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**Acknowledgements**

This is to thank UNIVERSITY GRANTS COMMISSION authorities for sanctioning the major research project on Bioprospecting fungal endophytes from *Terminalia* spp. for the production of certain cellulose degrading enzymes to me, and giving this opportunity to work on new group of organisms- Endophytes.

I am really thankful to Dr. S.K. Singh, Scientist Agharkar Research Institute, Pune for identifying the fungal organisms and to Prof. Sandhya Kiran, Head Department of Botany for providing laboratory facilities to Mr. PradeutDhar

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**UNIVERSITY GRANTS COMMISSION**

**BAHADUR SHAH ZAFAR MARG**

**NEW DELHI – 110002.**

Final Report of the work done on the Major Research Project.

1. Project report No. 3**Final Report**

 2. UGC Reference No.**F. No. 41 – 458/2012 (SR)**

 3. Period of report: from **1-7-12** to **31 – 12- 2015**

 4. Title of research project

**Bioprospecting fungal endophytes from *Terminalia* spp. for the production of certain cellulose degrading enzymes**

 5. (a) Name of the Principal Investigator **Prof Arun Arya**

 (b) **Botany Department Faculty of Science**

(c) **The Maharaja Sayajirao University of Baroda, Vadodara**

University/College where work has progressed \_\_\_\_\_\_\_\_\_\_\_\_\_

 6. Effective date of starting of the project **1-7-12**

7. Grant approved and expenditure incurred during the period of the

report:

 (a) Total amount approved Rs. **11,84,800=00**

 (b) Total expenditure Rs. **9,86,938 =00**

 **(c)** Balance to be recovered Rs. **2,61,138=00**

**8. Brief Objective of the Project**

Endophytes are reported to control the diseases and reduce the incidence of insects on the host plants. The potential benefits of endophytes to their hosts include increased tolerance to heavy metals, increased drought resistance, reduced herbivory, defense against pathogens, or enhanced growth and competitive ability (Saikkonen*et al.* 1998)Wood degrading enzymes comprise of a set of ligninolytic, cellulolytic and hemi cellulolytic enzymes. These enzymes are present in both in bacteria and fungi.

1. To isolate and identify the fungal Endophytes
2. To study the biological relationship between endophyte and its host
3. To study the effect of the plant extract on its own fungal endophyte
4. To isolate and characterize different enzymes produced by the activity of different Endophytic Fungi.

 **9. Report of the work done:**

i) Work done so far and results achieved and publications, if any,

resultingfrom the work ( Give details of the papers and names of the journals inwhich it has been published or accepted for publication.

The term “Endophytes” includes a suite of microscopic organisms which grow intra or intercellularly in the tissues of higher plants without causing any symptom on the plants in which they live, and have proven to be rich sources of bioactive natural products (Li *et al.*, 2008). Endophytes are known from plants growing in tropical, temperate and boreal forests from various habitats including extreme arctic alpine (Petrini, 1987), from halophytes of salt marsh (Mushin and Booth, 1987) and mosses as well as hepatics. Endophytes provide various benefits to the plants in the form of growth stimulation, pest resistance and stress tolerance. Microbiologists Gond *et al.* (2013) studied endophytes of bael(*Aegle marmelos*) leaves and isolated *Phoma herbarum.* Endophytic fungi have interest in researchers as an alternative source in controlling plant and human pathogens. Some of the earlier workers have documented the endophytic fungi residing inside the plant, exploring the biodiversity of hidden fungi. Webber (1981) demonstrated for the first time the role of endophytic *Phomopsisoblonga* in the protection of elm trees against the beetle *Physocnemumbrevilineum*. This report has generated interest in the role of endophytes in plant protection. Their beneficial role to plant as well as to humans is being considered. In this regard, a large number of antimicrobial compounds have been isolated from these endophytic microorganisms (Stroble, 2003: Zhang *et. al.,* 2006: Kharwar*et al.,* 2010). Endophytic fungi are now recognized as a new tool in the production of antimicrobials and pharmaceutical compounds.

The research work was undertaken to find out the association of endophytic fungi with following Five plants. All of these are members belonging tothe family Combretaceae and three of these are used extensively in Ayurveda.

**1.*Terminalia bellerica***(Gaertn.) Roxb, (Plate I, Fig D and Plate II, Fig D)

It isknown as "Bahera" is a large deciduous tree common on plains and lower hills in [Southeast Asia](http://en.wikipedia.org/wiki/Southeast_Asia), where it is also grown as an avenue tree. The [basionym](http://en.wikipedia.org/wiki/Basionym) is *Myrobalanusbellerica*Gaertn. ([William Roxburgh](http://en.wikipedia.org/wiki/William_Roxburgh) transferred *M. bellirica* to *Terminalia* as *"T. bellerica*(Gaertn.) Roxb". This spelling error is now widely used, causing confusion. The correct name is *Terminalia bellerica* (Gaertn.) Roxb.

Uses: In traditional Indian [Ayurvedic](http://en.wikipedia.org/wiki/Ayurveda) medicine, Beleric is known as "Bibhitaki" which in its fruit form it is used in the popular Indian herbal [rasayana](http://en.wikipedia.org/wiki/Rasayana) treatment [triphala](http://en.wikipedia.org/wiki/Triphala). The pulp of the fruit (*Belericmyrobalan*) is considered by Ayurvedic physicians to be astringent and laxative, and is prescribed with salt and long pepper in affections of the throat and chest. The fruit kernel is sometimes used as an external application to inflamed parts

**2.*Terminalia chebula***(Gaertn.) Retz. (Yellow Myrobalan or ChebulicMyrobalan)

It is native to Southern Asia from India and Nepal east to southwestern China (Yunnan) and south to Sri Lanka, Malaysia and Vietnam. This tree yields small, ribbed and nut-like fruits.

