A Correlation Study Of Aflatoxin B_1 And Ochratoxin A In Different Feed Ingredients In Agroindustries

By

J. Maheswari

A DISSERTATION SUBMITTED TO THE AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER EDUCATION FOR WOMEN - DEEMED UNIVERSITY, COIMBATORE - 641 043

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Certified as bonafied research work

S. Sivakamasundari 12/5/99

Signature of the Dean

&

Signature of the Head of the Department

S. Bhanumathy

Signature of the Guide

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CHAPTER I

INTRODUCTION

POULTRY INDUSTRY

Poultry production in India has made spectacular progress over the last three decades, evolving from backyard venture to a full-fledged commercial agro based industry.

Broiler industry in India witnessed a rapid upsurge in the output from 4 million broilers in 1971 to about 330 million (Projected) in 1995 (Anon., 1994). However, the availability of inputs, feedstuff in particular is not keeping its pace in par with the growing demand. It is hence essential to make the best possible use of any available feedstuff for the preparation of broiler feeds. Contamination of maize, groundnut and other feedstuff with significant levels of mycotoxin continues to be a major problem in many parts of the world. In developing countries like India, the problem of aflatoxicosis is more severe than in developed countries due to tropical and subtropical climatic conditions and poor handling of feeds and feedstuff. The aflatoxin contamination of feeds and feedstuff in India has been well documented by several scientists (Jagadish Kumar and Veeranarayana Gowda, 1979; Paul Gupta, 1985 and Selvasubramanian *et al.*, 1987).

A majority of farmers are involved in poultry farming (> 6000) especially layers. The population of layers is around 10 million in this area which produces 80,00,000 eggs daily. There are about 180 compounded feed

manufacturers who produce more than 1500 tonnes of feed / day. The annual turnover of poultry industry in Namakkal area is around 400 crores.

MYCOTOXINS IN DIFFERENT FEED INGREDIENTS

The terms 'Mycotoxin' is derived from the Greek word 'Mykes' meaning fungus and latin word 'Toxicum' meaning poison (Dr.B.S.Rao, 1998). The term mycotoxin is used to refer to all toxins derived from fungi.

The names of mycotoxins are based on the names of the fungi that produce them, actual chemical name and toxic manifestation of the toxin (N.J.Daghir., 1994).

There are some moulds/fungi which are beneficial and some others that are harmful which produce various toxins that are collectively called 'Mycotoxins'. These are toxins that are produced by fungus as metabolites during the metabolism of nutrients of various feed ingredients such as

- Cereal Source

  Maize, Wheat, Rice bran etc.

- Vegetable protein source

  Groundnut cake, sunflower meal, mustard cake, soya bean meal etc.

- Animal protein source and agro industrial by-products.

Layer mash (LM)

A balanced feed meant for layer type chicken from 21st to culling.

Deoiled sunflower cake (DOSFC)

Solvent extracted sunflower meal meant for protein supplementation in chicken.

Deoiled groundnut cake (DOGNC)

Solvent extracted groundnut cake meant for protein supplementation of chickens.

Maize

Maize is a cereal used in chicken feeds for energy content.

Soya

A protein supplement obtained after extraction of oil from soya bean.

The various crops can be invaded by harmful fungi during harvest, drying, transport, processing and storage under the right kind of moisture, relative humidity and temperature.

Mycotoxin contamination is a serious problem potential health hazard and particular concern to our country, where the climatic conditions are conducive for the growth of mycotoxin producing moulds. Further, the situation is aggravated by limited resources for prevention and controlling the mycotoxin in food chain (R.B.Sashidhar, Ramesh V.Bhar, 1991).

Mycotoxin contaminated feed was first recognized in the early 1960 in U.K with the discovery of aflatoxin of imported peanut as the cause of 'Turkey X disease'. The most important mycotoxins to the poultry industries today are

aflatoxins, Ochratoxins, Vomitoxins, Zearalenone, Oesporein, T-2 toxin, citrinin and fumonisins.

CHARACTERISTICS OF MYCOTOXINS

1. They frequently arise as veterinary problems whose true cause is not immediately identified.
2. The disorders are not transmissible from one animal to other, being neither infectious nor contagious.
3. Treatment with drugs or medicines has no effect on the cause of disease.
4. In field outbreaks the trouble is often seasonal, as particular climatic sequence may favour toxin production by the mould.
5. Careful study indicates its greater association with some of the foodstuff like maize, groundnut, rice etc.

In contrast various diseases that are caused in animal system are due to different virus and bacteria which are either infectious are contagious in nature and are treated by medicines, drugs or antibiotics.

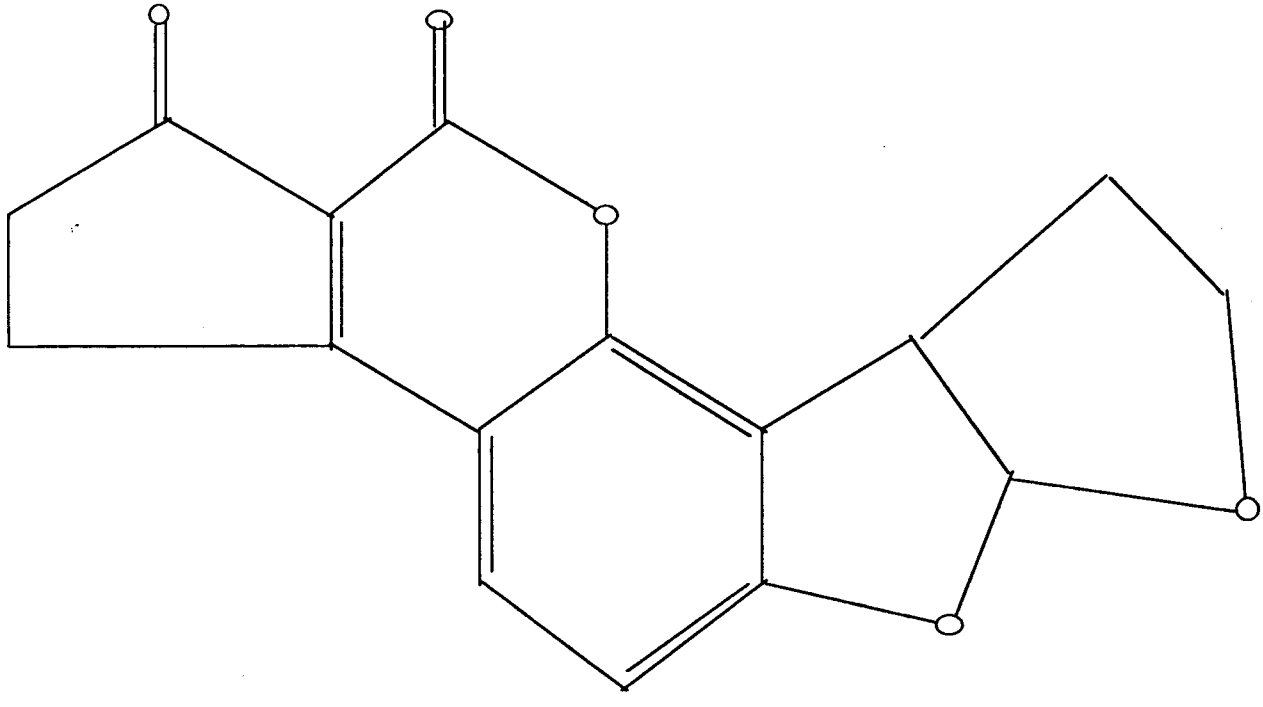
TYPES OF MYCOTOXINS

The common mycotoxins affecting poultry feeds are:

1. Aflatoxin
2. Ochratoxin
3. Citrinins
4. Trichothecenes
5. Zearalenone.

AFLATOXINS

STRUCTURE OF AFLATOXIN B_1



ETIOLOGY AND TOXICOLOGY

Aflatoxins are a group of closely related extremely toxic and carcinogenic fungal metabolites produced by *Aspergillus flavus* and *Aspergillus* parasites (Fredreic.J.Hoerr), which occurs as natural contaminants of poultry feed. These are stable compounds but sensitive to oxidizing agents like hypochlorite and reactive to U.V light at extreme pH of the four major forms of Aflatoxin B_1, B_2, G_1, G_2.

Aflatoxin B_1 is the most common, biologically active component and hepatotoxicity is the primary effect in nearly all animals. B_1, B_2, G_1, G_2 are identified with their blue and green color reaction to fluorescent light (365 nm wavelength) and their chromatographic R_f values.

Aspergillus flavus are relatively ubiquitous, can generate, grow and elaborate their toxins on a variety of substrates especially those commonly and mainly used as feed ingredients for poultry. Toxigenic strains of Aspergillus are best adopted to a high temperature ranging from 25 to 40°C and moisture contents as low as 10 percent. Besides these several other factors like prolonged storage, variation in environmental temperature damp floor and ill-ventilated store room also contribute for the high production of Aflatoxin during storage of poultry feeds.

Aflatoxins are gaining special significance in tropical countries like India, where the environmental conditions as well as pre- and post-harvesting

practices of grains and ingredients handling are not ideal and thus suitable for mould growth and toxin contamination (Devegowda, 1989) in poultry feed.

AFLATOXICOSIS

It is a serious toxicity in poultry resulting from the ingestion of feeds contaminated with Aflatoxin. This disease results in large economic losses to the poultry industry (Muirhead, 1989).

The symptoms of aflatoxicosis may be noted as

- High level of toxins :Causes sudden mortality without any symptoms
- Low level of toxins:Causes improper growth, impaired feed conversion, immuno suppression and signs of malnutrition.
- Hyper proteinemia (Brown and Abrams,1965)
- Loss of pigmentation and undigested feed particles in feces.
- Young birds are more susceptible than adults.
- Drop in feed consumption and Diarrhoea are also common.

The lesions noted are

- Liver enlarged, pale and bleeds in acute cases
- Degenerative changes in liver are present at low levels of toxins
- Atrophy of liver with distended gall bladder
- Spleen is also enlarged.
- Duodenum distended with catarrhal contents.
- Kidneys – Swollen, congested with degenerative changes and haemorrhages

Reasons for the interaction of aflatoxin with infectious diseases have been in part by findings that aflatoxin impairs the reticuloendothelial system (Michael *et al.*, 1973) and interferes with the ability of chicken to form antibodies (Thaxton *et al.*, 1974).

Aflatoxin also interacts with infectious agents. It interacts with certain vitamin deficiencies (Hamilton *et al.*, 1974) and high protein and high lipid diets have a protective effect against aflatoxicosis (Smith *et al.*, 1971; Hamilton *et al.*, 1972).

SOURCES

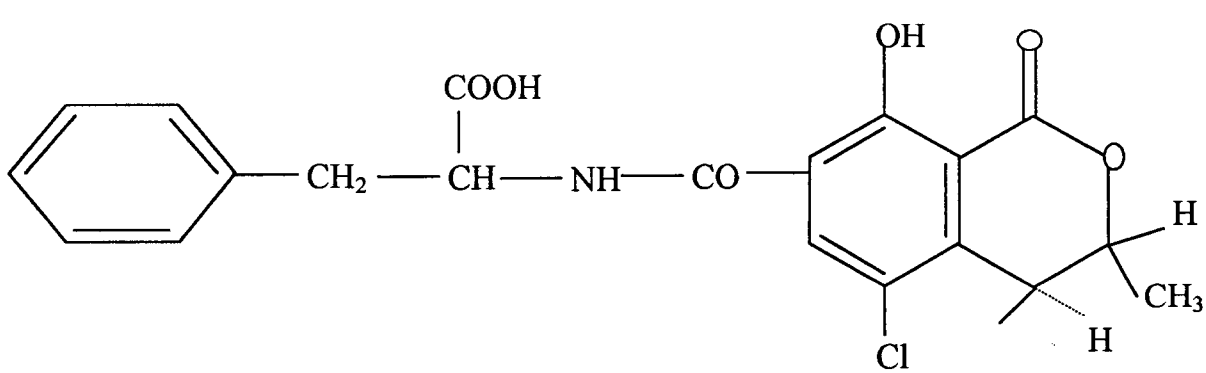
Groundnut cake, Soya bean, rice, Sorghum, corn.

TOXIC LEVEL

Above 0.02 parts per million.

OCHRATOXINS

STRUCTRUE



Ochratoxins are a group of structurally related, toxic metabolites produced by seven species of *Aspergillus* and six species of *Pencillium* (Chu, 1974). *Aspergillus ochraceus* which commonly occur on numerous grains and feed stuffs throughout the world. The wide spread occurrence of ochratoxin producing fungi, their ability to grow on a variety of feed stuff (Chu, 1974) and the natural occurrence of ochratoxin (Fishbach and Rodricks, 1973; Scott *et al.*, 1972; Krogh, 1973) combine to present a potentially great hazard to poultry.

Ochratoxins are isocoumarin compounds linked to L-beta phenylalanine and are designated A, B, C, D and their methyl and ethyl esters. Out of different types of ochratoxins the most common, highly toxic, important and thermostable one is ochratoxin-A. Ochratoxin A was first identified in the feed chain in 1969 as contaminant of corn.

The symptoms of ochratoxicosis may be noted as

* Similar to aflatoxins in chronic cases.
* Reduced renal function without any grass lesions.
* Reduced egg size, interior quality and shell specific gravity.

The lesions noted are

* Liver shows pale discolouration
* Enteritis is common

* Kidney is pale, shows nephrosis with swelling of tubular epithelial cells, tubular dilatation and proteinaceous material in the lumen in acute cases
* Kidneys show marked accumulation of urates in the ureters.
* Occasionally urate deposits are also seen on visceral organs
* Weights of liver and kidney increase, weight of lymphoid organs decreases in subacute toxicity.

SOURCES

Cereals and legumes

TOXIC LEVEL

0.5 ppm toxic level may even less than this

TRICHOTHECENES

Those toxins are produced by *Fusarium calonectria* and *Gibberella* species. Those toxins to poultry are T_2, DAS, DON and NIVALENOL.

The noted symptoms of trichothecenes toxicity are

- Reduced growth and marked depression. Blood diarrhoea.
- Necrosis of oral mucosa in the most common and important sign
- Oral lesions are white to creamy raised ulcers on the borders of the tongue and inside borders of upper and lower beak
- Necropsy shows reddening of the gastro intestinal mucosa.
- Mottling of liver and distended gall bladder
- Atrophy of spleen and visceral haemorrhages are common.

ZEARALENONES

Zearalenones are toxic metabolites produced by *Fusarium graminarum* and *Fusarium roseum*. There are 7 derivatives of this group, but zearalenone and zearalenol are toxic to poultry. Zearalenone grows well in corn and Sorghum.

The symptoms noted are

- Showed adverse effect in broiler breeders the form of reduced egg production but no effect on fertility and hatchability.
- The most common symptom is ascitis.
- Oviduct is distended with cysts and fibrinous material.

CITRININS

Citrinins are toxic metabolites produced by *Penicillium citrinin* and other species of *Penicillium* which are nephrotoxic.

Affected birds consume large quantities of water and show diarrhoea withdrawal of infested feed results in normally with 8 to 10 hrs.

The lesions noted is

Except enlargement of kidneys, no other is evident

OBJECTIVES

Determination of the following parameters in feed and feed ingredients.

- To estimate the amount of aflatoxin B_1
- To estimate the amount of ochratoxin A
- To correlate the presence of aflatoxin B_1 and ochratoxin A

- To obtain the minimum, maximum and average level of aflatoxin B_1 and ochratoxin A
- To discuss the toxicity level of aflatoxin B_1 and ochratoxin A
- To discuss the effect of atmospheric conditions and storage on toxicity of aflatoxin B_1 and ochratoxin A.

Review of Literature

CHAPTER II

REVIEW OF LITERATURE

MYCOTOXIN ANALYSIS IN DIFFERENT FEED AND FOODSTUFFS

Mora, M and Lacey, J examined above 3000 samples of maize from growing crop and storage to final sale. They reported that 80% of samples contained above 20 ng/g aflatoxins and the samples kept on the cob after harvest contained almost no aflatoxin while shelled samples were frequently highly contaminated. They observed that, in the initial survey, more aflatoxin contamination developed in shelled maize than in that handled on the cob during the period from harvesting to drying.

Cespedes, A.E and Diaz, G.J analysed different feed ingredients and feed stuff for the presence of aflatoxins using liquid chromatographic technique with a limit of detection of 1μg/kg for each aflatoxin (B_1, B_2,G_1 and G_2). They observed that aflatoxins were detected in 11 of 45 samples of sorghum, 4 of 33 of maize, 8 of 22 of rice meal, 12 of 30 of poultry feed and aflatoxins were not detected in soyabean. They reported that only 9 of 58 samples contained total aflatoxin levels exceeding maximum tolerable limits.

Azil *et al* reported that aflatoxin B_1 and Ochratoxin A production was considerably higher in the light than in the dark. The maximum aflatoxin B_1 and Ochratoxin A yield was obtained at pH 5.5, and by increasing the initial pH to near neutrality, both mycotoxin yield decreased. Iron copper and zinc

stimulated aflatoxin B_1 and Ochratoxin A production and enhanced growth rate of both A. flavus and A. Ocraceus.

Meerarani *et al* analysed 325 milk samples for the presence of aflatoxin M_1 by HPLC method during different months. They reported that aflatoxin M_1 was detected in 36 samples at concentration ranging from 0.1 to 1.0 μg/litre. Three samples had an aflatoxin M_1 concentration > 0.5 μg/lit.

Scudamore, K.A *et al* studied about the analytical methods for the reliable detection and estimation of 22 mycotoxins in maize gluten and other maize products used in the animal feed. They examined 40 samples of maize gluten and 27 samples of other maize products. They reported that the aflatoxins were not found above the reporting limit 1-5 μg / kg in any sample while Ochratoxin A was detected in only 2 samples of maize gluten at 2μg / kg.

Alip, M *et al* examined 47 maize samples for the presence of fungal and mycotoxin contamination. In that aflatoxin B_1 was found in 3 samples (12,18 and 20 ppb) and 36.2% of maize contained Ochratoxin A ranging from 120-3840 ppb.

Natural incidence of mycotoxins in 130 samples of poultry feeds was observed by Dalcero, A *et al*. They reported that the most significant mycotoxin isolated from poultry feeds was aflatoxin B_1 (AFB_1), found in 48% of the samples, at levels of 10 – 123 ng/g.

Pittet, A. reported the presence of six types of mycotoxin namely aflatoxins, Ochratoxin A, Patulin, fuminosins, deoxynivalenol and zearalenone and their occurrence in foods and certain feeds.

Halt, M analysed the level of toxigenic moulds and mycotoxins like aflatoxin, Ochratoxin and zearalenone in 62 samples of medical plant material and 11 herbal tea samples. He observed that the presence of Aspergillus Flavus in 11 of 62 medicinal plant samples and in 1 of 11 herbal tea samples. He reported that Ochratoxin was found in 1 of 7 samples analysed.

Scudamore, K.A *et al* examined 40 samples of rice bran used in animal feed industry for the presence of 20 mycotoxins. They reported that aflatoxin B_1 was present in 20 samples, Ochratoxin A at the level of 12 and 3 μg/kg was present in 2 samples.

According to Ahmed, I.A and Robinson, R.K, HPLC and post column derivatization procedures, the contaminants branch (CB) method are the best detection method for the detection of contaminating aflatoxins in Date fruits. They explained that using the best food extraction and purification method, aflatoxin recovery was 35% less than when using the CB method.

Kussak *et al* developed the simultaneous determination of aflatoxicol and aflatoxin B_1, B_2, G_1 and G_2 in human urine by immuno affinity column clean up and liquid chromatography.

Biancardi compared an immuno chemical method (ELISA) and chromatographic method for estimating aflatoxin M_1 in milk. He reported that

the ELISA method was unreliable method, suitable only for screening of large number of samples and cannot be used without the confirmation by HPLC method.

Danev, M *et al* investigated imported foods for the presence of aflatoxins B_1, B_2 and G_1, and Ochratoxin A. They found the presence of above mycotoxins in 58 (26.88%) samples out of 215 samples.

The mechanism of toxicity of aflatoxin B_1 which includes biotransformation and carcinogenisis and the possible role of aflatoxin B_1 in hepatocellular carcinoma were discussed by Fernandes De Oliveira C.A. *et al.*

According to Gordon S.H *et al*, maize variety, plant stress and susceptibility to infection, geographic location, weather insert vectors and handling and storage conditions are the epidemiological factors which used to predict *Aspergillus flavus* and other kinds of toxigenic fungal contamination in food grains. They also explained the fourier transform infra red photo acoustic spectroscopic method for the detection of toxigenic fungal contamination in corn.

Anuja Dudhe *et al* discussed about the occurrence, adverse biological effects due to aflatoxin toxicity, metabolic fate and transfer to products(milk, meat and eggs), analysis of aflatoxins (sampling, sample preparation, extraction, purification, qualitative detection and confirmation, and quantification), regulation of aflatoxins and control and detoxification (physical, chemical and biological methods).

Galvano, F *et al* reported that the aflatoxin M_1, level in milk and Yoghurt collected during November to April were about 4 times higher than those collected during may to October.

Dhand, N.K *et al* observed that out of 61 aflatoxin contaminated milk products the concentration of aflatoxins ranged from 0.5 to 1.0 μg/litre in 33 samples, from 1.0 to 2.0 μg/litre in 22 samples and from 2.0 to 4.0 μg/litre in 6 samples.

According to Giesbrecht, F.G *et al* both the compound gama and the negative binomial were the two distributions that have been used to model the occurrence of aflatoxin in groundnuts.

Domagala, J and Kisza, J, studied a method for parallel determination of aflatoxin precursors sterigmatocystin and O-methyl sterigmatocystin in milk by using gel permeation chromatography for extract purification and two dimensional thin layer chromatography on silica gel for separation of extract components.

According to Gloria, E.M *et al* the black light test for aflatoxin contaminated maize carried out in large food factory were evaluated against bidirectional thin layer chromatography (TLC) analysis for 286 samples of maize. They reported that all 286 samples were accepted by the black light test (<7 fluorescent points). However, the results from TLC analysis showed that 96 samples were contaminated and 14 showed aflatoxin B_1 contamination levels >20 μg/kg.

Czerivicki developed an optimized method HPLC for the determination of aflatoxin M_1 in milk at 370/418 – 700nm of fluorometric detection. The detection limit was about 0.01 μg/litre of milk.

Scudamore, K.A and Mac Donald, S.J, reported a collaborative study of an HPLC method for determination of Ochratoxin A in wheat using Immuno affinity column clean up. They concluded that the performance of the immuno affinity column method compared very favourably with results of other published collaborative studies on mycotoxins.

Chau *et al* observed the occurrence of aflatoxin B_1 and Ochratoxin A in 96 maize samples harvested in Vietnam. The highest level of aflatoxin B_1 was 130 ppb in maize kernels 20 samples of maize kernels were analysed for Ochratoxin A, only one of the 20 samples was contaminated with Ochratoxin A at the level of 90 ppb.

According to Shetty, P.H *et al* the rain affected samples and poultry feed samples were contaminated with aflatoxin B_1. 20% of normal sorghum and 89% of normal maize samples also contaminated contained aflatoxin B_1 in the range of 5 –125 and 0.38 – 109 μg/kg respectively.

CORRELATION AND REGRESSION STUDIES REGARDING MYCOTOXIN

Muhlemann, M *et al* examined different Ecuadorean foods and feeds for mycotoxin contamination by ELISA and HPLC method. According to them regression analysis of ELISA Vs HPLC results revealed a severe under estimation (50%) of naturally occurring Ochratoxin A by ELISA in maize. The

high contamination levels were found for Ochratoxin A in corn and beans (both upto 320 μg/g). A correlation between moisture content and fuminosin contamination was found. Again mould growth was not correlated with the presence of toxin.

Whitaker, T.B *et al.*, examined various groundnut grade like sound mature kernels plus sound splits (SMKSS), other kernels (OK), loose shelled kernels (LSK) and damaged kernels (DAM) contaminated with aflatoxin. According to them correlation analysis of the most accurate predictor of aflatoxin concentration in the lot is cumulative aflatoxin mass in the high 3 risk components OK + LSK + DAM (Correlation coefficient r =0.996). If the aflatoxin in the combined OK + LSK + DAM components was expressed in concentration units, r decreased to 0.936. Linear regression equations relating aflatoxin in OK + LSK + DAM to aflatoxin concentration in lot. They reported that the cumulative aflatoxin in the OK + LSK + DAM components were not an accurate predictor (r = 0.539) of aflatoxin in the SMKSS component.

Cantalejo, M.J *et al.*, examined different seeds and feeds for the presence of species of Penicillium, Aspergillus etc. They reported that there was a statistically significant correlation between the climatic conditions and the fungal contamination caused by yeast in the samples.

Ali, N *et al* analysed 16 maize samples for the presence of aflatoxins, fuminosins, Zearalalenones and trichothecenes using HPLC method. They observed that all of the aflatoxin contaminated samples were co-contaminated

with fuminosin. They reported the correlation between fuminosin producers and aflatoxin producers on kernel infection and contamination of there mycotoxins in maize.

Belmadani, A *et al* studied the sub-chronic effects of Ochratoxin A on young adult rat brain and partial prevention by aspartame (a sweetener). They correlated Ochratoxin A concentration in ng/g of brain in Y axis and duration of treatment in weeks in X axis. The correlation coefficient observed was r=0.989. They had also shown the regression curve for the accumulation of Ochratoxin A in whole brain with time as a function.

ADVERSE EFFECT OF MYCOTOXINS

Huff.W.E *et al* reported that both aflatoxin and Ochratoxin A significantly decreased body weights at 2 and 3 weeks age of broiler chickens. They also reported that the numerical increase in mortality with either aflatoxin or Ochratoxin A. Both these toxins have been shown to decrease the relative weight of spleen, pancreas and proventriculus. They reported that the most sensitive organ to the interactive effect of these mycotoxins was kidney.

Romoser *et al* showed that 30 mg/kg or high dietary Vanadium as calcium salt retarded the growth of chicks.

Kubena *et al* observed that the simultaneous occurrence of Ochratoxin A and vanadium might elicit toxic reponses not encountered when prevent singly and thus present an even more serious problem to the poultry industry.

According to Kubena L.F *et al* the body weight gains for the Ochratoxin – Vanadium combination were significantly lower than for the chicks receiving Ochratoxin A or Vanadium singly.

Lanza *et al* reported that the reduction in cholesterol and total protein concentration in broiler is the most sensitive indicator of aflatoxins in old broilers.

Michael *et al* found that the dietary aflatoxin caused in chickens a close related impairment of the reticuloendothelial system, which is responsible for the removal of the foreign particulate matter from circulation and for the protection of the tissues from invasion by noxious organisms.

According to Boon Bungearn Boonchuvit and Pat B Hammilton neither typhoid nor paratyphoid interact with aflatoxin to influence the growth rate.

Wifatt, R.D suggested that the serum glucose level of chicken decreased due to the presence of dietary aflatoxin level of 2.5 μg/g. They also suggested that aflatoxin caused a physiological stress in chickens.

According to Devurkar, U *et al* as the dose of dietary aflatoxins increased, the total serum protein could be decreased due to decrease in protein synthesis.

Huff, W.E and P.B Hamilton reported that Ochratoxin alters only potassium of the serum electrolytes and appears to affect the tubular portion of the kidney associated with maintaining the potassium but not the sodium and chloride balance in chickens.

Accordiong to Ubosi, C.O *et al* there was a large decrease in liver metabolism and a dramatic increase in heterophil, lymphocyte reactions when 5700 ppb of aflatoxin was fed, but no alteration in antibody production.

Pier and Heddleston found that as low as 250 ppb of aflatoxin B_1 reduced the immune response of turkeys to pasteurella multocida vaccination.

According to Giambrone, J.J *et al.*, turkeys given aflatoxin B_1 daily experienced morbidity and died within 18 days.

According to Hegazy *et al.*, clinical signs in chicken were difficult in breathing, fever, diarrhoea, mucoid discharge, decreased consumption, decrease in egg production, ruffled feathers etc.

Qureshi, M.A *et al* observed that the dietary aflatoxin level of 10 ppm reduced fertility, increased percentage of early dead and late dead embryos and reduced hatchability of fertile eggs in broiler breeders.

MYCOTOXIN CONTROL AND DETOXIFICATION

Reddy *et al* observed that the influence of certain food preservatives on the interaction of mycotoxigenic fungi and mycotoxin production. They reported that, in mixed culture, food preservatives were comparatively more efficient as the degree of inhibition of production of different mycotoxins was comparatively high.

Thanaboripat, D *et al* reported the detoxification of aflatoxin by streptococus lactis and lactic acid bacteria in commercial yoghurt. They observed that the yoghurt inoculated with S. lactis or commercial yoghurt lactic

acid bacteria together with 50 μg aflatoxin B_1/ml incubated at 45°C for 2-5 hours and stored 5-10°C upto 7 days. After 7 days *S. lactis* reduced aflatoxin B_1 concentration from 50 to 33.70μg/ml.

According to El-Nezami, H *et al*, physicochemical alterations enhance the ability of dairy strains of lactic acid bacteria to remove aflatoxin from contaminated media. They observed that the binding of lactic acid bacteria to remove aflatoxin by heat treatment is not effective as by the acid treatment and they reported that ethanol, UV radiation, sonication, alkaline or pH treatments has no effect on binding ability of lactic acid bacteria.

According to Njapau, H *et al.*, dehulling, followed by 24 hours soaking (steeping) and subsequent washing reduced the aflatoxin B_1 (AFB_1) content of maize flour from 900 to 150μg/kg. They also reported that boiling groundnut meal yielded a moderate reduction in the content of AFB_1 8600μg/kg to 1300 μg/kg.

Kececi,T *et al* reported that the effect of Polyvinyl poly pyrrolidone (PVPP), synthetic jeolite (SZ) and beutonite(BNT) when incorporated into the diet to reduce deleterious effects of aflatoxin in broiler chicken during aflatoxicosis.

Smith and Hamilton (1970) reported a threshold level of 1.25 ppm of aflatoxin for broilers, above which the growth depression was more evident. Feeding of aflatoxin at the dose of 10 ppm in the commercial broiler diet lowered the growth rate by about half (Smith *et al.*, 1971).

According to Mckenzie *et al* control and contaminated corn were treated for 92 hours with ozone at 200mg/min there is greater than 95% reduction of aflatoxin B_1 in contaminated corn. Alternations in the majority of relative organ weights, liver discoloration, serum enzyme activity, heamatological parameters and blood chemistry caused by AFB_1 were eliminated by treatment with ozone.

According to Edrington. T.S *et al* the addition of non-nutritive sorptive materials to the diet in order to reduce the absorption of mycotoxins from the gastro intestinal tract. A specific hydrated Sodium, Calcium alumino silicate has proven beneficial in alleviating the toxic effects of aflatoxin in growing poultry. They reported that the addition of dietary super activated charcoal is marginally effective in alleviating some of the toxic affects associated with aflatoxin.

According to Stephen. R, *et al.*, the supplementation of Tryptophan did not alleviate the adverse effect of aflatoxicosis in layers but appeared to enhance the severity of the disease.

The control and safety evaluations of mycotoxins like aflatoxin, Ochratoxin A in feed stuff was given by Alkins, D., Norman, F as mycotoxins present a potential threat to consumer safety it concluded that the continued vigilance is necessary to ensure that regulatory and advisory limits are complied with.

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

The study has been conducted in randomly chosen feeds and feed ingredients in agro industries in and around Namakkal area.

LOCATION AND CLIMATE

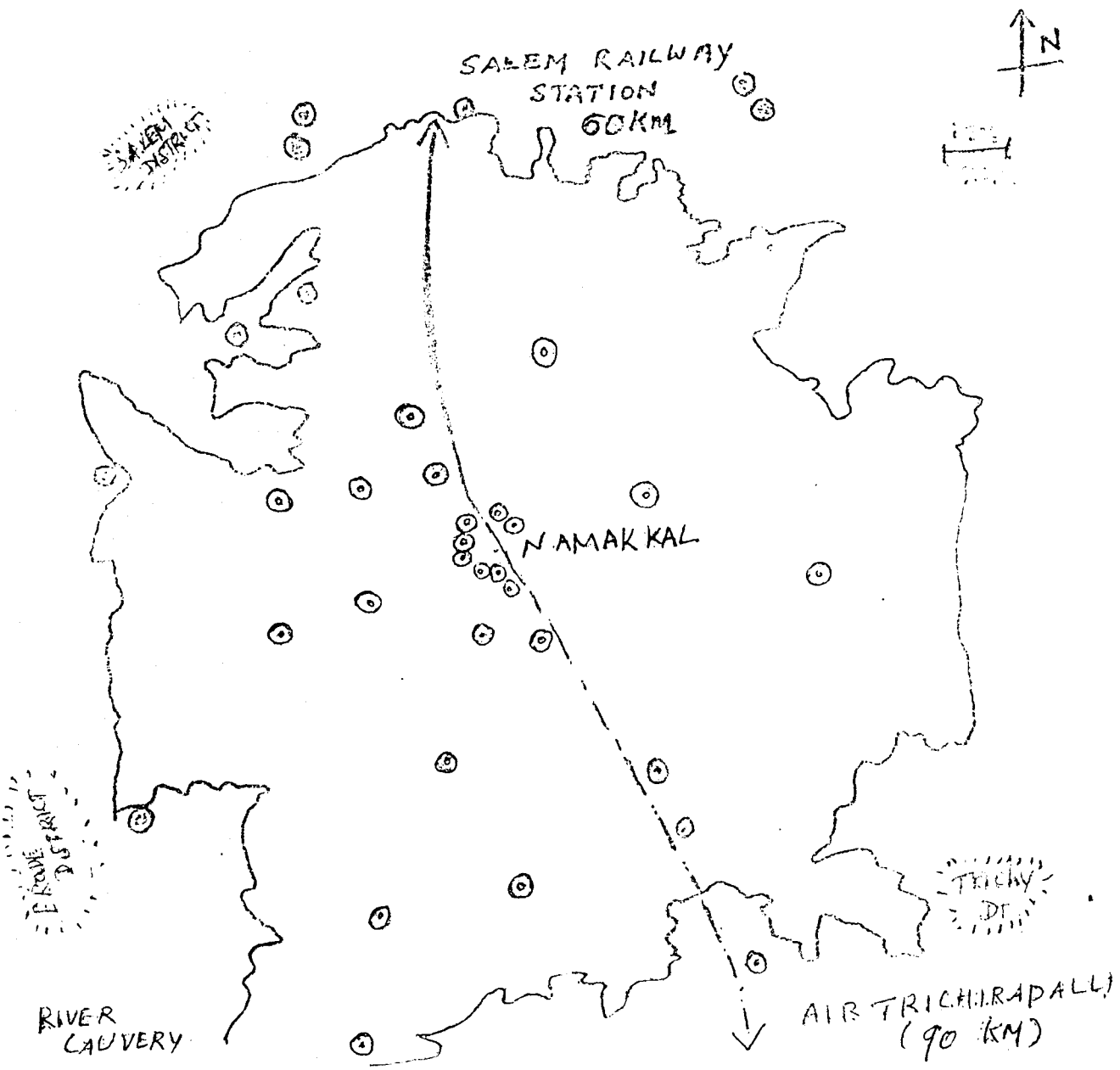
Namakkal is geographically situated at 11°N 15 latitude and 78.2°E 09 longitude with an altitude of 404 m. MSL and falls under north western agro climatic zone of Tamil Nadu State. The soil type is non calcarious red and brown soil and calcarious black soil.

According to Troll's approach Salem has been classified as tropical semi desert zone with a total annual rain fall of 871mm (Kulandaivelu *et al.*, 1986). There is no perennial river in Namakkal district except river Cauvery which runs in the border areas only. The major crops grown are sugarcane, tapioca and sorghum, under irrigated and rainfall conditions.

During the year 1998-1999, the forecast is received for 120days. During the period of study the average maximum and minimum temperature are recorded are 35.4°C and 23.5°C respectively. The maximum temperature is recorded during March (37.90°C) and the minimum temperature is recorded during January (19.22°C). Mean relative humidity is low throughout the study period and varied between 50.87 and 63.87 per cent. The number of rainy days are 15 during the study period.

FIGURE

LOCATION OF THE STUDY – NAMAKKAL DISTRICT



PERIOD OF STUDY

The period of study lasted from October 1998 to January 1999.

COLLECTION OF SAMPLES

Nearly six types of chicken feed ingrent samples are collected and analysed for the presence of mycotoxins like aflatoxin B_1 and ochratoxin A by two dimensional (2D) method. Samples taken for the study are maize, Deoiled groundnut cake (DOGNC), Soya, Chicken feed, Deoiled sunflower cake, Layer mash (LM).

The study is carried out with fifteen samples and the values are tabulated. The general average of these values of samples in the course of time is taken for the discussion.

METHODS OF MYCOTOXIN ANALYSIS

Various analytical approaches are available for determination of mycotoxin in food and feed stuff. There are two approaches possible for the detection and determination of mycotoxins viz. biological and chemical, Biological methods may be useful in screening toxins especially when the identity of the mycotoxin is not known. Analytical techniques such as TLC, HPLC, HPTLC, GC/MS and immuno-analysis have been developed for detection and quantitation of mycotoxins in food.

PRESUMPTIVE METHOD

A number of presumptive or screening methods for mycotoxins in food and feed stuffs have been developed. Minicolumn methods have been

developed for rapid detection and screening of mycotoxins (Holaday, 1981). This involves the use of a small reusable glass mini column, filled with certain absorbant materials to bind mycotoxins. When irradiated with long wave U.V light the absorbed mycotoxin in the mini column shows the characteristic fluorescence. The mini column method was developed to screen aflatoxin, Ochratoxin A, Zearalenone.

Recently, pressure mini column (PMC) techniques for detection of mycotoxins has been developed which employs commonly available syringes which are tightly packed with certain adsorbants. The adsorbed mycotoxins are visualized under the long U.V light.

QUANTITATIVE METHOD

(a) Physico-chemical methods

Chromatographic techniques are the methods of choice for quantitative analysis of mycotoxins. Thin layer chromatography is a common and popular technique for detection of mycotoxins. This technique is reliable, practical and relatively simple, with a wider area of application. Its two dimentional application offers especially good resolution, resulting in low limits of detection. The quantitation of mycotoxin in the TLC technique is achieved by densitometric detection (Van Egmond and Paulsch, 1986). High pressure thin layer chromatography (HPTLC) is as improvement of TLC procedure. Large number of samples can be analysed within short time. In recent years TLC procedures for mycotoxin analysis are replaced by high performance liquid

chromatography (HPLC) which is a very sensitive method. The detection of mycotoxin is achieved by ultra violet fluorescence, refractive index and amperometric detection. Reversed phase HPLC with a C18 column is most widely used method which analyses 18 mycotoxins (Coker and Jones, 1988). Trichothecene is difficult to be determined by TLC and HPLC method so gas liquid chromatography (GLC) is powerful technique for trichothecene group of toxins. GLC mass spectrometry with selected ion monitoring include electron impact (EI); negative ion chemical ionisation (NICI) and positive fast atom bombardment, are currently the most powerful analytical technique, for their commercial identity (Frieses *et al*, 1987; Uyakul *et al*., 1989).

(b) Immuno Chemical Methods

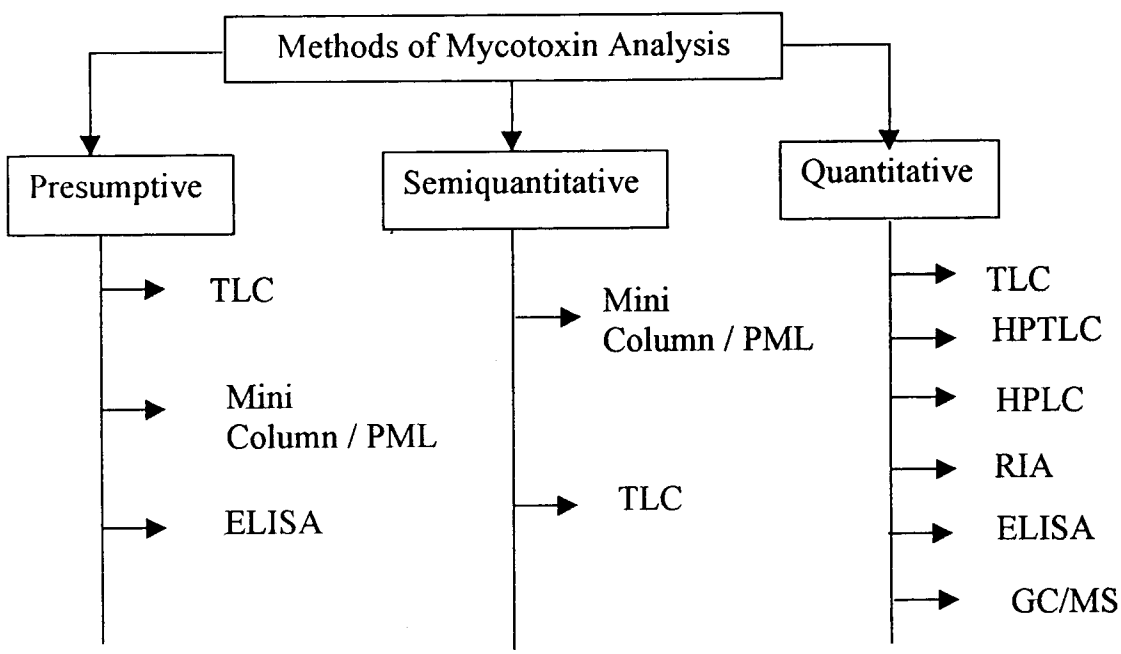
In the recent years newer, immuno analytical methods such as Radio immuno assay (RIA) and enzyme linked immunosorbent assay (ELISA) have been developed for various mycotoxins, such as aflatoxin B_1, Ochratoxin-A, T_2-Toxin (Chu 1984; Sashidhar and Rao, 1988).

Briefly, the immuno assay involves competition between a free mycotoxin and a labelled mycotoxin (radio labeled or enzyme labeled) for the binding site of anti body.

'Mycotoxin hazard' prevention by detection would be an appropriate approach, in view of the availability of a wide range of analytical methods for their detection. Application of simple and sensitive presumptive procedure in

conjunction with suitable quantitative method permits, practical means of achieving this goal.

SCHEMATIC DIAGRAM FOR METHODS OF MYCOTOXIN ANALYSIS



SEMI QUANTITATIVE TWO DIMENSIONAL TLC METHOD FOR THE ANALYSIS OF MYCOTOXINS (H. Egur, 1982)

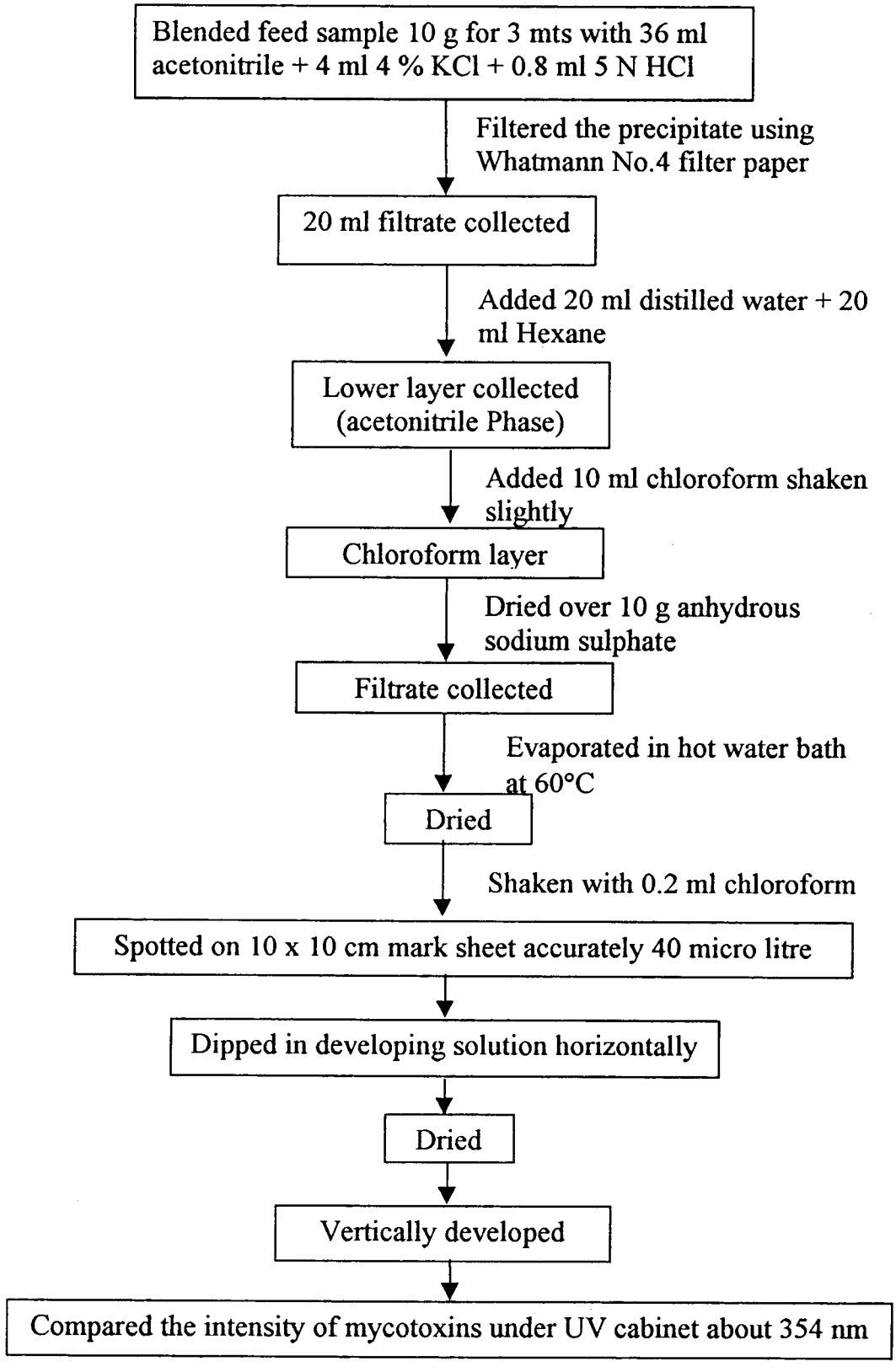


TABLE 1

ESTIMATION OF AFLATOXIN B_1 (PPB) IN CHICKEN FEED AND FEED INGREDIENTS

S.NO	*DOGNC*				*Maize*			
	October	*November*	*December*	*January*	*October*	*November*	*December*	*January*
1	175	145.8	58.4	116.6	51.02	28.5	40.8	0
2	93	93	116.6	116.6	81.6	8.2	40.8	12.24
3	35	116.6	116.6	233.3	12.24	4.1	30.6	12.24
4	87.5	93	145	175	20.41	28.5	24.46	0
5	58.3	93	145	58	20.41	4.1	16.3	12.24
6	93	116.6	93	35	12.24	20.4	20.4	12.24
7	93	145.8	35	35	4.1	4.1	8.2	8.2
8	116.6	116.6	87.5	145.5	40.8	4.1	16.3	10.2
9	116.6	58.3	87.5	35	51.02	8.2	28.8	40.8
10	116.6	116.6	58.3	116.6	20.4	4.1	32.6	20.4
11	70	116.6	58.3	87.5	4.1	12.2	32.6	12.24
12	23	81.6	46.6	81.6	20.4	20.4	40.8	12.24
13	46.6	93	35	70	40.8	10.2	4.1	12.24
14	116.6	116.6	93	35	51.02	20.4	12.24	40.8
15	70	87.5	81.6	58	81.6	8.2	12.24	20.4

TABLE 2

S. NO	*Soya*			*LM*		*Chicken Feed*		*DOSFC*	
	November	*December*	*January*	*October*	*November*	*November*	*December*	*October*	*January*
1	0	8.2	12.24	12.24	12.24	0	20.4	40.82	24.48
2	0	8.2	20.4	12.24	4.1	0	0	24.48	24.48
3	0	0	8.2	12.24	12.24	12.24	20.4	40.82	24.48
4	0	0	20.4	12.24	12.24	12.24	12.24	24.48	24.48
5	0	4.1	10.2	10.2	12.24	20.4	28.5	24.48	24.48
6	4.1	10.2	28.57	20.4	12.24	4.1	40.82	40.82	0
7	8.2	20.4	10.8	12.24	10.2	0	40.82	40.82	24.48
8	30.6	16.3	32.6	10.2	20.41	4.1	20.4	24.48	40.82
9	4.1	12.24	30.6	8.1	10.2	18.4	12.24	40.82	40.82
10	12.24	12.24	12.24	40.8	4.1	12.24	16.3	24.48	24.48
11	8.2	0	12.24	20.4	20.4	20.4	51.02	40.82	24.48
12	4.1	0	12.24	12.24	8.2	0	51.02	61.2	0
13	20.4	0	4.1	20.4	20.4	16.2	8.2	40.82	12.24
14	20.4	0	10.2	20.4	10.2	30.6	32.6	40.82	8.2
15	4.1	0	8.2	12.24	8.2	20.4	30.6	40.82	24.48

TABLE 3

ESTIMATION OF OCHRATOXIN A (PPB) IN CHICKEN FEED AND FEED INGREDIENTS

S.No	*DOGNC*				*Maize*		
	October	*November*	*December*	*January*	*November*	*December*	*January*
1	27.5	344.4	206.6	27.5	82.6	192.8	0
2	55.1	206.6	344.3	27.5	137.7	275.5	27.5
3	0	275.5	27.5	55.1	27.5	137.7	55.1
4	82.6	220.5	344.3	27.5	192.8	137.7	0
5	344.2	206.6	275.5	0	27.5	82.6	27.5
6	27.5	206.6	220.4	82.6	82.6	137.7	27.5
7	55.1	275.5	137.7	55.1	27.5	55.1	27.5
8	27.5	344.3	206.6	27.5	27.5	110.2	55.1
9	413.2	275.5	192.8	55.1	55.1	192.8	137.7
10	0	137.7	137.7	82.6	27.5	220.4	137.7
11	82.6	27.5	206.6	27.5	55.1	165.3	0
12	27.5	275.5	110.2	27.5	137.7	275.5	82.6
13	413.2	137.7	110.2	82.6	68.8	27.5	82.6
14	0	275.5	27.5	27.5	27.5	82.6	55.1
15	82.6	344.3	110.2	55.1	55.1	137.7	27.5

TABLE 4

S.No	Soya			LM		Feed		DOSFC	
	November	December	January	October	November	November	December	October	January
1	0	27.5	27.5	68.8	27.5	0	82.6	55.1	110.2
2	0	55.1	82.6	27.5	27.5	0	0	440.8	220.4
3	0	0	55.1	55.1	55.1	27.5	82.6	413.2	220.4
4	0	0	68.8	27.5	27.5	82.6	82.6	413.2	165.3
5	0	27.5	82.6	27.5	68.8	137.7	137.7	413.2	165.3
6	27.5	27.5	82.6	82.6	27.5	82.6	137.7	275.5	55.1
7	27.5	137.7	27.5	27.5	82.6	0	275.5	110.2	110.2
8	206.6	82.6	27.5	55.1	27.5	27.5	137.7	110.2	275.5
9	55.1	55.1	55.1	68.8	55.1	68.8	27.5	192.8	220.4
10	137.7	82.6	68.8	27.5	68.8	27.5	82.6	55.1	110.2
11	27.5	0	55.1	55.1	55.1	82.6	275.5	110.2	110.2
12	27.5	0	27.5	82.6	55.1	0	344.3	413.2	51.1
13	55.1	0	82.6	27.5	27.5	27.5	55.1	55.1	51.1
14	55.1	0	55.1	82.6	55.1	137.7	220.4	55.1	51.1
15	27.5	0	55.1	55.1	55.1	27.5	192.8	55.1	51.1

TABLE – 5

R_f VALUES FOR MYCOTOXINS

Mycotoxins	*Rf Values*	
	Solution I	*Solution II*
Aflatoxin		
B_1	0.62	0.22
B_2	0.56	0.20
G_1	0.48	0.15
G_2	0.08	0.12
Ochratoxin	0.00	0.46

CALCULATION

$$\text{Mycotoxin content in ppb} = \frac{S \times C \times d}{T} = x$$

$$= \frac{x}{E.W.} \times 1000$$

S - Standard which composes to sample in fluorescent intensity

C - Concentration of the standard

D - dilution factor

T - