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Isolation of fungus from mass mortality affected Rain tree (Albizia saman F. Muell) from Khulna region of Bangladesh

MAHMUDA SULTANA PAPIA

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DEDICATED

TO

MY BELOVED PARENTS

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At first of all, I am undoubtedly grateful to the Almighty Allah, the most benevolent who helped me to complete this study successfully.

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ABSTRACT

Rain tree (*Albizia saman* F. Muell.) belongs in the Fabaceae (Leguminosae) family, sub family Mimosoideae. It is a native of Central America and parts of Northern South America. *Albizia saman* is extensively cultivated both within and outside its presumed natural range in northern South America and Central America. It is a large tree, which has now become widespread throughout the humid and subhumid tropics. Although noted as a promising agroforestry species, there is little specific research that substantiates this potential. On the basis of a review of its biology, ecology and recorded uses, it is concluded that the most appropriate use for *A. saman* would be in extensive silvopastoral systems for cattle production. The tree provides excellent protective shade, and produces highly palatable pods that are suitable as a dry season feed supplement. Additionally, there are reports of enhanced grass production beneath its canopy indicating a potential role in maintaining or improving the productivity of tropical grasslands. It is concluded that the tree should receive more research attention, focusing particularly on its interaction with the herbaceous understorey and the identification of provenances adapted to a variety of environmental conditions. It has been recognised as a leguminous tree species with potential in agroforestry. It has been largely planted along roadside in the parts of India, Burma and Bangladesh. It is largely planted along the roads & highways for its excellent protective shade and aesthetic values in Bangladesh. Recently it is reported that in the south Bengal the plantation of rain tree faces a excessive amount of death, the cause of which is unknown.

In the present study, it is tried to find the causal factor of this massive mortality. For this purpose, some samples from different areas were collected and cultured in the laboratory. In the laboratory, on 2% MA medium the cultural procedure was done. From the culture of the collected samples, four types of fungi were isolated i.e. isolate A, B, C & D. From the four isolates,it is observed that the fungal isolate B was most dominant for each of the samples from both dying & dead rain tree. While the other isolates A, C & D had a very negligible amount of growth. So, it is clear that among the four type of isolates, isolate B has the higher chance of being the causal factor of this case.

ABBREVIATION

DFO	Divissional Forest Officer
et al.	With Others
e.g	Example
etc.	Etcetera
g / gm	Gramme
i.e.	That is
Max	Maximum
Mm	Milimeter
NaOCl	Sodium Hypochloride
PDA	Potato Dextrose Agar
pH	Negative Logaroithm of Hydrogen ion Concentration
p.s.i.	Pound Per Square Inch
ppm	Parts Per Million
spp	Species
UV	Ultraviolate
UK	United Kingdom
USA	United State of America
%	Percentage
°C	Degree in Celsius / Centigrade

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CHAPTER ONE

INTRODUCTION

1.1 Introduction

Albizia saman is a species of flowering tree in the pea family, Fabaceae (Leguminosae), sub-family Mimosoideae. It is a wide-canopied tree with a large symmetrical crown, about a height of 25m (82 ft) and a diameter of about 40m. It is commonly known as saman, rain tree, and monkeypod. The common name "Rain tree" is said to be derived from the honeydew-like discharge of cicadas feeding on the leaves (Wikipedia, 2017). Again the origin of the name "Rain tree" can be assigned to - leaflets of rain tree are sensitive towards the light and close together on cloudy days (as well as from dusk to dawn), allowing the rain to fall through the canopy to the ground, and/or the nectarines on the leaf petioles that excrete sugary juice that sometimes falls from the tree like rain drops (Staples and Elevitch, 2006). Soil & humidity determine the shape and size of the tree, in poor soil the growth of the branch is too low (Luna, 1996).

Albizia saman is native to Central America and parts of Northern South America where it grows mostly on riversides in the forest (FAO, 1998). It is largely distributed in the forest of tropical Asia (Zabala, 1990). Rain tree can be easily recognized because of its characteristics umbrella-shaped canopy. For this reason, it is most important in the Pacific as a shade tree on small farms , along roads, in parks and pastures (Staples and Elevitch, 2006). It was introduced in the west Bengal resion, Tamil Nadu, Kerala and Karnataka for the purpose of shading along the roadside and increasing the scenic value of avenues in 1880 (Luna, 1996).

As a member of leguminosae family, *Albizia saman* has the ability to fix atmospheric nitrogen with the association of bacteria by forming root nodules. Rain tree supplies the essential nitrogen to the soil that is very helpful for the growth of other species in agroforestry plantation, homestead plantation, and/or in shade tree plantation (UNDP, 1994).

Rain tree has a huge number of versatile use such as, firewood because of its high calorific value, animal fodder because of its edible and nutritious pod for livestock, as a living fence, etc. It has been largely planted in Bangladesh as an avenue & shade tree along the highways, parks & gardens, homesteads & village markets (Ahmed et al. 2009). It has also been successfully planted on raised coastal greenbelt. It is, however, seen more commonly in the southwestern part of Bangladesh more abundantly as compared to that in other parts of the country. Recently it is reported that the rain trees of the southern areas, i.e.. Khulna, Bagerhat, Mangla, Jessore, face the massive mortality. The reason of this massive mortality is yet unknown. So it is necessary, to find the causal factor of this massive mortality to prevent this situation and further problem.

Fig.1 : A healthy rain tree (*Albizia saman)* with elegant crown, big trunk and branches.

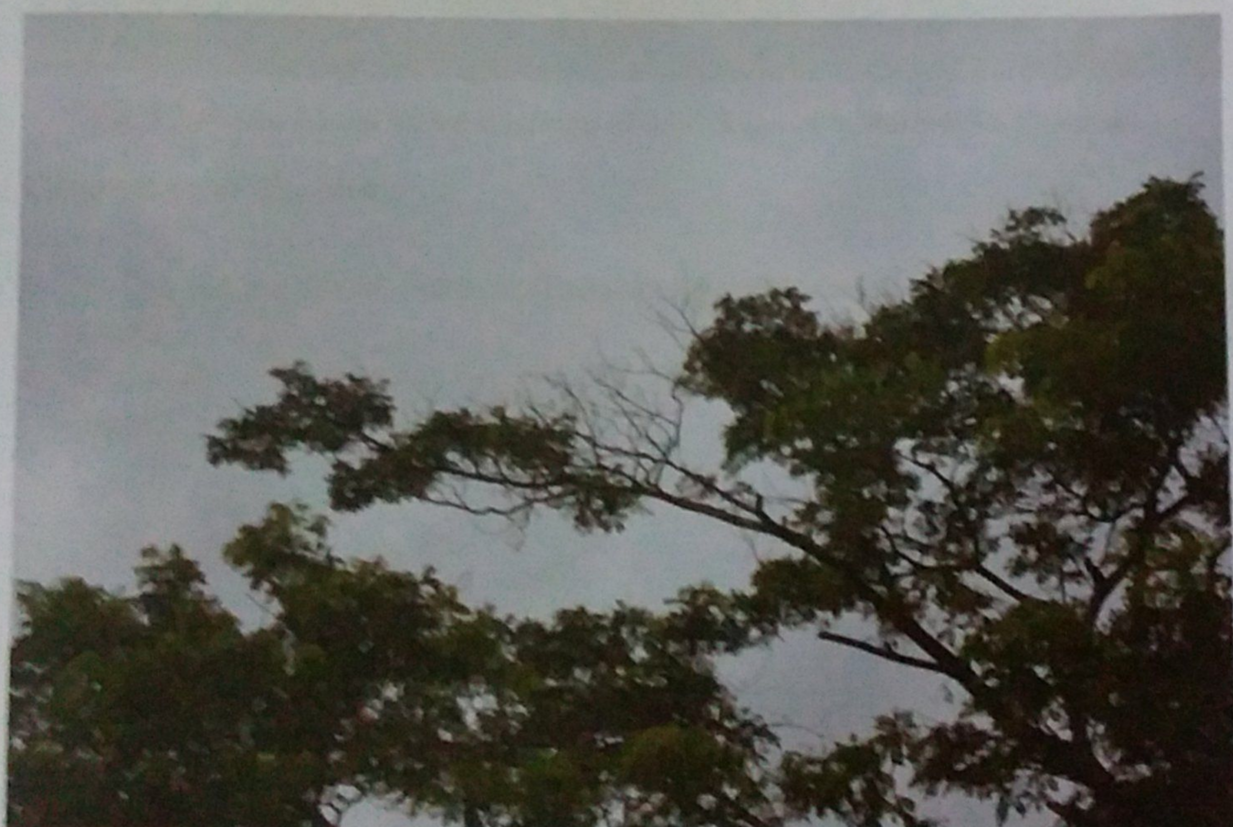

Fig.2 : Affected rain tree (*Albizia saman*) in the stage of dying from Voroshapur, Rupsha.