Uses: The fruits are picked when still green and then [pickled](http://en.wikipedia.org/wiki/Pickling), boiled with a little added [sugar](http://en.wikipedia.org/wiki/Sugar) in their own [syrup](http://en.wikipedia.org/wiki/Syrup) or used in [preserves](http://en.wikipedia.org/wiki/Fruit_preserves). The seed of the [fruit](http://en.wikipedia.org/wiki/Fruit), which has an elliptical shape, is an abrasive seed enveloped by a fleshy and firm pulp. The dry nut's peel is used to cure cold-related nagging coughs. Its fruit has digestive, anti-inflammatory, [anthelmintic](http://en.wikipedia.org/wiki/Anthelmintic), cardiotonic, aphrodisiac and restorative properties and is additionally beneficial in flatulence, constipation, [piles](http://en.wikipedia.org/wiki/Hemorrhoid), cough and colds.

***Terminalia bellerica*** and ***Terminalia chebula*** are both used for high [cholesterol](http://www.webmd.com/cholesterol-management/default.htm) and [digestive disorders](http://www.webmd.com/digestive-disorders/default.htm), including both [diarrhea](http://www.webmd.com/digestive-disorders/digestive-diseases-diarrhea) and [constipation](http://www.webmd.com/digestive-disorders/digestive-diseases-constipation), and [indigestion](http://www.webmd.com/digestive-disorders/indigestion). They have also been used for [HIV](http://www.webmd.com/hiv-aids/default.htm) infection.

**3.*Terminalia arjuna***(Roxb.) W.& A.**,**(Plate I, Fig E and Plate II, Fig F)

It is commonly known as arjuna or arjun tree in English usually found growing on river banks or near dry river beds in [West Bengal](http://en.wikipedia.org/wiki/West_Bengal) and south and central [India](http://en.wikipedia.org/wiki/India).

Uses: *Terminalia arjuna* has been used to balance the three “humors”: kapha, pitta, and vata. It has also been used for [asthma](http://www.webmd.com/asthma/default.htm), bile duct disorders, scorpion stings, and poisonings. It is also used as “a water pill,” and for [earaches](http://www.webmd.com/cold-and-flu/cold-guide/earache-cold-ear-infection), dysentery, [sexually transmitted diseases](http://www.webmd.com/sexual-conditions/default.htm) ([STDs](http://www.webmd.com/sexual-conditions/default.htm)), diseases of the urinary tract, and to increase sexual desire.

**4.*Terminalia catappa***L.(Plate I, Fig C)

It is a large [tropical](http://en.wikipedia.org/wiki/Tropics) tree in the [leadwood tree](http://en.wikipedia.org/wiki/Combretum_imberbe) family, [Combretaceae](http://en.wikipedia.org/wiki/Combretaceae), that is native to the tropical regions of Asia, Africa, and Australia. *Terminalia catappa*has corky, light fruit that are dispersed by water. The seed within the fruit is edible when fully ripe, tasting almost like almond. As the tree gets older, its crown becomes more flattened to form a spreading, vase shape. The leaves contain several [flavonoids](http://en.wikipedia.org/wiki/Flavonoid) (such as [kaempferol](http://en.wikipedia.org/wiki/Kaempferol) or [quercetin](http://en.wikipedia.org/wiki/Quercetin)), several [tannins](http://en.wikipedia.org/wiki/Tannin) (such as punicalin, punicalagin or tercatin), [saponines](http://en.wikipedia.org/wiki/Saponine) and [phytosterols](http://en.wikipedia.org/wiki/Phytosterol).

Uses: Due to chemical richness, the leaves (and the bark) are used in different [herbal medicines](http://en.wikipedia.org/wiki/Herbal_medicine) for various purposes. Keeping the leaves in an aquarium may lower the pH and heavy metal content of the water.

**5.*Terminalia crenulata*Roth**

It is a species of *Terminalia* native to southern and southeast Asia in India, Nepal, Bangladesh, Myanmar, Thailand, Laos, Cambodia and Vietnam etc. The [bark](http://en.wikipedia.org/wiki/Bark) is fire-resistant. The [wood](http://en.wikipedia.org/wiki/Wood) is coarse, fairly straight grained; dull to somewhat lustrous and without any smell or taste. The [heartwood](http://en.wikipedia.org/wiki/Heartwood) is moderately durable and the sapwood is liable to powder-post beetle attack.

Uses: The wood is used for [furniture](http://en.wikipedia.org/wiki/Furniture), cabinetwork, [joinery](http://en.wikipedia.org/wiki/Woodworking_joints), paneling, specialty items, boat-building, railroad cross-ties (treated), and decorative veneers.The bark is used medicinally against [diarrhea](http://en.wikipedia.org/wiki/Diarrhoea).

**Isolated Fungal Organisms**

A large number of fungi were isolated from healthy tissues of different Terminalia leaves. Description of few organisms is given here.

1. ***Aspergillus flavus*Link.**( Plate VI Fig. D)

Kingdom: Fungi, Phylum: Ascomycota Class:Eurotiomycetes,Order:Eurotiales

Family:Trichocomaceae,Genus:*Aspergillus*

Colonies are granular, flat, often with radial grooves, yellow at first but quickly becoming bright to dark yellow-green with age. Conidial heads are typically radiate, mostly 300-400 um in diameter, later splitting to form loose columns, biseriate but having some heads with phialides borne directly on the vesicle. Conidiophores are hyaline and coarsely roughened, the roughness often being more noticeable near the vesicle. Conidia are globose to subglobose (3-6 um in diameter), pale green and conspicuously echinulate. Some strains produce brownish sclerotia. It grows by producing thread like branching filaments known as hyphae. Filamentous fungi such as *A. flavus* are sometimes called molds. A network of hyphae known as the mycelium secretes enzymes that break down complex food sources. The resulting small molecules are absorbed by the myceilium to fuel additional fungal growth. It is predominately a saprophyte and grows on dead plant and animal tissue in the soil. *Aspergillus flavus* can also be pathogenic on several plant and animal species, including humans and domestic animals. The fungus can infect seeds of corn, peanuts, cotton, and nut trees.

**2*. Aspergillus niger* van Tiegh**( Plate III Fig. C and Plate VI E)

*Aspergillus niger* is classified as a member of the “deuteromycetes,” a “class” reserved for organisms with no known sexual state. Although they are considered a deuteromycete, modern taxonomy puts them in the phlyum of Ascomycota. Further taxonomy takes *A. niger* to the class of Eurotiomycetes, order of Eurotiales, family of Trichocomaceae, and genus *Aspergillus*.

*A. niger* is a ubiquitous fungus that grows very quickly. Strains can be isolated from many different ecological habitats such as soil, plant debris, rotting fruit, and even indoor air environments.

Macroscopically, this fungus can be identified growing on substrates producing colonies of felt like yellow to white hyphae, turning black with the formation of conidia.

 Microscopically, *A. niger* can be identified by its hyaline, septate hyphae. Asexual conidiophores can be identified by being long and globose at the tip, with what appears to be a hymenial layer of structures, each “ejecting” its own spore. While morphology provides a reasonable means of classification and assignment within the *A. niger* group, it is not a reliable means for identifying a given isolate from the field. The major distinction currently separating *A. niger*from the other species of *Aspergillus* is the production of carbon black or very dark brown spores from biseriatephialides (Raper and Fennell, 1965). Other features include the smooth and generally colorless conidiophores that are ó5um, globose, and have conspicuous ridges or spines not arranged in rows. *A. niger*isolates grow slowly on Czapek agar (Raper and Fennell, 1965).

**3*. Alternaria alternata***Fr. Keissl**.** ( Plate III Fig. B)

Kingdom: Fungi Phylum: Ascomycota Class: Euascomycetes, Order: Pleosporales

Family: Pleosporaceae, Genus: *Alternaria*

*Alternaria* is a cosmopolitan dematiaceous (phaeoid) fungus commonly isolated from plants, soil, food, and indoor air environment. The production of melanin-like pigment is one of its major characteristics. It grows rapidly and the colony size reaches a diameter of 3 to 9 cm following incubation at 25°C for 7 days on potato dextrose agar. The colony is flat, downy to woolly and is covered by grayish, short, aerial hyphae in time. The surface is greyish white at the beginning which later darkens and becomes greenish black or olive brown with a light border. The reverse side is typically brown to black due to pigment production (Collier *et al.* 1998). *Alternaria* spp. have septate, brown hyphae. Conidiophores are also septate and brown in color, occasionally producing a zigzag appearance. They bear simple or branched large conidia (7-10 x 23-34 µm) which have both transverse and longitudinal septations. These conidia may be observed singly or in acropetal chains and may produce germ tubes. They are ovoid to obclavate, darkly pigmented, muriform, smooth or roughened. The end of the conidium nearest the conidiophore is round while it tapers towards the apex. This gives the typical beak or club-like appearance of the conidia (Larone, 1995).

**4.*Fusarium oxysporum*Schltdl.**( Plate V Fig. C)

Macroscopic morphology may vary significantly on different media. Colonies are initially white, becoming tinged with salmon and lavender at maturity. Lavender to purple reverse. Salmon to orange sporodochia may be present. Hyphae are septate and hyaline. Conidiophores are short and simple, usually not branched. Macroconidia usually produced abundantly, slightly sickle-shaped, thin-walled, with an attenuated apical cell and a foot-shaped basal cell. They are three to 5-septate measuring 23-54 x 3-4.5 µm. Microconidia are abundant, mostly non-septate, ellipsoidal to cylindrical, slightly curved or straight, 5-12 x 2.3-3.5 µm occurring in false heads (a collection of conidia at the tip of the phialide) from short monophialides. Chlamydoconidia are present and often abundant, occurring singly and in pairs (Sutton *et al.* 1998).

**5.*Fusarium roseum***( Schweinitz) Petch.

Synonym: *Gibberellazeae(Fusarium roseum "Graminearum)*

Mycelium of the fungus *F. roseum* can be produced on potato dextrose agar. Two days after seeding, mass isolates from the edge of the colony were obtained, and the isolates were transferred to the growth medium. The cultures were then incubated for 3 days at room temperature on a rotary shaker at 200 rpm. The cells were harvested by filtration the solid culture mass (10 cm in diameter) was removed intact from the bottle and cut in half, and a disk 1 cm thick was sliced from the middle portion. From this disk 1 cm thick by 10 cm in diameter, four disks 1.0 cm in diameter were cut with a cork borer at random locations and used as controls. Four more disks were cut from the areas adjacent to those from which the control disks were taken, and these were used for the treatments; thus, each treatment was paired to a control the growth. The experimental results indicate that cAMP is present in growing cultures of *G. zeae*, it has a regulating effect on the production of perithecia when applied exogenously to mycelium, and it enhances the incorporation of [1-14C]acetate into zearalenone. It was also found that aminophylline mimics the effects of cAMP in zearalenone biosynthesis and that cyclic guanosine monophosphate has no sex-regulating effects on *G. zeae*.

