Wheat, are vulnerable as are other agricultural commodities to the fungal attack, including the group of *Penicillia*. Besides reducing the nutritive value of the food these microorganisms also produce insalubrious by products, so-called mycotoxins. The term mycotoxin was coined in 1962 in the aftermath of an unusual veterinary crisis near London in England, during which approximately 100,000 turkey poults died (Blout, 1961; Forgacs, 1962). Mycotoxins are bizarre molecules, generally being categorized into groups based on structural similarities (Pitt, 2000; Bennett and Klich, 2003), their major toxic effect, their biological origin and structure. The nature and quantity of mycotoxins produced is entirely influenced by interactions of several factors: types of substrate, moisture content, available nutrition, temperature and other associated factors (Anonymous, 1983; Coulumbe, 1993; Hendry and Cole, 1993; Viquez et al., 1994; Rao, 2001). Mycotoxins, the toxic secondary metabolites are produced by fungi during the late log-phase or stationary phase of the fungal growth (WHO, 1978; Schiefer, 1990; Calvo et al., 2002; Richard, 2007; Turner et al., 2009).

Food gets contaminated during handling, harvesting, transportation, storage, preparation and cooking. There are many factors that influence the growth of microorganisms like intrinsic factors (pH, moisture content, oxidation-reduction potential, nutrient status, antimicrobial constituents and biological structure) and extrinsic factors (storage condition, environmental conditions, etc). The consumption of food containing the toxic metabolite of the microorganism results in food poisoning.

The threat of mycotoxin contamination of food and feedstuffs destined for human and animal consumption, respectively, has become clearly evident since the discovery of the aflatoxin problem in 1960 (Goldblatt, 1969; Rodricks et al., 1977). Since then investigations of field and storage fungi led to the discovery of large number of mycotoxins including citrinin, that have been recognized in the etiology of human
and animal diseases (Uraguchi and Yamazaki, 1978). The Food and Agriculture Organisation (FAO) estimated that 25% of the world’s food crops are significantly contaminated with mycotoxins (WHO, 1991).

Alertness towards the citrinin toxicity originated from the strong implication of their involvement in diseases in living systems. The story of citrinin started when it was first isolated from *P. citrinum* in 1931 as an antibiotic (Hetherington, 1931). It has been of growing importance, ever since its presumable role in the occurrence of Balkan endemic nephropathy (BEN) came to be known.

The story doesn't end here. Several outbursts of food poisoning due to citrinin ingestion occurred at national and international levels. Citrinin was considered as one of the major cause of yellow rice mycotoxicosis in Japan (Saito *et al.*, 1971). It also played an important role in association with ochratoxin A in field outbreak of porcine endemic nephropathy in Denmark (Krogh *et al.*, 1973).

These instants and many others from all over the world have brought citrinin under core investigation and it has been included in the list of toxins to be examined by the screening subcommittees on natural toxins of the International Live Science Institute, European Branch (Frank, 1992). Mycotoxin contamination of food is unavoidable and at times unpredictable, which makes it a unique challenge to food safety. Decontamination of mycotoxin contaminated food is not fully successful, and control of mycotoxins is the need of the hour. A wide range of control measures (physical, chemical and biological) have been advanced owing to the risks associated with hazardous nature of citrinin on animals and humans, but none of them are fully satisfactory. Among the control measures used chemical methods have dominated. Chemical substances such as H₂O₂, ammonia, phosphate and nitrate fertilizers, amino acids, organic acids, phenolic compounds, pesticides, camphor and fatty acids are
officially recommended for the control of citrinin synthesis. All these official chemical elements are generally haunted with one or the other kind of trouble. Some are cost prohibitive while others pose environmental problems. Some of them leave harmful residues that exert long term effects on living systems. Still some have been reported to be mutagenic, teratogenic or carcinogenic. Moreover their excess use results in development of resistant varieties. All the above mentioned problems have alarmed the researchers to explore new alternatives, devoid of any risks and side effects. Eckert and Sommer (1967) also support this view and emphasized to search for new alternatives. Klingauf (1982) stressed on substituting the existing chemicals with new chemicals that are non toxic and environment friendly. He further suggested to explore the natural resources for the purpose. Committee of International Society for Plant Pathology on Chemical Control (Anonymous, 1980a) has also emphasized on the search for new controlling agents. The search for the new alternatives has not ended. Author in present work was convinced to use medicinal plant extracts, essential oils and homoeopathic drugs as alternatives of conventional chemicals. The use of plant extracts and essential oils as trials is common in comparison to homoeopathic drugs, but the decision to use homoeopathic drugs is courageous as homoeopathy is not considered “scientific” and is viewed as placebo therapy. Hence, the use of plant extracts, essential oils and homoeopathic drugs has been termed as unconventional substances. In order to appreciate the theme of the present study “Control of *Penicillium* toxin by unconventional substances” it will be relevant first to consider each component individually. Here it is-
1.1 The Host: Wheat

