**Introduction:**

Spices are the most important group of agricultural commodities, because of their aroma and taste they are widely used to flavour the food preparations. Spices are also having aromatic and medicinal properties; hence they are widely used in traditional medical practices such as Ayurveda, Siddha etc and they are also used in pharmaceutical, perfumery and cosmetic industries. Coming to the history of Spices India is known as “Home of Spices” The Persians are the first traders who transported these commodities from India to neighbouring Molucca islands. The Spice trade attracted peoples from western countries to India. Spices play a significant role in our national economy; Spices are mainly grown in states like Kerala, Karnataka, Andhra Pradesh, Maharashtra, Rajasthan and Bihar etc.

Spices have do not have much nutritive value but the importance of Spice in daily diet is due to the fact that they enhance the aroma and flavour of food preparations. Spices are well known appetizers, where they enhance the secretion saliva of gastric juice for this reason Spices are commonly known as food adjuncts. There is lot of heterogeneity found with respect to the plant parts used as a Spice viz. rhizome, bark, leaf, latex, bulb, bud, flowers, fruit, resin and seeds etc. Spices mainly contain carbohydrates, protein, fats and volatile oils etc.

India is one of the largest producers of Spices and condiments also India is one of the major contributor to the total world trade. The classification of the Spices and medicinal plant samples is mainly based on morphology and the plant part which is used for the purpose.

The variety and galaxy of fungi and their natural beauty occupy prime place in the biological world and India has been the cradle for such fungi, only a fraction of total fungal wealth has been screened and still, mycologists have to unravel the unexplored and hidden wealth. One third of fungal diversity of the globe exists in India. Out of which 1.5 million of fungi are characterized till now around 5–10% of fungi can be cultured artificially. Fungi are not only beautiful but play a significant role in the daily life of human beings. (Manoharachary et al., 2005); Although fungi are among the most important organisms in the world, only limited information is currently available for most species and current estimates of species numbers for fungi differ significantly. While the figure of 1.5 million
estimated fungal species is commonly used, critics have questioned the validity of this estimate. Data on bio geographic distributions, levels of endemism, and host specificity must be taken into account when developing estimates of global fungal diversity. (Mueller et al., 2007).

Fungi are ubiquitous inhabitants of soil and aquatic environments, and they may have either parasitic or symbiotic relationships with animals and plants. They play a major role in nutrient cycling, especially in organic matter decomposition and they are the major sources of biologically active compounds. (Orellana et al., 2013).

The Fungi comprise a diverse kingdom of eukaryotes that are characterized by a typically filamentous in nature but some are having unicellular vegetative form and heterotrophic, absorptive mode of nutrition. Their simple morphologies and variable ecological strategies have confounded efforts to elucidate their limits, phylogenetic relationships, and diversity. (McLaughlin, 2009).

The hypothesis that there are 1.5 million fungal species on Earth, of which only about 70,000 are described, remaining fungi are undescribed. The recognition that many new species have yet to be describe is the prime importance to plant pathologists, agronomists, and plant regulatory officials for new organisms which may be used as biological control agents or for their pharmaceutical importance. (Hawksworth, 1997).

Fungi are essential to the survival of some groups of organisms with which they form associations. (Blackwell, 2011); multiple uses of fungi are seen from in early historic times. Fungi were used for production of cheese, bread, wine, beer and other foodstuff. Based on these processes, fungi today have become one of the most important group of organisms in modern technology for their multiple applications in various fields. (Molitoris, 1995).

Fungal products are essential building blocks for sustainable future for our planet. In nature fungi are specialized group of organisms which helps in breaking down plant materials by the means of rich spectrum of enzymes. In industry such fungal products can be brought in use for converting biowaste and agricultural crop residues into bioenergy, biomaterials, biochemical’s, biofertilizer etc. But we
need to understand fungal biology and diversity better before; so that we can use it in efficient manner. (Lange, 2010).

It has been estimated that 25% of the world’s crops are affected by mould or fungal growth and as stable natural contaminants of the food chain; mycotoxin reduction requires a multifaceted approach, including farmers, government agencies, food processors and scientists. This can have a significant impact on the cost of food production. An international regulatory standard for mycotoxins in food commodities determines the extent of global trade in contaminated commodities. (Bryden, 2007).

There is an increasing knowledge and curiosity in understanding the role played by moulds in food Spoilage. Especially the discovery of mycotoxin production in foods has highlighted the importance of moulds in food quality. It is however, only within the last 5-10 years that major progresses have been made towards the prevention of spoilage caused by moulds. This is due to recent international agreements on taxonomy and analytical methods for food borne moulds, which has led to the discovery, that a specific, very limited mycobiota is responsible for the spoilage of each kind of food. (Filtenborg, et al., 1996). Food borne disease outbreaks caused by Spices have been increase in recent years. Spices are used for flavour, colour, aroma, taste and preservation of food and beverages but like any other object, they are also not free from microbial association. (Chaudhary, 2014).

