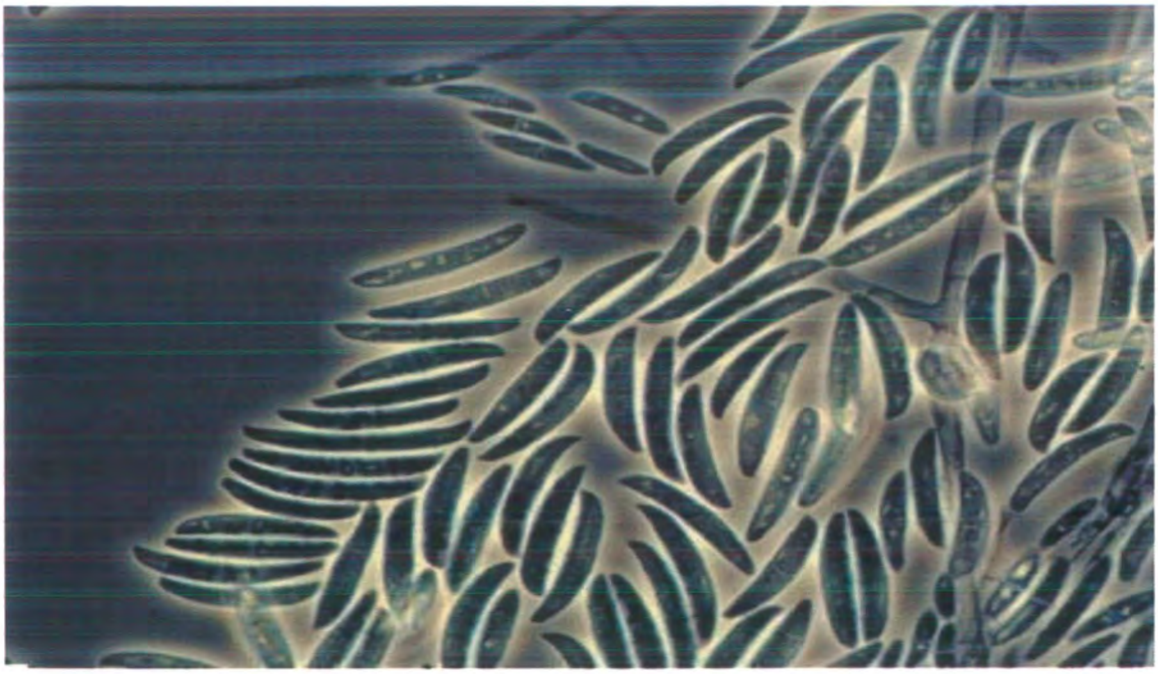


Fig 3.2-**Macroconidia and Microconidia of *Fusarium Solani.*** ***1000X***

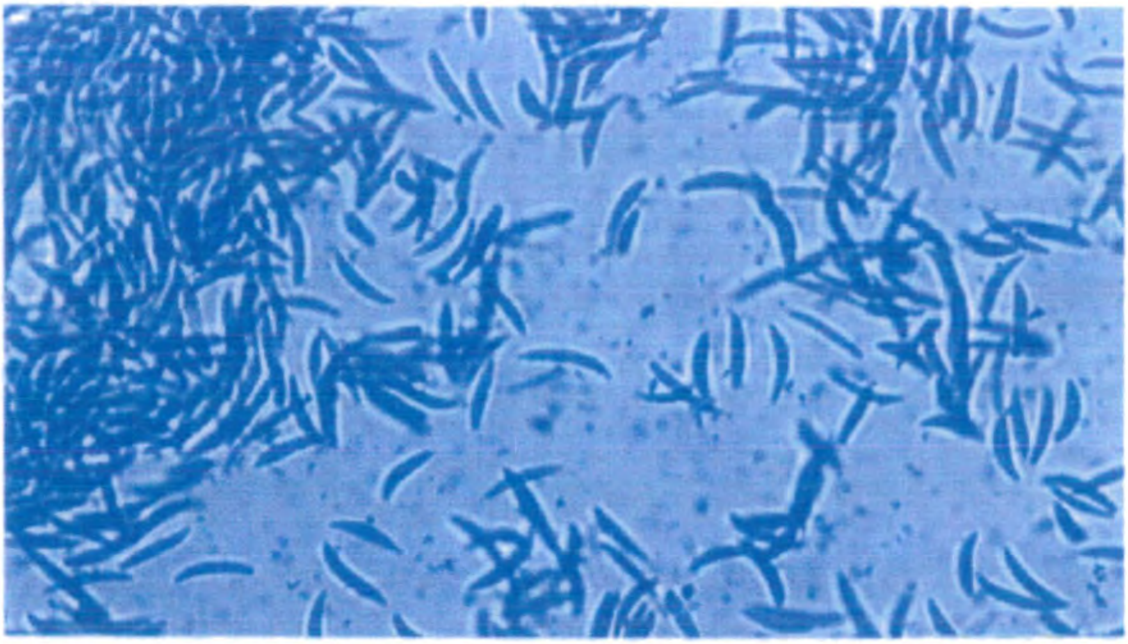


Fig 3.3-**Macroconidia and Microconidia of *Fusarium oxysporum.*** ***400X***

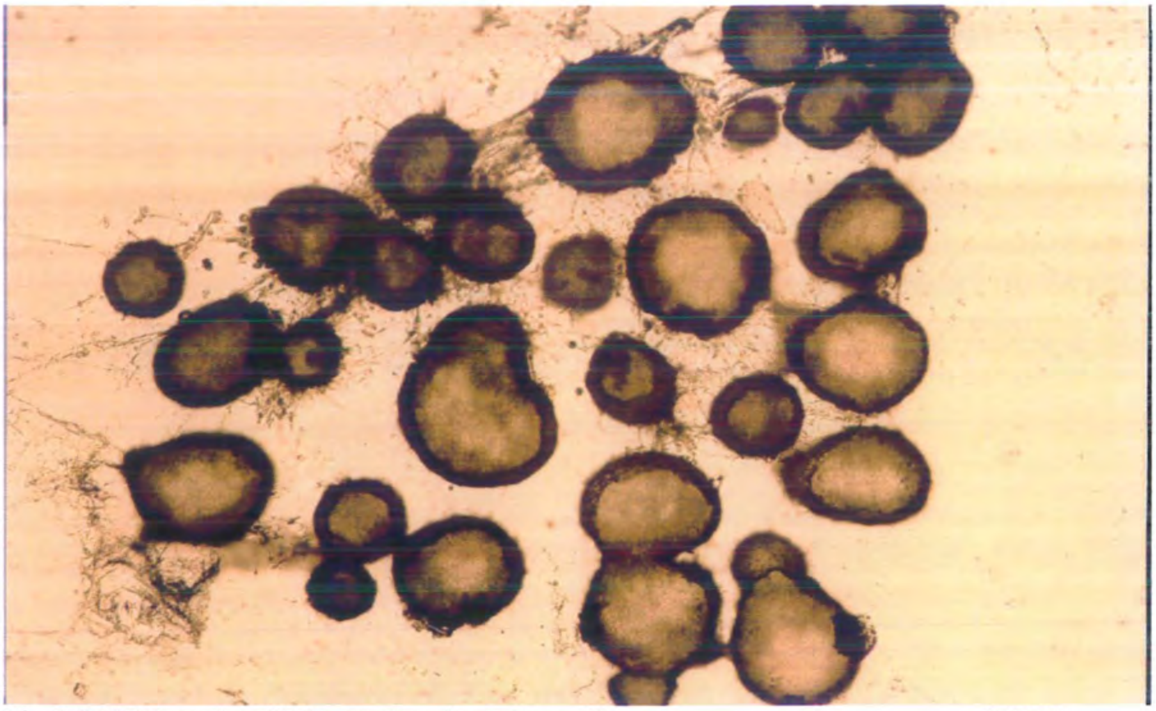


Fig 3.3-**Schlerocia of *Macrophomina phaseolina.*** ***1000X***

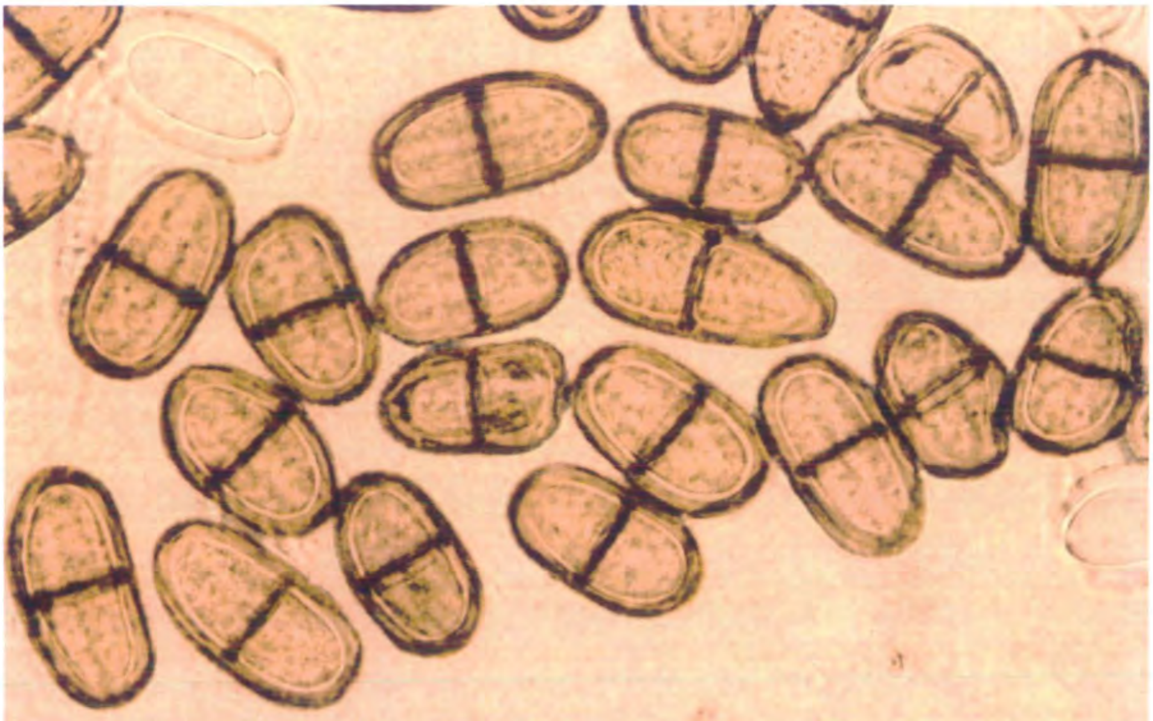


Fig 3.4-**Conidia of *Botryodiplodia theobromae*** ***1000X***

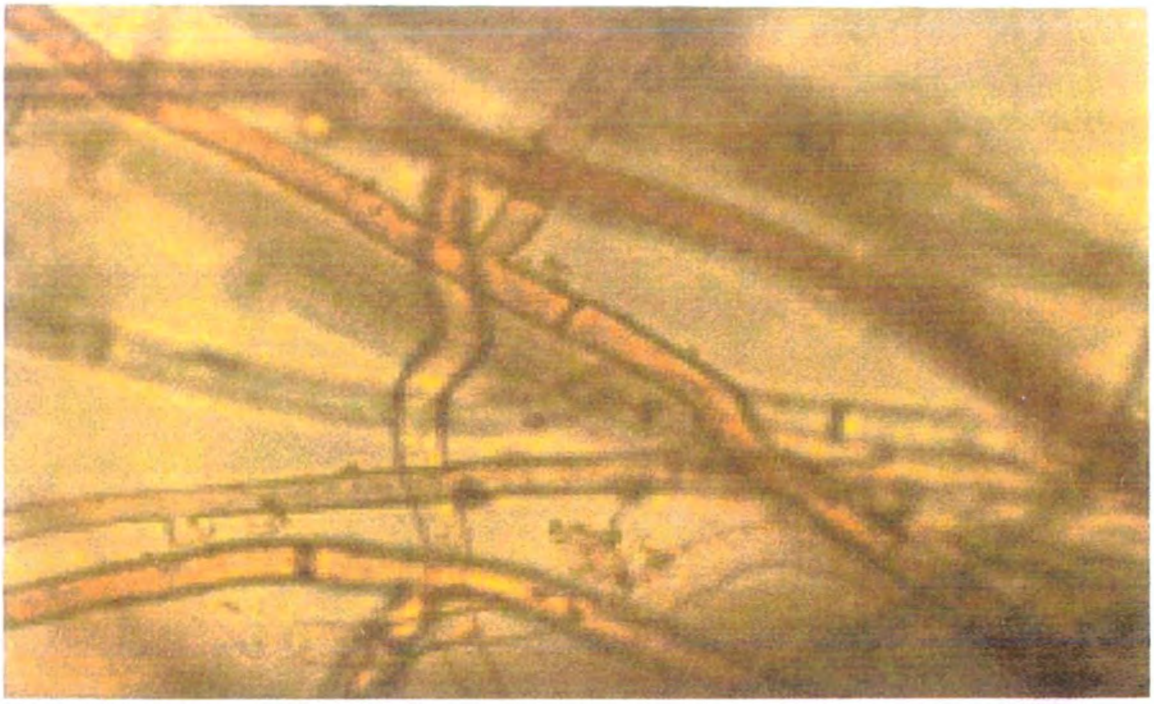


Fig 3.5-**Mycelium of *Rhizoctonia solani.*** ***1000X***

Fig 3.6: Healthy plant of *Dalbergia sisso*

Fig 3.7: Showing a numbers of plants under experiment

Fig 3.8: Showing method of Inoculation at stem junction