**6*. Chaetomium globosum*Kunze**( PlateIV Fig. B)

Kingdom: Fungi Phylum: Ascomycota Class:Euascomycetes, Order:Sordariales
Family, ChaetomiaceaeGenus: *Chaetomium*

*Chaetomium* is a dematiaceous filamentous Ascomycetousfungus found in soil, air, and plant debris. As well as being a contaminant, *Chaetomium* spp. are also encountered as causative agents of infections in humans (Sutton *et al.*1998). *Chaetomium* colonies are rapidly growing, cottony and white in color initially. Mature colonies become grey to olive in color. From the reverse, the color is tan to red or brown to black. septate hyphae, perithecia, asci and ascospores are visualized. Perithecia are large, dark brown to black in color, fragile, globose to flask shaped and have filamentous, hair-like, brown to black appendages (setae) on their surface. Perithecia have ostioles (small rounded openings) and contain asci and ascospores inside. Asci are clavate to cylindrical in shape and rapidly dissolve to release their ascospores (4 to 8 in number). Ascospores are one-celled, olive brown in color, and lemon shaped (Larone, 1995).

**7*. Curvulariapallescence*Boedijn**( Plate VI Fig. F)

Kingdom: Fungi Phylum: Ascomycota Class: Euascomycetes, Order: Pleosporales

 Family: Pleosporaceae, Genus: *Curvularia* Species: *pallescence*

*Curvularia* is a dematiaceous filamentous fungus. Most species of *Curvularia* are facultative pathogens of soil, plants, and cereals in tropical or subtropical areas, while the remaining few are found in temperate zones (Larone, 1995). *urvularia* produces rapidly growing, woolly colonies on potato dextrose agar at 25°C. From the front, the color of the colony is white to pinkish gray initially and turns to olive brown or black as the colony matures. From the reverse, it is dark brown to black (St-Germain, and Summerbell. 1996). Septate, brown hyphae, brown conidiophores, and conidia are visualized. Conidiophores are simple or branched and are bent at the points where the conidia originate. This bending pattern is called sympodial geniculate growth. The conidia (8-14 x 21-35 µm), which are also called the poroconidia, are straight or pyriform, brown, multiseptate, and have dark basal protuberant hila. The septa are transverse and divide each conidium into multiple cells. The central cell is typically darker and enlarged compared to the end cells in the conidium. The central septum may also appear darker than the others. The swelling of the central cell usually gives the conidium a curved appearance (Larone, 1995, Sutton *et al.* 1998).

**8*. Phomopsis*sp.**( Plate III Fig. E)

*Phomopsis*was characterized by darkpycnidia at the periphery of the culture, often with long, black setae up to 5 mm, andabundant comma shaped conidia, andovoid conidia, immersed in a white creamy liquid. Myceliumwas sparse, often white-yellowish, sometimesbrown, wrinkled in appearance, andseptate. Antagonism between colonies (asevidenced by pigmented zones of interaction)was common. Abundant dark pigmentswere deposited on the bottom of theplate (Miguel *et al*. 2005).

**9.*Lasiodiplodiatheobromae*** (Pat.) Griffon &Maubl.( Plate IV Fig. D )

**Synonyms:** *Botryosphaeriarhodina, Botryodiplodiatheobromae*

**Key Features:**Coelomycete, with pycnidia producing characteristic two-celled, dark brown, striated conidia.

*Lasiodiplodiatheobromae* is a well known plant pathogen reported from about 500 host plants, mainly confined to an area 40 degrees north to 40 degrees south of the equator.The only other known host of this fungus is cacao *(Theobroma cacao).*It has also been associated with ulcerated human cornea, lesions on nail and subcutaneous tissue.

**Morphological Description:**Colonies are greyish sepia to mouse grey to black, fluffy with abundant aerial mycelium; reverse fuscous to black. Pycnidia are simple or compound, often aggregated, stromatic, ostiolate, frequently setose, up to 5 mm wide. Conidiophores are hyaline, simple, sometimes septate, rarely branched cylindrical, arising from the inner layers of cells lining the pycnidial cavity. Conidiogenous cells are hyaline, simple, cylindrical to sub-obpyriform, holoblastic, annellidic. Conidia are initially unicellular, hyaline, granulose, sub-ovoid to ellipsoid-oblong, thick-walled, base truncate; mature conidia one-septate, cinnamon to fawn, often longitudinally striate, 20-30 x 10-15 µm. Paraphyses when present are hyaline, cylindrical, sometimes septate, up to 50 µm long.

**10***.* ***Nigrosporasphaerica* (Sacc.) E.W. Mason**( Plate IV Fig. A)

It may cause Foliar symptoms, characterized by grayish, round, semicircular or irregular-shaped, numerous spots (up to 9 mm in diameter) with dark brown borders and the appearance of black, granular structure within the dead leaf tissues. A fungus was consistently isolated from healthy leaf tissues on potato dextrose agar (PDA). Fungal colonies were initially white, becoming light to dark gray with the onset of sporulation with black, spherical to subspherical single-celled conidia (15 to 18 × 12 to 15 μm), which were borne on a hyaline vesicle at the tip of the conidiophore. These characteristics agree with published descriptions of *Nigrosporasphaerica* (Sacc.) E.W. Mason 1927.

**10**. **Ulocladium*chartarum*** (G Preuss) EG Simmons( Plate V Fig. E)

Ulocladium spp., phylogenetically are related to Alternaria, is a dematiaceous, filamentous fungus that inhabits the soil and decaying herbaceous plants. It was isolated from healthy petioles of *Terminalia cranulata*. It is widely distributed in nature and may also be isolated from paper, textiles, and wood. Ulocladium is commonly considered a contaminant. There are approximately 18 species in this genus, some of which are food spoilers and plant pathogens. The species also contains members that have potential as enzyme producers and bio-control agents. Ulocladium spp. is often found on dead vegetation; in soil, air and dust; but also on food and feedstuffs, and on water-damaged building materials. Some members of the genus can invade homes and are a sign of moisture, because the mould requires water to thrive. It is also found in mattress dust, and in air conditioners in dry climates. They can cause plant disease.

