Introduction
1. INTRODUCTION

Nutrients in foods offer a number of benefits to us including energy and building blocks of our body. Among nutrients, fat is an important source of energy and essential nutrients like fat soluble vitamins. General dietary guidelines for Indians recommend a moderate-fat diet consisting of 30% energy from total fat and less than 10% from saturated fat for everyone which can be obtained by 4-5 tsp of oil and cereals and pulses in 1600 Kcal diet. In the Western world, nutritional habits high in fat relate to the elevated rates of cancer of the breast, colon, prostate, pancreas, ovary and endometrium (Weisburger et al., 1996). No doubt, oil is the important source of fat and is a valuable product with universal demand. Hence special attention needs to be given to cooking fats and oils.

Edible oil or cooking oil is purified fat of plant origin. India is a leading player of edible oil which consumes around 10 million tons per year having 11 kg per capita. It is also one of the most important commodities used by Indians in day today life because of the associated traditional ayurvedic medicinal values. India is the fourth largest oilseed producing country in the world next to USA, Brazil and China. About 25 million tons of oilseeds are being produced in India against the world production of 250 million tons per annum. Many varieties of oilseeds are cultivated in India. Among these, the major oilseeds are groundnut, soybean, cottonseed, sunflower, rapeseed, sesame seed, copra, linseed, castor seed and palm kernels. The edible oil industry of the country comprises of 50,000 expellers, 600 solvent extraction plants, 300 vegetable oil refineries and 175 hydrogenation plants.

Groundnut (Arachis hypogaea L.), an annual legume is one of the world’s principal oilseed crops which occupies a unique position among oilseeds as it can be utilized in diverse ways and can be consumed directly as well. The groundnut (A. hypogaea) also known as the peanut or earthnut, is botanically a member of the Papilionaceae, largest and most important member of the Leguminosae (Shankarappa, et al., 2003). Mainly native to warmer climates, groundnuts frequently provide food for humans or livestock, and in the absence of meat, form a valuable dietary protein component. It is a very important oil seed and food crop around the globe for its nutritional and trade values (Olaomi, 2008). It is cultivated in 109 countries, in
tropical, sub-tropical, and warm temperate regions of the world. During 2003 it was
grown on 26.46 million hectare with an estimated total production of 35.66 million
 tonnes (groundnuts in shell) and an average productivity of 1.35 tonnes/ hectare
(Upadhyaya et al., 2006). Groundnut contributes to nearly 25% of total oil seed
production in India. Nearly 75% output occurs in June - September and the rest during
November - March known as khariff and rabi seasons respectively. The peanut
production is estimated to increase from 32.98 million tonnes in 2009-10 to 34.05
million tonnes in 2010-11 due to increase in acreage in India. About two thirds of the
total groundnut produced in the world are utilized in India, China and in the USA.
More than half of the world groundnut production is crushed for expulsion of oil,
which is diverted mainly as edible oil in Asian countries particularly in India (Carley
and Fletcher, 1995). The production and processing technologies of groundnut
witnesses’ radical changes due to the extensive research work carried out during
recent days (Patee and Stalker, 1995). Several innate factors that govern the culinary,
nutritive and organoleptic properties of groundnut have been identified (Owens, 1994;
Sanders, 1982). A number of physical and chemical factors were identified as the
determinants of quality, some of which are already being used as indicators of quality
by the groundnut processing industry for purchasing the raw stock. As a source of
edible oil, the physical qualities of kernels are not of much consequence and only the
oil content and its fatty acid composition determine the quality of groundnut (Misra,
2004). Hence in many countries, the contaminated kernels not suitable for direct
usage are directed towards oil expulsion.

Groundnut or peanut oil, an edible vegetable oil derived from the pods of
_A. hypogea_ L. forms an important component of the human diet in the world.
Groundnut or peanut oil, are the two terms used interchangeably to refer them which
have same meaning. The peanuts form a small portion of groundnuts. Groundnuts
give edible and pleasant tasting oil for direct human consumption and are used as
salad oil or for cooking. In South Asian, Chinese and Southeast Asian cuisine, this oil
is used in large quantities just as how olive oil is used in the Mediterranean. Apart
from its use in cooking, it is used as a source of fuel for diesel engine, also as an
ingredient in ear wax removing products, etc. The nutritional composition of
groundnut oil has come into prominence owing to the associated health benefits/ risks.
Among the various cooking oils, the ground nut oil is recognized as good since they have low amount of saturated, trans fat and high smoke point. Peanut oil is widely consumed in the US and holds greater nutritional significance in selected developing countries. Thus, its contribution to energy balance and total diet quality requires clarification (Iyer et al., 2006). The oil is also further processed to margarine or vanaspati in India, soaps, paints and cosmetics. The oil content of groundnut can contain up to 50% oil (although the usual range is 40% to 45%) and 25 % to 30 % protein (Hammos, 1994). Most vegetable oils are normally recovered by different processes including the grinding, cooking, expelling and pressing, or by solvent extraction of the raw materials (Gerald, 2009). Traditionally, oil is extracted from groundnuts by crushing between stones or a stone and an iron bar (Ajao et al., 2010). Groundnut oil could be extracted through either traditional means (mostly dependent on human energy with about 20-30% of the oil extracted) or mechanical means (over 90% of the oil can be extracted) (Olaomi, 2008). In cooking, the groundnut oils are mostly used for frying the foods.