Fig 3.9: Showing 2 inch cut in Inoculation at stem junction (cut)

Fig 3.10: Inoculation pressure applied through spraying

Fig 3.11: Showing Tooth picks technique

Fig 3.12: Showing Randomized block design experiment

Fig 3.13: Symptoms shown by plants after 2 weeks
Of treatment 2

Fig 3.14: Showing the fungal penetration in the section around the tooth pick

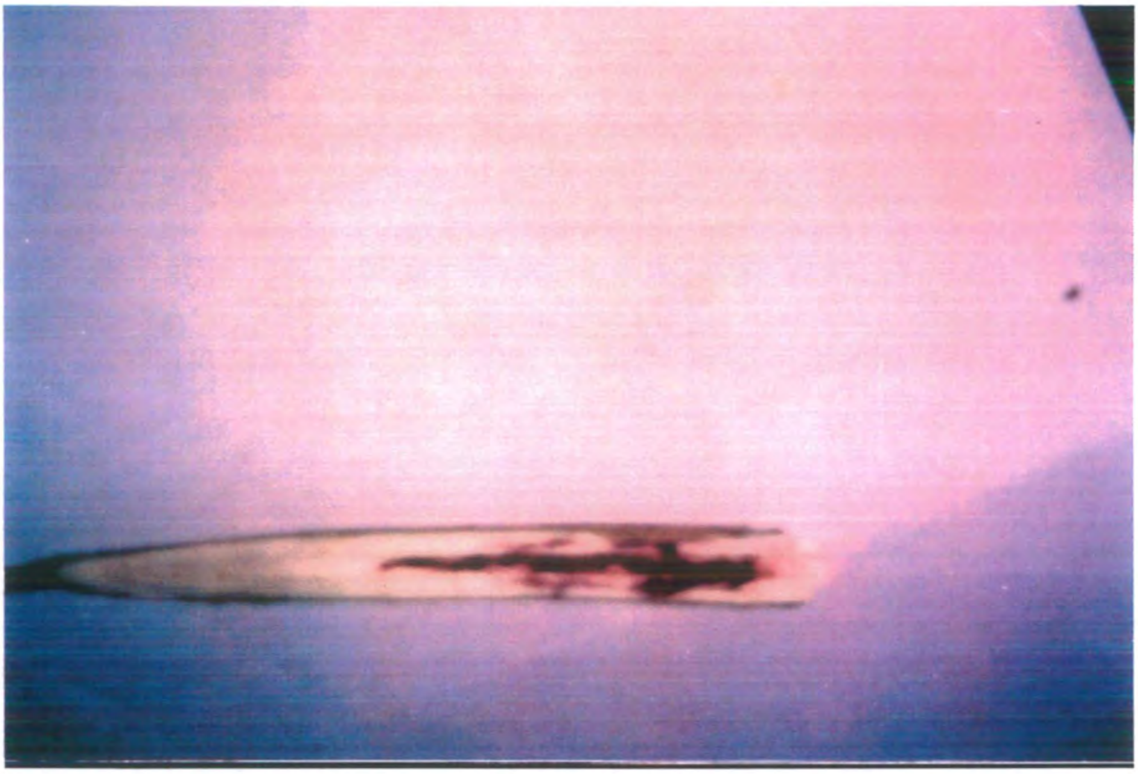


Fig 3.15: Showing blacking in L.S of infected part of plant inoculated with Macrophomina phaseolina under treatment 3 (tooth pick technique)

Fig 3.16: Photograph shows plants after the infection of *Fusarium solani*

Fig 3.17: Plant after inoculated showing *Fusarium solani* wilt

Fig 3.18: T 1 and T 3 results of *Fusarium* solani

T1 = Treatment 1= Inoculation at the junction of stem
T3 = Treatment 3= Tooth pick technique soil inoculation through spraying

Fig 3.19: Wilting shown by *Fusarium oxysporum*

Fig 3.20: Photograph shows plants after the infection of *Fusarium oxysporum*

Fig 3.21: Photograph shows plants after the infection of *Botryodiplodia theobromae*

Fig 3.22: T 1 and T 2 results of
Botryodiplodia theobromae

T1 = Treatment 1= Inoculation at the junction of stem
T2 = Treatment 2= Inoculation at the junction of 2 branches

Fig 3.23: Photograph shows plants after the infection of *Macrophomina phaseolina*

Fig 3.24: T 1 and T 2 results of
Macrophomina phaseolina
T1 = Treatment 1= Inoculation at the junction of stem
T2 = Treatment 2= Inoculation at the junction of 2 branches