Colonies of Ulocladium grow moderately rapidly. The growing colonies are woolly to cottony. From both the front and the reverse, the colour is olive-brown to black. No prominent beak is present as in *Alternaria.*

Species of Ulocladium resemble those of the genus Alternaria with which they were once included, but Ulocladium, unlike Alternaria, do not produce alternariols, tenuazonic acid, altersolanols, or macrosporin. Together with Alternaria and Stemphylium, it is considered one of the most common mould allergens in the United States.

1. **Survey and Field Observations**

**Survey**

**Fig. 1**

**Fig. 2 Photograph showing location of sites visited**

**Table 1.Survey of Terminalia spp. has been conducted in following six regions.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| No. | Area under study | District | Latitude  | Longitude  | Elevation |
| 1 | Arboretum, M.S.U | Vadodara | 22o19’14.64” N | 73o10’47.13” E | 129 ft |
| 2 | Botanical Garden, M.S.U | Vadodara | 22o18’36.08” N | 73o11’08.93” E | 131 ft |
| 3 | Virasat Van | Halol | - | - | - |
| 4 | Kadipani | Panchmahal | 22o00’29.23” N | 73o13’21.77” E | 1128 ft |
| 5 | Rajpardi | Bharuch | 21o43’08.86” N | 73o13’21.77” E | 147 ft |
| 6 | Ghoghamba | Panchmahal | 22o34’22.19” N | 73o38’46.40” E | 372 ft |

Leaf lamina, petiole, stem and bark samples have been collected from these areas.

**Materials and Methods:**

* Identification of suitable culture media

**Modified Asthana and Hawker’s Medium ‘A’** **Potato Dextrose Agar Medium**

Glucose 10 g Potato- 200 g

KNO3 3.5g D-Glucose 20g

KH2PO4 1.75g Agar 20g

MgSO4.7H20 0.75g Distilled water- 1000 ml

Distilled Water- 1000 ml

pH- 6.0

* **Isolation of Endophytic fungi**

Leaf samples were collected from survey area.

First the leaves were washed in running tap water for 10 minutes to drain off the dust particles present on leaf surface

The leaves were cut into 0.5 x 0.5 cm2 and surface sterilized in HgCl2 for 2 minutes

Then the leaves were dipped in NaOCl solution for 1 minute

The leaves were washed in sterile distilled water for 2 minutes

Then the leaves were inoculated in suitable culture media.

The growth of the organisms were observed after 7 and 21 days of inoculation

* Same technique was followed in 2 month old plants.
* **Screening of Celluloytic activity of selected fungal organisms**

The screening of cellulolytic activity of selected fungal organisms was done using CMC test of cellulase enzyme (Baines *et al.,* 2006).

**Procedure:**

* The screening of all species of fungi for their cellulolytic ability was done by substituting the malt extract agar medium with 0.5% CMC (Carboxymethyl cellulose) for test of cellulase.
* Streptomycin sulphate was added prior to sterilization to avoid bacterial contamination.
* After autoclaving, media was cooled and poured to sterile petriplates aseptically.
* On solidification, the plates were inoculated at the center with 0.5 cm dia. mycelial disc of different fungal cultures under study.
* The plates were incubated at 25±2oC for 5-7 days. 3 replicates were maintained for each treatment.
* The plates were flooded with visualizing reagent or dye, 0.25% Congo red for 15 minutes (Teather and Wood, 1982).
* Excess stain was drained off
* The enzyme activity was evaluated by observing the zone of clearance, if any, around the fungal culture.
* **Anatomy**

Free hand sections of 2 month old plants have been taken to study presence of endophytes in the inner tissues of stem, leaves as well as root. Sections were stained with Trypan blue.

**Work done:**

1. Isolation of endophytic fungi from leaves, petiole and leaf tip of 5 species of *Terminalia*
2. Isolation of endophytic fungi from 2 month old saplings
3. Screening of cellulolytic activity of selected fungal species
4. Observation on Growth of Plants from Seeds of *T. bellerica*and *T. arjuna*