The process of cooking foods by immersing them in edible oil maintained at the elevated temperature (~ 150-200°C) is known as deep-fat frying. It is a complex process and involves numerous factors that affect the quality of final product. These factors depend on either the frying process itself or the nature of the food or frying oil (Naghshineh and Mirhosseini, 2010). In the presence of oxygen, moisture, trace elements and free radicals at high frying temperature, the physiochemical reactions for example thermoxidation, hydrolysis, polymerization, isomerization or cyclization occurs. These reactions results in the decomposition of frying oil and formation of monomeric, polymeric compounds, primary and secondary oxidative compounds, accordingly affects the quality of oil and fried product. The oil deterioration also results in the physical changes. For instance the colour darkens, the viscosity increases and smoke appears. The repeated usage of frying oil may produce undesirable constituents compromising not only the quality of the food but also posing a potential risk to human health and nutrition. Considerable research has been carried out on oil frying for the possible changes in the physical, chemical characteristics and their associated health impacts. Also number of attempts by modifying the oil content or by blending it with others has been made for minimizing
these undesirable changes (Naghshineh and Mirhosseini, 2010). Apart from the constituents, the other components directly related to health deserves attention are allergens and the natural toxins, including the mycotoxins present in the oil. Cold-pressed or unrefined/ unprocessed (crude) oil may contain groundnut protein which can cause allergic reactions range from abdominal discomfort to anaphylactic shock in some individuals. At present, however, groundnut allergens are not considered a determinant of quality by the industry and the sensitive individuals are advised to eliminate groundnuts from their diets. So far there has not been much problem on this account as groundnuts are easily recognized in the food. Like most legume seeds, groundnuts also contain both trypsin and chymotrypsin inhibitors. These inhibitors interfere with the process of digestion and lower the digestibility of groundnut proteins. However, moist heat treatment destroys their inhibitory activity. Though the innate qualities of groundnut oil are of serious concern, the risk due to mycotoxin contamination elaborated by the storage fungi warrants due consideration.

The mold growth is implicated in the spoilage of many kind of foods and making them unfit to eat. Under favourable environmental conditions fungal growth occurs and it is associated with the production of secondary metabolites, many of which can be hazardous to vertebrates (animals and humans) (Ghitakou et al., 2006). Mycotoxins are secondary metabolites produced by microscopic (micromycetes) and macroscopic (macromycetes) fungi. The toxic effect of these metabolites in humans and warm-blooded animals is called as mycotoxicosis. As a secondary metabolite, these mycotoxins have no role in the normal metabolism of the producing organisms. Many mycotoxins are bizarre heterocyclic rings with molecular weight of scarcely 50, to groups of irregularly arranged six or eight member rings with total molecular weights 7,500, such small molecules induce no response in the human immune system (Quezada et al., 2000). These toxin elaborating moulds are ubiquitous in nature and are universally found where environmental conditions are apposite for their growth. Since these moulds are commonly present in soil and plant debris, easily spread by wind currents, insects and rain, they are frequently found in/ on foods together with their associated mycotoxins (Trucksess and Pohland, 2002). More than 300 mycotoxins exist but only few are practically important. The most commonly known in this group are the aflatoxins (Pohanka et al., 2007).
The growth and toxin elaboration by these fungi are influenced by number of biotic and abiotic factors. The production of mycotoxins particularly aflatoxins by these fungi on commodities in the field occurs normally under stress conditions or in storage when conditions such as high moisture and warm temperature (25°C-30°C) are encountered (Papp et al., 2002). In stored grain ecosystems, the most important abiotic conditions identified which influence growth and mycotoxin production are water activity (aw), temperatures and when grain is moist, gas composition. In particular, interactions between these factors can determine whether contamination increases and mycotoxins are produced (Giorni et al., 2008). Aflatoxin contamination can be divided into two distinct phases. First phase of contamination occurs due to birds, mammals, insects or mechanically. Second phase occurs when the nature crop is exposed to warm, moist conditions either in the field or during transportation and storage or use (Cotty and Jaime-Garcia, 2007).