Fig 3.25: T 3 and T 4 results of
Macrophomina phaseolina
T3 = Treatment 3= Tooth pick technique soil inoculation through spraying
T4 = Treatment 4= Spraying

Fig 3.26: T 3 and T 4 results of
Rhizoctonia solani
T1 = Treatment 1= Inoculation at the junction of stem
T3 = Treatment 3= Tooth pick technique soil inoculation through spraying

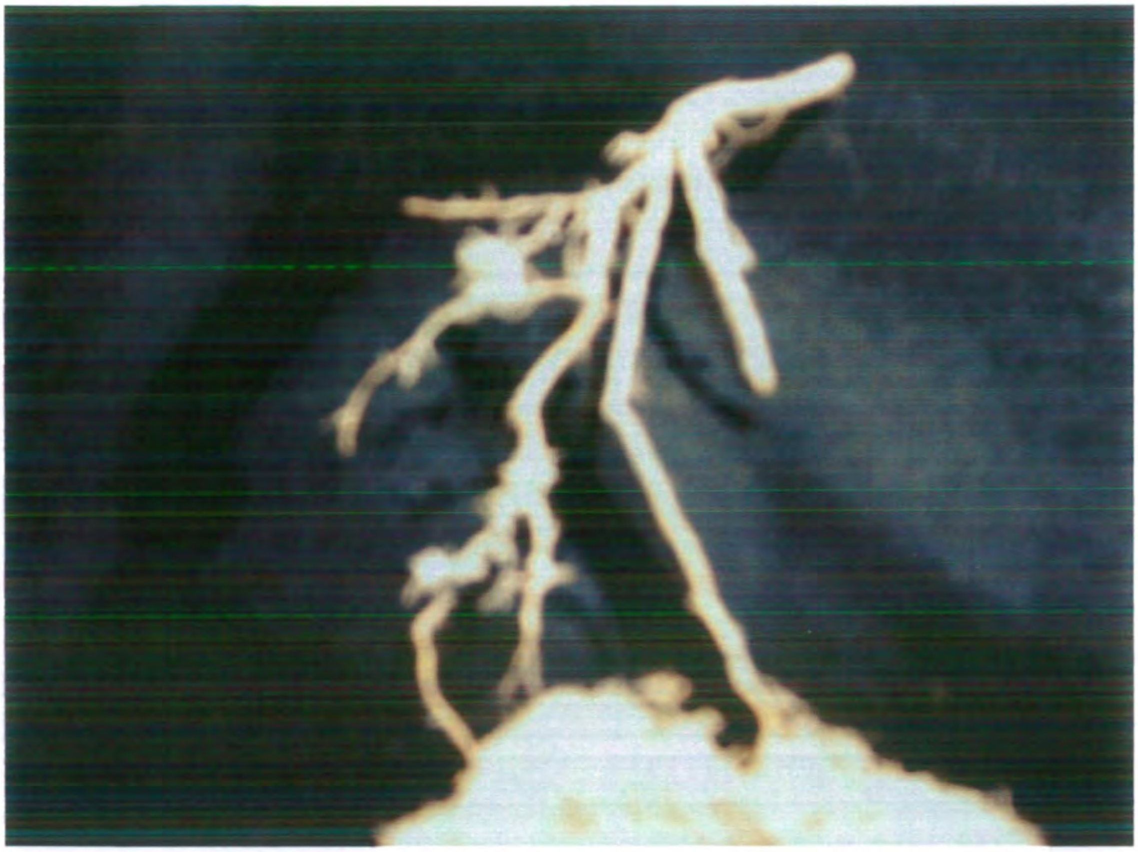


Fig 3.28: Roots damaged in T 4 on *Rhizoctonia solani*

Table 3.1: **FUNGI ISOLATED FROM ROOT AND STEM/BARK OF SHISHAM**

Sides	*Road sides*	*Canal side*	*Forest side*	*Farmer's field*
Locations	Kharian	Chishtian	Okara	Chishtian
Fusarium Solani	+	+	+	+
Fusarium Oxysporum	+	—	+	+
Fusarium equistei	+	—	—	+
Botryodiplodia theobromae	+	+	—	+
Macrophomina spp	+	—	+	+
Rhizoctonia solani	+	—	+	+
Curvularia spp	—	—	+	+
Cladosporium spp	+	—	+	—
Aspergillus niger	+	—	+	+
Aspergillus flavous	+	—	—	+
Aspergillus spp	—	+	—	+
Alternaria alternata	+	—	+	—
Alternaria spp	+	+	—	+
Penicillium spp	+	—	—	+
Mucor Spp	+	+	+	+
Rhizopus Spp	—	—	+	+
Scytalidium spp	—	—	—	+

Table 3.2: **PATHOGENIC FUNGI ISOLATED FROM ROOTS AND STEM/BARK OF SHISHAM PLANT.**

Sides	Locations	*Fusarium solani*	*Fusarium Oxysporum*	*Botryodiplodia theobromae*	*Macrophomina phaseolina*	*Rhizoctonia solani*
Road side	Kharian	+	+	+	+	+
Canal side	Chishtan	+	-	+	—	—
Forest side	Okara	+	+	—	+	+
Farmer's field	Chishtian	+	+	+	+	+

OTHER FUNGI ISOLATED

1. *Cladosporium spp*
2. *Aspergillus spp*
3. *Fusarium equistei*
4. *Alternaria alternata*
5. *Trichoderma spp*
6. *Mucor*
7. *Penicillium*
8. *Scytalidium spp*

Table 3.3: **TREATMENTS**

T1 R1 P1	T2 R1 P1	T3R1 P1	T4 R1 P1	
T2 R1 P2	T2 R2 P2	T3 R2 P2	T4 R2 P2	Control
T3 R1 P3	T3 R3 P3	T3R3 P3	T4 R3 P3	

Table 3: Shows the experiment arrangement

T1 = Treatment 1= Inoculation at the junction of stem
T2 = Treatment 2= Inoculation at the junction of 2 branches
T3 = Treatment 3= Tooth pick technique soil inoculation through spraying
T4 = Treatment 4= Spraying
R = Replication number
P = plant under experiment