**Table 2.Endophytic Fungal organisms isolated from leaves, petiole and leaf tip of 5 species of *Terminalia***

|  |  |  |  |
| --- | --- | --- | --- |
| **Name of Plant** | **Collected from****(Area/No. of Plant)** | **Plant Part** | **Isolated Fungal organism** |
| *T. bellerica*Baheda | Arboretum(2) | Leaf lamina | *Aspergillus flavus* Link. |
|  |  |  | *Alternaria alternata* Fr. Keissel |
|  |  |  | *Phomopsis*sp. |
|  |  |  | *Rhizopusstolonifer* (Ehthrenberg) Vuillemin |
|  |  |  | *Lasiodiploidia*sp. |
|  |  |  | *Pestalotiopsis*sp. |
|  |  |  | *Fusarium oxysporum*Schltdl. |
|  |  | Petiole | *Phomopsis*sp. |
|  |  |  | *Nigrosporasphaerica*(Sacc.) E.W. Mason. |
|  |  |  | *Aspergillus niger* van Tiegh |
|  |  |  | *Aspergillus flavus* Link. |
|  |  |  | *Pestalotiopsis*sp. |
|  |  | Leaf tip | *Rhizopusstolonifer* (Ehthrenberg) Vuillemin |
|  |  |  | *Phomopsis*sp. |
|  |  |  | *Chaetomium* sp. |
|  |  |  | *Aspergillus flavus* Link. |
|  | Botanical Garden (1) | Leaf lamina | *Alternaria alternata* Fr. Keissel |
|  |  |  | *Phomopsis*sp. |
|  |  |  | *Lasiodiploidia*sp*.*  |
|  |  |  | *Nigrosporasphaerica*(Sacc.) E.W. Mason. |
|  |  |  | *Rhizopusstolonifera* (Ehthrenberg) Vuillemin |
|  |  |  | *Aspergillus niger* van Tiegh |
|  |  | Petiole | *Phomopsis*sp. |
|  |  |  | *Nigrosporasphaerica*(Sacc.) E.W. Mason. |
|  |  |  | *Aspergillus niger* van Tiegh |
|  |  |  | *Aspergillus flavus* Link. |
|  |  |  | *Pestalotiopsis*sp. |
|  | Panchmahal forest (1) | Leaf lamina | *Aspergillus flavus* Link. |
|  |  |  | *Aspergillus niger* van Tiegh |
|  |  |  | *Alternaria alternata* Fr. Keissel |
|  |  |  | *Phomopsis*sp. |
|  |  |  | *Pestalotiopsis*sp. |
| *T. arjuna*Arjun Sadad | Arboretum(2) | Leaf lamina | *Aspergillus flavus* Link. |
|  |  |  | *Phomopsis*sp. |
|  |  |  | *Aspergillus niger* van Tiegh |
|  |  |  | *Alternaria alternata* Fr. Keissel |
|  |  |  | *Gliocladium*sp. |
|  |  |  | *Chaetomium globosum*Kunze |
|  |  | Petiole | *Aspergillus niger* van Tiegh |
|  |  |  | *Aspergillus flavus* Link. |
|  |  |  | *Colletotrichumgloeosporioides* (Penz.) Penz. &Sacc. |
|  |  |  | *Nigrospora*sp. |
|  |  |  | *Alternaria alternata* Fr. Keissel |
|  |  |  | *Pestalotiopsis*sp. |
|  |  |  | *Curvularialunata* (Wakker) Boedijn |
|  | Botanical Garden (1) | Leaf lamina | *Aspergillus flavus* Link. |
|  |  |  | *Phomopsis*sp. |
|  |  |  | *Aspergillus niger* van Tiegh |
|  |  |  | *Alternaria alternata* Fr. Keissel |
|  |  |  | *Pestalotiopsis*sp. |
|  |  | Petiole | *Aspergillus niger* van Tiegh |
|  |  |  | *Aspergillus flavus* Link. |
|  |  |  | *Nigrospora*sp. |
|  |  |  | *Alternaria alternata* Fr. Keissel |
|  |  |  | *Pestalotiopsis*sp. |
| *T. chebula*Harde | Arboretum (1) | Leaf lamina | *Aspergillus niger* van Tiegh |
|  |  |  | *Aspergillus flavus* Link. |
|  |  |  | *Phomopsis*sp. |
|  |  |  | *Alternaria alternata* Fr. Keissel |
|  |  |  | *Fusarium roseum* Link. |
|  |  |  | *Gloeosporium*sp. |
|  |  | Petiole | *Fusarium roseum* Link. |
|  |  |  | *Phomopsis*sp. |
|  |  |  | *Gloeosporium*sp. |
|  |  |  | *Alternaria alternata* Fr. Keissel |
|  |  |  | *Curvularia*sp. |
|  |  | Leaf tip | *Aspergillus niger* van Tiegh |
|  |  |  | *Phomopsis sp.* |
|  |  |  | *Fusarium roseum* Link. |
|  |  |  | *Alternaria alternata* Fr. Keissel |
|  |  |  | *Aspergillus flavus* Link. |
| *T. catappa*Desi badam | Arboretum (2) | Leaf lamina | *Alternaria alternata* Fr. Keissel |
|  |  |  | *Aspergillus niger* van Tiegh |
|  |  |  | *Aspergillus flavus* Link. |
|  |  |  | *Curvularia*sp. |
|  |  |  | *Lasiodiplodiatheobromae* (Pat.) Griffon &Maubl. |
|  |  |  | *Pestalotiopsis*sp. |
|  |  | Petiole | *Aspergillus niger* van Tiegh |
|  |  |  | *Aspergillus flavus* Link. |
|  |  |  | *Cladosporium herbarum* (Pers.: Fr.) Link, |
|  |  |  | *Penicilliumcitrinum*Thom. |
|  |  |  | *Drechslera*sp. |
|  | Panchmahal District (1) | Leaf lamina | *Alternaria alternata* Fr. Keissel |
|  |  |  | *Aspergillus niger* van Tiegh |
|  |  |  | *Aspergillus flavus* Link. |
|  |  |  | *Curvularia*sp. |
|  |  |  | *Lasiodiplodia*sp. |
|  |  |  | *Rhizopusstolonifer*(Ehthrenberg) Vuillemin |
|  |  |  | *Phomopsis*sp. |
|  |  | Petiole | *Aspergillus niger* van Tiegh |
|  |  |  | *Aspergillus flavus* Link. |
|  |  |  | *Cladosporium* sp. |
|  |  |  | *Penicilliumcitrinum*Thom. |
|  |  |  | *Drechslera*sp. |
|  | Vadodara roadsides (3) | Leaf lamina | *Aspergillus niger* van Tiegh |
|  |  |  | *Aspergillus flavus* Link. |
|  |  |  | *Curvularia*sp. |
|  |  |  | *Penicilliumcitrinum*Thom. |
|  |  |  | *Lasiodiplodiatheobromae* (Pat.) Griffon &Maubl. |
|  |  |  | *Fusarium oxysporum*Schltdl. |
|  |  | Petiole | *Aspergillus niger* van Tiegh |
|  |  |  | *Aspergillus flavus* Link. |
|  |  |  | *Curvulariapallescence*Boedjin |
|  |  |  | *Drecslerarostrata* |
|  |  |  | *Fusarium oxysporum*Schltdl. |
|  | Rajpardi, Bharuch (2) | Leaf lamina | *Alternaria alternata* Fr. Keissel |
|  |  |  | *Aspergillus niger* van Tiegh |
|  |  |  | *Aspergillus flavus* Link. |
|  |  |  | *Curvularialunata* (Wakker) Boedijn |
|  |  |  | *Chaetomium* sp. |
| *T. crenulata*Desi Sadad | Arboretum (1) | Leaf Lamina | *Aspergillus niger* van Tiegh |
|  |  |  | *Aspergillus flavus* Link. |
|  |  |  | *Alternaria alternata* Fr. Keissel |
|  |  |  | *Curvulariaprasadii*Boedign. |
|  |  |  | *Fusarium oxysporum*Schltdl. |
|  |  |  | *Chaetomium* sp. |
|  |  |  | *Trichoderma viride* Link. |
|  |  |  | *Phomopsis*sp. |
|  |  | Petiole | *Emericella*sp. |
|  |  |  | *Chaetomium fusisporale* J.N. Rai &Mukerji |
|  |  |  | *Alternaria alternata* Fr. Keissel |
|  |  |  | *Fusarium* sp. |
|  |  |  | *Trichoderma viride* Link. |
|  |  |  | *Phomopsis*sp. |
|  | Panchmahal District (2) | Leaf lamina | *Aspergillus niger* van Tiegh |
|  |  |  | *Aspergillus flavus* Link. |
|  |  |  | *Alternaria alternata* Fr. Keissel |
|  |  |  | *Curvulariapallescence*Boedjin |
|  |  |  | *Chaetomium* sp. |
|  |  |  | *Phomopsis*sp. |
|  |  |  | *Emericella*sp. |
|  |  | Petiole | *Chaetomium* sp. |
|  |  |  | *Ulocladiumchartarum*(G Preuss) EG Simmons |
|  |  |  | *Fusarium* sp. |
|  |  |  | *Trichoderma viride*Pers. |
|  |  |  | *Phomopsis*sp. |
|  |  |  | *Emericella*sp. |
|  |  | Leaf tip | *Aspergillus niger* van Tiegh |
|  |  |  | *Aspergillus flavus* Link. |
|  |  |  | *Phomopsis*sp. |
|  |  |  | *Trichoderma viride* Pers. |
|  |  |  | *Emericella*sp. |
|  |  |  | *Alternaria alternata* Fr. Keissel |

Scientists have found that non-balansiaceous fungi involved in asymptomatic colonisations of plants. These fungal endophytes represent a continuum of fungi with respect to physiological status, infection modus, colonisation pattern, altered secondary metabolism, change in life-history strategy, and developmental and evolutionary stages, but also with respect to the fungal and host taxa involved in the symbioses. It has been said that there are no neutral interactions, but rather that endophyte-host interactions involve a balance of antagonisms, irrespective of the plant organ infected. There is always at least a degree of virulence on the part of the fungus enabling infection, whereas, defense of the plant host limits development of fungal invaders and disease. It is also hypothesized that the endophytes, in contrast to known pathogens, generally have far greater phenotypic plasticity and thus have more options than pathogens: infection, local but also extensive colonisation, latency, virulence, pathogenity and (or) saprophytism. This phenotypic plasticity leads to evolution of species.

It is evident from above table and Fig. 3 that Number of isolated Fungi were more in *Terminalia bellerica* and *T.chebula* followed by *T. cranulata* and *T. arjuna*.







It was further observed that out of 16 fungi isolated 7 were from leaf lamina, 5 from petiole and 4 from leaf tip ( Fig. 4) More details are shown in Pie charts 5-8.

Endophytic fungi from Nyctanthes arbor-tristis were isolated and evaluated for their antimicrobial activity. A total of 19 endophytic fungi were isolated from 400 segments of healthy leaf and stem tissues of N. arbor-tristis. Eighteen endophytic fungi were obtained from leaf, while only ten from stem. Alternaria alternata had the highest colonization frequency (15.0%) in leaf, whereas Cladosporium cladosporioides ranked first in stem with a colonization frequency of 12%. The diversity and species richness were found higher in leaf tissues than in stem( Gond*et al*. 2012).

Warburgiaugandensis is a tree species with high ethnopharmaceutical relevance. In traditional local medicine the powdered bark is usually taken orally as aqueous infusion, smoked, or mixed with fat and applied externally as an ointment for treatment of a broad range of human diseases including measles and malaria (Beentje and Adamson, [1994](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3916764/#B6); Kokwaro, [2009](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3916764/#B29)). The existence of this tree species in its natural environment is however under severe threat. Deforestation and unsustainable use (harvest of roots and barks) results in drastic loss of these trees. Knowledge of the factors determining the variation in the patterns of drimanesesquiterpenes could help to identify individuals with high yield production traits for drimanesesquiterpenes in order to identify suitable genotypes or cultivation practices for plantations of this tree. This would substantially increase the value of this tree species for local farmers and facilitate preservation programs. However the actual reasons for the strong individual variations in the drimanesesquiterpene pattern in the pepper bark tree still remain obscure.