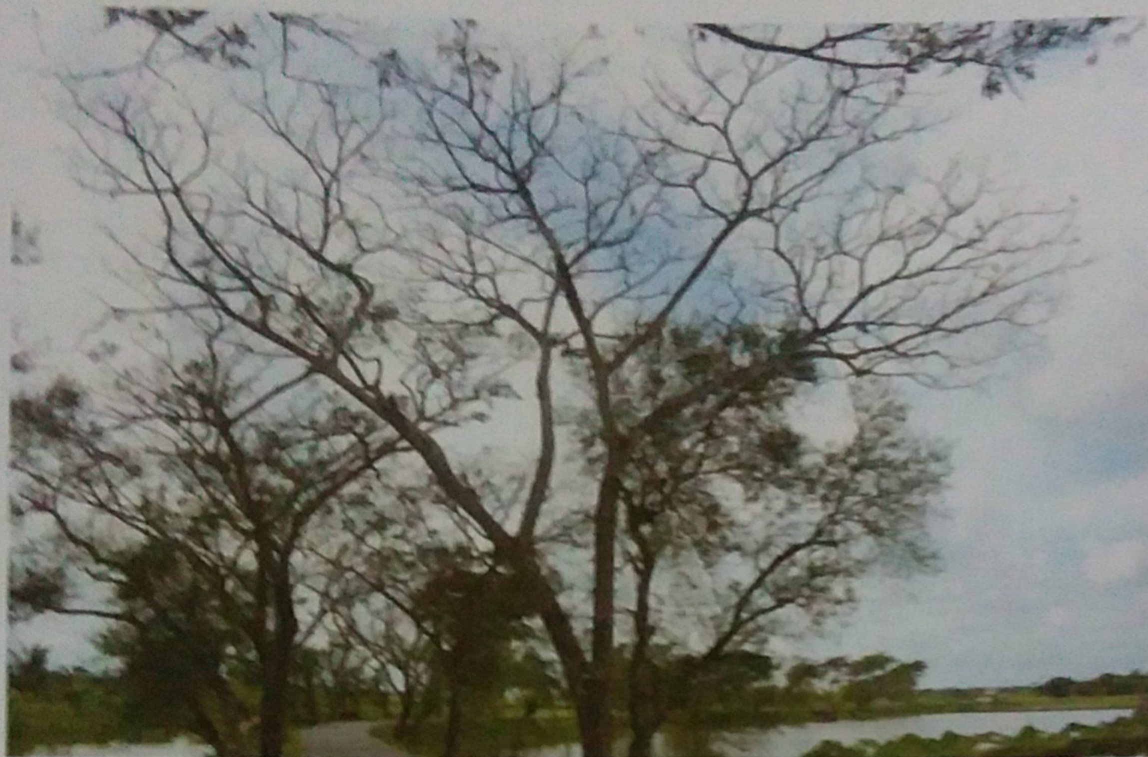

Fig.3 : *Albizia saman* in the condition of dead & dying in Bashundia, Dumuria.

1.1 Objectives of the study

- To find out the causal pathogen (fungus) associated with massive mortality in Khulna region.
- To find out most affected tree parts of *Albizia saman* that isolate highest amount of fungus.

CHAPTER TWO

REVIEW OF LITERATURE ON RAIN TREE (*Albizia saman*)

2.1 Taxonomy of rain tree

CLASSIFICATION

Hutchinson's (1959) system of Classification,

Domain: Eukaryota

Kingdom: Plantae

Phylum: Angiospermae

Sub-Phylum: Eudicotyledone (Dicotyledone)

Division: Lignosae

Order: Fabales

Family: Fabaceae

Sub-Family: Caesalpinioideae (Mimosoideae)

Genus: *Albizia*

Species: *Albizia saman*

(Wikipedia, 2017; Anon, 2017)

2.2 Geographical distribution of rain tree

Albizia saman F. Muell is native to northern South America, central America and Caribbean Islands. It is now spread and cultivated throughout the tropics (Schmidt, 2008). Rain tree is native from Southern Mexico and Guatemala, Bolivia and Brazil. It is neutralized throughout the tropics and has been introduced to sub tropical areas. It is not a forest tree (Hossain, 2015). It is widely distributed in the forest of tropical Asia (Zabala, 1991). It was introduced in 1880 in West Bengal, Tamil Nadu, Kerala and Karnataka for shade in plantations and roadside avenues and also for pastures. It was planted to a lesser extent in Gujrat, Bihar and Uttar Pradesh (Luna, 1996). Rain tree will not stand the colder part of northern India, but elsewhere it is not in particular as to soil and will thrive even in cooperatively dry climates as at Mandalay, though it grows best in moist climate. In the delta districts of Burma. It is capable of growing in some of the wettest places, rapidly killing out of grasses with its broad crown (Troups, 1921). After its introduction, it has become one of the most popular species for roadside plantation and also with agroforestry practices. It has been largely planted along roadsides and avenues in some warmer Indian region and in Bangladesh part. In Bangladesh, it has been introduced in the parts of Dhaka, Tangail, Mymensingh, Comilla, Dinajpur and also in the northern parts of Bangladesh (Zabala, 1990). It is extensively planted in Bangladesh as an avenue and shade tree along highways, parks and gardens, homesteads and village markets (Ahmed et al. 2009).

2.3 General characteristics of rain tree

Albizia saman is a conspicuous, semi-deciduous tree that can attain a height of 60 m, although it rarely exceeds 30 m and 4.5 m at DBH; crown dense, spreading, sometimes 30 m across; bole short, usually crooked, often with huge, widely spreading branches from low down. Bark distinctly grey-brown, yellow or cream-brown, smooth, becoming slightly to deeply fissured with age, peeling off in long, fibrous strips; slash yellowish-pink and fibrous beneath, exuding a brown gum; branches velvety. Leaves bipinnately compound, 15-40 cm long, velvety, with a circular gland at the base and usually between each of the pinnae; pinnae 4-6 opposite, 7-15 cm long, velvety, with small glands between most of the leaflets and a common stalk grooved on the upper surface; leaflets 4-8 pairs, opposite, progressively larger upwards, the end pair 4-5 cm long, 18-32 mm broad, unsymmetrical with the midrib curved inwards and the outer margin more curved than the inner; lower leaflets approximately in the shape of a parallelogram with the midrib running diagonally upwards, bright green, oblong, smooth, stalkless, finely hairy underside, almost glabrous topside, with prominent midribs and lateral nerves. Flowers white below, pink above, solitary or in small clusters in the leaf axils or clustered at the ends of shoots, forming subglobose heads are 5-7 cm wide, central flower different from the others, the heads on stalks 5-8 cm long; whole inflorescence finely hairy; stamens conspicuous. Pods more or less straight with conspicuously thickened edges, black or green and set in brownish pulp, 12-20 cm long, 1-2 cm long, 1.2 cm thick, indehiscent, containing numerous seeds embedded in the pulp (Orwa et al., 2009).

2.4 Morphological characteristics of rain tree

2.4.1 Flowers

In April-October, the flowers of rain tree (*A. saman)* are appeared (Ahmed et al., 2009). The showy flower heads composed of many narrow pink flowers (Hossain, 2015). The tiny flowers (12-25 per head) are massed in pinkish heads 5-6 cm across and about 4 cm in height. The long stamens are white colored in the lower half and reddish colored in the above. It gives the whole inflorescence a powder puff appearance. Usually only one flower per (rarely two) is pollinated and forms a fruit (Staples and Elevitch, 2006).

Fig.4 : Pinkish flower of rain tree (*A. saman*).

Fig.5: Pink-flowered rain tree pollinated by a black wasp.

2.4.2 Leaves

Leaves are bippinate and compound and about 25-40 cm long. Leaves are darkgreen colored in the above and light green colored in the below. The stalkless leaflets are arranged in pairs numbering from 12-32 (Little and Wadsworth, 1964). Leaves and leaflets both are progressively larger towards their terminal ends (Hossain, 2015).

Fig.6 : Leaves of rain tree.