Fungal contamination of food and feeds is a public health problem throughout the world. The members of Aspergillus section Flavi specially three species Aspergillus flavus link, Aspergillus parasiticus Speare and Aspergillus nomius Kurtzman have received major consideration as an economic and public health hazard especially in third world countries due to their aflatoxin production (Razzaghi-Abyaneh et al., 2006).

The mycotoxin production by commonly occurring fungi growing in foods and feeds has been established clearly in the last 30 years. These toxins were already known as causatives of major epidemics in man and animals during historical times. A major danger of mycotoxins in the human diet therefore resides in our inability to detect them biologically (Quezada et al., 2000). Many reports list the association between fungi with edible oil seeds (Suryanarayanan, 1990). Microorganisms play an important role in causing changes in the quality of vegetable oils. An important prerequisite for mould development is high moisture content. Inadequate drying or accidental wetting of the oil seeds usually leads to mould development of high free fatty acid in oils extracted from such mould infected seeds. The effects of lipolysis by moulds associated with some vegetable oils like groundnut oils, palm oil, palm kernel oil on the free fatty acid contents of the oils have been reported (Okpokwasili and Molokwu, 1996). Accordingly several investigations have been carried out to
enumerate the mycobiota of various kinds of foodstuff, oilseeds and their products. So, these oils always have an inherent threat of mycotoxin contamination particularly the aflatoxin.

Some important characteristics of these mycotoxins are their capacity for bioconcentration and bioaccumulation as well their great stability in different biotic and abiotic environments (Quezada et al., 2000). Mycotoxins have four basic kinds of toxicity: i) Acute, ii) Chronic, iii) Mutagenic and iv) Teratogenic. The most commonly described effect of acute mycotoxin poisoning is deterioration of liver or kidney function, which in extreme cases may lead to death. Some mycotoxins act primarily by interfering with protein synthesis and produce effects ranging from skin sensitivity or necrosis to extreme immunodeficiency. Others are neurotoxins, which in low doses may cause sustained trembling in animals, but at only slightly higher doses cause, brain damage or death. The prime chronic effect of mycotoxins is the induction of cancer, especially of the liver. Some toxins affect DNA replication and hence can produce mutagenic or teratogenic effects (Celik, 2000).

Hence mycotoxin analysis or inspection is essential to minimize the consumption of contaminated food and feed, for monitoring domestic and import surveillance programs, controlling quality of products, establishing regulatory standards and guidelines, validating decontamination procedures and preparing standard materials for use in toxicological studies (Trucksess and Pohland, 2002). Their human and animal hazardous nature also necessitates the need for establishment of control measures and tolerance levels by national and international authorities.

Aflatoxins are considered one of the most significant mycotoxins. They are natural toxins produced by fungi affecting several foods and feed commodities (Arranz et al., 2006). The Food and Agriculture Organization (FAO) estimates that among the mycotoxin which affects 25% of the world’s food crops, the most notorious are aflatoxins. The outbreak of the so-called Turkey X disease among turkeys in the UK in 1960, some 50 years ago, is the seminal event that led to the discovery of the aflatoxins and a proliferation in research on fungal toxins contaminating food and feed materials. The causative agent of Turkey ‘x’ disease, caused death of more than 1, 00,000 turkeys was finally traced to one of their feed
component peanuts. It turned out that the consumed peanuts were contaminated with *A. flavus* fungi whose metabolites, later called as aflatoxins, were responsible for the occurrence of the above-mentioned disease. In the word aflatoxin, the first syllable ‘a’ was derived from the genus *Aspergillus*, the second one ‘fla’, from the species *flavus* and the term, ‘toxin’, came from the adjective ‘toxic’ (Papp et al., 2002). The four naturally occurring aflatoxins produced by *Aspergillus* fungi are B1, B2, G1 and G2 (Diaz and Espitia, 2006). ‘B’ and ‘G’ refer to the blue and green fluorescent colours produced by the toxins under UV light, while the subscript numbers 1 and 2 indicate major and minor compounds respectively (Pitt, 2000). Many aflatoxins are highly resistant, and survive food processing, and therefore enter the food chain and provide a threat to human health (Liu et al., 2006).