Table 3.4: ***Fusarium solani***

Treatment\Replication	T_1	T_2	T_3	T_4
R1	+++	++	++	+++
R2	+++	++	++	+++
R_3	+++	++	++	+++

Table 3.4: Symptoms severity shown by *Fusarium solani*

+++= Strongly symptoms shown
++= shown symptoms
+ = Less symptoms shown
0 = No symptoms

Table 3.5: ***Fusarium oxysporum***

Treatment\Replication	T_1	T_2	T_3	T_4
R1	+++	++	++	++
R2	+++	++	++	++
R_3	+++	++	++	++

Table 3.5: Symptoms severity shown by *Fusarium oxysporum*

+++= Strongly symptoms shown
++= shown symptoms
+ = Less symptoms shown
0 = No symptoms

Table 3.6: ***Botryodiplodia theobromae***

Treatment\Replication	T_1	T_2	T_3	T_4
R1	+	+	+	++
R2	+++	+	+	++
R_3	+	+	+	++

Table 3.6: Symptoms severity shown by *Botryodiplodia theobromae*

+++= Strongly symptoms shown
++= shown symptoms
+ = Less symptoms shown
0 = No symptoms

Table 3.7: ***Macrophomina Phaseolina***

Treatment\Replication	T_1	T_2	T_3	T_4
R1	+++	+	++	+
R2	++	+	++	++
R_3	+	+	+++	++

Table 3.7: Symptoms severity shown by *Macrophomina phaseolina*

+++= Strongly symptoms shown
++= shown symptoms
+ = Less symptoms shown
0 = No symptoms

Table 3.8*:* ***Rhizocontonia solani***

Treatment\Replication	T_1	T_2	T_3	T_4
R1	++	+	+	+
R2	++	+	+	+
R_3	+	+	+	++

Table 3.8: Symptoms severity shown by *Rhizocontonia solani*

+++= Strongly symptoms shown
++= shown symptoms
+ = Less symptoms shown
0 = No symptoms

All the above tables show the effects of fungi on plants and the degree of symptoms shown by the plants.

Table 3.9: **PATHOGENIC FUNGI RE-ISOLATED FROM INFECTED PLANTS**

fungi	T 1	T 2	T 3	T 4
Fusarium Solani	+	+	+	+
Fusarium oxysporum	+	+	+	+
Botryodiplodia theobromae	+	—	+	+
Macrophomina phaseolina	+	+	—	+
Rhizoctonia solani	+	+	+	+

+ = fungi re isolate from infected plants

- =fungi don't reisolate from infected plants

Table 3.10: **% Mortality rate shown by different Pathogenic fungi**

Fungi/treatments	T 1	T2	T3	T4
Fusarium Solani	95	92	80	90
Fusarium oxysporum	85	92	75	90
Botryodiplodia theobromae	60	45	40	75
Macrophomina phaseolina	80	35	40	72
Rhizoctonia solani	55	40	35	80

Data based on the average percentage of symptoms shown by the plants.

Table3.11: **OTHER FUNGI RE-ISOLATED FROM INFECTED PLANTS**

fungi	T 1	T 2	T 3	T 4
Cladosporium spp	+	+	—	—
Aspergillus niger	+	+	+	—
Aspergillus flavous	+	+	—	+
Aspergillus spp	+	+	—	+
Alternaria alternata	+	—	+	+
Alternaria spp	+	—	+	+
Penicillium spp	+	—	+	+
Mucor Spp	+	+	+	+
Rhizopus Spp	+	—	+	+
Scytalidium spp	—	+	+	—

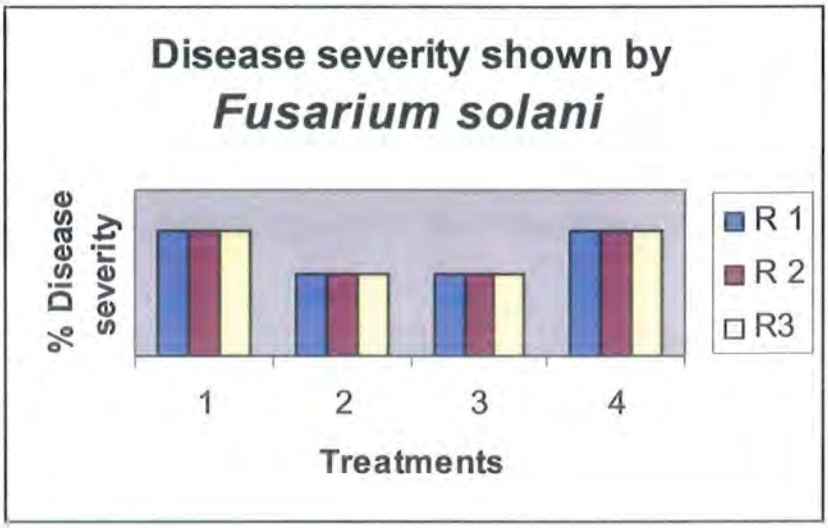
GRAPHS

These graphs show that which treatment shows the maximum and minimum symptoms of die back of *Dalbergia sisso*. To simplify the results the affectivity scale is changed into the numbers