2.4.3 Fruit

Fruits are dark brown to black in color. Pods are hard & thick and about 8-20 cm long and about 2 cm wide. The pods do not readily open and remain on trees for long periods on the tree (Hossain, 2015). Mature pods are black-brown in color and filled with a sticky, brownish pulp which is sweet & edible (Staples and Elevitch, 2006). Each fruit contains 15-20 seeds which is fatty, shiny, smooth and dark brown in color (Selvam, 2007).

Fig.7 : Fruits of rain tree.

2.4.4 Seeds

Seeds are 8-11 mm long and 5-7.5 mm wide. Seeds are smooth, dark glossy brown with a slenderly U-shaped yellowish marking on the flattened sides (Staples and Elevitch, 2006). Seeds with a scanty endosperm or without endosperm; straight embryo; radical shot (singh et al., 1994). One kilogram of seeds contain 4000-7700 seeds (Hossain, 2015). The seed has the typical hard impermeable testa of many legumes, and there is restricted germination if the pods remain where they fall under the canopy (Janzen, 1977).

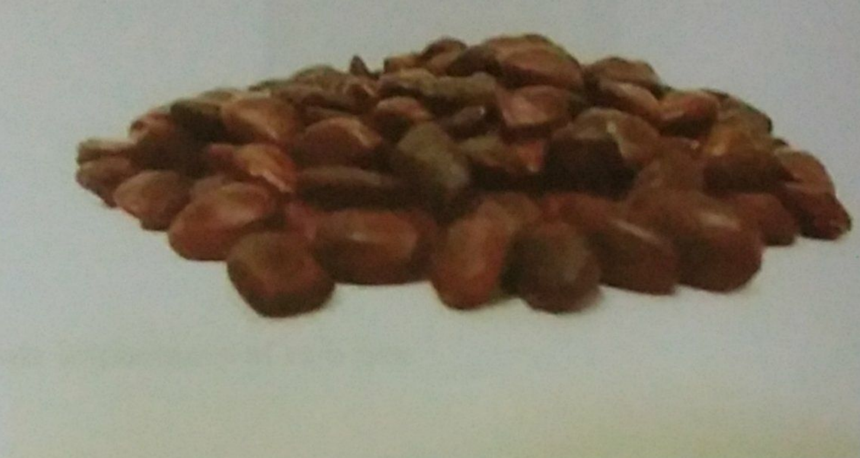

Fig.8 : Seeds of *Albizia saman*.

2.4.5 Bark

A. saman is a large deciduous tree with fissured, grey-brown to black bark (Little and Wadsworth, 1964). The bark of mature trees is gray, rough, and the bark of younger trees is smoother and paler gray to brownish in color (Staples and Elevitch, 2006).

Fig.9 : Mature bark of rain tree (*A. saman)*

2.5 Economic importance of rain tree

2.5.1 Fuel

A. saman wood produces about 5200-5600 kcal/kg when it burns and that it regrows vigorous after lopping or pollarding make it a valuable source of high-quality firewood and charcoal. However, where there is a strong market for wood carvings, the wood is considered too valuable to be used as fuel (Orwa et al., 2009).

2.5.2 Gum or resin

The bark is an abundant source of gums and resins (Orwa et al., 2009).

2.5.3 Tannin

Albizia saman is a potential suppliers of tannins for industrial purposes (Goncalves et al., 2001).

2.5.4 Animal fodder

The mesocarp of the pod of *A. saman* contains a sweet nutritious pulp which smells of honey when the pods are broken and which contains 12-18% crude protein (Chicco et al., 1973; Escalante, 1985; 1997) and is highly digestible (40% digestibility), making the pods a good source of proteins, carbohydrates and minerals for livestock (Flores, 2002)

2.5.5 Beverage/Drink/Tea

The pulp of the fruit of rain tree is used to make a beverage similar to tamarindo (made from tamarind pulp) in Latin America (Staples and Elevitch, 2006).

2.5.6 Wood products

The wood is highly valued for the manufacturing of furniture, cabinets, decorative veneers, and other handicrafts. The wood of *A. saman* is light-weight, strong, durable, works easily and takes a good finish. It shrinks so little that products made from green wood dry without warping (NAS, 1979).

2.5.7 Medicinal

The boiled bark is applied as a poultice for the treatment of constipation. A decoction of the inner bark and fresh leaves is used in the treatment of diarrhea in the Philippines. The seeds are chewed for sore throat in the West Indies (Staples and Elevitch, 2006).

2.5.8 Host

Albizia saman is a popular host plant for lac production in the North Bengal of Bangladesh (Hossain, 2015).

2.5.9 Yeast production

Candida utilis and *Saccharomyces cerevisiae* are grown on rain tree pod extract as sole carbon and energy sources. The pod extract is a suitable medium of cultivation of *Candida utilis* for single cell production (Geeta et al., 1990).

2.5.10 Agroforestry practices

Now-a-days rain tree is planted in the pastures for the enhancement of nitrogen in the soil, as a flowering ornamental plant in the parks and avenues and for shade along streets and highways. It is planted in the crop field like cocoa, vanilla, teak etc. for shading purpose (Staples and Elevitch, 2006).

2.6 Environmental requirements and tolerances

2.6.1 Climataic condition

A. saman is a truly tropical species. It thrives in both the seasonally dry and wet tropics (Allen and Allen, 1981). Now-a-days the species is successfully established in a wide range of climates both ever-wet (equatorial) and seasonally dry (monsoon) climates. (Staples and Elevitch, 2006).

2.6.2 Rainfall

The growth of rain tree (*A. saman*) where rainfall is greater than 1000 mm (Booth and Jovanovic, 2000). The mean annual rainfall is estimated to be 600 – 3000 mm (24-120 in) (Franco et al., 1995).

2.6.3 Elevation

Rain tree is known to grow on slightly elevated land just inland from mangroves (Francis, 2004). In the native range of rain tree, it grows mostly below 450m (1470 ft) (Staples and Elevitch, 2006).

2.6.4 Duration of the dry season

For better growth of rain tree, it needs a dry season duration of 0 - 6 months (Franco et al., 1995).

2.6.5 Temperature

Rain tree requires a mean annual temperature range of 20° to 30° C (68° to 100° F). Where the mean maximum temperature of hottest month is 24-38°C (75-100°F) and the mean minimum temperature of the coldest month is 18-20°C (64-68°F) (Staples and Elevitch, 2006).

2.6.5 Soil

Rain tree thrives in soils that are freely draining, and it tolerates impeded drainage. It can tolerate waterlogged soils for short periods of time in some cases (Staples and Elevitch, 2006). *A. saman* needs well-drained alluvial, fertile, neutral to moderately acid (>pH 4.6) soils for best growth (Franco et al., 1995).

2.6.6 Tolerances

It does not tolerate extended periods of drought. It can tolerate minimum temperature of 8°C (46°F). It cannot tolerate frost or low temperatures. Rain tree is light demanding species. It is rarely found in dense forest stands. Rain tree is intolerant of shade. At the seedling stage it can tolerate partial shade for 2-4 weeks.(Staples and Elevitch, 2006). Pavement, foundations, or shallow bedrock restrict root growth and make the species vulnerable to windthrow (Francis, 2004).

2.7 Growth rate of rain tree (*Albizia saman)*

Albizia saman is a promising fast growing species and is tolerant of heavy weed competition. It can reach an average diameter of 18 cm at age of 5 years. This species can reach 50 cm dbh in 60 years under favorable rainfall and soil conditions. In Bangladesh, most trials suggest that early growth is generally slower than commonly planted fast-growing species (Hossain, 2015).

Rapid early growth of S. saman on some sites, e.g. in Papua New Guinea (Brook et al., 1992), is comparable with other fast-growing legumes such as *Acacia angustissima*, *A. auriculiformis*, *Gliricidia sepium*, *Leucaena leucocephala* and *Schleinitzia novoguineensis*.

The performance of this species on eroded soils of northern dry zone showed that it is unsuitable for this type of land (Devaranavadagi and Murthy, 1999).

2.8 Silvicultural characteristics of rain tree

Albizia saman have short bole, extremely heavy branching and a wide, spreading crown. It is a strongly light demanding species. It generally has a very extensive shallow root system. This species is highly compatible with pasture and it is in extensive silvipastural systems that it fulfils its true potential (Raintree, 1987; Roshetko, 1995). It is a nitrogen fixing tree species (Hossain, 2015). It coppices quite well when young (Zabala, 1990).