The international agency for Research on Cancer (IARC 1993) has classified Aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2) as group I carcinogens. Among the 16 aflatoxin group compounds known, only AFB1, AFB2, AFG1, AFG2 and aflatoxin M1 (AFM1) are routinely monitored. AFB1, AFB2, AFG1 and AFG2 are chemical derivatives of difuracoumarin and they are most often analyzed in cereals, nuts, spices, cocoa beans, meat, milk products and eggs. AFB1 is the prevalent compound in samples and at the same time, is acutely toxic and most carcinogenic amongst them. The metabolic product of AFB1, AFM1 which can be found in milk and dairy products, is just as toxic as AFB1 (Papp et al., 2002).

Aflatoxins are known to be mutagen, carcinogen and teratogen compounds. Their high level exposure lead to an acute necrosis, cirrhosis, and carcinoma of the liver exhibited by haemorrhage, acute liver damage, edema, alteration in digestion and absorption and/or metabolism of nutrients (Groopman and Kensler, 2005). They are both acutely and chronically toxic to animals, including man, causing acute liver damage, liver necrosis, induction of tumours and teratogenic effects (Pitt, 2000). The intake of these toxins over a long period time in very low concentrations may be highly dangerous (Papp et al., 2002). Young animals and the pregnant of all species are very sensitive to them. Exposure during pregnancy has resulted in transplacental transfer of aflatoxin to foetus and immune dysfunction in the offspring (Bingham et al., 2004). Because of the low molecular weight of aflatoxins, once ingested, these
compounds are rapidly adsorbed in the gastro-intestinal tract through a non-described passive mechanism (Yiannikouris and Jouany, 2002) and quickly appear as a metabolite in blood after just 15 minutes (Moschini et al., 2008). Chronic aflatoxicosis results from continued consumption of low to moderate levels of aflatoxins, and the effects are usually subtle. The highest aflatoxin contamination is found in groundnuts, maize and other grains. Furthermore unborn children are exposed to aflatoxins in uterine and the AFB1-albumin adduct has been found in umbilical cord blood (Jolly et al., 2006). Food contaminated even with very small quantities of them can become unfit for animal or human consumption. Though occurs in low or sub micrograms per kilogram range, they are regulated by legislations throughout the world due to their potent human carcinogenic nature. According to United States (US) Food and Drug Administration (FDA) guidelines, grain crops for human or animal consumption must generally contain <20 μg aflatoxins/kg (Jaynes et al., 2007). About 20% food products, mainly of plant origin, are significantly contaminated with mycotoxins. The frequent occurrence, particularly of aflatoxins, has already led to temporary bans of certain “high risk” foods imported into Europe low levels. The current maximum levels for aflatoxins set by the European commission are 2ng/g for AFB1 and 4 ng/g for total aflatoxins in groundnuts, nuts, dried fruits and cereals (Gilbert and Anlkam, 2002). In European Union (EU) 2 μg/kg in foods is allowed for direct human consumption. The Rapid Alert System for Food and Feed (RASFF), a network involving the Member States, the European Commission and the European Food Safety Authority (EFSA) alerts when a residue of potential concern is detected in food of domestic or imported origin. These mycotoxins classified under the hazard category I received the highest number of notifications, of which 802 concerning aflatoxins. No region of the world escapes the problem of mycotoxins; however, in certain geographical areas of the world, some mycotoxins are produced more readily than others (Tedesco et al., 2008). Accordingly many countries regulate levels of aflatoxins in nuts. The European commissions set limits for maximum levels of total aflatoxins and AFB1 in groundnuts, other nuts and dried fruit to be subjected to sorting or other physical treatments before human consumption or use as an ingredient in food stuffs, the limits stand at 10 μg/kg for total aflatoxins and 5 μg/kg for AFB1 (Bacaloni et al., 2008).
AFB₁ is the most common and toxic among all aflatoxins and are classified as group I carcinogen in humans. Detection of AFB₁ from food sources is necessary to protect the public from chronic low dosage exposures which may play a role in production of certain cancers (Carter et al., 1997). It is considered to be one of the most potent hepatocarcinogens known for several mammalian species, including humans (CAST, 2003). AFB₁ has been shown to produce G-T transversions at codon 249 of the p53 tumor suppressor gene, whose altered sequence has been associated with a number of human cancers (Woloshuk and Prieto, 1998). These aflatoxins are firstly a hepatotoxin causing excessive build up of hepatic lipids with enlargement of the liver, proliferation of biliary ducts and hepatocellular carcinoma. Secondly, aflatoxin causes enlargement of renal tissue and might affect its function. It is biotransformed in the liver by monooxygenases, where the cytochrome P₄₅₀ turns them into an electrophilic highly active compound known as aflatoxin 8, 9 epoxide, can conjugate with glutathione and excreted through urine and bile (Quezada et al., 2000).