The study (Drage*et al.,* 2014) revealed that (1) the endophyte community of the tropical tree Warburgiaugandensis resembles at the genus level that of trees in temperate climates; (2) the endophyte community is not shaped by host drimanesesquiterpenes; (3) the diversity of the endophytic microflora in plantdoes not correlate with that of host chemicals**;** and (4) other factors rather than endophytic microbes might be responsible for the high variations in the content and composition of drimanesesquiterpenes in the pepper bark tree.

* **Anatomical Studies**

Free hand sections of 2 month old plants have been taken to study presence of endophytes in the inner tissues of stem, leaves as well as root. Sections were stained with Trypan blue.

No fungal hyphae of endophytes were observed the lamina of leaf tissues. The anatomical details are presented in Plate VIII Fig A,B and C.



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**Illustration of Photographs**

**Plate I**

Fig. A Botanical garden, The Maharaja Sayajirao University of Baroda

Fig. B Photograph of Virasat Van, Halol

Fig. C Tree of *Terminalia catappa*

Fig. D Tree of *Terminalia bellerica*(Gaertn.) Roxb,

Fig. E Plant of *Terminalia arjuna*(Roxb.) W. & A.

**Plate II**

Fig. A Leaves of *Terminalia bellerica*

Fig. B Flowers of *Terminalia arjuna*(Roxb.) W. & A.

Fig. C Leaf and fruit of*Terminalia bellerica*(Gaertn.) Roxb,

Fig. D Saplings of *Terminaliabellerica*(Gaertn.) Roxb,

Fig. E Fruits and F Young Plants of *Terminalia arjuna*(Roxb.) W. & A.

**Plate IIII**

Fig.A- F The Cultures of Endophytes grown in Petriplates

**Plate IV**

Fig. A. Photographs of conidia of *Nigrosporasphaerica*

Fig. B. Photographs of Perithecia and ascospores of *Chaetomium*

Fig. C. Photographs of conidia of *Pestalotiopsis*

Fig. D. Photographs of conidia of *Lasiodiplodiatheobromae*

**Plate V**

Fig. A. Photographs of conidia of *Curvularialunata*

Fig. B. Photographs of Conidia of *Fusarium oxysporum*

Fig. C. Photographs of conidia of *Fusarium roseum*

Fig. D. Photographs of conidia of *Penicilliumcitrinum*

Fig. E. Ulocladium*chartarum* (G Preuss) EG Simmons

Fig. F. Conidia of *Lasiodiplodiatheobromae*

**Plate VI**

Fig. A. Photographs of conidia of *Gloeosporium*

Fig. B. Photographs of Conidia of *Myrothecium roridum*

Fig. C. Photographs of conidia of *Chaetomium*

Fig. D. Photographs of conidia of *Aspergillusflavus*

Fig. E. Photographs of conidia of *Aspergillusniger*

Fig. F. photograph of Conidia of *Curvularia*

**Plate VII**

**Screening of Cellulolytic activity of different fungi**

**Plate VIII**

**Anatomical details of leaf and Petiole Sowing tissues**

**SUMMARY**

**Bioprospecting fungal endophytes from *Terminalia* spp. for the production of certain cellulose degrading enzymes**

Fungi colonize foliar surface and on twigs as epiphytes. Inside plants they enter asbiotrophs and necrotrophs. Biotrophic pathogens are parasites that have evolved the means to grow within living plant cells without stimulating plant defense mechanisms. The term endophyte has been used to denote a particular type of systemic, nonpathogenic symbiosis. The grass endophytes provide their hosts with a number of benefits, such as protection against herbivory and pathogens, that increase their fitness

Ecological roles played by endophytic fungi are diverse and varied (Saikkonen*et al. 1998*). Endophytes have been described as mutualistic that protect both grasses (Clay, 1990) and conifers (Carroll, 1991) against insect herbivory and many of these fungi produce biologically active compounds (Pelaez*et al.* 1998). Non grass endophytes produce antifungal (Pelaez*et al.* 1998) or antibacterial substances as well as insecticidal compounds. Endophytes not only provide disease resistance and improve plant’s ability to withstand environmental stresses (Sturz and Nowak, 2000) but also serve as a source of novel bioactive compounds (Strobel and Long, 1998).

**Isolation of Endophytic fungi and Enzyme Production**

Leaf samples were collected from survey area.First the leaves were washed in running tap water for 10 minutes to drain off the dust particles present on leaf surface. The leaves were cut into 0.5 x 0.5 cm2 and surface sterilized in HgCl2 for 2 minutes. Then the leaves were dipped in NaOCl solution for 1 minute. The Number of isolated Fungi were more in *Terminalia bellerica* and *T.chebula* followed by *T. cranulata* and *T. arjuna*.

It was further observed that out of 16 fungi isolated , of these 7 were from leaf lamina, 5 from petiole and 4 different fungi from leaf tip of *T. bellerica* ( Fig. 4) . anatomical sections did not showed the fungal hyphae in leaf and petiole tissue. Production of cellulolytic enzymes was also studied.

 iii. Has the progress been according to original plan of work and towards

achieving the objective, it not, state reasons

 iv. Please indicate the difficulties, if any, experienced in implementing

theproject\_*\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*

 V. If project has not been completed, please indicate the approximately

time by which it is **completed.**

 vi. If the project has been completed, please enclose a summary of the

finding of the study. **Report enclosed**

One bound copy of the final report of work done may also be sent to University Grants Commission.

 vii. Any other information which would help in evaluation of work

done on the project. At the completion of the project, the first report

should indicated the output, such as ( a).

Manpower trained (b) Ph.D. awarded (c) Publication of result (d)

 Other impact, if any **The student Mr. PradeutDhar is yet to submit his Ph.D. Thesis**

 **Published 2 Research Papers**

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

 REGISTRAR

 (Seal)