Studies on Fungal Deterioration of Certain Non- Wood Forest Produce and Their Control Studies

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Certificate

*The thesis entitled "**Studies on Fungal Deterioration of Certain Non-Wood Forest Produce and Their Control Studies"** submitted by **Mrs. Shraddha Olpadkar,** contains the original research work carried out by her in the Phytopathology Laboratory of the Department of Botany, The Maharaja Sayajirao University of Baroda. It has been prepared in accordance with the University norms under my direct supervision. It is further certified that this work has not been submitted earlier to any other University/Institute for any degree.*

(Prof. Arun Arya)
Guide and Head

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(Shraddha Olpadkar)

Contents

List of Tables

3.2.3	Showing Area of collection, Quantity, procure and cost in Kg of NWFP produce obtained in different locations	
3.3.1	Percentage occurrence of treated seed mycoflora of Madhuca, Emblica, Tamarindus and Terminalia in different culture media.	
3.3.2	Showing percentage germination,viability, in Emblica officinalis, Tamarindus indicus, T.bellerica, M.indica fresh seeds	
3.3.3	Showing percentage of germination and isolation of fungi in M.indica, E.officinalis, T.bellerica,T.indica.ungerminated seeds	
3.4.1	Changes in sugars in the flowers of M.indica, due to pathogenesis by Penicillium citrinum, Thielavia terricola, Fusarium oxysporum	
3.4.2	Changes in sugars in the fruits of M.indica,due to the pathogenisis by Penicillium citrinum, T.terricola, F.oxysporum	
3.4.3	Changes in sugars in the fruit of E.officinalis due to pathogenisis by P.citrinum, T.terricola, F.oxysporum	
3.4.4	Changes in sugars in the fruit of T.indica, due to pathogenisis by P.citrinum, T.terricola,F.oxysporum	
3.4.5	Concentrations of Amino acids in M.indica fruit as affected by injection with p.citrinum, T.terricola, F.oxysporum	
3.4.6	Concentrations of Amino acids in fruit , T.bellerica as affected by injections with p.citrinum, T.terricola, F.oxysporum	
3.4.7	Concentrations of Amino acids in fruit , T.indica as affected by injections with p.citrinum, T.terricola, F.oxysporum	
3.4.8	Showing Percentage incidence of different fungi on NTFPs	
3.4.9	Physico- chemical properties of madhuca indica oil	

3.5.1	Percentage occurrence of fungi and seed germination at different temperature and seeds stored in glass container, paper bags and poly bags (Blotter method) after 6 months of incubation	
3.5.2	Percentage occurrence of fungi and seed germination at different temperature and seeds stored in glass container, paper bags and poly bags (Blotter method) after 12 months of incubation.	
3.5.3	Percentage occurrence of fungi and seed germination at different temperature and seeds stored in glass container, paper bags and poly bags (Blotter method) after 18 months of incubation.	
3.5.4	Percentage occurrence of fungi and seed germination at different temperature and seeds stored in glass container, paper bags and poly bags (Blotter method) after 24 months of incubation.	
3.5.5	Percentage occurrence of fungi and seed germination at different temperature and seeds stored in glass container, paper bags and poly bags (Blotter method) after 24 months of incubation	
3.5.6	Percentage occurrence of fungi and seed germination (*Tamarindus*) at different temperature and stored in glass container, paper bags and poly bags (Blotter method) after 12 months of incubation.	
3.5.7	Percentage occurrence of fungi and seed germination (*Tamarindus*) at different temperature and stored in glass container, paper bags and poly bags (Blotter method) after 18 months of incubation.	
3.5.8	Percentage occurrence of fungi and seed germination (*Tamarindus*) at different temperature and stored in glass container, paper bags and poly bags (Blotter method) after 24 months of incubation.	

3.5.9	Effect of neem oil	
3.5.10	Effect of Nacl on Amla fruit	
3.5.11	Effect of grountnut oil and castor oil on Percentage occurrence of fungi on madhuca seed, Amla seed.	

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ABBREVIATIONS

µg/1	Microgram/litre
ºC	Degree in Celsius
h	hour
g	Grams
mg	Milligram
ml	Millilitre
mm	Millimeter
PDA	Potato Dextrose Agar (medium)
pH	Potentials of Hydrogen Ion
wt	Weight
WLS	Wild Life Sanctuary
ppm	Parts per million
km	Kilometer
TTC	Triphenyl Tetrazolium Chloride
NWFP	Non- Wood Forest Produce

MATERIALS & METHODS

Survey of markets and Forest areas

A large number of microorganisms may be associated with post harvest phase of different MFPs. To access the percentage occurrence of fungal infection, a survey was conducted in forests and markets of Ratlam, (M.P.), Ratanmahal, Godhra, Halol, (Panchmahal), Varnama, Jaspur, Kadipani, and Chhota-Udepur (Vadodara) and samples were collected and brought to the laboratory assayed for the occurrence of deteriorating fungi percentage losses were also calculated and details are recorded in tables .

Testing of Seed Viablity

Before the seeds of different forest trees were kept for isolation of the seed mycoflora they were tested for the viability. For seed viability test, four replicates of 100 seeds each were fully immersed in distilled water for 18 h to start activity of dehydrogenase enzyme and for the facilitation of the penetration of tetrazolium solution (0.5% of TTC was used) for three hours at room temperature i.e. 25 ± 2 ºC in dark. After three hours the solution was decanted the seeds were then rinsed with water and evaluated. Individually the seeds were assessed for their viability. On the basis of staining of embryo, staining of cotyledon, as well as assessment of necrosis, and the color intensity was also done. The percentage viability of four different variety of seeds was calculated and Results are recorded in Table 3.4.5. The method suggested (Khare and Bhale 2000) is used.

Collection of Healthy and Diseased fruits

Samples of diseased and healthy fruits of *M. indica, T. bellerica, E. officinalis, T. indica* were collected from various fruit and vegetable shops and store houses in the

pre-sterilized polyethylene bags and were then brought to the laboratory for isolation of the rot producing fungi.

Isolation and purification of fungi from Healthy and Diseased fruits

Isolation of the pathogens causing post-harvest decay of fruits of four forest produce was made within 24 hours of their collection. Small pieces of the diseased tissue were aseptically removed from the middle and margin of the lesions, these was then surface sterilized with 0.01% mercuric chloride solution, washed in sterile distilled water (SDW) and cut into small pieces. These pieces were placed on potato dextrose agar (PDA) medium supplemented with streptomycin sulphate (0.06g/1) as a bacteriostat. The inoculated petridishes were incubated petridishes was incubated at 25 ± 2ºC until proper growth of the pathogens was recorded at regular intervals. All the fruit rot fungi, thus isolated were purified by streaking or mass inoculation technique and axenic cultures were obtained.

Pathogenicity test (Confirmation of Koch's Postulates)

Tests were conducted to confirm the pathogenic ability of the isolated rot inducing fungi. For these tests, fresh fruits of similar size and approximately same maturity were obtained from the market. These fruits were washed with running tap water, surface sterilized with 70% alcohol and then inoculated with the isolated fruit rot fungus. Inoculation was done by pinprick method (Tomkins & Trout,1931).The inoculum used in each case was 5 days old pure culture grown on PDA at 28 ± 2ºC were placed in 100 percent relative humidity. Pathogenicity of the organism was considered established only when Koch's postulates were fully satisfied.

Identification and maintenance of fruit rot pathogens:

Identification of the fruit rot causing fungal species was done by studying their macro – and micro morphological characters. Relevant literature and appropriate keys were used for their identification (Raper and Fennel, 1965 ; Tandon, 1968; Rifai, 1969 ; Booth, 1971; Ellis, 1971; Sutton, 1980). Identity of some of the fungal isolates was also confirmed from Agarkar Reseach Institute, Pune and by Dr. P.N. Choudhary, New Delhi. Preservation and maintenance of identified isolates was done as recommended by the International Mycological Institute, U.K. (Smith & Onions, 1983).

Blotter Method

In this method the Petri dish was lined with 2 layers of filter paper and then moistened with distilled water, the seeds were then placed and the dishes are kept at 25 ± 2^{o} C temperature and the germination of seeds was recorded on 4^{th} and 7^{th} day. Altogether 400 seeds are used for this experiment (ISTA). If any fungal mycelium was seen with seeds, it was sub cultured under sterile conditions.

Agar Plate Method

In this method 2 different types of media were used which were semi- synthetic media and the synthetic media. In the semi- synthetic medium potato was used along with the dextrose and agar and this medium was known as Potato Dextrose agar medium. 200 g of potato were peeled and sliced into small pieces. It was then boiled and autoclaved for 40 min in 500ml of distilled water and filtered through a cheese cloth. Twenty grams of dextrose (D-glucose) was added and the total volume was raised to 1000ml. Agar (2%) was used as a solidifying agent.

In agar plate method, potato dextrose agar medium and Asthana and Hawkers medium were prepared. All the contents were mixed and autoclaved and then poured in pre autoclaved petriplates in the laminar flow and the medium was allowed to solidify. After that seeds were kept in the petriplates in which there were two different sets of which were treated and untreated seeds. Seeds were treated with NaOCl 0.5% for 1 min and repeated washing with sterile distilled water and then placed on the medium; the petriplates were then kept in the incubator at 25 ± 2^{0}C and the data was recorded for occurrence of fungi after eight day or earlier. If any fungi were found growing on the seeds it was scrapped with the help of an inoculating needle and transferred on the PDA petriplates for further multiplication. After four to five days again it was transferred to culture tubes and the process was repeated. Purified by single spore and single hyphal tip methods and maintained under laboratory conditions (Riker and Riker 1936). The percentage incidence of dominant mycoflora was calculated by the following formula (Arya and Mathew, 1991):

$$\text{Percentage incidence} = \frac{\text{Number of seeds bearing fungal colonies}}{\text{Total number of seeds examined}} \times 100$$

Staining Technique

Some of the fungal mycelium was placed on a slide and stained with cotton blue. Excess stain was removed by lactophenol and a cover-slip was placed on the slide and observed under the microscope.

Isolation of fungal organisms

Isolation of fungi was done in 4 different media. These included Asthana and Hawker's medium modified Asthana and Hawker's medium, Czapek's medium, Potato Dextrose Agar medium.

Two different types of media were selected for this purpose.

1. Semi- synthetic medium.

2, Synthetic media.

Semi- synthetic medium (Potato Dextrose Agar medium)

200 g of potatoes were peeled and sliced into small pieces. It was boiled for 40 min in 500 ml of distilled water and then filtered through a cloth. 20 g of dextrose (D glucose) and 20 g agar was mixed by boiling the medium. Total volume was raised to 1000 ml.

Synthetic media

Asthana & Hawker's medium 'A'

D – Glucose : 5 g

KNO_3 : 3.5 g

KH_2PO_4 : 1.75 g

$MgSO_4.7H_2O$: 0.75 g

Distilled water : 1000 ml

Agar : 20 g

Modified Asthana and Hawker's medium 'A'

D – Glucose : 10 g

KNO_3 : 3.5 g

KH_2PO_4 : 1.75 g

$MgSO_4.7H_2O$: 0.75 g

Distilled water : 1000 ml

Agar agar : 20 g

Czapek's Medium

Sucrose : 30 g

$NaNO_3$: 2 g

KH_2PO_4 : 1 g

KCl : 0.5 g

$MgSO_4.7H_2O$: 0.5 g

$FeSO_4.7H_2O$: 0.01 g

Distilled water : 1000 ml

Isolation was done in different media as described earlier to access the maximum number of fungi present on forest produce. Agar plate method was used having different media.

Field Experiment (Germination test)

To access the germination an experiment was set up in which 50 seeds each of four plants were taken and sown in 10 pots. The experiment was set up in Botanical garden of M.S. University of Baroda. Seeds of *M. indica, T. bellerica, E. officinalis and T. indica* were taken and in each pot 5 seeds were sown. After seven days the germination percentage was observed. Seeds which were not germinated were collected and analyzed in the lab. Isolation of the fungi was done from those seeds which failed to germinate.

Changes in Biochemical contents after pathogenesis

The quality of oil expressed from seeds depends largely on the conditions under which they have been stored. Even under the best conditions of storage, the concentrations of free fatty acids increases. Mahua oil tends to get rancid during storage, due to the presence of oxidisable constituents in the unsaponifiable fraction.

Fresh oil of mahua is yellow in color without an unpleasant taste. Commercial oils is generally greenish in color with an offensive odor and disagreeable taste. Seeds of *M. indica* were collected from Kavat (Gujarat) and brought to the laboratory for isolation of fungi. The seed mycoflora was studied using agar plate method with PDA as routine medium recommended by ISTA (1976). Seeds were surface sterilized with 0.5 percent NaOCl for 1min and repeated washing with sterile water. Observation was made after 4 days of incubation. The fungi were isolated on agar slants. The percentage incidence of dominant mycoflora was calculated.

M. indica seeds from which oil are subsequently derived grows naturally over a wide range of geographical regions and under a variety of physical and climatic conditions. The kernel of seed contains about 50% oil. The oil yield in an expeller is nearly 34% - 37% (Bhatt *et al*. 2004) which can be extracted by variety of processes or combination of processes, such as hydration processes, continuous screw presses and solvent extraction. However, the most satisfactory approach is hot pressing using a hydraulic press, followed by solvent extraction.

The *M. indica* seeds were processed as follows for extraction. The unit of operations involved is;

Clearing; The *Madhuca* seeds had some foreign materials and dirt which is separated by hand picking.

Drying; the cleaned *M.indica* seeds were sun dried in the open until the threshing is done and fruit sheds the seeds. The seeds were further dried in the oven at 60º C for 7 h to a constant weight in order to reduce its moisture content, which was initially at about 5 to 12%.

Winnowing; The separation of the shell from the nibs (cotyledon) was carried out using ray to blow away the cover in order to achieve very high yield.

Grinding (size reduction); Mortar and pestle were used to crush the seeds into a paste (cake) in order to weaven or rupture the cell walls to release *Madhuca* fat for extraction.

Oil analysis

The seeds were stored in dark glass bottles at room temperature and were inoculated with 5 test fungi. Extraction was done after 15, 30, 60, 90, days respectively.

Operation of Soxhlet Extraction

Seeds were Soxhlet extracted using normal Hexane. The soxhlet was heated at 40-60 ^{o}C and analyzed by standard method for oil and fat analysis (Ramachandra *et al.* 2008) .When the solvent was boiling , the vapour rises through the vertical tube into the condenser at the top. The liquid condenser drips into the filter paper thimble in the centre, which contains the solid sample to be extracted. The extract seeps through the pores of the thimble and fills the siphon tube, where it flows back down into the round bottom flask. This was allowed to continue for 30 min again to determine the amount of oil extracted.

The physico-chemical properties of seeds oil were analyzed for specific gravity, acid value and pH of oil by using ASTM (American Standard Testing Method).

Determination of Specific Gravity

Density bottle was used to determine the density of the oil. A clean and dry bottle of 10ml capacity was weighed (Wo) and then filled with the oil, stopper inserted and then filled with the oil, stopper inserted and reweighed to give (W1) .The oil was substituted with water after washing and drying the bottle and weighed to give (W2). The expression for specific gravity (Sp.gr) is Sp.gr = W1-Wo / W2-Wo = Mass of the substance / Mass of an equal volume of water.

Determination of Acid Value

25 ml of diethyl ether and 25ml of ethanol was mixed in a 250ml beaker. The resulting mixture was added to 10 g of oil in a 250 ml conical flask and few drops of phenolphthalein were added to the mixture. The mixture was titrated with 0.1 M NaOH to the end point with consistent shaking for which a dark pink colour was observed and the volume 0f 0.1M NaOH (Vo) was noted. Free Fatty Acid (FFA) was calculated as : Vo/Wo2.82.100, where 100ml of 0.1M NaOH = 2.83g of oleic acid, Wo = sample weight: then acid value = FFA.2.

Determination of pH

Two g of the sample was poured into a clean dry 25ml beaker and 13 ml of hot distilled water was added to the sample in the beaker and stirred slowly. It was then cooled in a cold – water bath to a temperature of 25ºC. The pH electrode was standardized with buffer solution and the electrode immersed into the sample and the pH value was recorded.

Percentage loss of the products by fungi

Different fruits (diseased and healthy) and other produce were collected from different markets of Vadodara. The Purchasing rate and selling price were recorded and loss was calculated. Isolation was done from healthy and diseased fruits. Percentage loss of these products by fungi was estimated.

Changes in different Amino acids Sugars due to fungal invasion:

The physiological conditions of the plant as influenced by its age nutritional conditions, environmental factors etc. play a great role in the establishment and spread of the diseases. The successful infection of a host by the pathogen may result in

balanced parasitism on the one end and restricted infection or death of the host tissue on the other end. In case of obligate parasitism both the pathogen and host establish a cordial relationship so that either of them is not killed in process.

An analysis of changes in such biochemical (amino acids and sugars) contents of the host tissues was done in order to assess the damage caused by three species *Penicillium citrinum, Fusarium oxysporum, Theilavia terricola,* under study.

For the study of the utilization of different sugars by chromatographic technique was studied. The mixture was spotted at about 2 cm height from the base and the solvent migrates up against the gravitational pull. The samples were spotted using capillaries or micropipettes. Special capillary tubes (glass tubes drawn to a capillary with a narrowed tip) were used. The spot should not exceed 0.5cm in diameter. The quantity of various sugars was used in experiment drops of known volume (0.5ml) were taken from the extract every five days 5, 10, 15, days and were placed on the chromatograms by a micropipette at a position located for this purpose. The running solvent was n-butanol-acetic acid- water (4: 1: 5 : v/v) . A mixture of 5 vol of 4% aniline was used as spraying reagent for the detection of sugars. Chromatograms were developed after drying at room temperature by heating in an electric oven at 100ºC for 90 seconds. The background color of the developed chromatograms was reduced by dipping them 6% hypo ($Na_2S2_2O_3.5H_2O$) solution. The method had the advantage that the bands developed were prominent, sharp and brown. The position of a spot in a chromatogram was recorded as its Rf value. The Rf values were calculated by the following formula:

$$Rf = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent}}$$

Utilization of amino acids was also studied chromatographically The technique was same used in utilization of sugars, except that the chromatograms were sprayed with 0.1% ninhydrin in n-butanol (w/v) and after drying at room temperature the bands were developed at 80ºC for 20 min. To detect free and bound amino acids, 7 days old each three species (*Penicillium citrinum, Thielavia terricola, Fusarium oxysporum*) was taken. Three fungi were inoculated in fruits of *M. indica, T. indica, T. bellerica,* for 5, 10, 15, days respectively. The material was thoroughly crushed in a ground glass homogenizer with 25 ml of 80% ethanol. The crushed substance was boiled over an electric water bath for half an hour and subsequently filtered in an evaporating dish. The process was repeated twice in order to ensure complete extraction of the amino acids. The alcoholic extract, thus obtained, contained the soluble amino acids and amides. The extract was evaporated to dryness and the residue was dissolved in 1 ml of 20% ethanol so as to adjust 100 mg fungal mat / ml vol. The whole residue was transferred to a 15 ml centrifuge tube by means of a policeman and centrifuged at 2000 rpm for half an hour. The clear supernatant liquid was decanted and was used for the chromatographic analysis of free amino acids.

To release the amino acids from the proteins, the alcohol extracted residue on the filter paper and the colloidal protein from the centrifuge tube were combined and hydrolyzed with the help of 6N HCl in an autoclave at 15 lbs pressure for 30 min. A pinch of stannous chloride was added to avoid humin formation by destruction of amino acids in the presence of carbohydrates. The hydrolyzed residue was filtered through a Buchner funnel, using Whatman filter paper No 1 and hydrolysate obtained was so adjusted as to contain 100mg fungal mat per ml. After centrifugation, the supernatant was used for chromatographic analysis of protein bound amino acids.

Two dimensional ascending paper chromatographic technique as described by (Consdon *et al.* 1994) was used for detection of amino acids. Solvents used were, Phenol:ammonia : water (80: 3: 20 v/v) in the first direction and n-butanol : acetic acid: water (4: 1: 5 v/v) in the second direction (right angle to the first). The chromatograms were sprayed and developed as suggested earlier.

Detection of Aflatoxin by Qualitative Method

Fungal contamination of stored products has been one of the major problems of mankind. Aflatoxins have been found associated with various diseases, like aflatoxicoses in live-stock, human and domestic animals. The Food and Agricultural Organization of United Nations has set a tolerance level of 30 ppb for mycotoxins in foods. Aflatoxins are some structurally related toxic compounds produced primarily by some strains of *Aspergillus flavus*, *A. flavus* strains produce numerous large sclerotia on Czapek's agar medium & also form all the aflatoxin (Hesselhine *et al*., 1968). Fungus *Aspergillus* belongs to family Eurotiaceae Colonies of *A. flavus* on Czapek' s agar medium spreading rapidly with floccosity limited to scanty growth of sterile hyphae in older and dryer areas among crowded conidiophores.

Preparation of TLC Plate

Silica gel G was taken in conical flask by weighing it in gram equal to the width of the glass plate in inches. Glass plate was cleaned with acetone and fixed with Johonson plaster for required thickness of plate. Slurry is prepared by adding two parts of by weight of H_2o to one part of dry silica gel G and was shaken vigorously. This slurry was immediately coated on the glass plate. The plates were allowed to dry and then activated at 60º C for overnight.

Thin layer chromatography was the first analytical method used for aflatoxin detection and is still a very important, simple, versatile and economic analytical tool. It can quantify aflatoxin levels as low as 1 mg/g. Hundred g of healthy plant material was taken and *A. flavus* was inoculated aseptically. The Polythene bags were tied properly and were kept for 21 days. After 21 days the plant material was dried and powdered. From this 40g of finely ground homogenized plant material was extracted in 200 ml of chloroform for 4 hours. The filtrate was collected and concentrated. The concentrate was spotted on TLC. The solvent system used was Toluene: Iso-amyl alcohol: methanol (90:32:3). After the TLC plate had run, it was dried and observed under uv light (240- 280nm) The Rf of the band was taken. For spectra, the silica was scrapped off from four replicates of TLC plates and mixed with methanol, heated and filtered. Spectra was taken between 240-400nm.

Confirmative Test for Aflatoxin

To confirm that florescent band developed aflatoxin, the plates were sprayed with 25% sulphuric acid. The blue fluorescent band changed to yellow fluorescent band, thus indicating the presence of aflatoxin.

Production of Aflatoxin *in vitro*

Fifty ml of Czapek Dox's medium was taken in 5 different flasks (250 ml) and *A. flavus* was inoculated in this medium. The flasks were incubated at 28 ± 1°C in an incubator for 21 days. After 21 days the culture filtrate was obtained by filtering through Whatman's filter paper No.1 Culture filtrate was used for isolation of aflatoxin. 50 ml culture filtrate and 20 ml of chloroform were taken in a separating funnel and shaken. Then it was allowed to settle. The lower chloroform layer was taken for extraction of aflatoxin. This chloroform layer was distilled to dryness in a

water bath. One ml of chloroform was added to the concentrate and spotted on TLC. Solvent system used was Toluene: Isoamyl alcohol : methanol (90: 32 : 3) The TLC was observed under UV (240-280 nm) for the florescent band.

Control Studies

Poisoned food technique (Nene and Thapliyal, 1979) was used to test the efficacy of different oils. Neem oil was used in three different concentration, 1%, 5%,10%. The results are presented in Table - 3.6.1. Fig. 9. The oil was mixed in PDA and colony diameter was recorded. The effect of Groundnut, neem and castor was recorded for 7 different fungi. Results are recorded in Table 3.6.2.

OBJECTIVES

MAIN OBJECTIVES OF THE Ph.D. PROGRAM

Include study of fungal deterioration of flowers, fruits, and seeds of Mohwa, fruits of Bahera, Aonla, and Tamarind during post harvest phase and suggest possible storage and control methods.

1. Survey of forest, store houses, and markets in different parts of Gujarat will be undertaken.

2. Fungi will be isolated from the seeds, fruits and flowers of Mohwa (*Madhuca indica*) fruit of Bahera (*Terminalia belerica*), Aonla (*Emblica officinalis*) and Tamarind (*Tamarindus indica*).

3. Percentage loss of these products by fungi will be estimated.

4. Purification of the fungal cultures will be done. Identification of fungi will be made.

5. Changes in different biochemical compounds will be observed due to fungal invasion in fruits of Mohwa, *Terminalia*, *Tamarindus* and Aonla.

6. Production of Aflatoxins will be observed *in vitro.*

7. Efforts will be made to find out proper storage conditions for these produce and control measures will be suggested against several fungal deteriogens.

INTRODUCTION

Dependence of human beings on plants for their existence dates to the beginning of the human race. In the early days man had only limited needs like, food, shelter, and clothing but with the advancement of civilization his requirements also grew. Non Wood Forest Produce (NWFP) extracted from Indian forests, play an important role in rural and forest or economics is immense (Shahabuddin and Prasad 2004). In India, there are about 15,000 plant species out of which nearly 3000 species (20%) yield NWFPs, however, only about 126 species (0.8%) have been commercially used (Murthy *et al.* 2005).

Importance of NWFPs

Non-wood forest products (NWFPs) have been defined as "all goods of biological origin other than wood in all its forms, as well as services derived from forest or any land under similar use." In many parts of the world, these products still play an important biological and social role in local food systems. They can contribute substantially to nutrition, either as part of the family diet or as a means to achieve household food security. They can also improve health through the prevention and treatment of diseases. Poor households residing in and around forest areas particularly landless people, women and children depend to a greater or lesser extent on the exploitation of common property forest resources in their everyday life or in periods of crisis.

“In the past, policy makers, forest economists, and foresters have viewed forests primarily as a source of national revenue with timber as the dominant product” (Tewari 1994). However, in an era of fast-declining old grown forest products besides timber, that is non-timber forest products (NWFP) are commercially exploited Such forest products range from exudates (gums, resins and latex) to canes, fruits, flowers,

seed derivatives, entire plants, leaves, root, or stem bark. Additionally, unlike timber that brings profits to state treasuries, economic benefits provided by NTFP are largely at the local level (Lele 1994; Panayotou and Ashton 1992). The potential role of NTFP in forest conservation was further supported by several studies that demonstrated that long term financial return from sustainable NTFP harvest could far outweigh the net economic benefits of timber production or conversion of the same area of land to agricultural fields(Chopra 1997; Nepstad and Schwartzman 1992; Peters *et al*. 1989; Pinedo- Vasquez and Zazin, 1992).

Forest provide numerous products to human society for its sustenance. Besides timber and wood, a variety of products, food, spices, medicines, essential oils, gums and resins, tans and fibers, flosses, honey, lac, silk, minerals and so on emanate from forests. These variety of products termed as Non-Wood or Non –timber Forest Products (NWFP/NTFP) are not only the main supplementary source of income for the rural communities and forest dwellers including tribals, but are also the main revenue earner for the developing countries. World wide interests in NTFPs have launched significant advances in forests based enterprises development.

The utility and beauty of growing forest trees is beautifully expressed in the following Gond Folk Song

"Plant the mango, plant the tamarind and the plantain:
Clusters of fruit will weigh their boughs.
Plant ten kachner trees for flowers.
In a garden set the tulsi.
Water them unweariedly,
but they will always wither.
But the trees in the forest which depend on God alone,
Never wither and die.
The forest tree grows always."

(http://edugreen.teri.res.in/misc/poem/poem)

Massive forest fire caused unprecedented forest destruction in Brazil in 1987, which caught the imagination of world public opinion (Kaimowitz, 2003). The world bank's

1991 forest policy, issued on the eve of Rio "Earth Summit" in Rio de Janeiro, 1992 declared an end to tropical deforestation as its central goal. The catch phrase of Rio was "sustainable development". Most of the 240 million people in forested regions in developing countries are poor and depend heavily on forest trees (World Bank, 2003). 'Sustainable development' is a key concept in discoveries of the global environment. Plant based fungicides offer a new possibility for safer alternative of future disease control programme. A plant product which has no mammalian toxicity and whose fungitoxic property is broad spectrum and persists for a longer time can be exploited for fungal control in vivo (Muliya and Arya, 2007).

Extensive researches in these sectors can create and expand the opportunities for combining income generation with biodiversity conservation and developmental strategies. Besides, most of such enterprises are a small scale and this would create very little environmental hazards as almost no toxicant is liberated in the atmosphere. The forest food plants offer best option to supplement nutritious food. The potential of NTFP items such as chironji, (*Buchanania lanzan*) mahua flower (*Madhuca* species) aonla (*Emblica officinalis*), honey, safedmusli (*Chlorophytum* species) and several other edible items is immense, particularly during droughts or famines (Tiwari, 1986; FAO, 1989; Dobhal, 1994). In families living below poverty line, women are able to support the household if forests are rich in biodiversity because women often manage NTFP activities more than men.

In the state of Madhya Pradesh, revenue obtained from collection and the sale of tendu leaves (*Diospyros melanoxylon*) used as wrapper to country-smoke -Beedi fetches the state government a revenue INR. 4,500 million. If revenue from other non nationalized NTFP items is also added, it may reach a mark of INR. 10,000 million. The total forest revenue from timber is less than INR.5,000 Million. In some state

governments, NTFPs are diverted to industries for maximizing revenues and in some states, it is being supplied to industries on long-term agreement basis. In a village called Soliya in South Gujarat, villagers collect NTFP worth INR.1.2 million per year. This is the only cash income to villagers (Tewari, 1994). Chopra (1994) reported average annual income of a household of Rs.45,065.29 from collection and sale of 9 items of NTFP in Raipur district of Chhatisgarh (based on average of 50 households). Other estimates suggest that some 35% of the income of the tribal households in India is obtained from collection of unprocessed NTFP. Also, since NTFPs involves a large variety of products available on year-round basis, returns are more frequent and relatively continuous, when compared to long gestation timber crops. Local processing of NTFP can increase rural employment opportunities. Small-scale forest based enterprises, many of them based on NTFP provide up to 50% of income for 20-30% of the rural labour force in India. There are 3000 to 5000 plant species yielding various useful products. However, the information about them is very scanty. As a first step, it is desirable to have inventory of the available NTFP resource in all 16-forest types of India. It may appear to be a difficult task, but a beginning has to be made by inventorying the NTFP rich sites and hot spots. Local communities have been traditionally collecting and using a number of NTFPs from their surrounding forests. We may take their help in enumerating the available resource. Participatory forest resource assessment (PFRA) is an important tool in increasing people's awareness of the benefits of conserving biodiversity. The economic valuation of NTFP is difficult for several reasons. Community collectors in villages adjoining the forests gather NTFPs. These are sold in raw form to small traders and middlemen who usually visit the villagers and buy the produce from the collectors. The collectors sometimes carry the produce to the nearby places for sale and barter with the other

household goods such as rice, kerosene, salt, cloth, etc. The middlemen at the village level sell the produce to larger intermediaries who in turn supply the raw material requirements of the manufacturers and large industries. The final links in the marketing chain are consumers in bigger towns, and cities. NTFP markets are by and large imperfect, and the collectors do not get a fair return for their labor. Middlemen have a dominant position in the marketing of NTFPs produce at the village level: The present marketing system can be altered through simple interventions that promise to substantially increase the returns to the people. Organizing community groups in to societies and facilitating the value addition and marketing of NTFPs through these can eliminate several links in the marketing chain. Studies have indicated that value additions at primary collector's level could significantly enhance the return from the sale of the same amount of NTFP. Better incomes -from NTFP collection, processing and marketing hold the key to the economic development of forest dependent populations, particularly the women and children (Prasad, 1999).The simplest value-adding step is the grading of the produce. Equally important is storage capacity. Many NTFPs deteriorate fast in the absence of proper good storage facilities, and the bargaining power of the collectors can be increased and their returns can be further improved if proper storage facilities are provided. Arranging such facilities is an important issue for management at the village level. NTFP items now provide substantial revenue to the state, in many cases more than timber revenue. Collection of non-timber forest produce particularly in central India contributes to the cause of wild fire to a large extent. Significant among them are collection of beedi leaves (*Diospyros melanoxylon*) and the mahua (*Madhuca indica*) flower. Collectors of tendu leaves cause deliberate fire in summer months to promote better flush softer and tenderer leaves. Mahua pickers burn the dry leaves under mahua trees to get a clean

patch of floor to facilitate collection of its edible flowers which is of considerable value tothe poor villagers and tribals particularly in the years of short ages. The mahua trees are generally found scattered in the tropical dry deciduous forests in many parts of northern and central India, and though the intention of the people lighting such fires is only to have a small patch of clean floor under the mahua trees, they, however, do not extinguish the fires which escape in to the forest generally by negligence. Since collection of mahua flowers is done in summer months, the hot dry weather aggravates the situation and it results into large fires. NWFP has emerged as a potentially significant source of income of the tribal people and forest dwellers. Table 1.1 shows the production of Tendu leaves in past 12 years in Chattisgarh (having highest collection).

Table 1.1: Showing Collection and Sale of Tendu leaves in last twelve years from Chattisgarh

Year	Collected Quantity (Standard bags)	Collection Wages (Rs Crores)	Sale Value (Rs Crores)
2001	16.67	75.53	165.22
2002	19.58	88.92	198.71
2003	18.12	82.18	173.25
2004	18.86	84.92	148.50
2005	14.92	67.17	135.06
2006	14.72	66.31	140.02
2007	17.18	85.96	325.59
2008	13.79	82.77	197.61
2009	14.67	95.33	256.41
2010	15.45	108.15	335.30
2011	13.57	108.52	355.31
2012	17.15	188.66	646.90

Source**:** www.cgmfpfed.org

NWFP are used by rural communities for food, fodder, fibre, bio-fuels and medicine. They make the industrial raw material for processing, marketing and export. We have to optimize the production of NWFP with adequate investments in regeneration/plantation, collection, processing and marketing. The Eleventh Finance Commission has also supported this cause through grant-in-aid for forest maintenance and increasing the production of timber and NWFP. In the 73rd amendment of the Constitution, NWFP has been allocated to the PRIs. Sustainable management of these resources, with the development of value addition chains will definitely help to

improve the income of the PRIs as well as that of primary collectors and processors. Where government alone does marketing, it is inefficient; and where it is left to private trade, it may be worse and equally exploitative (Saxena, 1999). In many cases, forest development and tribal development corporations as well as other such state undertaking had entered into the collection and trade of NTFP with an objective to eliminate middlemen and help poor gatherers to get remunerative price. However, in practice, the middlemen were not eliminated but instead replaced by agents appointed by government who were as exploitative as the earlier middlemen. With state monopolies, expanding to cover a large number of NTFPs the problem of gatherers in respect of their access to resources, freedom to sell to buyer who pays more have been greatly restricted. In order to maximize their margins, government agencies buy only better quality NTFP, thus reducing official collection and also reducing the earning of gatherers. In Madhya Pradesh for example, collection of Tendu leaves averaged over the period 1989-96 was 43% less than the period 1981-88 (Prasad, 1999). In India NWFP is mostly collected by the inhabitants of economically backward hilly tracts (Vidyarthy and Gupta 2002). These are mostly household based and low volumes are involved. These are labor intensive and use simple technologies which are accessible particularly to the women and children (Campbell *et al*., 1995). Godoy *et al* (1995) have done an analysis of the effects of income levels on the kinds and quantities of non-wood forest products extraction by various forest communities. Ravishanker (1989) found that the tribal people have been conserving plant and crop genetic resources as well as the knowledge on their utility without expecting any return. The presence of abundant forest resources has made the forests a major source of livelihood (tribals have the right to collect FPs from government forest). They collect a variety of FPs from their own or forest lands, to sustain their living, notable among

which are saal-seeds, 'chirongi', tendu-leaf, harra, mahua- flower/seed, mango kernels, bamboo, gums, etc. They do not pursue other economic activities like poultry (except for domestic consumption) or dairying. They almost never go to a 'mandi' to sell goods, small quantities of FPs and the distance to the mandis being the disincentives. Sometimes, they walk long distances to reach even a 'haat'(Ganguly and Chaudhary 2003). In Baster District, 75% of the people depend upon forests, they supplement their food with tubers, flowers and fruit all year round (Solanki, 1984). NTFPs collected by the forest villagers mainly for commercial gain, while *Madhuca latifolia* (mahua) is used both for commercial as well as domestic uses (Giri *et al*. 2005). There is an increase in demand for NWFP as raw material for a number of products such as cosmetics, medicine and seed for oil extraction. Cutting of trees is banned from forests and wild life sanctuaries but the forest department is trying to get revenue from collection and sale of NWFPs and medicinal and aromatic plants (MAPs).

Handling of Minor Forest Produce in Gujarat

To procure more profits Minor Forest Produce are handled by the Corporation. This had substantial bearing on tribal economy. The state provided due legitimacy and support to it by promulgating the Gujarat MFP trade Nationalization Act, 1979. The Article 19 (6) (II), Fundamental Rights and Article 46 Directive principles of state policy, constitution of India provide for this. Through this Act, the trade of four MFPs viz., The Timru leaf, Mohwa flower Doli, and Gums was nationalized in Gujarat. In the wake of 73rd constitutional amendment to the Gujarat Panchayats Act in 1998, corporation now trades in MFP from scheduled areas on behalf of Gram Panchayat /Sabhas on no profit no loss principle. The business of Minor Forest Produce i.e. Timru leaves, Mohwa flowers, Doli and all types of gums have been nationalized by the state Government by passing the Gujarat Minor Forest Produce Trade

Nationalization Act ,1979. Gujarat state Forest Development Corporation Ltd. has been appointed as the sole agent of Government to purchase, sell and transport these M.F.Ps in the state. Collection rates have been increased progressively by taking into consideration prevailing market rates, demand and supply of M.F.Ps collection rates of 13 Nationalized M.F.Ps. are fixed by the government on recommendation by the Advisory committee constituted under Gujarat Minor Forest Produce Trade Nationalization Act, 1979 and published in government Gazette during December for each calendar year through Notifications and collection rates of Non- nationalized M.F.Ps are fixed by the Managing Director on recommendation of the Technical committee of the corporation. M.F.Ps are also collected rates and commission charges to the primarily collectors through Direct purchase centres.

Gujarat has established the Gujarat state Forest Development Corporation (GSFDC), which procures non wood forest products. GSFDC is also looking after collection and sale of NWFPs in Gujarat. Its main objective was to organize the NWFP trade and stop the exploitation of tribals. The Corporation trains tribals in improved methods of collection and processing. The corporation has its retail counters at at Vadodara, Baria, Rajpipla, Godhra and Rajkot. Value addition steps in Mohwa include grading of flowers and proper drying. Aonla powder either individually or mixed with Harde and Behada (Trifla mixture) can also add value to the produce (Mohapatra and Sinha 1997). With the prevalent poverty in these backward areas of Jharkhand, various NWFPs provide very useful food components as well as a source of income to the villagers, though these were never quantified earlier.

Dhanvantari

Dhanvantri project was set up in the year 1991- 92 with an investment of Rs. 8 Lacs which includes cost of building, machinery and various ancillary items. The project

has capacity to manufacture various powders, Tablets manufacturing of said items is done in three processes. Firstly the raw materials are cleaned and graded and then they are sent for powdering, Pulverizing and mixing then finally they are packed in air tight container of 100, 200 and 500 g. Various MFPs like Amla, Harde, Behada , Mushli, and various types of Gums like Babul, Guggal etc are also graded and packed in 100, 200, and 500g in polythene bags. Table 1.2 shows various commercial formulations marketed by Dhanvantri and their retail price. Dhanvantri unit is a leading supplier of Honey to various dairies viz, Sugam dairy (Vadodara) Vasudhara dairy (Chikhali Dist. Navsari), Banas Dairy (Palanpur) for ice-cream manufacturing.

It has been noted that states such as Orissa have classified forest produce as minor forest (MFP) and NTFP with latter being larger group. The recently enacted Forest Rights Act, 2006 has defined " Minor forest produce" to include all non- timber forest produce of plant origin including bamboo, brush, wood, sumps, cane, tussar, cocoons, honey, wax, lac, tendu, or Kendu leaves, medicinal plants and herbs, roots tubers. As a result the distinction between MFP and NTFP may no longer be relevant. A large number of rural own-account workers are dependent on the forest base activities for survival; many are from forest-dependent communities living in and around the forest. A large number of NTFP workers are women, who supplement their agricultural activity with collection of NTFPs. NTFPs are collected for direct selling orfor use in handicraft and cottage enterprises such as bidi rolling, matches, silkworm rearing etc. Today in India, at the national level, over 50 percent of forest revenue and about 70 percent of forest export revenue comes from NTFPs, mostly from unprocessed and raw forms (Tiwari and Campbell 1997). Chopra 1997 estimates the average value of NTFP per hectare for India to be1671.54 rupees. Non-timber forest products are estimated to generate 70 per cent of all employment in the Indian

forestry sector (Udyogini. 2004). "NTFP collection generates over 2 million person years of work annually and this could possibly be increased to 4.5 million person employment years'. Other studies have highlighted the important contribution of NTFPby underlining its role in filling in cash-flow gap, and in helping to poor cope with leanperiods in subsistence agriculture.The regulatory framework which governs their purchase and trade, divides NTFPinto two groups: nationalized and non-nationalized. Nationalization effectively means that state government monopolizes the purchase and sale of the specified NTFPs. Several justifications for nationalization are given, including: better returns to the collector, sustainable extraction and revenue to the state. In most cases, the state-owned institutions such as state forest development corporations, federations, and cooperatives control trading.

Table 1.2: Showing price list of Dhanvantari Brand products and Minor Forest Produce in retail package (2011-2012).

S.No	Name of MFP	Botanical Name	Rate Inclusive of Tax (Rs)		
			100g	200g	500g
1	Amla	*Emblica officinalis*	-	-	43
2	Aritha	*Sapindus emarginata*	-	-	16
3	Behada	*Terminalia bellerica*	-	-	10
4	Harde	*Terminalia chebula*	-	-	19
5	Bavalgum	*Acacia arabica*	22	54	-
6	Salaidhoop	*Boswellia serrata*	-	36	89
7	Dhavada gum	*Anogoissus latifolia*	-	37	93
8	Kher gum	*Acacia catechu*	-	22	55
9	Gugal gum	*Commiphora wightii*	50	90	220
10	White musli	*Chlorophytum tuberosum*	207	-	-
11	Himaj	*Terminalia chebula*	-	15	37
12	Jamun seeds	*Syzygium cumini*	-	9	20

Nationalized NTFP

Tendu was the first NTFP to be brought under state control collection by MadhyaPradesh in 1964, followed by Maharashtra (1969), Andhra Pradesh (1971), Bihar (1973), Gujarat (1979) and Orissa (1981). Other NTFPs to be nationalized were sal seed, gums and myrobalan.

Non- nationalized NTFPs

The collection and trade of some NTFPs are not state controlled and ostensibly, there is space for market forces to operate in the area of price fixation. In reality, the institutional mechanisms (market system) emerge as a result of a complex interaction of a large number of factors – e.g. supply-demand, information availability, other policy and legal constraints and availability of capital and credit etc. Two examples will high light this issue. *Mahua* is collected both for self consumption and for sale. Its flowers are used to brew country liquor and its flowers and seeds which have medicinal value are collected and dried. One single mature tree can provide an annual income of Rs.1500 from its flowers and seeds. The collection period is only 15-20 days during which each person is allowed to collect a specified amount of flowers from the mahua trees. The flowers are dried over for four to six days and sold to local traders. Consumption is often limited to domestic and local markets. Distress selling and improper weighing is common. Often local traders make an advance payment and subsequently buy at abysmally low prices. Mahua is a non- nationalized product yet it has different policies governing its trade. It is not a freely tradable item due to its classification as an oxidant under the Bihar and Orissa Excise Act. Besides, several taxes are imposed in many of the states - VAT, *mandi* (market for sale of agricultural commodities), excise and sales tax. There is restriction on storage and inter state transport too, and all these taxes have acted as deterrent for community access for larger markets, within and outside the state. Medicinal plants are the other NTFP in great demand in the herbal produce trade.

For herbals villagers have a high dependency on forest resources. About 63% of their total income and 59% of their cash income comes from forest resources. (Gunatilake, *et al*., 1993)

An attempt has been made to describe the "traditional use" of forest products at the national level. An analysis of these studies suggests that four basic prepositions emerge to explain the main relationship between NTFPs and the communities that use them (Wickramasinghe *et al*., 1996).

(a) NTFPs are still a relevant part of the economy of communities living on the periphery of the forest.

(b) The collection of NTFPs allows in most cases for the maintenance of the forest cover and most of its species.

(c) The extent and type of use of the forest are heavily influenced by the distance from the forest to collectors' homes.

EU notification on guar gum: The Government of India, Department of Commerce, has decided that wide sampling of the entire chain of guar gum needs to be done to find out presence of dioxins and pentachlorophenol (PCP) in food–grade guar gum exported from India. The step has initiated to clear certain batches with contamination for exports to fetch higher values.

Other R & D Projects: The Ministry of Commerce, Government of India, has recently approved a project on R & D proposed by Shellac and Forest Based Export Promotion Council in order to develop high –yielding, quality seeds for enhancing India’s guar gum exports under the Market Access Initiative scheme (MAI). The project is set to increase exports of guar gum in a big way. A strategic objective of R & D project is to maximize arid land utilization by bringing them under guar cultivation.

Vishesh Krishi Upaj yojana: A new scheme called Vishesh Krishi Upaj Yojana announced under the new Foreign Trade Policy by the Ministry of Commerce and Industry, Government of India, provides a host of incentives to boost exports of minor forest products and their value added products.

Fungi: Microbes responsible for spoilage of NTFPs

Fungi is big heterogeneous group of organisms spread over three kingdoms: Fungi, Chromista and Protozoa . The members of first two kingdoms are much similar in their thallus construction and absorptive nutrition, in which the protozoan fungi are much different. Nevertheless, the unity in diversity is remarkable and they are inseparable by their morphology, ecology, and nutrition.

Nutrition Fungi are heterotrophic, dependent on external supply of food like animals and some bacteria. These are chemoorganotrophic, derive their energy from oxidation of organic substances. Ingestion of food is rare and found only in slime molds which in the somatic phase are represented by myxamoebae and plasmodia. The plasmodia can also absorb external nutrients. The extracellular enzymes convert the insoluble food into soluble form which is then absorbed.

Thallus: The thallus of fungi usually consists of microscopic tubular filaments, called hyphae; mass of hyphae constituting the thallus is called mycelium. The hyphae are branched and the branches ramify on or inside the substratum to form three dimensional network. Cytoplasmic streaming in fungal hyphae is unidirectional towards the tip where growth takes place. Hyphae grow entirely at their tips. The thin and plastic hyphal tip 50-100 µm in the apical region is the zone of elongation and is filled with protoplasm.

Cell Wall: Fungal cell walls contain 80-90% carbohydrates, the remainder being proteins and lipids A typical feature of fungal cell wall is the presence of chitin and

not cellulose. It does occur in the cell walls of *Oomycetes* and *Hyphochytridiomycetes*. Chitin in the wall is accompanied by glucose polymers other than cellulose, and mannans. The composition of the cell wall is variable and is modified by age and environmental factors.

Fungi reproduce both asexually and sexually. In some fungi, e.g. fungi imperfecti, reproduction is by asexual methods only. In some others, it is parthenogenetic. But in majority of fungi, reproduction occurs in two well marked phases – the asexual and sexual. Conidia are of two types, Thallospores are formed by transformation of pre-existing cells of the thallus and are detached by decay of the hypha, Conidiospores are formed as new structures on the thallus. Arthrospores are formed by close septation of the distal end of hypha in a basipetalous succession. Chlamydospores are formed by terminal or intercalary cells of the hypha and are released after its death. In *Fusarium* chlamydospores are formed on the macroconidia. Conidiospores are common in *Ascomycota* and lower fungi some genera form true conidiospores, while in some others *e.g. Albugo* and downey mildews, the sporangia in dry weather, behave like conidia and germinate directly through a germ tube, rather than forming zoospores.

Phylum *Chytridiomycota* are the smallest, simplest and most primitive fungi.The thallus may consist of a simple spherical structures which is entirely converted into a reproductive organs.

Phylum *Neocallimastigomycota* includes obligate anaerobic flagellated fungi that thrive saprobically in the rumen of herbivore mammals and some reptiles. These organisms are highly fibrolytic and produce polysaccharide degrading enzymes.

On the basis of importance of various NTFPs and their occurrence in the area surveyed, it was thought desirable to undertake detailed studies on following four plants and their produce.

1. *Madhuca indica* Gmel.
2. *Terminalia bellerica* Roxb.
3. *Emblica officinalis* Gaertn.
4. *Tamarindus indica* L.

Madhuca indica Gmel.

A genus of trees distributed from India to Australia and Polynesia and belongs to family Sapotaceae. Mahua is a medium –sized to large deciduous tree, usually with a short bole and large rounded crown. It is found in mixed deciduous forest usually of a some what dry type, often growing on rocky and sandy soil and thriving on the Deccan trap. It is common throughout Central India, Maharashtra, Gujarat and Andhra Pradesh. It is common in drier type of Sal forests in Madhya Pradesh. *M. indica* and *M. longifolia* are two major species of mahua found in India These two are closely related species (Bhatt *et al*., 2004) both are valued for their seeds which yield fatty oil known in commerce as Mahua Butter or Mowra fat. *M. indica* tree has immense value to village people, especially the tribals as a source of food, liquor and cash through sale of seed and flowers. The importance of *M. indica* tree is recognized by the Forest Department and its protection is recommended as long as it did not interfere with the growth of more valuable tree species.

M. indica seeds available in the trade contain 5-12% moisture. Seeds containing more than 7-8% moisture are liable to fungal attack when stored. Analysis of seed kernel gave the following values: fatty oil 51.1; protein 8.0; N-free extr., 27.9; fibre 10.3 and ash 2.7%. Mahua oil is used mainly in the manufacture of soaps; particularly laundry soaps (Anonymous, 1964). Fresh oil from properly stored seeds is yellow in colour, while commercial oils are generally greenish yellow with a disagreeable odor and taste (Bhatt *et al*., 2004). The storage condition of kernel determines the quality of

expelled oil, as the kernels are susceptible to both fungus *Aspergillus flavus* and *Rhizopus* sp. (*H:/intro.mohwa.htm*). It has been reported that treatment of oil with steam before saponification and addition of gum benzoin along with tobacco or clove extract during processing renders the oil suitable for the production of high quality soaps. The potential availability of *M. indica* fat in India is nearly 50,000 ton per annum (Awasthi *et al*.,1975). Mahua oil has emollient properties and is used in skin diseases, rheumatism and headache.

Mahua flowers are largely used in the preparation of distilled liquors. The freshly prepared liquor has a strong smoky odor which disappears on aging. Mahua flowers are rich source of sugars, contain 72.9%, Protein, 4.4% Fat, 0.5%, Calcium 150 mg, Iron 15 mg/ 100 gm, magnesium, copper and vitamins (Benerji *et al*., 2010). The total sugar content of the corollas is maximum when the flowers are mature and ready to fall. In the growing stage, fructose is present in greater amount than glucose, and in the ripe stage, the quantities are almost equal. In India various parts of Andhra Pradesh, Maharashtra, Chhattisgarh, some tribal communities cultivating and harvesting mahua flowers for alcoholic beverages using traditional methods (Yadav *et al*., 2009). Sarkar and Chatterjee (1984) studied structural features of the polysaccharides of mahua flowers. Mahua flower is abundant in India and it is having good keeping qualities. If the utilization of mahua flower as a substrate for the production of ethanol though submerged fermentation is done, it will become a great economic advantage in the Indian context. Mahua flowers are regarded as cooling, tonic and demulcent. They are used in cough, colds and bronchitis and show anti-bacterial activity against *Escherichia coli.*

Mahua fruit is orange brown ripe fleshly berry. It is 25 to 50 mm long and contains one to four shining seeds . The seeds can be separated from the fruit wall by pressing.

Ripe fruit showed the following values: moisture 73.64%, Protein 1.37% fat (ether extract) 1.61%, carbohydrates 22.69%, and mineral matter 0.69% , Calcium 45mg; Phosphorous 22mg, iron 1.1mg; carotene (as vitamin A) and ascorbic acid 40.5 mg / 100g. Tannins are also present. The fruit collected during the ripening stage are rich in starch; the starch is hydrolyzed into sugars within 2-3 days after plucking. The fruit pulp may be used as a source of sugar for alcoholic fermentation. Fruit fallen on the ground are easily attacked by insects and ants and thus become unfit for human consumption.

***Terminalia bellerica* Roxb.**

The trade name Behada is based on the Indian name of the tree. The word *Bellerica* is taken from the scientific name of the tree and distinguishes this myrobalan from the other one (Chebulic myrobalan) . *T. bellerica* is also known in Sanskrit as – Vibhitaka, in English Belliric myrobalan, in Hindi Baheda.

T. bellerica is large tree, up to 40 m high. Leaves are petiolate, broadly elliptic, clustered towards the end of the branches. Flowers greenish yellow, in solitary, simple, axillary spikes. Fruits globular, 1.5-2.5 cm in diameter, obscurely 5 –angled when dry. *T. bellerica* is found in deciduous forests throughout the greater part of India, but not in the arid regions in areas of upper Gangetic plain, Chota Nagpur, Bihar, Orissa, West Bengal, Chittagong, Konkan, Deccan and most of the parts of South India In Himachal Pradesh, the species is confined to low hills and valley areas (Troup 1921).

T.bellerica plant contains different chemical constituents in different parts such as stem bark contains arjungenin and its glycosides, belleric acid, bellericosides. Fruits contains hexahydroxydiphenic acid ester, β- sitosterol, gallic acid, ellagic acid, ethyl

gallate, galloyl glucose, chebulagic acid, mannitol, glucose, galactose, and rhamnose (Meena *et al*., 2010).