AFB₁ consumption contributes significantly to the high incidence of hepatocellular carcinoma (HCC), the sixth most prevalent cancer worldwide with a higher incidence rate within developing countries especially in individuals infected with hepatitis B or C virus. Moreover AFB₁ could also interact with HIV/AIDS. Preliminary evidence suggests that there may be an interaction between chronic aflatoxin exposure and malnutrition, immunosuppression, impaired growth, and diseases such as malaria and HIV/AIDS. The chronic incidence of aflatoxin in diets is evident from the presence of AFM₁ in human breast milk. Aflatoxin exposure in children is also associated with stunting and neurological impairment increase. The manifestation of chronic or acute toxicosis as well as carcinogenicity depends on the dose, duration of exposure, and rate of metabolism to less toxic metabolites (Tedesco et al., 2008). When present even in small amounts as a feed contaminant, AFB₁ causes decreased feed conversion and weight gain, impairment of the immune system and marked histological changes in the liver such as fatty infiltration, focal necrosis and biliary hyperplasia (Guarisco et al., 2008).

Hence the fungal contamination of food and feeds is a major public health catastrophe all over the world. Due to the harmful effect of aflatoxins on human and
animal health as well as their consequence in international food trade, the contaminated food with aflatoxicogenic fungi has received worldwide attention (Carter et al., 1997). These toxic fungal secondary metabolites can be found in a wide range of food and feed commodities including cereal grains, oil seeds, dried fruits, apple juice, wine and meat products from animals fed contaminated meal. These aflatoxins are highly resistant, and survive food processing, and therefore enter the food chain and provide a threat to human health (Sun et al., 2003). The aflatoxins producing fungal species *A. flavus* and *A. parasiticus* are common saprophytes and opportunistic pathogens, occurring extensively in wide range of agricultural commodities and food matrices of the tropics and semi-tropics. Of these two species, *A. flavus* produces AFB$_1$ and AFB$_2$ whereas *A. parasiticus* produces these B aflatoxins as well as AFG$_1$ and AFG$_2$ (Shephard, 2008). *A. flavus* is the predominant species responsible for aflatoxin contamination of crops prior to harvest or during storage. The acute toxicity of aflatoxins and the carcinogenic property of aflatoxins produced by them were established and recognized for over 40 years (Yu et al., 2004). Hence *A. flavus* and *A. parasiticus* are of agronomic importance and cause annual losses of approximately $270M (Price et al., 2005).

The presence of mycotoxins in food products and the amount produced depend entirely on the ecological and processing parameters of the food stuff (Ghitakou et al., 2006). The *A. flavus* growth and production of aflatoxin in natural substrates are found to be influenced by a number of factors like type of substrate, moisture content of the substrate, presence of minerals, relative humidity of the surroundings, temperature and physical damage of kernels. The temperature range for aflatoxin production is 28-30°C. The minimum moisture content of peanuts necessary for *A. flavus* growth is 8-10% at 82% relative humidity, and aflatoxin production on peanut is optimum at 15-35% moisture content. Immature, broken, undersized, loose-shelled, rancid and discoloured peanut kernels are most likely to be contaminated (Rustom, 1997). Earlier work assumed that invasion was primarily a function of inadequate drying or improper storage and these factors are certainly important in the occurrence of aflatoxins in the humid tropics. Invasion of peanuts occurs as a result of drought stress and related factors (Cole et al., 1995).
Aflatoxin producing fungi are native to warm arid, semi-arid and topical regions in which changes in climate results in large fluctuations in the quantity of aflatoxin produced. *A. flavus*, which produces only B aflatoxins, is present on crops in virtually all areas examined (Cotty and Jaime-Garcia, 2007). *A. flavus* has a particular affinity for nuts and oil seeds. Peanuts, maize and cotton seed and their by products are the three most important crops affected (Masoero *et al.*, 2007).