Growth of commonly occurring filamentous fungi in foods may result in production of toxins known as mycotoxins, which can cause a variety of ill effects in humans and also create allergic responses. The most important mycotoxins are Aflatoxins, Ochratoxin, Fumonisins, Trichothecenes and Zearalenone, (Pitt, 2000); during harvesting, processing and handling practices food become contaminated with a wide range of microorganisms. Subsequently, during transport, distribution and storage a small fraction of these will develop and cause serious deteriorations or damages. Microbial growth and biochemical changes depends upon nature of food and Environmental factors. (Jos et al., 1996).

Mycotoxins are small, toxic chemical products formed as secondary metabolites by a few fungal species that readily colonise crops and contaminate
them with toxins in the field or after harvest (Turner, et al., 2009). Mycotoxins likely have existed for as long as crops have been grown but recognition of the true chemical nature of such entities of fungal metabolism was not known until recent times. Evidence of their periodic, historical occurrence exists until the recognition of Aflatoxins in the early 1960s. At that time mycotoxins were considered as a storage phenomenon whereby grains becoming mouldy during storage allowed for the production of secondary metabolites proven to be toxic when consumed by man and other animals. (Richard, 2007)

Mycotoxicoses are diseases caused by mycotoxins, although they occur more frequently in areas with a hot and humid climate, favourable for the growth of moulds, they can also be found in temperate zones. Exposure to mycotoxins is mostly by ingestion, but also occurs by the dermal and inhalation routes. (Peraica et al., 1999). Moulds and associated mycotoxins are important factors adversely affecting foods and feed production. Mycotoxins are toxic to humans and animals, which explains the major concern of food and feed industries in preventing them from entering the food chain. (Jouany, 2007).

The major types of mycotoxins are Aflatoxins, Trichothecenes, Fumonisins, Zearalenone, Ochratoxin, and Ergot alkaloids. The aflatoxins are produced by *Aspergillus flavus* and *A. parasiticus* and they are important agents of disease. The fumonisins are produced by *Fusarium verticillioides*, these toxins are capable of causing significant disease in some domesticated animals. The Trichothecenes are a large class of mycotoxins produced by several fungal genera. *Fusarium* species and some species of *Stachybotrys* most commonly occurring Trichothecene is Deoxynivalenol. Zearalenone is produced primarily by *F. graminearum* causes estrogenic responses in swine. The Ochratoxins are produced primarily by *Penicillium verrucosum* and cause disease in animals Ergot alkaloids are produced primarily by several species of *Claviceps* that are plant pathogenic, and Ergotism is one of the oldest recognized mycotoxicoses. (Cast, 2003)

Contamination of cereal commodities by moulds and mycotoxins results in dry matter, quality and nutritional losses and represents a significant hazard to the food chain. Cereal quality is influenced by a range of abiotic and biotic factors viz. stored grain ecosystem, grain contamination, insect pests, rodents and the
environmental factors such as temperature, water availability etc. and type of preservatives added (Dantigny et al., 2007).

Mycotoxin contamination in certain agricultural systems has been a serious concern for human and animal health. Mycotoxins are toxic substances produced mostly as secondary metabolites by fungi; they grow on seeds and feed in the field, or in storage. The major mycotoxin producing fungi are species of *Aspergillus, Fusarium* and *Penicillium* and the important mycotoxins are aflatoxins, fumonisins, Ochratoxins, Cyclopiazonic acid, Deoxynivalenol, Nivalenol, Patulin and Zearalenone (Vinod Kumar et al., 2008). Spices were contaminated by some fungi that might constitute health hazards for humans (Elshafie, 2002).

Mycotoxins are undesirable compounds often found in cereal grains and forages. Production of mycotoxins is dependent on environmental conditions and agricultural practices. Moisture level ranges between 13%-18% and temperatures between 20\(^{\circ}\) C and 30\(^{\circ}\) C can increase fungal growth rate during growing seasons as well as during transport and storage. (Majeed, 2013). India, despite being the largest producer of Spices, there is great potential for increasing export of Indian Spices. To realize this potential there must be improvement of productivity and quality (Sumanth, 2010).

The Spices including black pepper, fennel and cumin are extensively utilized by majority of world’s population in response to multifold usage as food, medicine, flavouring and colouring agent. In storage, seed borne contaminants not only deteriorate the seeds but also alter the physiochemical properties of seeds leading to increase the chances of consuming toxic elements, produced by storage mycoflora. (Hedawoo, et al., 2014).

Spices and herbs are natural products or their blends that must be free of extraneous matter. Conventional production of these products implicates a number of hygienic problems so Spices and herbs may be exposed to a wide range of microbial contamination during pre and post harvest practices and they can present high microbial counts. (Sospedra, et al., 2010)

The moulds occurs in soil, decaying vegetation, hay and grains undergoing microbiological deterioration and invades all types of organic substrates whenever
and wherever the conditions are favourable for their growth. Favourable conditions include high moisture content and high temperature (Rajarajan, 2013).

The crop is often attacked and deteriorated by a number of pathogens; many of these are seed borne fungi, which produce adverse effects on the growth and development of plants. (Zaidi, 2012). Fungal pathogens adversely affect the production and quality of Spices. The seed borne pathogens are one of the major causes of serious diseases in growing crops because of poor health and quality of the seeds (Hedawoo et al., 2011).

Spices and herbs are susceptible to microbial contamination. Growing conditions, harvesting and processing methods, storage conditions, and post-harvest treatments should be carefully controlled to prevent potential food spoilage and food-borne illnesses due to contaminated Spices and herbs (McKee, 1995).

Spices and medicinal plants may be high risk products and therefore, more studies are necessary to find methods of decontamination. (Abou Donia, 2008); Upgrading the conditions of spice processing, storage, transport, and continuous mycological and mycotoxicological control prior to food processing is necessary to lower the risks from sanitary incompatibility of products in order to efficiently protect human health. (Dimic, 2008).

The concern with the quality of the natural products is due to the potential fungal contamination and the risk of the presence of mycotoxins. (Bugno, 2006); Insufficient hygiene conditions during drying, transport and storage stages in the production of red pepper could cause microbiological and mycological growth which could result in the formation of mycotoxins. (Aydin et al., 2007). Mycotoxins contamination in some agricultural food commodities seriously impact human and animal health and reduce the commercial value of crops. (Gnonlonfin, 2013).

Mycotoxins produced as contaminants on food and feed commodities are considered to be economically and toxicologically important worldwide. Aflatoxins, Ochratoxins, Citrinin, Fumonisins, Zearalenone and Trichothecenes are important mycotoxins which have been analysed as natural contaminants in various Agricultural commodities. Many developed countries have laid down
specific regulations for import and export of those items in terms of economic implications (Chaudhary, 2010).

Mycotoxins are causing acute and chronic adverse effects in humans and animals. The most mycotoxin exposures are chronic generating irreversible effects as cancer or immune suppression, so that mycotoxicoses are sometimes difficult to diagnose. Acute poisoning can be lethal. Some mycotoxins are genotoxic, immunotoxic, allergenic, carcinogenic, mutagenic or teratogenic. (Oancea et al., 2008), Aflatoxins are classified as (Group1) carcinogen and Aflatoxin M1 (Group 2B) (IARC, 1993). also Ochratoxin-A as (Group 2B) carcinogens (IARC, 1993).

The worldwide contamination of foods and feeds with mycotoxins is a significant problem. Some moulds are capable of producing more than one mycotoxin and some mycotoxins are produced by more than one fungal species. Often more than one mycotoxin is found on a contaminated substrate. Exposure to mycotoxins is mostly by ingestion, but also occurs by the dermal and inhalation routes. (Zain, 2011)

The presence of mycotoxins, such as Aflatoxins, Ochratoxin-A, and Patulin, in fruits and their processed products is of high concern for human health due to their properties to induce severe acute and chronic toxicity at low-dose levels. Currently, a broad range of detection techniques used for practical analysis and detection of a wide spectrum of mycotoxins. Many analytical methods have been developed for the determination of each group of these mycotoxins in different food matrices, but new methods are still required to achieve higher sensitivity and address other challenges that are posed by these mycotoxins (Yang et al., 2014).

Fungal contamination causes considerable damage to the Spices and other stored grains viz. decrease in germination capacity, decrease in quality, destruction of viability, discoloration, abnormalities in growth, Effect on flavour and odour, Biochemical changes, Rotting and caking, absolute weight loss, preparation of the materials attack by other agents, production of mycotoxins (Christensen, 1957, Oyeniran, 1980).
By keeping all the above points in view for the present study dharwad is selected as study area to screen and analyse the mycoflora and mycotoxin (Aflatoxins B1, B2, G1 G2 and Ochratoxin-A) concentration in selected Spices.

Dharwad is selected as the study area for screening spice mycoflora, the area is located at $15^\circ 44'$ North latitude $74^\circ 99'$ East longitude in Karnataka state (India), because of the rich vegetation and conducive atmosphere for fungal growth it harbours different groups of fungi. The sites selected for the present investigation is market area and different places of Dharwad.

In the present study following five Spices were selected which are having medicinal properties viz.