The timber is yellowish brown coarse grained and not durable being subject to insect attack and in consequence is of little use. The *T. bellerica* grows well on poor soil and in dry places, when most trees are stunted and the choice of suitable plant as a shade trees. For propagation the fruits may be sown and if kept watered the seeding should appear in one to two month. Seedlings may be transplanted in the second rainy season. Its bark is mildly diuretic and is useful in anaemia and leucoderma. Fruits are anti- inflammatory, antihelmintic, expectorant, ophthalmic, antipyretic, antiemetic. Fruits are useful in cough, asthama, bronchitis, dropsy, dyspepsia, cardiac disorders, Skin diseases, leprosy, ulcers and myocardial depressive activity and also used in eye diseases and scorpion sting. The fruits of *T. bellerica* are reporated to be used as substitute in tanning industry.

Emblica officinalis Gaertn.

Emblica fruits are one of three "myrobalans," a term deriving from the Greek for acorn, which is a well-known astringent used in tanning. The green fruit is described as being exceedingly acidic. The dried fruit is sour and astringent. The flowers are cooling and aperient. The bark is astringent Nadkarni and Nadkarni (1999). There are two forms of Amla, the wild one with smaller fruits and the cultivated form sometimes called "Banarsi" with larger fruits Thakur *et al*. (1989) The use of amla as an antioxidant has been examined by a number of authors (Bhattacharya *et al*.1999) and Chaudhuri 2004). Amla has been considered the best of the Ayurvedic rejuvenative herbs, because it is *tridosaghna*. Uniquely, it has a natural balance of tastes (sweet, sour, pungent, bitter and astringent) all in one fruit, it stimulates the brain to rebalance the three main components of all physiological functions, the water,

fire, and air elements within the body Bajracharya (1979). The tree is common in the mixed deciduous forests of India ascending to 4,500 meters on the hills. It is also often cultivated in gardens and homeyards.

The Ayurvedic description of Amla

The fruit has these properties using the Ayurvedic classifications:

Rasa (taste): sour and astringent are the most dominant, the fruit has five tastes, including sweet, bitter, and pungent

Veerya (nature): cooling

Vipaka (taste developed through digestion): sweet

Guna (qualities): light, dry

Doshas (effect on humors): quietens all three doshas: *vata*, *kapha*, *pitta*, and is especially effective for *pitta*

Because of its cooling nature, amla is a common ingredient in treatments for a burning sensation anywhere in the body and for many types of inflammation and fever; these are manifestations of *pitta* (fire) agitation (Williamson, 2002).

E. officinalis is usually propagated by seeds. It may also be propagated by budding, cutting and in arching. The plant is sensitive to frost and drought. Flowers usually appear in the hot season and fruits ripen during the following winter.

The plant is reported to be affected by a leaf rust caused by *Phakopsora phyllanthi* Diet, and a ring rust caused by *Ravenelia emblicae* Syd.

The fruit is a very rich source of vitamin C according to most if not nearly all references; this is probably not the case [Ghosal, *et al.*, 1996]. The fruit juice contains nearly 20 times as much vitamin C as orange juice. It was proposed that superior effect of the mistaken

"Vitamin C" component is actually the more stable and potent anti-oxidant effect of the tannins that appeared to be the vitamin. The antiscorbutic value is well conserved by preserving the fruits in salt solution or in the form of dry powder .The dried fruit loses only 20% of its vitamins in 375 days when kept in a refrigerator, but loses 67% in the same period when stored in a same period when stored in a room temperature. A repeated laboratory test showed that every 100g of fresh fruit provides 470 – 680 mg of Vitamin C. The vitamin value of amla increased further when the juice was extracted from the fruit. The dehydrated berry provided 2428 - 3470mg of vitamin C per 100g.

Its mineral and vitamin contents include calcium, phosphorous, iron, carotene, thiamine, riboflavin, and niacin. The seeds of the Indian gooseberry contain a fixed oil, phosphatides, and an essential oil. The fruits bark and the leaves of this tree are rich in tannin. The fruits, leaves and bark are rich in tannins. The root contains ellagic acid and lupeol and bark contains leucodelphinidin. The seeds yield a fixed oil (16%) which is brownish-yellow in colour. It has the following fatty acids: linolenic (8.8%), linoleic (44.0%), oleic (28.4%), stearic (2.15%), palmitic (3.0%) and myristic (1.0%) [Thakur *et al*]. Akhund *et al*. (2010) reported fruit borne mycoflora of amla. They find occurrence of genera like *Aspergillus, Alternaria, Cladosporium, Curvularia, Fusarium* and *Penicillium* in fruits collected from three localities in Pakistan. Total 19 genera were recorded. Lal *et al*. (1982) recorded occurrence of *Phomopsis phyllanthi* on amla fruits.

***Tamarindus indica* L.**

Tamarindus indica L. belongs to dicotyledonous family Leguminosae, sub-family Caesalpiniaceae, which is the third largest family of flowering plants with a total of 727 genera and 19,327 (Lewis *et. al.*, 2005). Fruit pulp is used for the preparation of

beverages in different regions. (Khanzada *et al*., 2008). Large segments of human population and animals in developing countries suffer from protein malnutrition.(Conway & Toenniessen 1999). *T. indica* is also high in level of protein with many essential amino acids which help to build strong and efficient muscles. *T. indica* is high in carbohydrate, which provides energy rich in the minerals, potassium, phosphorus, calcium and magnesium. It also provide smaller amount of iron and vitamin A. The plant is also identified as a rich source of tartaric acid and polysaccharides gum with valuable qualities.

Fresh fruits are often dried using small scale dehydrators, lower in most countries rural household dry pods in the sun. The shells, fibres and seeds are removed and pulp is stored in plastic bags or earthenware pots.

The occurrence of various fungi has been observed on these minor forest produce, and the result are presented in table 1.3.

Table 1.3: Showing Occurrence of mycoflora associated with three different fruits *Emblica officinalis, Tamarindus indica and Terminalia bellerica* as reported by earlier workers

***Terminalia bellerica* Roxb./ Fruit Rot**			
S.No	**Fungi**	**Place**	**Author**
1	*Penicillium frequetans*	Panchmarhi hills	Chandra & Gupta (1984)
2	*P. variabile*	of M.P.	"
3	*Aspergillus flavus, A.niger, A.Tamarii, Rhizopus* sp.	Baroda	Chauhan & Gupta (1985)
4	*Aspergillus awamori, A.niger, A. flavus, Rhizopus sp., Monilia* sp.	Bhavnagar	Nair (2002-03)
5	*Aspergillus niger, A.flavus, A.tamarii, Aspergillus Sp., Rhizopus* sp	Jamnagar	"
***Tamarindus indica* L. Seeds / Fruit Rot**			
1	*Phomopsis tamarindi*	Baroda & Allahabad	Arya et al., (1999**)**
2	*Aspergillus niger, Aspergillus* sp., *Chaetomium sp., Rhizopus stolonifer*	Sagar (M.P.)	Purohit et al.,(1996)
***Emblica officinalis* Gaertn. Fruit Rot**			
1	*Pestalotia cruenta*	Allahabad	Srivastava et al.,(1964)
2	*Penicillium citrinum*	Gwalior (M.P.)	Chauhan & Gupta (1985)
3	*Phomopsis phyllanthi, A. ustus*	Baroda	Arya (1994)
4	*Aspergillus niger, A.flavus, A.awamori, Rhizopus stolonifer*	Baroda	Nair (2002-03)
5	*Aspergillus niger, A. flavus, Aspergillus sp., Rhizopus sp, ,Mucor heimalis, Syncephalastrum sp.*	Bhavnagar	"
6	*Aspergillus niger, A.flavus, A. tamarii,*	Jamnagar	"
7	*Rhizopus* sp.	Baroda	Arya and Arya (2004)
8	*Aspergillus terreus*	----	Prakash 2009
	Syncephalastrum racemosum Schr		