The protein rich groundnuts, the seeds of *A. hypogaea* aflatoxin contamination pose serious problems for both producer and consumer (Roch *et al.*, 1995). Peanuts of all varieties in all geographical locations are liable to contamination with aflatoxins in the field before and after harvest, during curing and storage (Rustom, 1997). Aflatoxin contamination of peanuts (*A. hypogaea* L.) results from growth of peanut kernels by toxigenic strains of fungi, *A. flavus* link and *A. parasiticus* Speare. These fungi are commonly found in soil where peanuts are grown and many strains of *A. flavus* and most strains of *A. parasiticus* produce aflatoxins. Contaminated peanuts cannot be used for human consumption and therefore represent great economic losses for the peanut industry (Dorner and Cole, 2002). *A. flavus* dominates the mycoflora of peanut field soils, where the kernels develop and mature beneath the surface (Rustom, 1997). The fatty acid content of seed lipid bodies composed mainly of palmitic, oleic and linoleic acid supports the growth of *Aspergillus* spp. (Leontopoulos *et al.*, 2003). Peanuts usually grown under dry culture, drought stress before harvest is a major factor causing aflatoxin production. In bad seasons, *i.e.* seasons when inadequate rainfall causes temperature and moisture stress to peanut plants before harvest, more than half of the crop may be affected to some degree (Pitt and Hocking, 1997). An aflatoxin concentration in excess of 1,000,000 ng/g for individual peanut kernels was reported (Whitaker, 2003). Once a lot is contaminated, it could spread fungus and their toxins to uncontaminated foods upon mixing and/or contact (Proctor *et al.*, 2004).

Most developing countries lie in the tropics, where temperatures and relative humidity often favour mould growth and facilities for monitoring groundnuts and associated products for aflatoxin contamination are limited or non-existent. Various surveys conducted in different parts of India have revealed that groundnuts and their products are high-risk commodities for aflatoxin contamination; this contamination
affects 1.8 million tons of groundnuts each year (Kumar et al., 2010). The groundnut cake, a by-product of the crushing industry, is generally used as livestock feed supplement. In India, many commercial groundnut cakes and seed samples tested positive for aflatoxin contamination, with levels reaching as high as 8000 mg kg/l leading number aflatoxicosis incidences.

In most part of Asia, especially in India the contamination rate of grains and other food commodities is often higher than the global norm due to climatic and other post harvest handling processing, etc. Subsequent to the finding of the carcinogenicity of the aflatoxins, the immunosuppressive nature of the aflatoxins and the discovery that the aflatoxins not only pose a storage problem in grains but actually contaminated certain crops pre-harvest, the total mycotoxin problem became a multidisciplinary issue involving analytical chemists, microbiologists, medical and veterinary practioners and producers (Richard, 2007). According to Mishra and Das (2003) the mycotoxin should be destroyed or transformed to non-toxic compounds, fungal spores and mycelia should be destroyed so that new toxins are not formed, the food or feed material should retain its nutritive value and remain palatable, the physical properties of raw material should not change significantly, and the detoxification process should be economically feasible, that is the cost of detoxification should be less than the value of the treated commodity. Aflatoxins have high decomposition temperatures ranging from 237°C to 306°C. AFB1 is quite stable to dry heating at temperatures below its thermal decomposition temperature of 267°C. Normal home cooking conditions such as boiling and frying failed to destroy AFB1 and AFG1 (Rustom, 1997). Aflatoxin occurrence in food products is public health concern because of its possible involvement in the aetiology of human liver cancer (Oliveira et al., 2000).

Three possibilities are available to avoid harmful effects caused by aflatoxin. They are i) prevention of aflatoxin producing fungal attack at preharvest stage, ii) detoxification of aflatoxin-contaminated food and agricultural commodities iii) inhibition of absorption of aflatoxin in consumed food in the digestive tract (Mishra and Das, 2003).

Current control measures intending fungal growth and mycotoxin formation control includes many physical, chemical and biological methods. They comprise the
aeration, cooling, and modified atmospheres like physical methods, and chemical treatments with ammonia, acids and bases or with food preservatives and various biological methods. These control strategies require sophisticated equipment and expensive chemical, or reagents. Since most of them require expensive chemicals, technology, and technical experts to monitor physical parameters, they are not feasible for use by rural subsistence farmers (Atanda et al., 2007). Also amongst numerous physical and chemical aflatoxin detoxification and control methods which have been tested so far, none really fulfils the necessary efficacy, safety, and cost requirements. Physical and chemical methods adopted for aflatoxin removal have certain limitations like nutrient loss and toxicity (Tripathi and Mishra, 2010). The widely used chemical strategy (pesticides and fungicides), excessive use of chemical treatments has many undesirable consequences including (1) marked environmental pollution (2) resistant pathogenic populations’ increase and (3) presence of chemical residues in food commodities. Thus several research groups are now considering “light” or “natural” food grade products with a high efficacy in the inhibition of mycotoxin production but with a low impact on the environment and on human health (Reverberi et al., 2005).