Wheat (*Triticum aestivum*) is an annual plant (Family Poaceae or Gramineae) growing upto 1.5 m (5ft) tall. It is in flower from June to July, and the seeds ripen from August to September. The flowers are hermaphrodite and are pollinated by wind.

Well drained loams and clay loams are ideal for wheat cultivation. However, good crops are raised in sandy loams and black soils also. Soil pH below 6.5 and above 7.8 is not suitable. Being low temperature loving crop, it is grown in winter from end of October to February under rain fed conditions and from middle of November to March/April under irrigated conditions. Total duration of the crop ranges from 110 to 120 days under irrigated conditions and 100-110 days under rain fed conditions.

India is the second largest producer of the wheat after China with 25 million ha area and 70 million tonnes production. Wheat is the most important food grain crop in India, occupying approximately 230 million ha world over. About one tenth of the global production is from India. Punjab, Haryana, Uttar Pradesh, Bihar, Rajasthan, Madhya Pradesh and Maharashtra are the major wheat growing areas in India.

1.1.1 Nutritive Value

It has high nutritive value and plays an important role in human diet. Wheat has high protein content than maize, corn, rice and other major cereals (Belderok *et al*, 2000). It also contains carbohydrate, fat, fiber and iron. Much of the carbohydrate fraction of wheat is starch. Wheat starch is an important commercial product of wheat, but second in economic value to wheat gluten (International Starch Institute, 2008). A wheat-based meal is highly nutritious due to high fiber content than other diet (Curtis, 2002; Victor, 2011).
1.1.2 Uses and Applications

Wheat as a major diet component is consumed worldwide. Its main use is for flour and bread-stuffs. The popularity of foods made from wheat flour creates a large demand for the grain, even in economies with significant food surpluses. The seed sprouts are antibilious, antivinous and constructive. They are used in the treatment of malaise, sore throat, thirst, abdominal coldness, spasmodic pain, constipation and cough.

Due to poor management practices and climatic conditions Indian wheat is likely to get contaminated with fungi like *Penicillium citrinum* which is the potent producer of neurotoxin, i.e., citrinin.

1.2 *Penicillium citrinum*

*P. citrinum*, the toxigenic mould of present study is ubiquitous in nature with a worldwide distribution and it may well be one of the most commonly occurring eukaryotic life forms on earth (Pitt, 1979). This species has been isolated from various substrates such as soil, (tropical) cereals, spices and indoor environments (Samson *et al.*, 2004). *Penicillium* is a genus of ascomycetous fungi of major importance in the natural environment as well as food and drug production. The secondary metabolites produced by *P. citrinum* include citrinin, cyclopiazonic acid (CPA), ochratoxin A (OTA), patulin (PAT), penicillic acid (PIA) and roquefortine C (RQC) (Modestas, 2004).
Colony of *P. citrinum* is blue gray and yellow in reverse. Conidiophores are bi-verticillate, 50-200 x 2-3 µm, smooth-walled with 3-5 divergent metulae in a whorl and flask-shaped sterigmata. Metulae are 12-20 x 2-3 µm in size, each bearing 6-10 phialides. Conidia are globose and usually smooth (Mislivec, 2004). Phialides are flask-shaped, 8-10 x 2-2.5 µm. Conidia are produced in columns, globose to subglobose, smooth-walled, or finely rough, hyaline to greenish, 2.5-3.0 µ.

