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PREVALENCE OF FUNGI IN AN OPEN ENVIRONMENT AND PRODUCTION OF LOW COST CULTURE MEDIUM FOR FUNGAL CULTIVATION USING AGROINDUSTRIAL WASTES

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Abstract

Fungi grow on diverse habitats in nature and are cosmopolitan in distribution requiring several specific elements for growth and reproduction. In laboratory these are isolated on specific culture medium for cultivation, preservation, microscopical examination and biochemical and physiological characterization. A wide range of media are used for isolation of different groups of fungi that influence the vegetative growth and colony morphology, pigmentation and sporulation depending upon the composition of specific culture medium, pH, temperature, light, water availability and surrounding atmospheric gas mixture. However, the requirements for fungal growth are generally less stringent than for the sporulation. Microorganisms require carbon, nitrogen, minerals, sometimes growth factors, water and oxygen if aerobic, as elements for cell biomass, energy, biosynthesis and cell maintenance. The maximum production of some metabolites requires the incorporation of specific inhibitors in the medium either to minimize formation of metabolic intermediaries or to prevent further metabolism of the desired product. The prime ingredients of the media are water, energy sources, sources for carbon, nitrogen and minerals, chelators, growth factors, buffers, precursors and inhibitors. The present review was focused on the prevalence of fungi in an open environment and production of low cost culture medium fungal cultivation using Agroindustrial wastes.

Key words: Open environment, Prevalence, Fungi, Culture medium and Agroindustrial wastes

1. Introduction

India is agriculture based country and the farming system have its special place in the universe from the ancient days. Among the various agricultural products produced in India, vegetable cultivation has occupied the major role due to its daily requirement for cooking purposes and the presence of various nutrients.

India ranked second in the production of vegetables and marching towards the first rank because of the highest need due to the increase in population. Besides its beneficial role as a nutritive source in food, the peel wastes generated from the vegetable creates nuisance to the environment. The management of wastes has been considered as hot topic in various debates during recent times. The wastes generated from unspoiled vegetables are not harmful to the human health and environment but it is very essential to find a way for utilizing the vegetable peel wastes in a beneficial way. The disposal of unwanted agriculture wastes in the beneficial way is very

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challenging in the present scenario. It is sure that the designed research work will provide one best way for utilizing the waste vegetable peels in a beneficial way.

Microorganisms are the invisible creates which are ubiquitous in nature and present everywhere in this universe. Various researchers narrated the microorganisms as the "Double edged sword" because of its beneficial and harmful activities. The major categories of the microorganisms in this universe are bacteria, archaea, fungi, algae and protozoa. Among the all microbial types, the fungi take part an imperative role in the productions of various industrial products which are repeatedly used by human beings in their routine life. Fungi are the ubiquitous eukaryotes present in air and soil. Based on its, cellular nature, fungi are classified into two major groups *viz*., Molds and Yeast. The molds are multicellular and the yeast is unicellular in nature. The mold is widely spread in the air and soil in the form of spores and the presence of yeast is very less in an environment.

Medicinally, the fungi belong to mold and yeast has an equal importance in causing acute and chronic infections in human beings. The molds are responsible for causing various diseases *viz*., respiratory tract infection, skin infection, hair infection and nail infection. Commonly the fungal molds inhabit the respiratory tract then moves to lungs and results in various respiratory disorders. The Dermatophytes are well known for its infections in skin, hair and nail regions. The harmful effect of mold was extended to plant species also. The fungi have the ability to synthesize the toxin Mycotoxin which causes serious health issues in human beings and agricultural crops. Every year the phytopathogens belong to the mold category is resulting in the cause of severe economic loss of various agricultural crops by causing diseases during various growth stages. The harmful effect of yeast was reported against humans only and not against plant or animal species. The common harmful dimorphic fungal yeast namely, *Candida albicans* has showed severe effect against human beings particularly female community by causing Urinary tract infection, Nosocomial infection and Pulmonary candidiasis.

The percentage of beneficial role of fungi is equivalent to the harmful effects. The word "Double edged sword" is more suitable for the fungi when compared to other microorganisms. The fungal mold has finds its special role in the production of antibiotics, industrial enzymes, organic acids, organic solvents, biopesticides, biocontrol agents against phytopathogens and several polymers (Saranraj *et al*., 2010). The first antibiotic Penicillin was discovered from the fungi *Penicillium notatum* by Sir Alexander Fleming, *Trichoderma viride* act as a biocontrol agent and *Beauveria bassiana* & *Metarhizium* sp. act as a Bioinsecticide. The Basidophytic fungi Mushrooms are well known for its role as a food for human diet and the presence of various bioactive compounds which was regularly used by the pharmacological industries. The role of fungal mold in the bioremediation of various wastewaters and textile dyes were investigated by various researchers. Unfortunately, the usage of fungal mold was limited in the wastewater treatment due to the production of mycelial mats and replaced by the bacteria due to its high efficient bioremediation in short duration.

The fungal yeast *Saccharomyces cerevisiae* is commonly called as Backer's yeast and Brewer's yeast due to its active role in the production of bread, bioethanol, beer and wine. The role of *Saccharomyces cerevisiae* in alcoholic fermentation was reported in early days by the French scientist Louis Pasteur who was the frontier of Microbiology research and named as Father of Modern Microbiology (Saranraj *et al*., 2017). The yeast also fills its role in the category of food and feed. Some species of yeasts like *Saccharomyces cerevisiae, Amoco torulo, Candida tropicalis, Candida utilis, Candida novellas* and *Candida intermedica* can act as a Single cell protein (SCP) and used as a food for human beings and feed for cattle.

Culture medium is an environment which favors the growth of microorganisms by providing various chemical nutrients and growth factors. Based on its properties, culture medium is classified into six types *viz*., Basal medium, Enriched medium, Enrichment medium, Differential medium, Selective medium and Transport medium. The nature of the culture medium was liquid, semi-solid or solid and technically the solid medium are referred as the agar medium and the liquid medium are called as broth. Sabouraud's dextrose agar which was familiarly abbreviated as SDA is the common culture medium used for the cultivation of fungi. Other than SDA, the commonly used culture medium are Martin Rose Bengal agar (RBA) and Potato dextrose agar (PDA) (Tharmila *et al*., 2011). All the culture medium are rich in the chemical nutrients like carbon sources (usually carbohydrate sugars), nitrogen sources, phosphorous sources and sulphur sources for the utilization of microorganisms. The carbohydrate sugar Dextrose is considered as an important ingredient of fungal culture medium. The cost of commercial culture medium is rising day by day due to the highest need of researchers and less availability of raw material sources. In developing countries like India, tax system like Goods Service Tax (GST) increases the cost of culture medium and causing economic troubles for researchers and industrialists. For managing the economical issues, we are in need to find an alternative source for the cultivation of microorganisms. Utilization of enormous agricultural waste materials for the cultivation of microorganisms particularly industrially important fungi is the correct remedy for the replacement for synthetic culture medium. On that line, the present research was designed (Mateen *et al*., 2012; Ravimannan *et al*., 2014).