2.9 Diseases and pests of rain tree *(Albizia saman)*

2.9.1 Dampling off

Dampling off is a very well known disease in our country and also the other parts of the world which is caused by the infection at root and collar region by the fungi named *Pythium spp.*; *Fusarium spp.*; *Rhizoctonia solani* and other soil inhabiting fungi. If seed germination is prevented, it is then called pre-emergence dampling off. It is called post-emergence dampling off when the disease rots the seedling at the soil level, in this case, the seedling collapse at collar region due to the death of roots. For the treatment of the dampling off diseases, tiram, captan and copper-oxycholoride, drenching with formalin 1.0-1.5 gallon per squre yard are used (Rahman, 1982). An effective measure against dampling off caused by *Fusarium palidoroseum* in Malay-pradesh in India were found named 'Topsin and Bavistin' treatment (Harsh, 1993).

2.9.2 Leaf spot

A disease of rain tree (*Albizia saman*) which is caused by *Colletotrichum spp.* This pathogen caused leaf spot on rain tree (Borah et al., 1998).

2.9.3 Stem canker

This disease is caused by *Fusarium semitectum* (*Fusarium pallidoroseum*). This disease can be controlled by spraying with 0.2% Bavistin (Carbendazin) (Dadwal and Jamaluddin, 1991).

2.9.4 Collar rot

This disease is caused by *Rhizoctonia bataticola*. In this disease, the collar region of the affected tree is appeared sunken and discoloured black by *Sclerotia* under the bark. And the above leaves can be shedded (Siddaramaiha et al., 1979).

2.9.5 Root gall

Seedling of *Albizia saman* exhibits symptoms of wilting, stunted growth and numerous root galls in nursery soils. This disease is caused by *Mincogita incogita* (Mahali, 1999).

2.9.6 Powdery mildew

This disease is caused by *Erysiphe communis*. In the seedling stage *Albizia saman*, this disease is observed (Quiniones and Dayan, 1987).

2.9.7 Dieback

This disease is caused by the *Botryodiplodia theobromae* fungas. Because of the attack of the fungi the tree gradually faces dying condition day by day (Hossain, 2004).

2.9.8 Bark eating caterpillar

The bark eating caterpillar *Indarbela quadrinotata* attacks on the rain tree (*A. saman*) and the tree gradually dies (Babu et al, 2000).

2.9.9 Mealy bugs attack on the rain tree

Recently in Mumbai, India, it has been observed that many Rain trees were attacked by mealy bugs in roadside rain tree plantation. Mealybugs are small, soft-bodied insects which have sucking mouthparts. They are white or whitish-pink in color and are generally covered in a white waxy material. They have filaments around the edge of their bodies and tails. Mealybugs occur in all parts of the world. Most occur naturally only in warmer parts. Mealybugs are insects in the family Pseudococcidae. They are considered pests as they feed on plant juices and also act as a vector for several plant diseases. Mealybugs are often found in clusters on stems and leaf whorls. They produce honeydew like secreation which often leads to sooty mould growth. Large colonies can weaken the plant because of the amount of sap being sucked which can result in yellowing leaves and defoliation. Mealybugs can build up in huge numbers in a very short time and cause considerable damage. The waste product of the mealy bug feeding process is a perfect growth medium for sooty mould fungi. These moulds/fungus damage plants by covering leaves and reducing light available for photosynthesis resulting the death of plants. In order to control mealy bugs, predator insects like Australian lady bird beetle (*Cryptolemus montezuari*) which directly feeds on mealybugs. Dusting or spraying of Biopesticide containing Parasite fungus Metarizium Verticilium Lecani , which creates disease on mealybug and feeds on it (Anon, 2017).

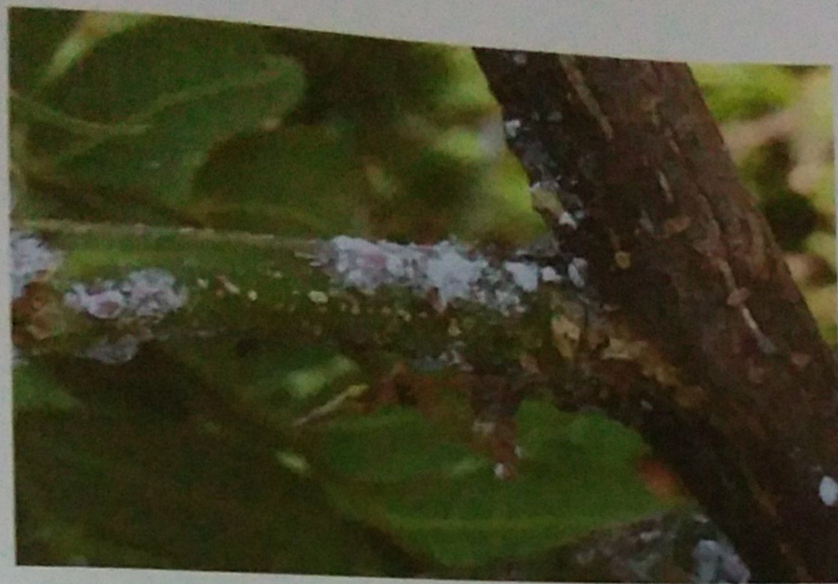

Fig.10 : Rain tree attacked by Mealy Bug.

2.9.10 Other Pests

A number of minor insect pests affect *A. saman* in different areas, but none of these have so far caused serious problems. Two psyllid insects, *Heteropsylla cubana* and *Psylla acacia-baileyanae* (*Acizzia acaciaebaileyanae*), are minor defoliators causing curling of leaves, minor defoliation, stunted shoot growth and development of numerous branches where young shoots die back (Braza, 1987; 1990; Braza and Calilunga, 1981).

In the native range, *A. saman* resprout shoots from cut stumps often have clusters of bright red, orange and blue-black nymphs of a coreid bug feeding on the shoot tips, leafcutter ants occasionally harvest some leaves, and howler monkeys eat flowers and young leaves (Janzen, 1983).

The bean maggot, *Hylemya platura (Delia platur)a*infests cotyledons of *A. saman* seedlings and may kill them in nurseries in Haiti (Timyan, 1996).

Seeds of *A. saman* in Costa Rica are predated by two bruchid beetles, *Merobruchus columbinus* and *Stator limbatus* which oviposit on the fruits as they reach full size and may kill 50-70% of the seeds (Janzen, 1977).

In Costa Rica, parrots may harvest up to a third of the expanded green fruits of S. saman (Janzen, 1982).

In Bangladesh, 18 insect pests from rain tree is reported (Baksha, 2008).

2.9.11 Nematodes

Heavy nematode infestation is observed on rain tree (Sabori et al., 1992). *Pammene theistis* is a common nematode on rain tree. *Holotrica fissa* is also a common pests on the species (Ghorpade and Patil, 1991).

CHAPTER THREE

MATERIALS And METHODS

3.1 Study area for sample collection

At first information about the extent and area of dominance of rain tree was collected from the divisional forest officer (D.F.O.) of Khulna, Bagerhat districts along with information about the occurance of mortality of rain tree in different parts of these districts. A reconnaissance survey of the major rain tree growing areas in Khulna, Bagerhat was conducted to identify the areas having worst mortality of rain tree.

Fig.11 : Affected *Albizia saman* in the study area.

3.2 Sample collection and preservation

Firstly, some dying rain trees were selected from different areas where rain tree mortality was severe. Then samples were collected from the dying and dead trees. The samples were collected from Bashundia under Dumuria thana, Khulna district ; Voroshapur under Rupsha thana, Bagerhat district and Digraj under Mangla thana, Bagerhat district where the severe mortality of rain tree (*A. saman*) was reported. From dying rain tree, the collected samples were root, stem bark, stem-heartwood, stem-sapwood, branch, and affected (damaged) leaf samples. And from dead rain tree of those area, the collected samples were root, stem bark, stem-heartwood, stem-sapwood and branch samples. Samples were collected and conveyed to the laboratory. Then the samples were kept in the deep freezer for the preservation purpose.

Fig.12 : Affected leaf sample of dying *Albizia saman*.

Fig.13 : Stem-wood sample of dying *Albizia saman*.

3.3 Materials required

Instruments like Laminar Air Flow (Bio-Safety Cabinet), Autoclave (Sterilizer), Digital Balance, Hallogen Hot-plates (Induction Cooker), Incubator etc.

Chemicals like Agar powder, Malt extract, Distilled water, Rectified Spirit, Streptomycin Sulphate solution etc.

Glasswares like petridishes, conical flusk, beaker etc.

Some other tools like burner, scalpel (surgical knife), inoculation loop, forcep, niddle, anti-cutter, hand gloves, aluminium foil paper etc also required.