Fusarium solani

Graph 3.1: Disease severity shown by

Fusarium solani

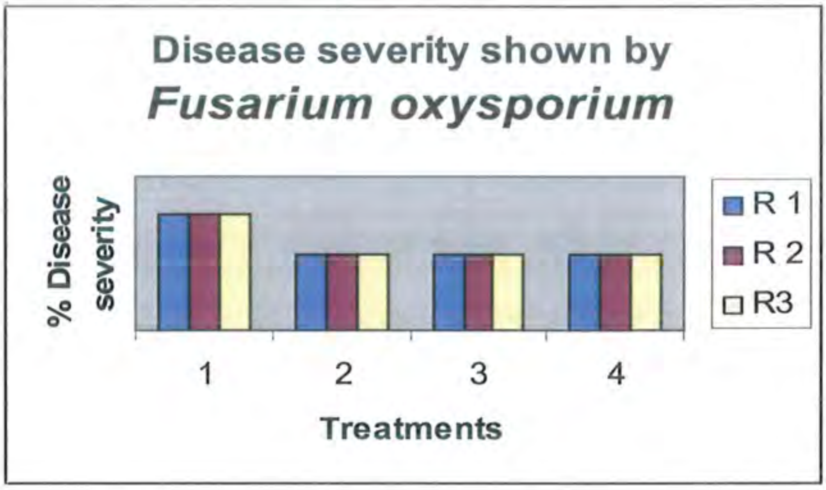


R 1 =Replication 1
R 2= Replication 2
R 3=Replication 3

Fusarium Oxysporum

Graph 3.2: Disease severity shown by

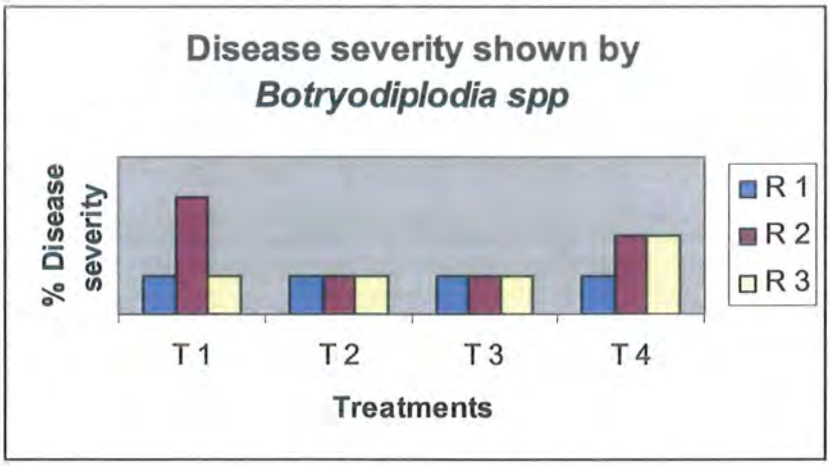
Fusarium Oxysporum



R 1 =Replication 1
R 2= Replication 2
R 3=Replication 3

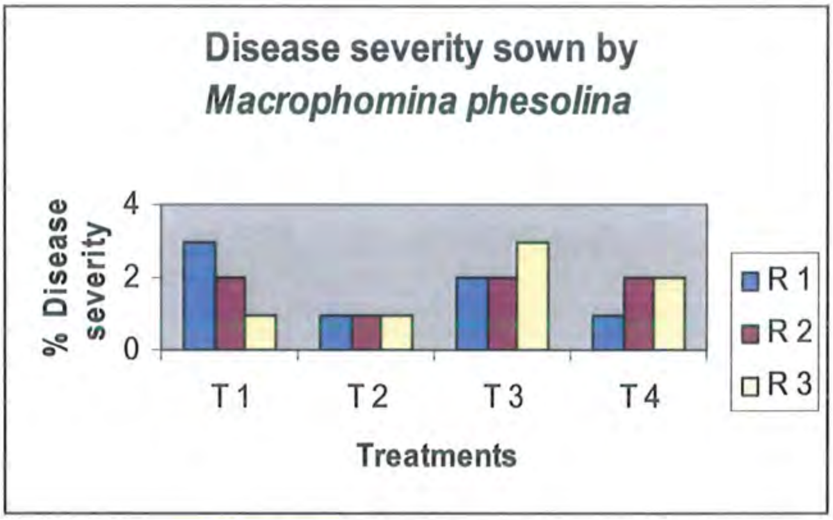
Botryodiplodia theobromae
Graph 3.3: Disease severity shown by

Botryodiplodia theobromae



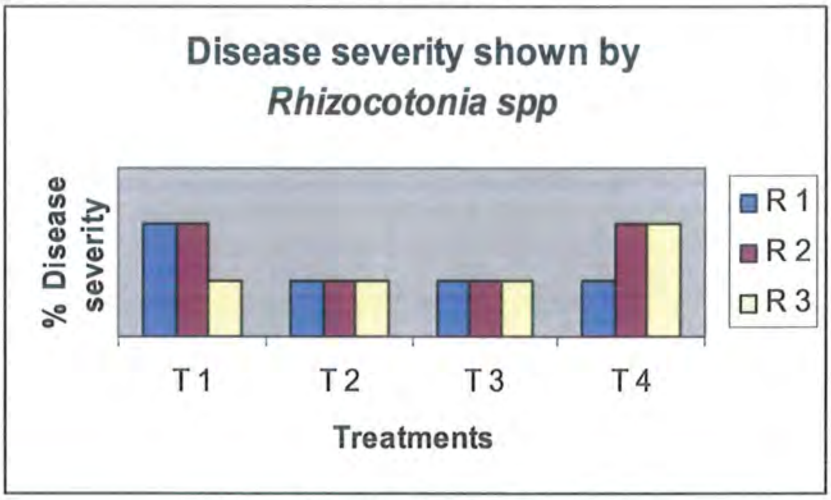
R 1 =Replication 1
R 2= Replication 2
R 3=Replication 3

Macrophomina phaseolina
Graph 3.4: Symptoms severity shown by
Macrophomina phaseolina