2. Fungi

Fungi grow on diverse habitats in nature and are cosmopolitan in distribution requiring several specific elements for growth and reproduction. In laboratory these are isolated on specific culture medium for cultivation, preservation, microscopical examination and biochemical and physiological characterization. A wide range of media are used for isolation of different groups of fungi that influence the vegetative growth and colony morphology, pigmentation and sporulation depending upon the composition of specific culture medium, pH, temperature, light, water availability and surrounding atmospheric gas mixture (Kuhn and Ghannoum, 2003; Kumara and Rawal, 2008). However, the requirements for fungal growth are generally less stringent than for the sporulation.

Nowadays, fungal taxonomy is in a state of rapid flux, because of the recent researches based on molecular approaches, that is DNA comparisons of selected strains either isolated locally or obtained from culture collection centre, which has changed the existing scenario of fungal systematic and often overturn the assumptions of the older classification systems (Hibbett, 2006). Different concepts have been used by the mycologists to characterize the fungal species, out of which morphological (phenetic or phenotypic) and reproductive stages are the classic approaches and baseline of fungal taxonomy and nomenclature that are still valid (Diba *et al*., 2007; Zain *et al*., 2009). It seems evident that in near future, modern molecular techniques will allow most of the pathogenic and opportunistic fungi to be connected to their corresponding sexual stages and integrated into a more natural taxonomic scheme.

Physical and chemical factors have a pronounce effect on diagnostic characters of fungi. Hence, it is often necessary to use several media while attempting to identify a fungus in culture since mycelial growth and sporulation on artificial media are important biological characteristics (St-Germain and Summerbell, 1996). Furthermore, findings for one species are not readily extrapolated to others, particularly for filamentous fungi, where significant morphological and physiological variations exist (Meletiadis *et al*., 2001). With these perspectives, the present study was undertaken to observe the influence of three different culture media on the mycelial growth, colony characters and sporulation patterns of ten

dominant fungi isolated from decaying vegetable wastes.

Fungi are ubiquitous in indoor environments and are responsible for a wide range of diseases, from localized non-invasive pathologies to invasive and disseminated infections. These infections occur predominantly among highly immunosuppressed patients (patients with acute leukaemia, haematopoietic stem cell or solid organ transplantation) and can have devastating consequences. *Aspergillus* remains the most common mould to cause invasive infections, but other fungi are emerging as serious pathogens and threats in immunosuppressed patients. Most invasive fungal infections are acquired from air. It is therefore imperative to adopt, in clinical environments, preventive measures in order to reduce airborne fungal concentrations and, concomitantly, the risk for development of a fungal infection. At present, there are no methods and equipments that can completely eliminate fungi from indoor medical environments. Exposure to moulds in medical units is inevitable but the presence of air filtration systems, isolation, and adoption of environmental protective measures do mitigate patient exposure. Airborne mycological investigations should inform about indoor air quality and therefore should be routinely carried out in hospitals or other institutions where immunosuppressed individuals are treated. It is important to improve the methods already available to study indoor fungi in clean environments, and it is critical to define indicators of indoor air quality in medical environments.

3. Fungal growth, Sporulation and Adaptation

Fungi from the atmosphere and indoor environments are influenced by temperature and humidity (atmospheric relative humidity and substrate moisture content). The optimum temperature for growth and sporulation is usually around 25 - 30 °C. Lower or higher temperatures result in lower growth and sporulation rates. A remarkable exception comprises fungi that can infect humans, such as those involved in Aspergillosis and Candidiasis, which display an optimum temperature around 37 °C (Araujo and Rodrigues, 2004). Temperature is usually not limiting in indoor environments, since most indoor fungi can grow in a wide range of temperatures (Douwes, 2009).

Humidity is the most important factor determining fungal growth in indoor environments (Nielsen, 2003). Atmospheric relative humidity influences directly the release of conidia from conidiophores, and concomitantly, the concentration of spores in the atmosphere. Different patterns are displayed by *Cladosporium* sp. and *Penicillium* sp. Whereas in *Cladosporium* sp., spore release is favoured by low humidity, the opposite behaviour is displayed by *Penicillium* sp. These differences influence the seasonal patterns of outdoor fungi. *Cladosporium* sp. has maxima in the summer, but *Penicillium* sp. display higher concentrations in the wetter months (Sautour *et al*., 2009).

Fungal growth in building materials is more dependent on the moisture content of the substrate than on atmospheric relative humidity. The minimum moisture content of building materials allowing fungal growth is near 76 % (for atmospheric relative humidity, this value is near 82 %). Wood, wood composites (plywood, chipboard), and materials with a high starch content are capable of supporting fungal growth, at the lowest substrate moisture content. Plasterboard reinforced with cardboard and paper fibres, or inorganic materials coated with paint or treated with additives that offer an easily - degradable carbon source, are excellent substrates for fungal growth when substrate moisture content reaches 85 - 90 % (Nielsen, 2003). All fungi need nutrients for growth and sporulation. When growing in indoor substrates such as food, nutrients are not limiting, but on the surface of certain building materials, nutrients may limit fungal growth. Local differences in ventilation and surface temperature can generate microclimates with very high substrate moisture content, although the room can have a low atmospheric relative humidity. For this reason, a measurement

of indoor atmospheric relative humidity is a poor predictor of indoor fungal growth (Nielsen, 2003).

Xerophilic fungi are well adapted to indoor environments, since these fungi grow and sporulate with low atmospheric relative humidity and substrates with low moisture content. Indeed, the majority of *Aspergillus* sp. and *Penicillium* sp. are xerophilic and able to growth in substrates with water activity lower than 0.80. Most of the other indoor fungi (namely *Cladosporium* sp., *Stachybotrys* sp., *Chaetomium* sp., *Trichoderma* sp. and *Ulocladium* sp.) are much less tolerant to xerophilic conditions. Because of their low water activity requirements (compared with bacteria), fungi are the principal contaminant in various types of indoor substrates. They tend to colonize a wide variety of humid building materials wetted by floods or by plumbing leaks (Dacarro *et al*., 2003).

4. Fungal fragments and Allergicity

Until 1990 - 2000, it was thought that indoors fungi exist only as spores and hyphae. Work published by several teams showed that fungi from the atmosphere, growing in culture media or building materials, subjected to air currents, release cellular fragments (presumably hyphal and spore fragments). The presence of these fragments in indoor air was confirmed experimentally. For three common species from the atmosphere, growing in culture medium or building material, Gorny *et al*. (2002) showed that when the colonies were subjected to air currents, the number of released fragments was higher that the number of spores. Fragments released from fungi growing in culture medium were not influenced by air velocity. Kildeso *et al*. (2003) reported the release of spores and fragments from colonies of three different species. When *Penicillium chrysogenum* was subjected to air currents, only spores were released from the colonies, but with *Aspergillus versicolor*, 1 µm fragments were also released, in addition to individual spores. With *Trichoderma harzianum*, three types of particles were released from the colonies: groups of spores; individual spores; and fragments. The release of fragments and spores from indoor fungi (*Aspergillus versicolor* and *Stachybotrys chartarum*) growing on the surface of white ceiling tiles, wall-papered gypsum board and culture medium, and subjected to air currents, was recently reported by Seo *et al*. (2009). One month-old cultures released more spores than fragments, but after six month incubation, the number of released fragments exceeded the number of spores. The mass of released fragments and spores (assessed by the amount of glucan) generally increased with age of the cultures.

The presence of fungal fragments in indoor atmosphere, predicted by these studies carried out *in vitro* (in laboratory conditions), was confirmed by field determinations. Reponen *et al*. (2007) reported a study carried out in five mould-contaminated single houses in Louisiana and Southern Ohio. Indoor total spore concentrations were very high and higher than outdoor concentrations (both in winter and summer). Assessed by the 1,3- β -D-glucan concentration, the ratio between fragments and spores ranged from 0.011 to 2.163, the highest average (1.017) being for indoor samples collected in the winter. Considering that fragments are much smaller than spores, the corresponding number of fragments in indoor air in these houses was certainly much higher than the number of spores. It was concluded that, in mouldy houses, fungal fragment mass can be as high as spore mass, and fragment number can exceed total spore number.

Long term mould damage in buildings may increase the contribution of sub-micrometer sized fungal fragments to the overall mould exposure. The health impact of these particles may be even greater than that of spores, considering the strong association between numbers of fine particles and adverse health effects reported in other studies (Reponen *et al*., 2007; Seo *et al*., 2009). However, there are at present no detailed morphological and cultural studies of these fragments released by fungal colonies subjected to air currents, and therefore important questions remain open. Are these particles, fragments of spores or of hyphae? Are they viable and able to grow in culture media and in the respiratory tract? It has been

demonstrated that *in vitro*, depending of the fungal species and tested antibody, immunological reactivity of fungal fragments is 2 to 5 times higher than conidia (Gorny *et al*., 2002). In several moulds responsible for releasing airborne allergens, Green *et al*. (2005) found that many of the allergens were in hyphal fragments. Germinated conidia and hyphae may be more allergenic than fungal conidia, but personal exposure to fungal allergens may be difficult to evaluate (Gorny *et al*., 2002; Green *et al*., 2005). Common fungal allergens described in the literature include *Aspergillus* Asp f 1, Asp f 3, Asp f 6, and *Alternaria* Alt a 1 (Chapman *et al*., 2001; Crameri and Blaser, 2002).

Enzyme Linked Immunosorbent Assays (ELISA) are commercially available for quantification of these allergens in environmental and house-dust samples. Very often, the allergens are not detected by available immunological methods and protocols. Chapman *et al*. (2001) reported that in order to detect allergens in spore suspensions, it was necessary to use heavily concentrated suspensions (>100,000 conidia × ml^{-1}). This may hampered the direct detection of allergens in atmospheric sampling. In the human body, mucociliary clearance represents the first strategy for removal of airborne fungi from the respiratory tract. This can be followed by the activation of innate and adaptive immune responses. Occasionally, inflammation occurs and individuals may suffer mucous membrane irritation, chronic bronchitis and organic dust toxic syndrome. The most common inflammatory reactions to fungi are non-allergic, but an allergic response or a hypersensitivity pneumonitis can occur in individuals exposed to conidia, hyphae or fungal fragments (Green *et al*., 2006; Eduard, 2009).

More sensitized individuals may suffer from allergy following exposure to fungi. These patients usually present high IgE values and increased release of some inflammatory mediators. Houba *et al*. (1998) described baking workers with high IgE against common allergens. These professionals presented an increased risk for mould occupational allergy. Allergic bronchopulmonary aspergillosis (ABPA) is also an allergic response, but specific to *Aspergillus fumigatus* allergens present in the environment. The disease is more frequent among patients with asthma or with cystic fibrosis. The usual complains are breathless, pulmonary infiltrates, bronchiectasis and fibrosis (Stevens *et al*., 2003). Patient serum display high levels of total IgE, specific *Aspergillus fumigatus* IgE and IgG antibodies, IL-2 receptor, and precipitins to *Aspergillus fumigatus*.

Besides an allergic response, hypersensitivity pneumonitis can occur upon exposure to fungi. This pathology, as described by the European Academy of Allergy and Clinical Immunology is generally associated with high IgG antibodies concentrations in response to alveolar or bronchiolar inflammation caused by fungi or other allergens. On the contrary of allergy, this type of hypersensitivity to fungal allergens does not seem to be mediated by IgE. The patients may present neutrophilic inflammation with increased production of TNF - α and IL - 6, and symptoms such as fever, chilliness, dry cough, dyspnoea, changes in nodular bilateral x-ray, fatigue and headache (Eduard, 2009). In some asthmatic patients, fungi seem to exacerbate symptoms, but in others this effect has not been found.

Newson *et al*. (2000) described an association between airborne total fungal counts and incidence of severe asthma in England's Trent region. However, no specific fungal species were implicated. A twofold reduction of airborne exposure to allergens has been reported to reduce the risk of developing asthma and asthma severity (Peat and Li, 1999). Other studies reported no evidence of association between airborne fungi and asthma (Richardson *et al*., 2005). Thus, further studies are needed in order to clarify this problem.

5. Prevalence of fungi in air and inanimate materials

Fungi are heterotrophic eukaryotes that are usually filamentous, devoid of chlorophyll and chitinous cell wall and produces spores. They are

found in the soil, water, air, on vegetation, on humans and everywhere in the environment. Fungi mostly present in the air and causes allergy are called Aeroallergens. Fungal density in the air varies in accordance with geographical regions and seasons. Besides, climatic parameters such as wind, humidity, temperature, precipitation, altitude and flora combination may also affect the type and amount of fungi in the air. The shape and size of conidia of fungi along with the meteorological factors determines its speed and dispersal (Bush and Portnoy, 2001).

Fungi are ubiquitous in all atmospheres. In general, both outdoor and indoor atmospheres are dominated by *Cladosporium* sp., *Penicillium* sp., *Aspergillus* sp. and *Alternaria* sp., and by yeasts and Mycelia Sterilia. *Cladosporium* sp. is always the dominant fungus in outdoor atmospheres and in indoor atmospheres of normal and healthy buildings (except hospitals where *Aspergillus* sp. and *Penicillium* sp. are usually dominant). The abundance of the other fungi varies with the season and place. In relation to outdoor environments, indoor atmosphere typically display lower diversity and abundance of fungi (Dacarro *et al*., 2003). The following genera can be represented indoors, but are always in clear minority: *Absidia* sp., *Acremonium* sp., *Arthrinium* sp., *Aureobasidium* sp., *Beauveria* sp., *Botrytis* sp., *Candida* sp., *Chaetomium* sp., *Chrysosporium* sp., *Epicoccum* sp., *Fusarium* sp., *Gliocladium* sp., *Mucor* sp., *Nigrospora* sp., *Paecilomyces* sp., *Phoma* sp., *Rhizopus* sp., *Scopulariopsis* sp., *Sporobolomyces* sp., *Stemphylium* sp., *Syncephalastrum* sp., *Trichoderma* sp., *Ulocladium* sp. and *Verticillium* sp. (Shelton *et al*., 2002; Dacarro *et al*., 2003; Horner *et al*., 2004; Martinez *et al*., 2004; Jo and Seo, 2005; Sautour *et al*., 2009).

The prevalence of respiratory allergy to fungi was estimated at 20 % to 30 % among atopic individuals and up to 6 % in the general population. The major allergic manifestations induced by fungi are asthma, rhinitis allergic bronchopulmonary mycoses and hypersensitivity pneumonitis. These diseases can result from exposure to spores, vegetative cells or metabolites of the fungi. Some fungi like *Alternaria* sp., *Aspergillus* sp., *Cladosporium* sp. and *Penicillium* sp. are generally considered to be important causes of both allergic rhinitis and allergic asthma. *Cladosporium* sp. and *Alternaria* sp. exist more commonly in the atmosphere in periods of warm air while *Aspergillus* sp. and *Penicillium* sp. exist more intensively in cool periods (Centre for Disease Prevention and Control, 2000).