### 1.3 Citrinin

Citrinin is one of the potent mycotoxins and shall be the focus of investigation. It is one of the well-known quinone methide mycotoxins, which is possibly spread all over the world. *P. citrinum* is the potent producer of citrinin. Other citrinin producing fungi include *Aspergillus niveus*, *A. niger*, *A. oryzae*, *Monascus ruber* and *P. camemberti*. It mainly occurs in wheat, rice, barley, maize, oat, peanut, rye and fruits and may occur as co-contaminant in cereals and is regarded as an important mycotoxin which may be ingested by men and animals (Krogh *et al.*, 1973). Occurrence of citrinin on wheat is supported by the incident that occurred in Tunisia where 200 samples of wheat that can last up to three months got contamination with citrinin (Chiraz, 2012). Enormous
literature is available documenting the contamination of wheat grains by \textit{P.citrinum} and occurrence of citrinin toxin produced by it.

1.3.1 General Characteristics of Citrinin

![Fig.3: Chemical Structure of Citrinin](image1)

![Fig 4: Yellow crystals of citrinin toxin](image2)

Citrinin (C_{13}H_{14}O_{5}) is 4,6-dihydro-8-hydroxy-3,4,5-trimethyl-6-oxo-3H-2-benzopyran-7-carboxylic acid. It is a simple, acidic, low molecular weight compound that crystallizes as lemon coloured needles melting at 172°C. Alternatively citrinin is also known as antimycin. Its molecular weight is 250.2 g/mol and density 1.335 g/cm$^3$. Mass fraction of citrinin contains 62.4% carbon, 5.64% hydrogen and 32% oxygen. It is sparingly soluble in water but soluble in dilute sodium hydroxide, sodium carbonate, sodium acetate, methanol, acetonitrile, ethanol and most other polar organic solvents (Deshpande, 2002). Some photodecomposition occurs in fluorescent light both in solution and in the solid state. It can be degraded in acid or alkaline solution, or by heat.

1.3.2 Toxicity and Stability of Citrinin

Citrinin is reported to be cytotoxic, genotoxic and nephrotoxic in all the species in which it has been tested (Hanika \textit{et al.}, 1983; Bilgrami \textit{et al.}, 1988; Berndt, 1990). However, it would seem unlikely in normal circumstances that citrinin presents much
risk to humans as it is unstable in cereal processing so that the greatest risk is probably to livestock, particularly pigs, feeding on contaminated cereal products.

1.4 Impact of Citrinin on Living Systems

Citrinin is known to cause a wide range of abnormalities in a number of animal systems belonging to mammals, fishes, birds, insects and even micro-organisms. However, susceptibility varies with sex, age and dosage. Citrinin acts as a nephrotoxin in all species in which it has been tested (Heperkan et al., 2006). Kidney is the preliminary target of the citrinin toxin. High levels of citrinin may affect the liver in addition to the kidney. There can be an exacerbation of the effect of citrinin when it occurs in combination with ochratoxin A in grains and animal feed, because of the similarity of the effects of both toxins.

1.4.1 On Animals

Citrinin exerts toxic effect on turkeys and ducklings by alteration in nephrosis that is more severe in turkeys. Tubular necrosis is the dominant lesion at 3-72 hours in turkeys and at 6-24 hours in ducklings. Hepatic and lymphoid lesions occur in both (Mehdi et al., 1986).

The toxic effects of citrinin in chick embryos include growth retardation of foetuses, microphthalmia, cleft beak, deformalities of the limb etc. A strict additive effect was seen in combination with ochratoxin A (Vasela and Velinek, 1983).

Citrinin is embryo/fetotoxic and embryocidal in mice and rats. Depending upon the concentration, effects include reduction in yolk sac diameter, crown-rump length, sornite number, protein and DNA contents. No embryonic demorphogenesis was observed (Yang et al., 1993).
Significant effects of citrinin toxin are also reported in rats and mice like decrease in DNA, RNA and protein content in kidney as well as in liver. Liver glycogen was significantly lowered. Surviving animals showed decreased body weight, food consumption, per cent liver to body weight and liver glycogen. (Hood et al., 1976; Phillips and Hayes, 1978). Nephrotoxicity caused by citrinin in rodents like rats and mice is due to slight stimulation of orotic acid incorporation into liver and kidney RNA in the early stage while in later stage orotic acid incorporation is inhibited (Sansing et al., 1976). Citrinin also produces renal tumours in male rat by oral administration in diet. When administered after N-nitrosodimethyamine an increased incidence of renal tumour was observed. In rodents, embryotoxicity occurred after injection of maternally toxicity dose of citrinin (Citrinin. IARC, 1986).