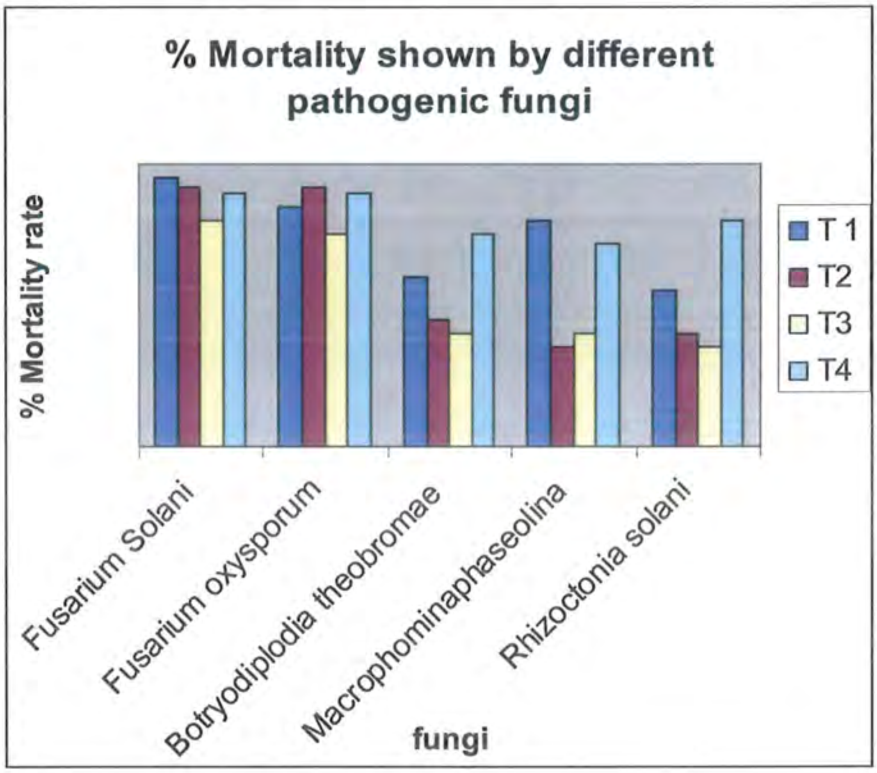
R 1 =Replication 1
R 2= Replication 2
R 3=Replication 3

Rhizoctonia solani
Graph 3.5: Symptoms severity shown by
Rhizoctonia solani

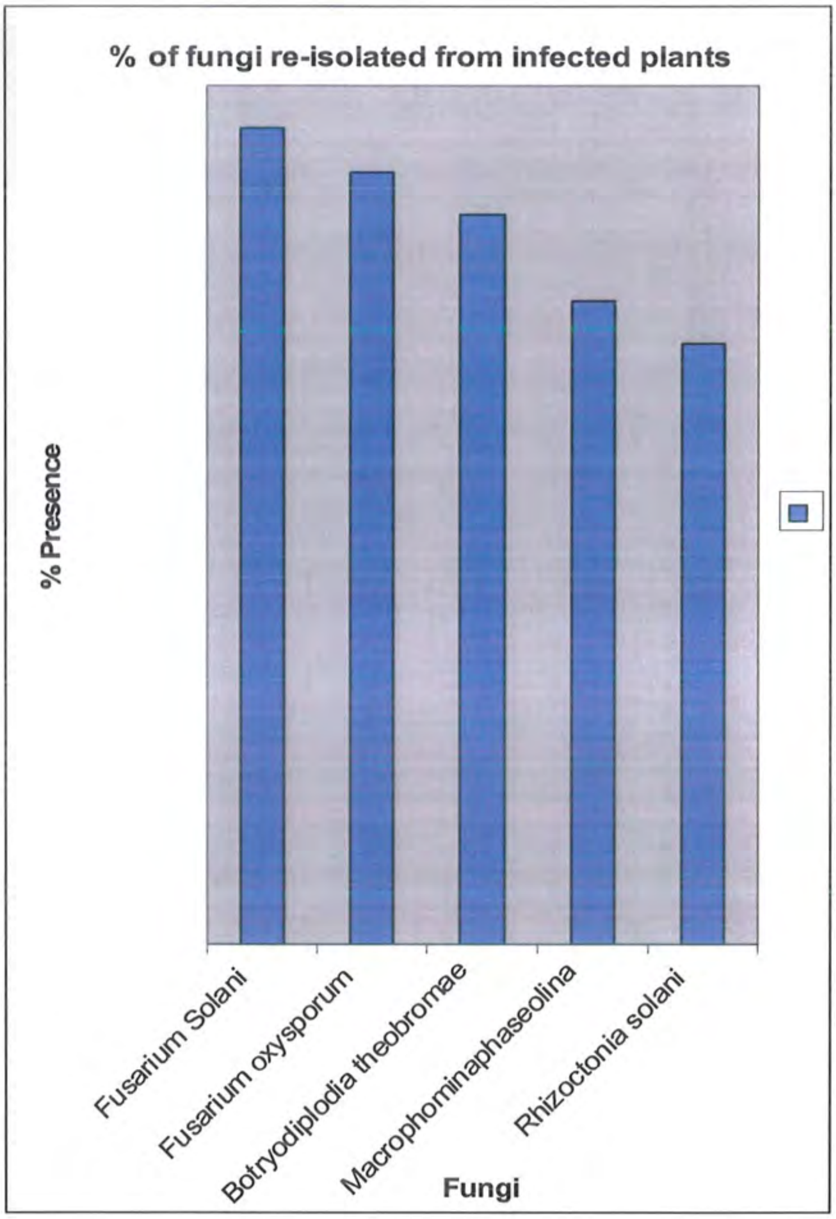


R 1 =Replication 1
R 2= Replication 2
R 3=Replication 3

Graph 3.5: % Mortality rate shown by different pathogenic fungi



Graph 3.6: Fungi re-isolated from infected plant samples



DISCUSSION

Discussion

Decline is a term used to describe a tree that is generally deteriorating. The deterioration may be caused by many factors. The symptoms often results because the translocation system of the tree has been disturbed. The root system may be restricted or damaged .The trunk tissue may be blocked, wounded or infected by some agents. Decline also results when a tree food reserves are depleted. The Dieback was reported during early 1900 and this problem is getting severe day by day. *Dalbergia sissoo,* a medium to large, deciduous, long-lived tree with a spreading crown and thick branches, Dieback is a common Problem in countries like Pakistan, Nepal, India, Bhutan and Bangladesh (Sahim, 2001). In Pakistan quick Wilting, Drying, Decline and Die-back came to lime light in 1998. Now days Shisham trees in Pakistan are found to be attacked with two types of disease; Wilting and Dieback, where as die back is more Prevalent than Wilting (Bajwa *et al.* 2004). Dieback is entirely different disease as compare to Wilting as die back is characterized with thinning of leaves and crown, drying up of the ends of branches, table topped condition and stag-hardness in extreme conditions (Khan, 2000).