3.4 Sterilization of laboratory glass wares

Petridishes were washed and dried well. Then wrapped with aluminium foil paper. After wrapping, they are placed into a metallic container. The container along with the petridishes, beakers, tissue paper etc needed for isolation were sterilized by autoclaving for 20 minutes at 15 p.s.i. at 121°C temperature. Other laboratory equipments such as knife, forceps, scalpels etc were also sterilized with rectified spirit and subsequent burning over flame of a spirit lamp. The chamber i.e. Laminar Air Flow in which the sterilized inocula were placed and other transferring work were done was also sterilized. The chamber was sterilized with ultraviolet (UV ray) for at least 30 minutes. The bench was also wiped with cotton soaked in rectified spirit. The hands were wiped with cotton soaked in rectified spirit up to the elbow.

3.5 Preparation of culture media

Cultivation of fungi is an important part for stydying their nutritional requirements, response to physical, chemical and biotic environments and also for proving their pathogenicity on specific host plants. A natural medium is entirely composed of complex natural products such as extracts of potato, maize or oat meal, leaves, fruits, etc. Selection of a satisfactory media for stimulating the growth and sporulation of particular fungus is important. Any rich carbohydrate source will support the fungal growth but PDA (Potato Dextrose Agar), MA (Malt Agar), Corn Meal Agar, Oatmeal Agar etc. are the most commonly used media. In the present study, 2% MA media has been used. For the preparation of MA media 20 gm of malt, 20 gm of agar powder and 1 litter distilled water were used. At first malt was added into the water and then boiled until it dissolved. Then agar powder was added into the suspension and again boiled. The medium was autoclaved then for 20 minutes at 15 p.s.i. at 121°C temperature.

3.6 Prevention of bacterial contamination

In culturing fungi, the inhibition of bacterial contamination and growth is a major problem. This problem was overcome by using antibiotics such as streptomycin sulphate (200 ppm) solution which was added to the medium just before pouring the media on the petridishes.

3.7 Cutting of the selected samples into inocula

For the purpose of isolation small portions of decayed samples that were stored in a deep freezer were taken and washed with distilled water to remove any adherent dirt or soil. After washing in distilled water, the samples were surface sterilized by using sodium hypochloride (NaOCl) for 2 minutes. Then the inocula were cut from the sterilize samples by the sterilized knife. The inocula were about 1mm to 1.5 mm in size. The cutting of inocula was done into the sterilized chamber.

3.8 Plating of inocula

After cutting the inocula from the sterilized samples, the inocula were placed on the sterilized media that was previously poured and solidified into the sterilized petridishes. The whole works were done into the sterilized chamber in order to conduct a contamination free experiment. The inocula were put on the media with the help of sterilized forceps about 1 cm far from the edge of the petridishes. About 3 inocula were inoculated into each petridish. The petridishes were then wrapped with brown paper and incubated in an incubator at 25°C -30°C temperature, which is appropriate for fungal growth.

3.9 Observation of inocula for fungal growth

After 2 days the inoculated plates were observed to see the yielded number of inocula. The yielded numbers were then counted and then the procedure was further followed at 2 days interval up to 10 days, which is the optimum range for maximum fungal growth after inoculation. Four types of fungi were isolated from the inoculated plates.

Fig.14 : Culturing of fungi.

CHAPTER FOUR

RESULTS And DISCUSSION

4.1 Fungal isolation from the samples of Bashundia,Dumuria area

4.1.1 Fungal isolation of affected tissues of dying *Albizia saman*

From the dying tree samples of Bashundia, four (4) types of fungi were isolated. Percentage of fungal isolates from the affected tree parts are presented in the Fig.15.

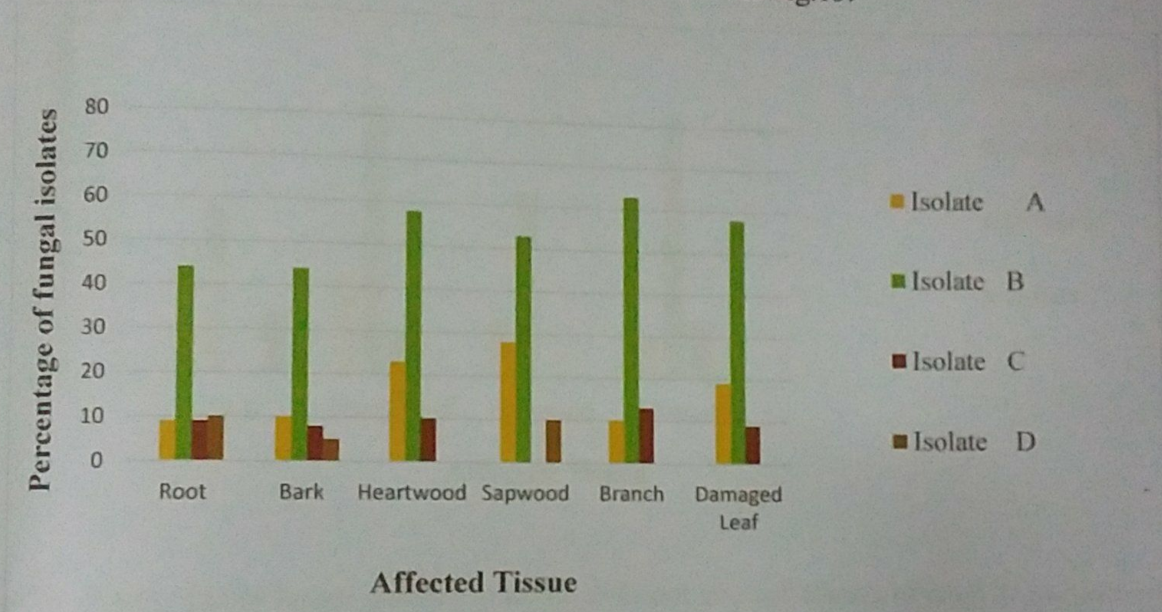

Fig.15 : Percentage of fungal isolates from different affected tissues of dying *A. saman* trees from the Bashundia area.

From the Fig.15, it is observed that isolate B showed the higher yielding for every affected tissues. In case of the root portion, the isolate B yielded about 44%, while isolate A, C and D yielded 9%, 9% and 10% of fungus cultures respectively. From the stem bark, isolate B yielded 44%, while isolate A, C and D yielded 10%, 8% and 5% of fungus cultures respectively. In the Stem-Heartwood, isolate B yielded 58%, while isolate A and C yielded 23% and 10% respectively and isolate D yielded 0%. Again the Stem-Sapwood, isolate B yielded 53%, while isolate A and D yielded 28% and 10% respectively and isolate C yielded 0%. Again from the Branch portion, isolate B yielded 63%, while isolate A and C yielded 10% and 13% and isolate D yielded 0%.

And from the Damaged Leaf, isolate B yielded 58%, while isolate A and C yielded 19% and 9% and isolate D yielded 0%.

So overall, the yielding of isolate B is higher in the different affected tissues of dying *A. saman* tree.

4.1.2 Fungal isolation of affected tissues of dead *Albizia saman*

From the dead *A. saman* tree samples of the Bashundia area, four types of fungi were isolated. Percentage of fungal isolates from the dead tree parts are presented in Fig.16.

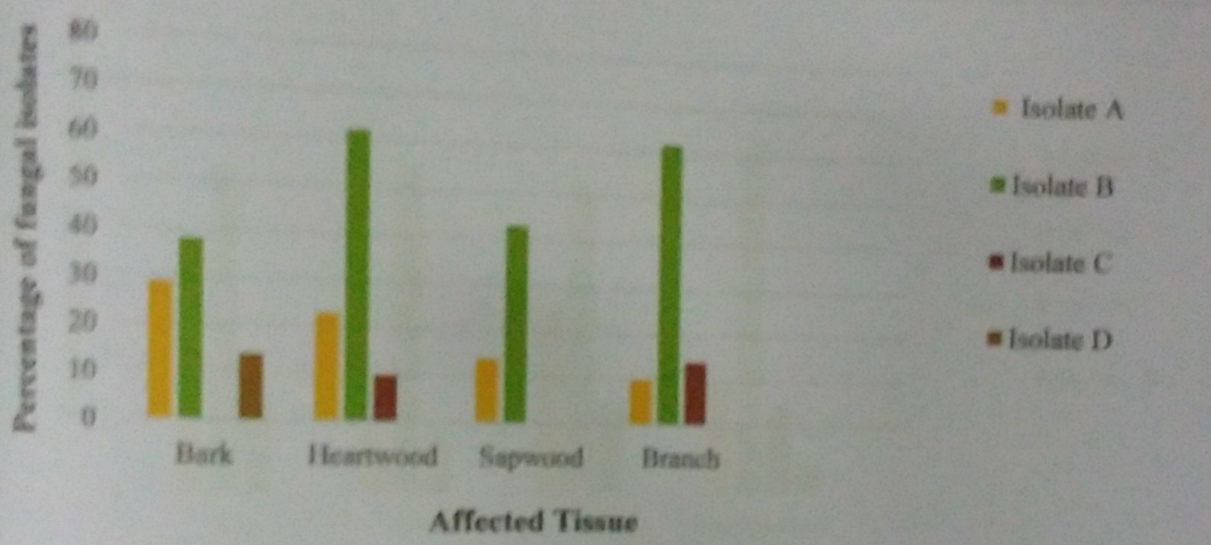

Fig.16 : Percentage of fungal isolates from the different affected tissues of dead *A. saman* trees from Bashundia area.

In Fig.16, it is observed that the isolate B showed the higher percentage of yielding for every affected tissue. In case of Stem-Bark, isolate B yielded 38%. In this tissue, isolate A yielded moderately high i.e. 29%. While isolate C and D yielded 0% and 14% respectively. In the Stem-Heartwood, isolate B yielded 62%, while isolate A, C and D yielded 23%, 10% and 0% respectively. From the Stem-Sapwood, isolate B yielded 43%, while isolate A, C and D yielded 14%, 0% and 0% respectively. Again from the Branch samples, isolate B yielded 62%, while isolate A, C and D yielded 10and, 14%and 0%.

4.2 Fungal isolation from the samples of Voroshapur,Rupsha area

4.2.1 Fungal isolation of affected tissues of dying *Albizia saman*

From the dying rain tree samples of Voroshapur, four (4) types of fungi were isolated. Percentage of fungal isolates from the dying tree parts are presented in the Fig.17.

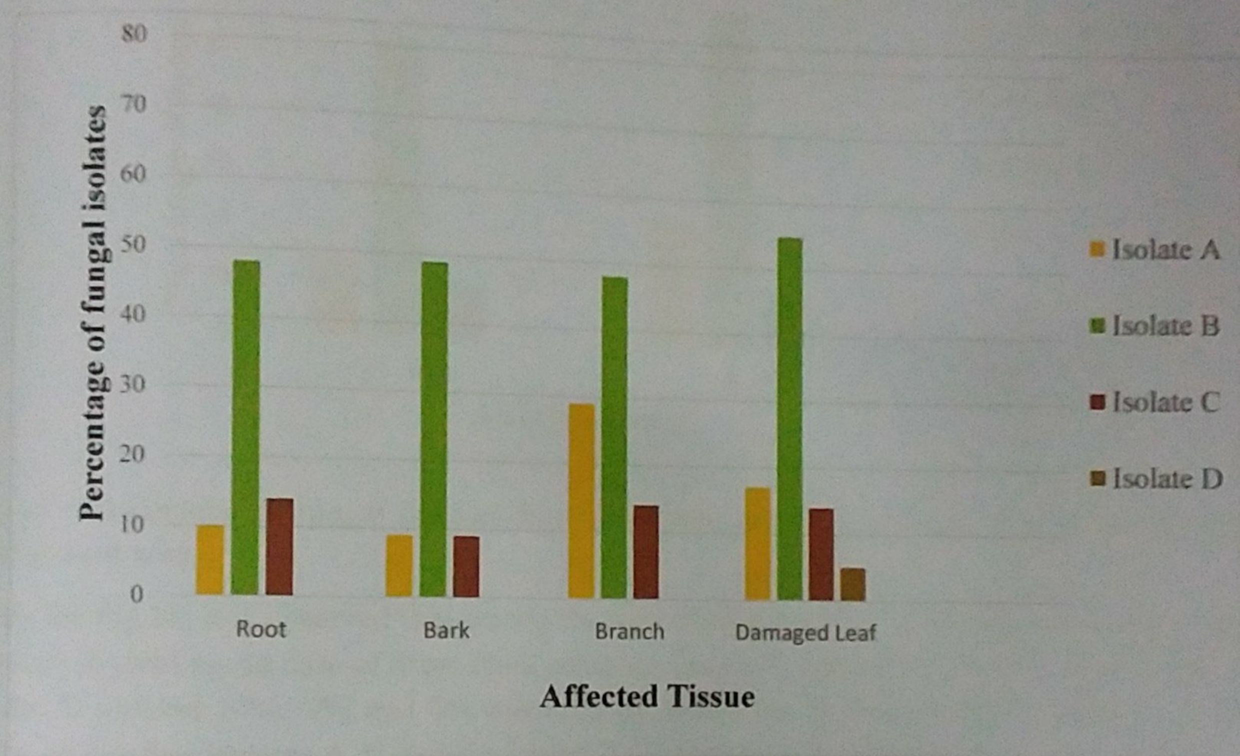

Fig.17 : Percentage of fungal isolates from the different affected tissues of dying *A. saman* trees from Voroshapur area.

In Fig.17, isolate B showed the higher yield, while the other isolates A, C and D showed a negligible amount of yield. From the Root samples, isolate B yielded 48%, while isolate A, C and D yielded 10%, 14% and 0% respectively. In case of the Stem-Bark samples, isolate B yielded 49%, while isolate A, C and D yielded 9%, 9% and 0% respectively. Again from the Branch samples, isolate B yielded 48%, while isolate A, C and D yielded 29%, 14% and 0% respectively. And in the Damaged Leaf samples, isolate B yielded 55%, while other isolates A, C and D 17%, 14% and 5% respectively.

4.2.2 Fungal isolation of affected tissues of dead *Albizia saman*

From the dead rain tree samples of Voroshapur, four (4) types of fungi were isolated. Percentage of fungal isolates from the dead tree parts are presented in the Fig.18.

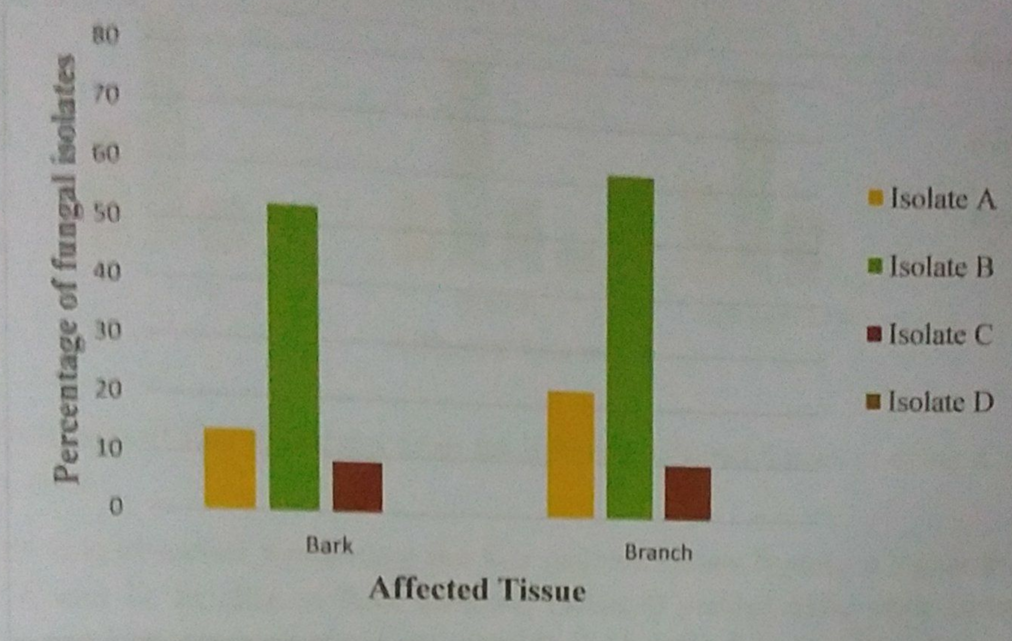

Fig.18 : Percentage of fungal isolates from the different tissues of dead *A. saman* trees from Voroshapur area.

From the Fig.18, it is observed that among the four (4) isolates type, isolate B showed the highest amount of yielding. In case of Stem-Bark samples, isolate B yielded 53%, while other isolates A, C and D yielded 14%, 9% and 0% respectively. From the Branch samples, isolate B yielded 62%, while other isolates A, C and D yielded 23%, 10% and 0% respectively.