The interparitontial injection of citrinin in Rohu (Labeo rohita) fingerlings showed damage of kidney, liver and intestine along with clinical signs of depigmentation and congestion of caudal fins and mortality (Sahoo et al., 1999).

The aneuploidogenic and the clastogenic potential of citrinin was studied by determining the inhibition of microtubule assembly under cell free condition and by measuring the induction of mitotic arrest and micronuclei in cultured Chinese hamster V79 cells. Citrinin inhibited cell free microtubule polymerization in concentration dependent manner (Pfeiffer et al., 1998).

The primary effect of citrinin on established cell line of baby hamster kidney cells was the adherence of the cells to culture bottles. The cells were originally elongated and flattened and became swollen and round. Transplasma membrane redox system is inhibited to 81% by citrinin. This is not only dependent on toxin concentration but also on time of cell exposure (Generoso et al., 1994).
Citrinin was found positive when tested for DNA-attacking ability in the rec assay using combination deficient mutant of *Bacillus subtilis* M45 (rec') and parent strain M17 (rec') (Yoshio and Kiyoshi, 1976).

Citrinin alone and in combination with ochratoxin A induces drastic effect on white rabbits. Predominant and consistent lesions were recorded in the proximal convoluted tubules (PCTs) lining cells. Distal convoluted tubules (DCT) were unaffected in citrinin treatment, however, mild to moderate lesions were observed in ochratoxin A and in combination with citrinin (Kumar *et al.*, 2007).

Citrinin toxicity is also reported in dogs. It adversely affects renal function and ultrastructure. They have tenesmus and prominent serous nasal discharge and lacrimation. These dogs have elevated blood urea nitrogen, decreased specific gravity, glucosuria, mild proteinuria and urinary casts. At necropsy, prominent gross alterations were found in kidneys, which were pale and swollen (Carlton *et al.*, 1974; Kitchen *et al.*, 1977a; Mark *et al.*, 1995).

### 1.4.2 On Microorganisms

Citrinin and ochratoxin A interfere with iron metabolism in *Neisseria meningitides*, since a marked growth inhibition by both toxins was also observed in the presence of iron. One function of ochratoxin A and citrinin in nature could consequently be to affect the iron uptake of other competing microorganisms (Størmer and Hoiby, 1996).

Citrinin is reported to be a broad spectrum antibiotic especially against gram positive bacteria (Raistrick and Smith, 1941; Wang *et al.*, 1947; Blanc *et al.*, 1995). Citrinin is also found to be fungicidal against *Fusarium spp.* and *Aspergillus niveus* (Devi *et al.*, 2009).
In a study citrinin, produced by *P. citrinum*, was found to have a newly identified function of inducing bacterial motility by transcriptional activation of some genes related to the expression of flagella. The swarming motility of *Paenibacillus polymyxa*, a gram-positive low-G1C spore-forming soil bacterium, belonging to the plant growth-promoting rhizobacteria was greatly induced by citrinin in a dose-dependent manner. The development of lateral flagella by citrinin toxin has also been reported in other bacterial cells with swarming motility (Merino et al., 2006).

Organic solvent tolerant micro-organisms (OSTM) are extremophiles that have developed resistance to withstand solvent toxicity. They play important role in biotransformation of organic compounds. *Moraxella* sp. MB₁ was used for biotransformation of citrinin into decarboxycitrinin in a biphasic system. The antibiotic activity of both citrinin and decarboxycitrinin is also reported (Naik and Rodrigues, 2006).

1.4.3 On Plants

Citrinin toxicity in plants is not much reported. The sole reference in this respect is that of pathological effect and ultrastructure changes in young leaves of cultivar Nijisseiki and Chojuro of Japanese pear. Citrinin caused necrosis and permeability changes in Nijisseiki pear but no effect was seen in Chojuro pear (Nishimura *et al.*, 1974).

1.4.4 On Humans

The International Agency for Research on Cancer (IARC) classifies citrinin in group 3 because of limited evidence on carcinogenicity for animals and no data for humans (Castegnaro and McGregor, 1998). However, the presence of citrinin and other toxic metabolites in food, regardless the concentration, must be considered a potential human health hazard.
In humans the renal system is primarily affected by citrinin and the mitochondrial respiratory chain was identified as a possible sensitive target for this toxin.

Exposure to citrinin diminishes the numbers of T-helper type 1 (Th1) cells in the peripheral blood of children and is a risk factor for the development of allergic diseases. It is hypothesized that citrinin being a mycotoxins is responsible for this effect (Wichmann et al., 2002).

Citrinin also affected human neutrophils with regard to O$_2$ generation. It stimulated the superoxide anion production in resting neutrophils in whole blood but inhibited it in isolated cells. In neutrophils stimulated with zymosan or PMA (Propylene Glycol Monomethyl Ether Acetate), mycotoxins showed inhibitory effect on both isolated and in whole blood cells. The behaviour of resting isolated neutrophils, identical to that of stimulated cells, was caused by manipulations to isolate cells that activated O$_2$ production. This effect was not dose dependent which may be connected to variability of individual sensitivity to these compounds. (Adriana et al., 2003).

Evidence supporting a role of mycotoxin, in particular ochratoxin A and citrinin, in the etiology of Balkan endemic nephropathy (BEN) and associated urinary tract tumours (UTT) is reviewed. Both diseases occur in subjects born and/or living in certain rural areas where home-produced and home-stored stable foods were found to be more frequently contaminated by the ochratoxin A and citrinin. (Castegnaro et al., 1999).

The seriousness of the agony caused by ingestion of citrinin contaminated food by wide range of organisms cannot be overlooked and hence, it becomes necessary to cope up with the situation. Apart from various practices to preserve the food items they
still get contaminated. This calls for therapeutic measures. A lot of such measures are in use. They will be described briefly in ensuing chapter.

1.5 Medicinal Plant Extracts

India is endowed with a rich wealth of medicinal plants. These plants have made a good contribution to the development of ancient Indian materia medica. The curative properties of drugs are due to the presence of complex chemical substances of varied composition (present as secondary plant metabolites) referred to as phytochemicals present in one or more parts of these plants.

1.6 Essential Oils

An essential oil is a concentrated hydrophobic liquid containing volatile aromatic compounds from plants. Essential oils derived from plants also possess medicinal properties and are used in pharmaceutical industry. Their antimicrobial activity is assigned to a number of small terpenoides and phenolic compounds (thymol, carvacrol and eugenol) which also in pure form demonstrate high antimicrobial activity (Conner, 1993; Vazquez et al., 2001).

As naturally occurring antimicrobial agents they have found application in the field of pharmacology, pharmaceutical botany, phytopathology, medical and clinical microbiology, food preservation, etc.

1.7 Homeopathic Drugs

Homoeopathy is a system of alternative medicine originated in 1796 by Samuel Hahnemann based on the doctrine, similta similibus curenturl (“like cures like”) according to which a substance that causes the symptom of a disease in healthy people will cure that disease in sick people (Hahnemann`, 1833). Homoeodrugs contain very small quantities of substances called potencies prepared in a special way following
unique method of potentization. The base of homoeopathic medicines is alcohol, water or lactose (sugar of milk). Homoeopathic remedies are prepared by repeatedly diluting a chosen substance in alcohol or distilled water followed by forceful striking on an elastic body, called succussion (Hahnemann, 1921). Dilution usually continues well past the point where none of the original substance remains (Ernst, 2005). Homoeopathy uses many animal, plant, mineral and synthetic substances in its remedies. Some modern homoeopaths considered esoteric bases for remedies known as “imponderables” because they do not originate from a substance but from electromagnetic energy such as X-rays and sunlight, presumed to have been captured by the vehicle, alcohol or lactose (Lee and Thompson, 2007a).

It therefore implies that there is a need to revise dogmatic approach. In view of a section of plant pathologists these unconventional substances could fulfil the prerequisites of becoming a promising plant protection device devoid of risks of side effects, adverse reactions and iatrogenic diseases. Impressed by such ideas, the author became motivated to use them to control the growth of P. citrinum and citrinin production.