Global assessment of the genetic diversity in a given environment has been studied using several molecular techniques. The metagenome description of the microbial communities in Sargasso Sea is still not concluded but a vast amount of new data was obtained (Venter *et al*., 2004). Metagenome fingerprinting techniques, such as automated ribosomal Intergenic spacer analysis (ARISA), terminal restriction fragment length polymorphism (TRFLP) and denaturating gradient gel electrophoresis (DGGE), have been employed worldwide for measuring fungal species richness in communities. However, these methods may not reflect the actual microbial diversity, as they tend to identify only the dominant members of the community (Bent *et al*., 2007). ARISA is a high-resolution, highly reproducible, automated technique that uses the variability in the length of the intervening transcribed spacer regions (ITS) of rRNA genes in order to separate several samples into operational taxonomic units (OTUs). ARISA allows the characterization and distinction of fungal communities and has been employed to distinguish fungal soil communities from distinct cities and countries (Ranjard *et al*., 2001). The other two methods (TRFLP and DGGE) employ restriction enzymes or specific primers and non-automated gel electrophoresis for identification of microbial OTUs. TRFLP allowed a good characterization of fungal communities isolated from air samples collected from an urban area of Seoul (Korea) and soil samples in UK (Schutte *et al*., 2008; Lee *et al*., 2010).

Recently reported fungal metagenomic studies found that Ascomycota (Dothideomycetes, Eurotiomycetes, Leotiomycetes, and

Sordariomycetes) and Basidiomycota (Agaricomycetes) were the most represented Divisions in outdoor atmospheres (Frohlich Nowoisky *et al*., 2009; Lee *et al*., 2010). Reports on fungal metagenome of indoor environments have been included in studies screening complete microbial communities (Angenent *et al*., 2005), but these are still very incomplete. The construction of metagenomic libraries is nowadays possible, although technically demanding and economically expensive. The future will bring new technologies and cheaper alternatives for sequencing large number of OTUs and these will allow knowledge of the composition of complete communities. The study of hospital metagenome can allow physicians, researchers and other medical staff a full knowledge of the microbial communities present inside medical wards. This is expected to give information on the presence of certain fungi in highly-restricted areas where critical patients are admitted, and therefore to have a considerable impact on public health.

Climate and human activities are the main factors that influence the composition of outdoor atmosphere. In the temperate climates, these display a typical pattern around the year. On the contrary, climate is not determinative in the mycoflora of indoor atmosphere, but human activities and the quality and maintenance of the building do play a major role in these environments. For these reasons, dominant fungi indoors vary between buildings and can be used as monitors of indoor air quality (Araujo *et al*., 2008). In the atmosphere, fungi are present in bioaerosols. Bioaerosols contain bacterial and fungal cells and cellular fragments, and products of microbial metabolism. Fungal spores constitute a significant fraction of bioaerosol microbial particles, and are often 100-1000 times more numerous than other bioparticles, like pollen grains. The particulate fraction in a bioaerosol is generally 0.3 - 100 µm in diameter. Fungal spores larger than 10 µm are deposited in the nasopharynx and can unchain nasal and ocular disorders. The respirable size fraction of 1 – 10 µm is of primary concern. Spores and fragments smaller than 10 µm (especially those smaller than 6 µm) can be transported to the lower airways and lungs, and trigger allergic reactions or infect tissues (Martinez *et al*., 2004; Stetzenbach *et al*., 2004).

Bioaerosols that range in size from 1 to 5 µm generally remain in the air, whereas larger particles are deposited in the surfaces. Physical and environmental factors affect the settling of bioaerosols. Air currents, relative humidity and temperature are the most important environmental parameters affecting bioaerosol settling. The most significant physical parameters are particle size, density and shape (Martinez *et al*., 2004; Stetzenbach *et al*., 2004). A human inhales on average 10 m^3 of air per day, and spends 80 - 95 % of their time indoors. Indoor air pollution is therefore frequently reported to cause health problems (Dacarro *et al*., 2003).

The mycoflora composition of outdoor and indoor atmosphere displays high variability. Fungi in the atmospheres vary along the year and during the day. For this reason, a reliable estimate of fungal levels in the atmosphere demands multiple determinations carried out in different seasons (Jantunen *et al*., 1997). Temporal variability is a major problem in assessing human exposure to indoor fungi. This variability is mainly due to the release of fungi from carpets and walls or other surfaces. This release depends on the type and degree of activity of occupants in the dwelling or building. All activities in buildings disturb settled fungal particles, but cleaning, constructional work and any other major dust-raising activities have a particular impact (Flannigan, 1997). To circumvent this temporal variability of indoor mycoflora, it has been suggested that floor dust should be sampled instead of the air, since it provides a long-term accumulation of previously airborne fungi. However, although house dust fungi reflect atmospheric populations, there are qualitative differences between these two mycofloras, probably resulting from the differences in the environments. Sampling of dust should not be used as a substitute for air sampling. In addition, viable counts for settled dust are much higher than corresponding air sampling counts for

aerosolized dust, suggesting that many microbes in dust either form aggregates or are carried on dust particles which settle very rapidly (Flannigan, 1997).

Jaffal *et al*. (2000) using a mechanical air samples for the enumeration of fungal CFU in residential environments, found five groups of fungi, mainly members of the genus *Aspergillus*. The authors concluded that although their high numbers, the fungal cells presented little effect on human health. Pasanema *et al*. (2000) investigated fungal growth and the maintenance of its viability in building materials under controlled humidity. The materials were subjected to various environmental conditions with varying absorption of water and relative humidities. After appropriate treatment, the authors observed the proliferation of fungi and actinomycetes, after two weeks of incubation. The results showed that when water was absorbed by capillary action, fungal growth was faster in early wood-based materials under 20 % (w/v) humidity. Condensation under varying humidity and temperature was responsible for the the rapid growth of different fungal populations, particularly under high humidities. It is noteworthy that the fungal species were particularly tolerant to fluctuations in temperature and humidity, with very little effect viability.

Hyvarinena *et al*. (2002) studied the diversity of fungi and actinomycetes in spaces designed with building materials of different natures, amenable to turn into microenvironments for their proliferation. In particular, cellulosic materials and ceramics, paints and plastics, in a clear state of decomposition, were evaluated for the presence of microbial populations. The authors found approximately 100 cfu g^{-1}, with the largest microbial populations associated with the presence of lignocellulosic and paper based materials. The authors also observed that bacterial populations were lower than fungal populations, in contrast to the great fungal diversity, particularly of the genus *Penicillium* sp., as well as a great number of yeasts. In paper based materials the main fungal genera found were *Cladosporium* and *Stachybotrys*; in glues and paints the most prevalent genus was *Acremonium* and the species *Aspergillus versicolor*. According to the authors, the main contribution of that research was to show the association between microbial growth and its occurrence in building materials of different nature. In particular, the authors highlighted a certain degree of specificity between the type of material and the predominant fungal genera.

Shelton *et al*. (2002) examined 12,026 fungal air samples (9,619 indoor samples and 2,407 outdoor samples) from 1,717 buildings located across the United States; these samples were collected during indoor air quality investigations performed from 1996 to 1998. The most common cultivable airborne fungi, both indoors and outdoors and in all seasons and regions, were *Cladosporium*, *Penicillium*, non-sporulating fungi, and *Aspergillus*. *Stachybotrys chartarum* was identified in the indoor air in 6 % of the buildings studied and in the outdoor air of 1 % of the buildings studied. Kulcsar Neto and Siqueira (2003) suggested reference standards for microbiological quality in interiors, both in terms of quality and quantity. The authors state that it is not acceptable the presence of the following pathogenic species or toxigenic fungus: *Histoplasma capsulatum, Cryptococcus neoformans, Paracoccidioides brasiliensis, Aspergillus fumigatus, Aspergillus parasiticus, Aspergillus flavus, Stachybotrys atra* and *Fusarium moniliforme*.

Nielsena *et al*. (2004) studied the influence of relative humidity and temperature on the growth and metabolism of selected fungal species in various types of building materials. The authors evaluated the microbial metabolic diversity, after incubation of several samples of building materials based on wood, starch and composite materials, at temperatures varying from 5 to 25 °C, under 69 to 95 % relative humidity, during seven months. The authors observed a high diversity of species present on the materials, with a prevalence of the genera *Penicillium* sp., *Aspergillus* sp., and *Eurotium* sp., all of them mycotoxins producers.
Bortoletto (2005) reports the presence of fungal contamination in a large library due problems related to control of the heating and air

conditioning system and air humidity, which gave rise to outbreaks of occurrence of fungi, whose removal was being done in a manner concomitant with the repair of the cooling system. After the outbreaks structural interventions, corrective and preventive actions have been suggested, which included fumigation of environments for inactivation of fungal structures, complete cleaning of the ducts by mechanical cleaning, followed by a new fumigation after washing. Books in the collection found in the monitored environment presented fungal cells of *Aspergillus* sp., *Penicillium* sp., *Cladosporium* sp. and *Trichoderma* sp., all present in the environments selected from the library, with populations of about 800 fungal and bacterial populations cfu/m^{-3} of 500 cfu/m^{-3}, respectively.

Burge *et al*. (2006) collected volumetric culture plate air samples on 14 occasions over the 18 month period immediately following a building occupancy. On each sampling occasion, the authors collected duplicate samples from three sites on three floors of the building, and an outdoor sample. Fungal concentrations indoors were consistently below those outdoors, and no sample clearly indicated fungal contamination in the building, although visible growth appeared in the ventilation system during the course of the study.

Aira *et al*. (2007) found some fungi in the architectural complex of the Cathedral of Santiago de Compostela in Spain, observing the presence of 35 different genera, mainly *Alternaria* sp., *Aspergillus* sp., *Cladosporium* sp. and *Penicillium* sp.. Interestingly, the authors did not find differences between populations inside and outside the Cathedral, with a maximum occurrence between spring and summer. The amount of fungi found was relatively small at various points of the central nave of the Cathedral, while in Corticela Chapel this number reached 6,500 cfu/m^{-3}. There was also a higher incidence of microorganisms around 13:00 hrs (around 400 cfu/m^{-3}), where the flow of visitors reaches a peak. Giannantonio *et al*. (2009) observed the presence of incrustations on concrete surface, under controlled laboratory conditions, due to the direct action of the fungal genera *Alternaria* sp., *Cladosporium* sp., *Epicoccum* sp., *Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Pestalotiopsis* sp. and *Trichoderma* sp. on the concrete. Mesquita *et al*. (2009) used advanced techniques of molecular biology to elucidate the fungal morphology and to evaluate the infection of historical documents. The researchers identified a wide diversity of fungi, on parchment, laid and wood pulp paper. Authors identified fourteen genera of fungi, the most frequent *Cladosporium* sp., *Penicillium* sp. and *Aspergillus* sp., and less abundantly the presence of the genera *Alternaria* sp., *Botrytis* sp., *Chaetomium* sp., *Chromelosporium* sp., *Epicoccum* sp., *Phlebiopsys* sp. and *Toxicocladosporium* sp.

According to Mesquita *et al*. (2009), *Rhizopus* sp. is known to cause organic dust syndrome. The concentration of mycoflora was recorded highest from the month of August to January (during monsoon and winter season) and gradually declined towards May (summer season). Abe (2010) found fungal contamination of materials stored in an art museum, which was monitored according to a biological index related to climatic parameters, giving an indication of the environmental capacity to maintain and proliferate fungal cells. To determine this index, fungal spores were encapsulated, followed by the observation of the germination of spores, and measurements of the extent of fungal hyphae. The authors identified a predominant occurrence of *Aspergillus* sp. and *Eurotium penicillioides*. A number of other microbial populations was reported in the literature, specific to intrinsic characteristics of materials where populations grow, as well as with environmental factors that regulate proliferation.

Hoang *et al*. (2010) evaluated the susceptibility of "green" building materials to biodeterioration by *Aspergillus nige*r, an indoor reference fungus. The detection of spores and the presence of external compounds acting as nutrients contributed to the growth of *Aspergillus niger* on walls and ceilings gypsum. The authors

found a strong correlation between the content of the mixture and organic materials by observing the time for coating of 50 % of surface area by fungi. The results suggested that the presence of organic matter in a given material appears to be an important factor for the diagnosis of fungal susceptibility to a subsequent possible biodeterioration. Not only are the materials responsible for the spread of fungal spores and bacteria, but also the climatic conditions that regulate the environment, internally or externally.

According to Hoang *et al*. (2010), *Aspergillus* and *Alternaria* are showing high prevalence in air in store houses. Spores of *Alternaria, Penicillium* and *Cladosporium* play a significant role in causing allergic asthma2. In our survey these species are showing moderate to high occurrence. Many fungus spores can survive in difficult conditions like low temperatures in winter and high temperatures in summer and can be transported by air. The five sampling sites shown high moisture conditions in surrounding areas which might be one of the reasons for high prevalence of fungi. Fungal density in the air reaches the highest level in monsoon and winter compared to summer. The increase in fungal density in October – January plays a significant role in seasonal distribution and highest fungus isolation was observed in this month.

6. Detection of fungi in an open environment

Atmosphere sampling for bioaerosols has been conducted for decades with classical monitoring that relies on collection using forced air samplers and analysis by either culture media or microscopy (Stetzenbach *et al*., 2004). Quantitative microbiological methods for atmosphere analysis witnessed important developments in the 1940 - 1960. K. R. May' s cascade impactor, described in 1945 (May, 1945), was one of the first instruments that allowed the detection of fungal cells, since collected all particles with 0.6 – 20 µm. The cascade impactor consisted of a system of four air-jets and sampling slides in series. The slits were progressively narrower, so that the speed jet and therefore the efficacy of impaction of particles increase from slide to slide. Particles impacted on glass slides covered with an adhesive substance, and, at the end, were counted by optical microscopy. The instrument allowed discrimination of the particles by size due to the four successive stages (Burge and Solomon, 1987).

An improvement of May's device was carried out by J. M. Hirst, in 1952 (Hirst, 1952). The instrument was also a slit sampler based on impaction on an adhesive surface, but allowed monitoring during a whole day (achieved by the slow and constant displacement of the slide underneath the slit) and with strong winds and rain. The equipment was reliable for capturing large spores. Small spores such as those of *Aspergillus* and *Penicillium* were underestimated (Martinez *et al*., 2004).

May and Hirst slit impactors allowed no distinction between viable and dead cells, and, very importantly, did not enabled a rigorous identification of the fungal spores, since morphological characteristics of these cells only allow an identification at a genus level, and only for a restricted group of fungi (Stetzenbach *et al*., 2004). These drawbacks were resolved in the slit sampler developed by Bourdillon and collaborators in the 1940s (Bourdillon *et al*., 1941). Using the same principle of air suction through a narrow slit, a Petridish with culture medium was placed underneath. The dish slowly rotated during sampling, so that an annular ring trace was formed in the agar. Bacteria were collected with very high efficiency (Henningson and Ahlberg, 1994).

A great step forward was given by Andersen in 1958, with the design of a six-stage impactor, with collection of particles on culture medium (Andersen, 1958). Air sucked passed six successive aluminium plates drilled with decreasing size holes. Underneath each plate was placed a Petri dish with culture medium. The decreasing size of the holes forced air to accelerate from the upper to the lower stage. The upper stage collected the biggest particles and the lowest stage the smallest cells. Between these, increasingly

smaller cells were collected. Andersen sampler allowed discrimination of the particles by size, the determination of the concentration of culturable cells, and, after observation of the colonies, the identification of the fungi at species level (Martinez *et al*., 2004; Stetzenbach *et al*., 2004).

May, Hirst, Bourdillon and Andersen samplers were based on impaction on a solid surface - the projection of particles onto the surface of a glass slide or culture medium. By the time of design of these samplers, impingement - blowing the particles into a liquid by the use of glass impingers – was also improved in order to be used in microbiological analysis. Impingement is based on the suction of the air through a narrow capillary tube, and projection of the air jet into a liquid. Particles present in the atmosphere, such as fungi, are forced to enter the liquid.

From impinger models adapted to microbiological uses, stands out the all-glass impinge AGI-30 described by Malligo and Idoine (1964) and the three-stage impinger described by K. R. May, in a paper published in 1966 (May, 1966). AGI-30 impinger was developed from the AGI-4 model - the Porton impinger. The inlet was designed to simulate the human nose. The jet nozzle was raised above the liquid in order to get an impingement surface softer than the glass bottom of the flask. The collection efficiency for bacteria was very high (Eduard and Heederik, 1998).

The multi-stage liquid impinger of May (1966), built in thick walled Pyrex glass, had three superimposed chambers. In the first two chambers, air-jets impacted vertically on to glass discs filled with sampling liquid. The third chamber was a bowl-shaped swirling impinge (Martinez *et al*., 2004). Impingement has some advantages over impaction on solid surfaces: 1) if the concentration is too high, the liquid can be diluted; 2) affords, simultaneously, total cell counting (by microscopy) and culturable cell counting (by culturing aliquots on nutrient media); 3) different culture media can be used, at the same time, to study a given sample; 4) collection of the cells in a liquid avoids desiccation resulting from impaction on solid surfaces, especially on glass slides; 5) cell clusters, kept intact when using impaction of agar medium, are dissociated in their individual cells; 6) the particle retention efficiency is very high; 7) the equipment is compact and inexpensive (Martinez *et al*., 2004; Stetzenbach *et al*., 2004). The method has however some limitations: 1) it is not appropriate for clean atmosphere, since a reduced number of cells will be present in a relatively large volume of liquid; 2) after certain time of operation, the liquid, which is under low pressure, evaporates appreciably; 3) the efficiency for collecting bacteria is higher than for spores (Eduard and Heederik, 1998).

In addition to impaction and impingement, other methods have been used in the study of fungal populations in atmospheric bioaerosols. In filtration methods, filters collect particles through impaction and interception mechanisms. Filter materials commonly used for air microbiological sampling include glass fibre filters, mixed cellulose esters, polytetrafluoroethylene, polyvinyl chloride, gelatine, and polycarbonate (Martinez *et al*., 2004). Advantages of filter sampling include the simplicity of collection and sample handling procedures, the ability to perform different analyses on the same extraction solution, and the relatively inexpensive cost. Membrane filters can be placed directly on the surface of culture medium, or washed with a liquid, and this added to culture medium. Certain filters are dissolvable in warm liquids, and the resulting suspension can be platted on agarized medium. Two disadvantages for filter sampling are the low extraction efficiency from the filter material, and the dehydration of microorganisms, which reduces their cultivability (Martinez *et al*., 2004).

Sedimentary sampling is generally carried out using the settle plate method. Open Petridishes with appropriate culture medium are left open during a given time (minutes, hours or days depending on the air contamination load). After a certain period of incubation, colonies are counted and identified. Sedimentary sampling has several advantages: 1) it is simple and inexpensive; 2) allow a cumulative assessment over a prolonged

exposure times. The cfu collected on settle plates are like a photocopy, or a mirror of what was going on at a particular point, during a period of time. Long sampling periods may increase measurement significance and reproducibility (Pasquarella *et al*., 2000). The method suffers however from several limitations: 1) no known volume of air is analyzed, it is therefore not quantitative; 2) the rate of deposition of cells can be affected by air turbulence; 3) small cells tend to be under-estimated (Pasquarella *et al*., 2000).

Pasquarella *et al*. (2000) argued extensively about the advantages of the sedimentary methods for hospital indoor microbial analysis. A new index was defined - the Index of Microbial Air Contamination (IMA), determined with the following procedure: A standard petridish 9 cm in diameter containing plate count medium is left open to air according to the 1/1/1 scheme, for 1 h, 1 m from the floor, at least 1 m away from walls or any relevant physical obstacle. After 48 hrs incubation at 36 ± 1 °C the colonies are counted. The number of colonies is the IMA. The IMA classes and maximum acceptable levels of IMA were defined empirically. Five classes of IMA were devised: 0- 5 very good; 6-25 good; 26 -50 fair; 51-75 poor; >76 very poor. Maximum acceptable values of IMA were established, related to different infection or contamination risks. These were 5, 25 and 50, in places with very high, high and medium risk, respectively. For example, hospital operation rooms, with very high risk, should have a maximum IMA value of 5, corresponding to 9 cfu × dm^{-2} × h^{-1}. The authors also provided a comparison between IMA classes and several international standards.

Two standard incubation temperatures are used: 25 – 27 °C for growing the great majority of species, and 35 – 37 °C, for human-related species such as *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger* (Araujo *et al*., 2008). Spores of *Aspergillus* and *Penicillium* may survive for long periods, even years, whilst the cultivability or viability of others may decline very rapidly. The use of culture-based analysis methods underestimates populations in bioaerosols owing to the detection of only those fungi that grow in culture media, while non-culturable (live or dead) organisms go undetected. As with other environments, most of atmospheric fungi appear to be in a non-culturable sate (Flannigan, 1997).

Fungi are capable of causing health effects whether they are in the culturable or non-culturable but viable state. However, these effects are expected to be very different, but are poorly known. Can a live but non-culturable fungal spore, hypha or fragment grow on the surface of our respiratory epithelium? Enumeration of total fungi by microscopy lacks identification specificity, unless accompanied by specialized staining or immunological assay. Specific antibodies with heavy metals bind only to specific microbes that are viewed under scanning electron microscopy. Epifluorescence and electron microscopy has also been used. With fluorescence microscopy, microbes are stained with fluorochromes and are viewed with fluorescent light. Fluorescein diacetate (FDA) has been used for viable fungi. Scanning electron microscopy is useful for studying fungal spore surface characteristics, but is not routinely used for microbes identification (Martinez *et al*., 2004).

In addition to these methods, biochemical assays that detect fungal specific molecules such as (1,3)- β -D-glucans, chitin, and ergosterol have been to estimate total fungal bioaerosol loads. These are particularly important for the quantification of fungal fragments, which are non-culturable and difficult to recognize by microscopy (Martinez *et al*., 2004; Stetzenbach *et al*., 2004). As Flannigan (1997) wisely remarked, most microbiological investigations of indoor air still employ culture-based methods, but sufficient attention is seldom given to four important issues: sampler performance, temporal variability, culture media and accurate identification. Too many studies identify only to the genus level and disregard the diversity of species, their ecology and potential significance for health, especially in important genera such as *Aspergillus* sp. and *Penicillium* sp.

7. Agroindustrial wastes

Agricultural - based industries produced the vast amount of residues every year. If these residues are released to the environment without proper disposal procedure that may cause to environmental pollution and harmful effect on human and animal health. Most of the agro-industrial wastes are untreated and underutilized, therefore in maximum reports it disposed of either by burning, dumping or unplanned landfilling. These untreated wastes create different problems with climate change by increasing a number of greenhouse gases. Besides this, the use of fossil fuels also contributing the effect on greenhouse gases (GHG) emission (Bos and Hamelinck, 2014). So, now it is a worldwide concern to dictating the improvement of alternative cleaner and renewable bioenergy resources (Okonko *et al*., 2009).

The agroindustrial wastes cause a serious disposal problem (Rodriguez – Couto, 2008). For examples, the juice industries produced a huge amount of waste as peels, the coffee industry produced coffee pulp as a waste, and cereal industries produced husks. All over the world approximately 147.2 million metric tons of fiber sources are found, whereas 709.2 and 673.3 million metric tons of wheat straw residues and rice straws were estimated, respectively, in the 1990s (Belewu and Babalola, 2009). As per the composition of these agro-industrial residues are concerned, they have high nutritional prospective, therefore they are getting more consideration for quality control and also categorized as agro-industrial by-products (Graminha *et al.,* 2008).

Various studies reported that different kinds of waste such as pomegranate peels, lemon peels and green walnut husks can be used as natural antimicrobials (Katalinic *et al*., 2010; Adamez *et al*., 2012). Wastes from the organic compounds although a risk to the atmosphere, but they represent a possible source for making of mushrooms as foodstuffs and other bio-based products like bio-energy and biofertilizers. Some of the agricultural residues are used for animal food. However, such wastes contain variability in composition like high amount of proteins, sugars, and minerals. Due to high nutritional composition, these residues not described as "wastes" but considered as raw materials for other product formation and developments.

The availability of these nutrients in raw materials offers appropriate environments for the growth of microorganisms. These microorganisms have got the ability to reuse the raw materials with the use of fermentation processes. The agro-industrial residues are used for solid support in Solid state fermentation (SSF) developments for making different beneficial products. It also helps for the production of fermentable sugars by reducing the production cost on the basis of food crops. Various studies were carried out to know the conversion of agricultural waste into sugars by using different microorganisms (Nguyen *et al*., 2010).

8. Types of Agroindustrial wastes

Agricultural Residues

Agriculture residues can be divided into field residues and process residues. Field residues are residues that present in the field after the process of crop harvesting. These field residues consist of leaves, stalks, seed pods, and stems, whereas the process residues are residues present even after the crop is processed into alternate valuable resource. These residues consist of molasses, husks, bagasse, seeds, leaves, stem, straw, stalk, shell, pulp, stubble, peel, roots, etc. and used for animal feed, soil improvement, fertilizers, manufacturing, and various other processes. Huge amount of field residues are generated and most of them are underutilized. Controlled use of field remains can enhance the proficiency of irrigation and control of erosion. In Middle East region, wheat and barley are the major crops. In addition to this, various other crops like rice, lentils, maize, chickpeas, fruits, and vegetables are also produced all over the world. Agricultural residues are differentiated on the basis of their availability as well as characteristics that can be different from other solid fuels like charcoal, wood, and char briquette (Zafar, 2014).

Industrial Residues

A huge amount of organic residues and related effluents are produced every year through the food processing industries like juice, chips, meat, confectionary, and fruit industries. These organic residues can be utilized for different energy sources. As the population increases continuously, the requirement of food and their uses also increased. So, in most of the countries, different industries of food and beverage have increased remarkably in that region for fulfillment of need of food. Approximately, 20 % of the production of fruits and vegetables in India are going waste every year (Rudra *et al*., 2015) because in India a large amount of apple, cotton, soy bean, and wheat are produced. So as the production increased in the country, it also increased the percentage of waste produced from them. Similarly, the waste produced from food industries contains high value of BOD, COD, and other suspended solids. Most of these wastes are left unutilized or untreated, which caused adverse effect on environment as well as human and animal health but the composition of these wastes contains a large number of organic compound that produced a variety of value-added products and also reduced the cost of production.

Especially in oil industries, huge amount of processed residues are produced after oil extraction from the seeds; these residues are known as oil cakes. These industries cause air, water, and solid waste pollution because these residues contain high concentration of fat, oil, grease, suspended solids, and dissolved solids. Oil cakes have variabilities based on their substrate. Oil cake is of different types like canola oil cake, sunflower oil cake, coconut oil cake, sesame oil cake, mustard oil cake, palm kernel cake, soy bean cake, groundnut oil cake, cotton seed cake, olive oil cake, rapeseed cake (RSC) (Ramachandran *et al*., 2007).

9. Production of low cost culture medium for fungi using waste materials

India is the second major producer of vegetables in the world and contributes 14% of total world vegetable production. Taking estimated production of fruits and vegetables in India at 150 million tons, the total waste generation comes to about 50 million tons per annum. Fruits and vegetables wastes are more prone to spoilage than cereals due to their chemical composition. This creates unhygienic condition leading to spread of diseases and loss of resources. The wastes produced from these vegetables are a rich source for nitrogen and carbohydrate but are not fit for consumption. This resource can be utilized for the production of not so fastidious mushroom such as the oyster variety. In the recent times the management of waste disposal has created a hot of topic for debate. Proper management and execution of waste disposal practices have become the need for the hour. The inappropriate management of waste has led to many problems such as rapid spread of infectious diseases, development of new varieties of diseases and inability of the common man to cope with them. The exponential increase in the present amount of waste produced brings to notice an immediate requirement of an answer to the threat. Managing the day to day wet waste produced at a home seems to be the only probable solution (Singh and Singh, 2012).

Microorganisms require carbon, nitrogen, minerals, sometimes growth factors, water and oxygen if aerobic, as elements for cell biomass, energy, biosynthesis and cell maintenance. The maximum production of some metabolites requires the incorporation of specific inhibitors in the medium either to minimize formation of metabolic intermediaries or to prevent further metabolism of the desired product (Martin *et al*., 2004). The prime ingredients of the media are water, energy sources, sources for carbon, nitrogen and minerals, chelators, growth factors, buffers, precursors, inhibitors etc.

Large amount of wastes are generated every year from the industrial processing of agricultural raw materials and individual homes. Most of these wastes are used as animal feed or burned as alternative for elimination. However, such wastes usually have a composition rich in sugars, minerals and proteins, and therefore,

making them useful for other processes directly or indirectly. The presence of carbon sources, nutrients and moisture in these wastes provides conditions suitable for the development of microorganisms and this open up great possibilities for their reuse. The economical aspect is based on the fact that such wastes may be used as low - cost raw materials for the production of other value - added compounds, with the expectancy of reducing production costs. The environmental concern is because most of the agro-industrial wastes contain phenolic compounds and other compounds of toxic potential which may cause deterioration of the environment when the waste is discharged to the nature. The need to develop alternative media to various culture media has become imperative as the conventional media used are either not readily available or relatively expensive in most developing countries of the world (Barnett *et al*., 2000).

Microbiological studies based on the ability of grow and maintain microorganisms under laboratory conditions by providing suitable culture media that offer favourable conditions. Appreciable minerals, amino acids and carbohydrates which are the essential requirements in a suitable fungi cultivation medium were present in the agro wastes materials used for the alternative fungi growth medium. According to Sudha and Abraham (2003), these compounds are essential to support the growth of microorganisms without supplement. Media containing high carbohydrate source, nitrogen source are required for the growth of fungi at pH range of 5 to 6, and a temperature range from 15 to 37 °C. The results obtained in this study with Plantain glucose agar (PGA) and Yam glucose agar (YGA) showed optimal spore growth which may be as a result of the nutritional constituents of the agro wastes.

The ideal medium for reference testing is mandated to be totally defined, reproducible, free of antagonists or boosters of antimicrobial action, well buffered to maintain pH, and available in both liquid and solid formulations. These criteria were met by the YGA and PGA. However, pineapple glucose agar could not gel on addition of agar No 1 to use for susceptibility test in solid form.YGA and PGA was exceptionally naturally buffered based on the results obtained without further micronutrients enhancement. The uncertainties associated with agar such as influencing the results of susceptibility testing and the substances that can stimulate growth of certain microorganisms which include biotin, thiamine, and other unknowns (Barnett *et al*., 2000), susceptibility testing with YGA and PGA were of interest as reference medium for susceptibility testing.

Studies of various researchers revealed that the agrowastes contains minerals and nutrients that can meet the nutritional requirements of fungi, thus they can be utilized as an alternative material in the formulation of culture media for the *in vitro* cultivation of fungi. An important advantage of the food crop peels used in formulating the various media is there readily availability, cheap if at all they are to be purchased and need no further enhancement with supplements to give maximum fungi growth. Using agro wastes for fungal cultivation media will help in reducing the amount of waste in the environment to avoid the menace wastes constitutes in some areas. It was highly recommended that the researchers and students could make use of these agro wastes in media formulation for fungi isolation.

Okunowo *et al*. (2010) also observed least sporulation and minimum mycelia growth of *Myrothecium roridum* on Czapek's Dox agar which may be due to the presence of chloride ion in the test medium. The mycelia growth of the organism on different nitrogen sources was found to be highest on sodium glutamate containing medium and lowest on ammonium chloride containing medium. However, in LCA despite the presence of chloride ion, as in case of CYA, mycelial growth and sporulation. Several workers have recognized the importance of reproductive structures for inoculums production and studies have been conducted on the effects of various media components along with important

physiological parameters that lead to maximum sporulation (Kim *et al*., 2005; Saha *et al*., 2008). Type of culture media and their chemical compositions significantly affected the mycelia growth rate and conidial production of *Phoma exigua* (Zhae and Simon, 2006).

Potato Dextrose Agar (PDA) is one of the most commonly used culture media because of its simple formulation and its ability to support mycelial growth of a wide range of fungi. Several workers stated PDA to be the best media for mycelial growth (Maheshwari *et al*., 1999; Saha *et al*., 2008). Most fungi thrive on PDA, but this can be too rich in nutrients, thus encouraging the mycelial growth with ultimate loss of sporulation (UKNCC, 1998). In the present study, *C. funicola* showed heavy perithecia formation in LCA. Osono and Takeda (1999) stated that LCA because of its low glucose content suppresses the overgrowth of fast growing species and induces sporulation, hence this medium is useful for fungal identification. The fungal systematic is still based mainly on morphological criteria as observable characteristics. Hence, fungi are recognized and identified basically by their phenotypes (Zain *et al*., 2009). Moreover, the variations in colour of spores, especially among *Aspergillus* and *Penicillium* species, are one of the main criteria used widely for their identification and taxonomic placement (St-Germain and Summerbell, 1996) which seems to be mainly attributed to the constituents of a medium.

A growth medium is a liquid or gel designed to support the growth of microorganisms. The commercially available media are very costly. Routine practical require large amount of media on regular basis for streak plate, pour plate, spread plate experiments. Availability of low cost media rich in nutrients, giving comparative results is the need of the day. The search for alternative, cheap media for use in laboratory agents for routine microbiological experiments is going on. Recent research has been focused on finding alternatives to gelling agents of media, agar in particular, and media, in general, because of its exorbitant price (Mateen *et al*., 2012).

Microorganisms are omnipresent and very diverse. Preparation of suitable culture media is one of the necessary to study them. Different microorganisms grow in different environments and have variety of growth requirements; like nutrients, pH, osmotic conditions and temperature (Nichols, 2007). The current limitations of cultivation of microbes in lab need to be addressed by formulation of newer media. Microbial culture media can be of different types, depending on the nutritional growth requirements of the microorganisms. Microorganisms require various macro elements namely Carbon, Hydrogen, Oxygen, Nitrogen, Sulphur, Phosphorous, Potassium, Calcium, Magnesium and Iron. For Carbohydrates, Lipids, Proteins and Nucleic acids synthesis Carbon, Hydrogen, Oxygen, Nitrogen, Sulphur and Phosphorous are used, while Potassium, Calcium, Magnesium and Iron exist in the cell as cations, playing a variety of roles. In addition to macro elements, all microorganisms require several microelements like Manganese, Zinc, Cobalt, Molybdenum, Nickel and Copper. These are generally part of enzymes and cofactors. Microorganisms also require organic compounds as growth factors.

Different media for the growth and isolation of organisms have been reported from different substrates (Basu *et al*., 2015). Some vegetables and fruits have been used to cultivate both fungi and bacteria, such as Gooseberry, Carrot, Tomato, Cabbage, Pumpkin etc (Pham and Kim, 2012) with easily available low cost material as substitutes for Nutrient Agar. Some others have used cow pea, green gram and black gram as starch and protein substitutes to reduce the cost of microbial media (Tharmila *et al*., 2011).

Mondal *et al*. (2012) used cucumber and orange peels to evaluate the production of single cell protein using *Saccharomyces cerevisiae* by submerged fermentation. The authors state that the bioconversion of fruit wastes into single cell protein production has the potential to solve the

worldwide food protein deficiency by obtaining an economical product for food and feed. Fruit wastes rich in carbohydrate content and other basic nutrients could support microbial growth. Apple, turnip, papaya and banana peels were used for alcohol fermentation and biomass production by Kondari and Gupta (2012). The use of legume seeds as alternative nutrient media for bacteria and fungi has been reported (Ravimannan *et al*., 2014).

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