- *Piper nigrum*, L. (Pepper)
- *Coriandrum sativum*, L. (Coriander)
- *Cuminum cyminum*, L. (Cumin)
- *Elettaria cardamomum*, Maton. (Cardamom)
- *Cinnamomum tamala*, Nees & Eberm. (Indian cassia)

The study deals with screening the mycoflora of five Spices which are having medicinal properties and analysis of mycotoxins (Aflatoxin B1, B2, G1, G2 and Ochratoxin –A) were studied from Dharwad. The Spice samples were collected from different localities of Dharwad. Mycoflora of Spices were isolated and classified. Analysis of mycotoxin was carried out by using High Performance Liquid Chromatography.
Five Spices selected for present study based on Spice, medicinal properties and their usage.

1. *Piper nigrum*, L. (Pepper)  
   **Family:** Piperaceae.

   It’s a climbing perennial shrub mainly cultivated in hot and moist climatic regions of India. Bearing stout branches, roots at nodal regions, leaves entire acute at apex and rounded base, usually dioecious, fruits ovoid to globose in shape, appears bright red when ripe, when the seeds reaches maturity turns brownish black shades. Peppers are classified into types viz.

   1. Black pepper: Consists of the dried fully developed unripe fruits, characteristic seed coat with deep set wrinkles because of drying, usually appears dark brown to black in colour.

   2. White pepper: It consists of dried fruits without the pericarp.

   **USES:** Pepper is commonly used as a Spice and medicinal plant. It is used as a medicine for cough also as a food preservative etc. The aromatic odour is due to the volatile oil and the pungent taste is because of the presence oleoresin, pepper stimulates the secretion of the saliva.

2. *Coriandrum sativum*, L. (Coriander)  
   **Family:** Apiaceae.

   It is an aromatic herb native of the Mediterranean, It is extensively grown in Russia, central Europe region now cultivated throughout India, plant herbaceous, generally 2-3 feet in height bears white/pinkish flowers the lower leaves are broad segmented while upper leaves are narrow; fruits small oval, aromatic in nature; fruit cremocarp type, consists of 1-2 seeded carpels, mericarp with numerous oil ducts.
USES: The fruit is having medicinal properties aromatic such as stimulant, carminative, antispasmodic, diuretic etc. The watery paste of seeds is used to treat mouth and throat ulcers, and also used as flavouring material in the various food preparations.

3. *Cuminum cyminum*, L. (Cumin)
   **Family:** Apiaceae.

   This is an annual aromatic herb measures about 1-3 feet in height. It is native of Mediterranean region; commonly cultivated in Punjab and Uttar Pradesh especially Unja is well known for its cultivation and also the market, bears aromatic oval fruits which appears brown in colour at maturity.

   USES: The aromatic fruits are used as a Spice and condiment. They form an ingredient of various food preparations. The fruits contain essential oil which is having active constituents like Cymol and Cuminol and also Thymol. It is used as a medicine to treat, diarrhoea; it’s aromatic odour and taste is due to the presence of volatile oil.

4. *Elettaria cardamomum*, Maton. (Cardamom)
   **Family:** Zingiberaceae.

   This is tall herbaceous perennial plant with subterranean branching from root stock which bears number of upright leafy shoots; plant measures about 5-8 feet in height, bearing alternate elliptical to lanceolate shaped sheathing leaves. Flowers born in panicle 2-4 feet long arising from the base of vegetative shoots. Flowers are 1.5 inch long, white to pale green in colour. Fruits trilocular capsules, fusiform to ovoid in shape pale green to yellow colour containing 15-20 hard brownish to black seeds covered by
mucilaginous membrane. It is native of moist evergreen forests of south India. Cardamom is exclusively cultivated in the states like Kerala, Karnataka, Tamil Nadu and Maharashtra.

**USES:** It is used as Spice also as masticator. The seeds posses a pleasant aroma and pungent taste used to flavour food preparations, liquors, coffee and tea. Seeds are aromatic stimulant, carminative they are useful in treating some of the respiratory diseases and throat infections.

5. *Cinnamomum tamala*, Nees & Eberm. (Indian cassia)

**Family:** Lauraceae.

It is a moderate sized evergreen tree found on high altitudes of the Himalayas it’s also cultivated in Bengal, Khasi hills and Assam etc. It is popularly known as Indian cassia or “Tejpat”. The stem bark and root bark used as a Spice and condiment.

**USES:** The bark and dried leaves are used to flavour the food preparations viz. pulao and also curries. The leaves are as stimulant, carminative Leaves and bark aromatic; leaves contain essential oil contains d- Phellandrene and 78% Euginol as an active constituents.

**The following are the objectives of the research work:**

- To isolate the Mycoflora of five Spices having the medicinal properties from Dharwad.
- Taxonomy and Ecology of the isolated mycoflora of selected Spices.
- Percentage and Seasonal occurrence of isolated mycoflora of selected Spices.
- Analysis of Mycotoxins from five selected Spices by chromatographic techniques viz. HPLC.