4.3 Fungal isolation from the samples of Digraj,Mangla area

4.3.1 Fungal isolation of affected tissues of dying *Albizia saman*

From the dying *A. saman* samples of Digraj, four (4) types of fungi were isolated. Percentage of fungal isolates from the dying tree parts are presented in the Fig.19.

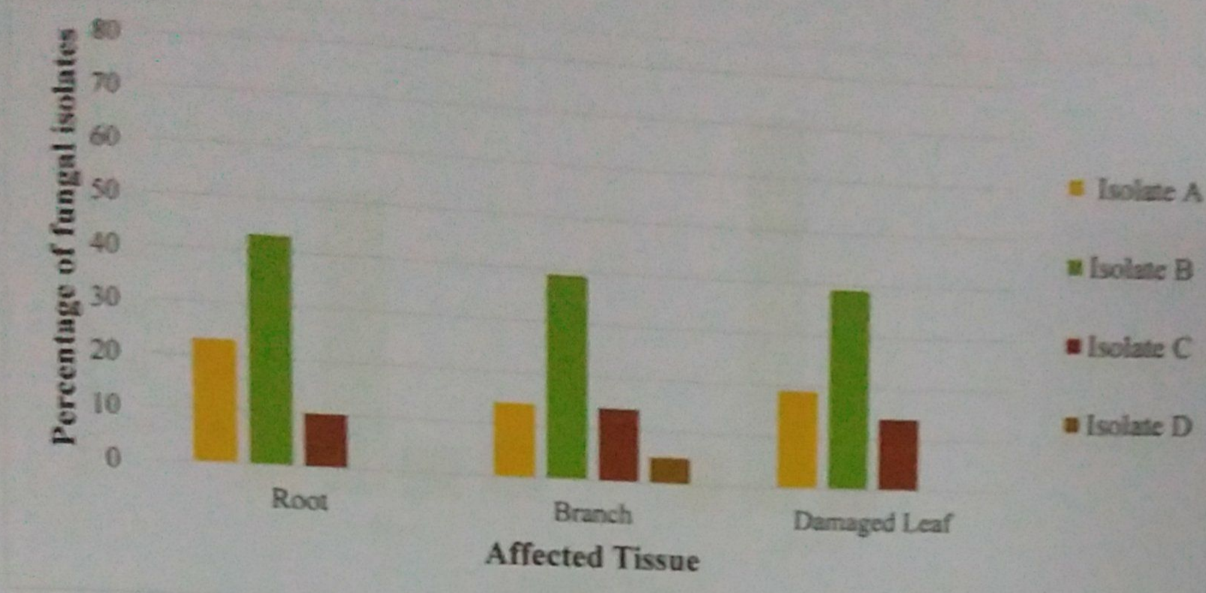

Fig.19 : Percentage of fungal isolates from the different affected tissues of dying *A. saman* trees from Digraj area.

From Fig.19, it is observed that among the four isolates, isolate B yielded higher than the other isolates A, C and D. In case of Root samples, isolate B yielded 43%, while isolate A and C yielded 23% and 10% respectively. And isolate D yielded 0%. From the Branch samples, isolate B yielded 39%, while isolate A, C and D yielded 14%, 14% and 5% respectively. Again from the Damaged Leaf samples, isolate B yielded 39%, while isolate A and C yielded 19% and 14% respectively. And isolate D yielded 0%.

4.3.2 Fungal isolation of affected tissues of dead *Albizia saman*

From the dead *A. saman* samples of Digraj, four (4) types of fungi were isolated. Percentage of fungal isolates from the dead tree parts are presented in the Fig.20.

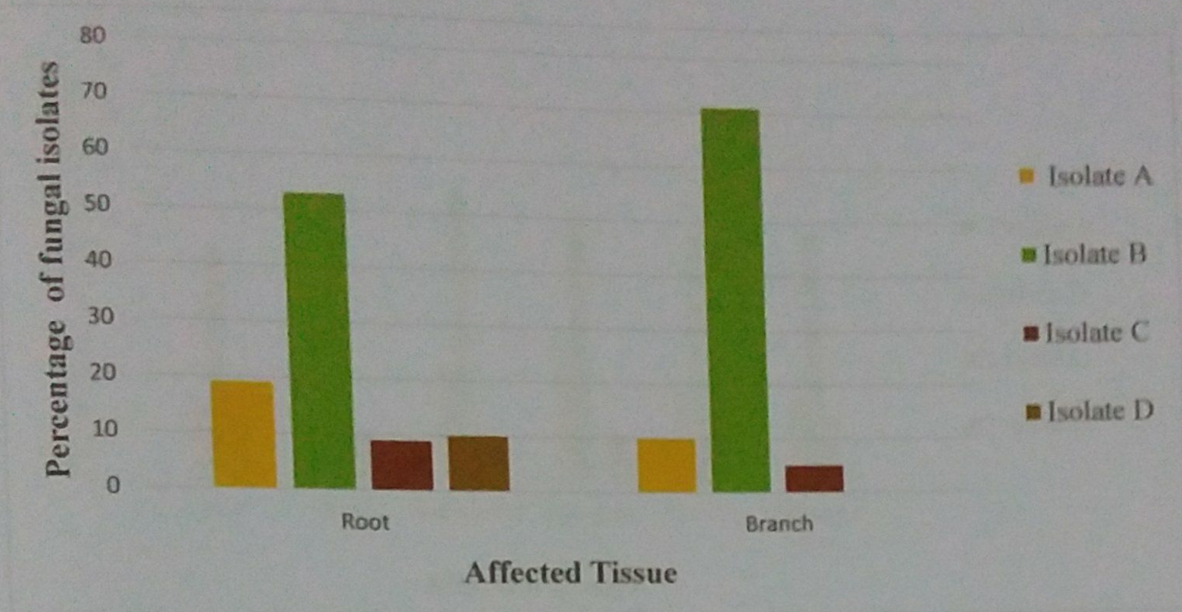

Fig.20: Percentage of fungal isolates from the different tissues of dead *A. saman* trees from Digraj area.

From Fig.16, it is observed that among the four isolates, isolate B yielded higher than the other isolates A, C and D. In case of Root samples, isolate B yielded 53%, while the other isolates A, C and D yielded 19%, 9% and 10% respectively. And from the Branch samples, isolate B yielded 71%, while isolate A and C yielded 10% and 5% respectively. And isolate D yielded 0%.

4.4 Overall fungal isolation from the samples of *Albizia saman* from the different areas

4.4.1 Overall fungal isolation from the dying *A. saman* samples

From the dying rain trees samples from different area, overall four types of fungi were isolated, where isolate B yielded with higher percentage for every affected tissue than the other isolates. The percentage of fungal isolates overall from the dying rain trees are presented in the Fig.21.

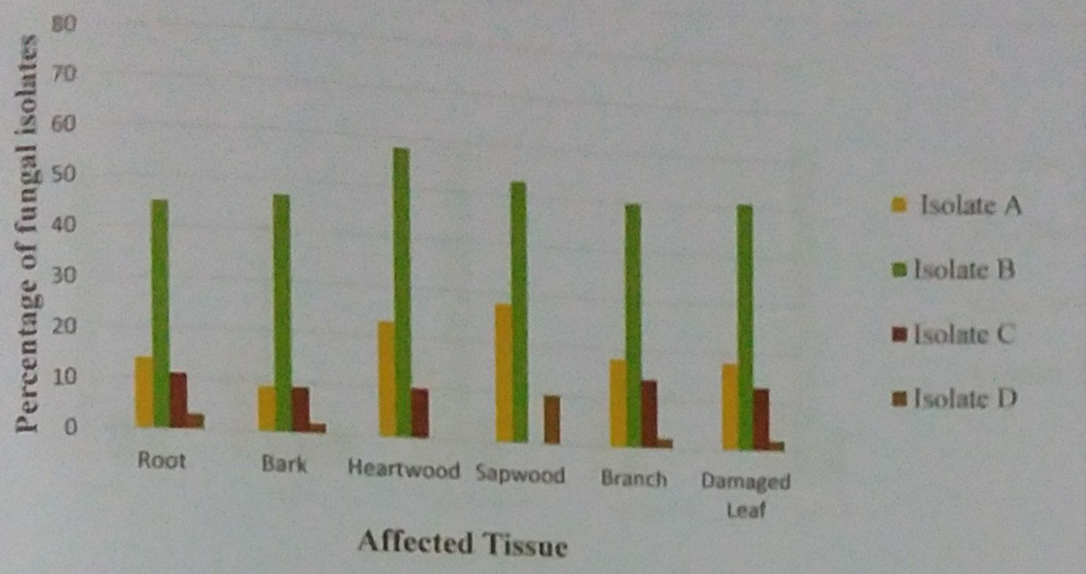

Fig.21 : Percentage of fungal isolates from the different affected tissues of dying *A. saman* trees.

In Fig.21, it is observed that isolate B yielded higher for every affected tissues i.e.. root, stem-bark, stem-heartwood, stem-sapwood, branch and damaged leaf and the percentage is 45%, 47%, 58%, 53%, 50% and 51% respectively. While the other isolate A, C and D yielded with a negligible amount. Among all the affected part of the dying rain trees, the yielding of B is highest in the stem-heartwood portion i.e. 58%.

4.4.2 Overall fungal isolation from the dead *A. saman* samples

Again the dead rain trees samples from the different area, overall four types of fungi were isolated, where isolate B yielded with higher percentage for every affected tissue than the other isolates. The percentage of fungal isolates overall from the dead rain trees are presented in the Fig.22.

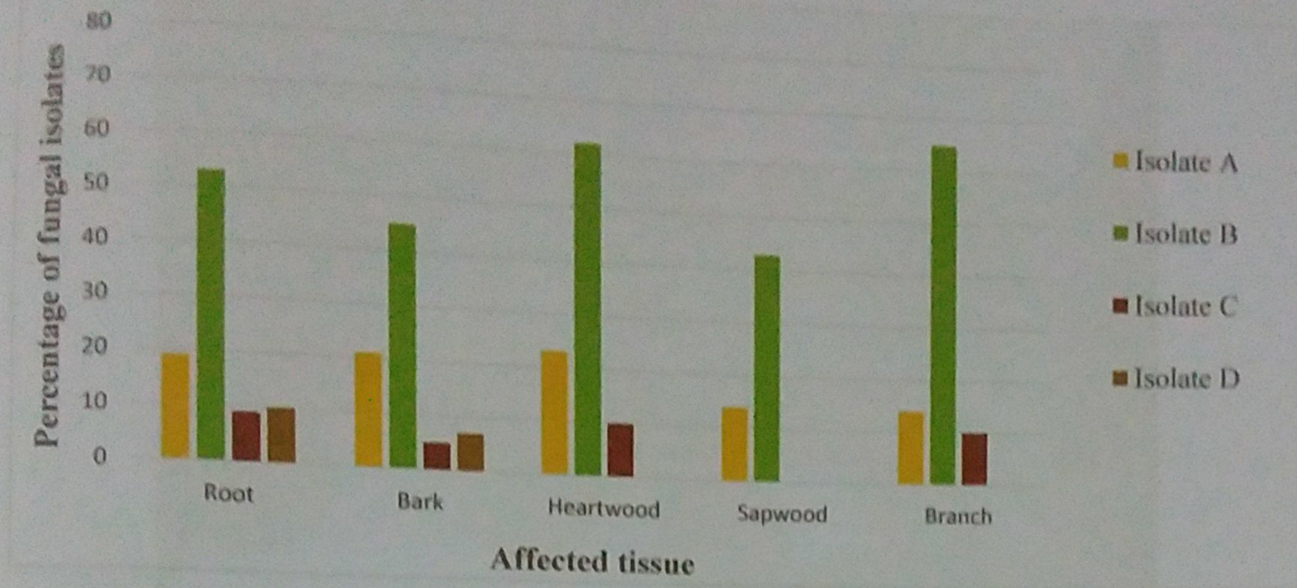

Fig.22 : Percentage of fungal isolates from the different affected tissues of dead *A. saman* trees.

In Fig.22, it is observed that isolate B yielded higher for every affected tissues i.e. root, stem-bark, stem-heartwood, stem-sapwood and branch and the percentage is 53%, 45%, 62%, 43%, 65% respectively. While the other isolate A, C and D yielded with a negligible amount. Among all the affected tissues of dead rain tree, the yielding of B is highest in the branch i.e. 65%.

Fig.23 : Yielding of fungi.

Fig.24 : Isolate B cover the whole media plate

CHAPTER FIVE

CONCLUSION And RECOMMENDATIONS

5.1 Conclusion

Rain tree (*Albizia saman*) is planted along the roadsides and highways now-a-days for its various economical, environmental and aesthetic values. In the south region a huge number of rain tree faces the massive mortality. To find the actual cause of this massive mortality this study is the key.

In the present study, four types of fungal isolates (A, B, C and D) have been isolated where it is observed that the isolate B is the dominant in every part.

For the dying rain trees samples, it is observed that the stem-heartwood sample showed the higher yielding of isolate B than the other affected part. And in case of dead rain tree samples, it is observed that the branch sample showed the highest percentage of isolate B than the other affected part.

So, it is clear that the isolate B has the chance of being the main causal factor for this massive mortality situation of rain tree (*A. saman*).

5.2 Recommendations

Further study is needed as it is the first study in this area. Study about the isolate B with different parameter such as temperature, light, dark etc will be needed. The identification of the isolate B will be needed for knowing the nature and types of the isolates specifically. The growth of the isolate B is needed to be studied in different condition. For prevention of further mortality careful study about the isolate B is needed. The pathogenicity test of isolate B is also needed for prescribing the right dose of appropriate insecticide / fungicide necessary for control measure. Also the study about the soil quality of those areas will be needed. The pH value of the soil will need to be analyzed. A detailed study on the mode of infection should be carried out. The control measure of the causal factor will also be needed to study.

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Appendix 1: Results of isolation of different types of isolates of fungi on 2% Malt Agar medium from dying & dead rain tree samples from Bashundia, Dumuria, Khulna.

Tree Condition	Affected Tissue	No. of MA plate used	No. of inocula plated	Yield of fungi from inocula	Percentage inocula yielding fungi	Percentage isolation of fungal isolates			
						A	B	C	D
Dying	Root	7	21	15	72	9	44	9	10
	Bark	7	21	14	67	10	44	8	5
	Heartwood	7	21	19	91	23	58	10	0
	Sapwood	7	21	19	91	28	53	0	10
	Branch	7	21	18	86	10	63	13	0
	Damaged Leaf	7	21	18	86	19	58	9	0
Dead	Bark	7	21	17	81	29	38	0	14
	Heartwood	7	21	20	95	23	62	10	0
	Sapwood	7	21	12	57	14	43	0	0
	Branch	7	21	18	86	10	62	14	0

Appendix 2: Results of isolation of different types of isolates of fungi on 2% Malt Agar medium from dying & dead rain tree samples from Voroshapur, Rupsha,Bagerhat.

Tree Condition	Affected Tissue	No. of MA plate used	No. of inocula plated	Yield of fungi from inocula	Percentage inocula yielding fungi	Percentage isolation of fungal isolates			
						A	B	C	D
Dying	Root	7	21	15	72	10	48	14	0
	Bark	7	21	14	67	9	49	9	0
	Branch	7	21	19	91	29	48	14	0
	Damaged Leaf	7	21	19	91	17	55	14	5
Dead	Bark	7	21	16	76	14	53	9	0
	Branch	7	21	20	95	23	62	10	0

Appendix 3: Results of isolation of different types of isolates of fungi on 2% Malt Agar medium from dying & dead rain tree samples from Digraj, Mangla, Bagerhat.

Tree Condition	Affected Tissue	No. of MA plate used	No. of inocula plated	Yield of fungi from inocula	Percentage inocula yielding fungi	Percentage isolation of fungal isolates			
						A	B	C	D
Dying	Root	7	21	16	76	23	43	10	0
	Branch	7	21	15	72	14	39	14	5
	Damaged Leaf	7	21	15	72	19	39	14	0
Dead	Root	7	21	19	91	19	53	9	10
	Branch	7	21	18	86	10	71	5	0

Appendix 4: Results of overall isolation of different types of isolates of fungi on 2% Malt Agar medium from dying & dead rain tree samples from different areas.

Tree Condition	Affected Tissue	Percentage of fungal isolates			
		A	B	C	D
Dying	Root	14	45	11	3
	Bark	9	47	9	2
	Heartwood	23	58	10	0
	Sapwood	28	53	0	10
	Branch	18	50	14	2
	Damaged Leaf	18	51	13	2
Dead	Root	19	53	9	10
	Bark	21	45	5	7
	Heartwood	23	62	10	0
	Sapwood	14	43	0	0
	Branch	14	65	10	0