The species has grown for decades away from its natural ecological habitat as multipurpose trees. *Dalbergia sissoo* has been widely planted outside its natural range. It seen, along roadsides and canals, and around farms and Farmer fields. The debarking and heavy lopping for fuel wood has induced stress conditions in the species resultantly inviting insects/pest attack as a natural sequence of events. (Khan and Bokhari, 1970).

It is well established fact that Shisham is facing severe threat of die back, yet not identified. However, there are some reports available in literature those indicate, die back is perhaps due to collar rot and root decay. In this situation the top leaves of the infected plants turn light yellow and withering of individual branches takes place from top to down. Bark at the collar region turns brown and gives rot like appearance, often it splits away, separated from cambium which can be easily pealed off. There are holes in the bark and galleries in the cambium. This disease attacks the plant regardless of its age. (Zentmyer, 1980).

Diseased and Healthy Shisham Sampling was conducted from four sites of different locations of Punjab Province. Isolations were made from the collected Samples. The isolated fungi which were Pathogenic and selected for Pathogenicity were *Fusarium oxysporum, Fusarium solani, Macrophomina phaseolina, Botryodiplodia theobromae Rhizoctonia solani.*

The frequency of *Fusarium* species were maximum as compared to other fungi in both root and stem/bark diseased samples. In which *Fusarium oxysporum, F. solani*, were isolated frequently as compare to other *Fusarium spps* the frequency of *Macrophomina Phaseolina* and *Botryodiplodia spp.* and *Rhizoctonia solani* were also higher as compare to other fungi so results indicate that these are the more pathogenic and have rapid growth system. On the basis of their frequency of occurrence they were selected for Pathogenicity test.

According to the results *Fusarium* may be the possible cause of Dieback because it was found in root and stem/bark of the infected trees with high percentage; however the frequency percentage of *Fusarium oxysporum* & *Fusarium solani* were highest. Pathogenicity showed that more than 90 % mortality trough Fusarium solani and almost 85% results through the Fusarium oxysporum. *Macrophomina Phaseolina, Botryodiplodia spp,* and *Rhizoctonia solani* were also showed mortality in the Shisham

(*Dalbergia sissoo* Roxb.). Almost same fungi were re-isolated by Tabassum (1996) from Shisham Pods and Seeds. Gill *et al.* (2001) conducted research on plants of Shisham infected of Dieback and reported the Presence of *Rhizoctonia* and *Fusarium* along with other fungi.

The Isolation of Fungi from roots Infected by Dieback was carried out by Shakier *et al.* (1999) and also reported the Presence of *Fusarium.* Sabir *et al.* (2004) isolated similar fungi from Shisham roots, stem and branches. Of these, *Botryodiplodia spp* was the most abundant fungus that was isolated from all the locations as well as from all the plant parts while *Fusarium solani* was the second most frequent fungus isolated from the roots. Further more it was also concluded that there was an invariable association of *Botryodiplodia spp* with aerial as well as under ground parts. Manandhar and Shrestha (2000) examined five diseased samples of *D. sissoo* and *Botryodiplodia theobromae* and *Fusarium solani* were found associated with the samples. The *Phytophthora spp* was not isolated due to the fact that general purposes Medias were used.

Fungus appears to be causative agents of Shisham Dieback. Gill *et al.* (2001) conducted the study on *Dalbergia sissoo* Dieback and reported that fungi were involved in infections and Dieback in both young and old trees and pathogens can be from soil or environment. Joshi and Barel (2000) conducted a study in 1998-99 in Nepal and recorded several fungal pathogens as the major factors of Sissoo mortality. Khan (2000) concluded that Sissoo Dieback could be due to the attack of root and stem rotting parasites.

Out of the four treatments used to find out the pathogenic nature of the *Fusarium* species maximum plants show the symptoms after 45 days .Thus it seems that genus *Fusarium* is involved in the diseased development in fields besides other reasons. Similar reports were shown by the Bakshau and basak (2000) reported a wide spread mortality of sisso tree in varying ages in Bangladesh and assumed that *F.solani* and *shot hole* borer may be the cause of this disease .Manandhar and Shrestha (1999) isolated *Botryodiplodia sp.* and *Fusarium solani* from 5 root samples received from department of Forest Research and survey. Ivory (1995) observed the symptoms expressed by dying tree and suspected similar to Wilt caused by *Fusarium oxysporum.* In the light of the present study we are able to conclude that the *Fusarium solani* and *Fusarium oxysporum* may be the causative fungi as said by the Ivory (1995)

In the Pathogenicity test of *Fusarium* species, symptoms started appearing in 30 days after inoculation and finial data were collected after 45 day. Inoculated plants show almost 90 % declined symptoms while uninoculated remained healthy. The symptoms pathogen on plants observed were wilting, yellowing and shedding of leaves.Reisolation were made from the infected plants to fulfill the requirements of the Koch's postulates. The pathogens were identified and found that besides other reasons Fusarium is also the cause of Shisham decline

Besides *Fusarium solani* and *Fusarium oxysporum. Macrophomina phaseolina* also showed the mortality rate which was not as higher than the *Fusarium. Rhizoctonia solani* was re isolated from the infected plants but the symptoms shown by him were not very strong.

Gibson (1975) observed vascular wilt caused by *Fusarium oxysporum* in *Dalbergia sissoo.*Wilt caused by *Fusarium solani and Fusarium oxysporum* were also recorded in India.

The main cause of Shisham Dieback is still unknown. The workers stressed physically injury, construction injury, soil compactation, water conditions, water logging, salinity and use of herbicides as major factor for tree Dieback.

It is possibility that adverse biotic pressure, high susceptibility and Decline in management practices are the main causes of this menace. Over mature and damaged trees are more effected by the attack of disease, they must be looked carefully as this problem is getting severe day by day. Moreover it should but kept in mind that the new plantation should start as the age factor is also one of the main factors besides all other factors.

The result of present studies are in close confirmation to those of the Manandhar and Shrestha (2002) who also recorded similar symptoms (Shisham decline)and found that after artificial inoculation with Fusarium solani, Fusarium oxysporum and Botryodiplodia theobromae caused this disease.

It is clearly indicated that the Shisham decline is caused by the Fusarium solani and Fusarium oxysporum where as Rhizoctonia solani showed no significance role in disease development.

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