Adsorbents are widely used as a means to decrease the concentration of free aflatoxins. This approach consists of adding nonnutritive inedible components to the food ration of cattle and birds. These components bond the toxin and prevent its intestinal absorption. Fodder additions based on mineral clays (zeolitic, bentonitic, and aluminosilicate), complex carbonic hydrates, and activated carbon are the most efficient among them. Binding AFB₁ by various aluminosilicates in vitro can achieve 90–100%, depending on the affinity to mycotoxin, the pH of the environment, and the relative concentration of the components. Lower values (about 40%) are received for esterified glucomannans. However, the result obtained in vivo testifies to the fact that the effectiveness of the above mentioned sorbents is sharply reduced (up to 30–60%). It is also emphasized that existing sorbents are destined only for animals (Puzyr et al., 2010).

Because of the adverse effects of these mycotoxins on human and animal health and failure of preventive measures, practical and effective detoxification strategies are highly desirable (Mckenzie et al., 1997). Since it is impossible to
completely avoid aflatoxin contamination, there is a need to develop some technologies for its detoxification from foodstuffs (Tripathi and Mishra, 2010).

Any decontamination process must be technically and economically feasible if it is to be applied practically. The FAO requirements for acceptable decontamination process stipulate that the procedure must

- Destroy, inactivate or remove aflatoxins
- Not produce nor leave toxic and/or carcinogenic/mutagenic residues in the final products or in their food products.
- Not significantly alter the important technological properties.
- Destroy fungal spores and mycelium that could proliferate and produce new toxins under favourable conditions (Piva et al., 1995).

Natural products consumption is showing an upward surge in recent times probably stimulated by cultural or psychosociological factors. People become aware of possible health benefits of natural products. Also restrictions imposed by the food industry and regulatory agencies on the use of some synthetic food additives have led to renewed interest in searching for alternatives, as natural antimicrobial compounds, particularly those derived from plants. Over 6000 traditional medicinal plants used in India, a large portion of them are used in food industries since purified plant products in addition to enhancing the flavour, exhibits antibacterial and antifungal activity. One among the natural plant food stuff is spices which are widely used in combination with foods. The antimicrobial activity of spices and essential oils is well recognized. Several studies have reported their preservative action (Guyonnet et al., 2002). Spices are used in food preparation throughout the world for the ultimate reason to help cleanse foods of pathogens and thereby contribute to the health, longevity and reproductive success of people who find their flavours enjoyable. Natural antimicrobials have demonstrated important antifungal properties (Lopez-Malo et al. 2000).

Indian cooking is so different from other cuisines due to its variety of style used for all dishes. It is regarded as one of the most diverse cuisines in the world. Curries are one of the well-liked ingredients in Indian cooking. The word curry actually means gravy instead of spice. Frying spices and vegetables in vegetable oil
often makes curry. Groundnut oil is preferred for making curries in most part of India particularly in North and West India. The essential flavour of added spices is contained in their oils. Those oils are often bound up with the rest of the seed. Most spices are seeds, as opposed to herbs, which are made of leaves. Spices can also be made of bark, like cinnamon, or root, like ginger. Heating the seed/bark/root helps release the essential oil from the mass of the seed, where it can be more easily transferred to cooking item. When fried in oil, spices impart flavor to the oil, which in turn impart flavor to other ingredients. Generally, there needs to be an oil component, since the oils dissolve more easily in other oils than in water. It is used as a garnish to perfume a dish and is essentially seasoning in sizzling oil; *i.e.* the frying of spices in hot oil to release their fragrance and aromas, which is then added to the dish just before serving thus luring one to its seductive and enticing flavours.

Ancient people wanted ways of extending the storage life of foods. They discovered various methods and applied them that would increase the storage life of food. It was well-known that spices provide important preservation qualities. Garlic has been used since the time of ancient Egyptians to control the spread of viruses and bacteria - even before existence of such things was known. Although they were not aware about the causatives of food spoilage, they learned by trial and error how to use spices such as garlic, cinnamon, clove etc to control or prevent food loss. Spices and flavouring plants part rich in supposedly health promoting phytochemicals which are currently receiving much attention as a possible source of cancer chemopreventive compounds since number of experimental evidences supports their anticarcinogenic possessions (Banerjee *et al.*, 2006). Attempts made to harness the naturally occurring antimicrobials for food preservation receives increasing attention now due to consumer awareness of natural food products and a growing concern of microbial resistance towards conventional preservatives. The challenge is to isolate, stabilize and incorporate natural antimicrobials into foods without adversely affecting sensory, nutritional and safety characteristics (Murugan *et al.*, 2012). Numbers of medicinal plants extracts are receiving attraction and approval as a functional material, and it is attracting more interests from functional food industry as well as research fields for novel functionality. Natural products may regulate the cellular effects of aflatoxins
and evidence suggests that aromatic organic compounds of spices can control the production of aflatoxins.

The use of spices in preserving food products has been traditional and they are cultivated in many countries such as India, Japan and Russia. Spices occupy a prominent place in the traditional culinary practices and are indispensable part of daily diets of millions of people all over the world. They are essentially flavouring agents used in small amounts and are reported to have both beneficial effect and antimicrobial properties. Their antimicrobial properties have been found to be mostly due to the presence of alkaloids, phenols, glycosides, steroids, essential oils, coumarins and tannins (Atanda et al., 2007). Spices are desirable food ingredients to create and explore new tasty products. Extracts of plants spices play an important role in promoting human health by their anticancer, antioxidative and anti-inflammatory and anticarcinogenic properties (Ceylan and Fung, 2004).

Spices with strong antimicrobial activity such as garlic, onion are used more frequently. Garlic compounds such as allyl sulfides affect aflatoxin B₁ carcinogenicity (Berges et al., 2004). Certain organic acids and some herbal compounds are known to suppress growth of Aspergillus fungus and reduce aflatoxin production (Gowda et al., 2004).

Though the outcome of numerous studies conducted to determine the effects of various food additives, preservatives, chemical and environmental condition to inhibit growth and aflatoxin production are effective (Bluma and Etcheverry, 2008), most failed to satisfy the consumers. It was found that locally available garlic is cheap source of peroxidase which has AFB₁ detoxifying properties. It was inferred that the partially purified peroxidase from garlic can be used efficiently to degrade AFB₁ from red chili powder with minimal or no quality changes up to 70.0% under optimized conditions. Enzymatic degradation involves complex mechanism which is not known completely but is thought to be through formation of some less toxic compounds from AFB₁ after its oxidation (Tripathi and Mishra, 2010).

Recent investigations of the molecular mechanism of AFB biosynthesis by some Aspergillus species showed that the genes required for biosynthesis are in a 70 kb gene cluster. They encode a DNA-binding protein functioning in aflatoxin pathway
gene regulation, and other enzymes such as cytochrome P450-type monooxygenases, dehydrogenases, methyltransferases, and polyketide and fatty acid synthases. Information gained from these studies has led to a better understanding of aflatoxin biosynthesis by these fungi. The characterization of genes involved in aflatoxin formation affords the opportunity to examine the mechanism of molecular regulation of the aflatoxin biosynthetic pathway, particularly during the interaction between aflatoxin-producing fungi and plants (Bhatnagar et al., 2003).

Though the mold and mycotoxin contamination of groundnut is recognized, its extracted product oil aflatoxin contamination does not appear high on the priority list for regulatory authorities in internal or domestic markets due to various problems which include the technical ones. The only time and place where aflatoxins were considered a quality parameter was during export to external markets, where the regulatory authorities, viz. the Agricultural and Processed food products Export Development Authority (APEDA) and the Indian oil products export authority (IOPEA) were involved. APEDA and IOPEA were concerned with aflatoxins at the marketing stage and ignored the problem of contamination at the production, processing and during storage.

A large portion of the natural plant products are used in food industries since purified plant products in addition to enhancing the flavour of added food also exhibits antibacterial and antifungal activity. One among the natural plant food stuff is culinary plants spices. Spices are widely used in combination with foods e.g. leaves of Murraya koenigii (L) spreng are commonly used as flavouring agent in Indian curry preparation since ancient times. The antimicrobial activity of spices and their essential oils is well recognized. Several studies have reported their preservative action. Traditionally numbers of spices were added with oil while frying, a common practice meant for flavour enhancement and detoxification. Now days a number of studies established the antioxidant potentials of these spices and suggest them as good natural replacements for synthetic ones in food industry. These spices have not been evaluated for their role in reducing the mycotoxin associated risk. The threats associated with storage fungi, mostly xerophilic ones have not been well established in ground nut. Since at present the herbal mixed products dominates the consumer markets and number of spices are known to have antimicrobial activity, the present
research aims to determine the level of contamination, screening, molecular characterization and ecological adaptation of aflatoxigenic xerophilic fungi in groundnut and its oil. It also aims to employ spices, which are considered as safe by GRAS standards, to reduce the aflatoxigenic fungi associated risk.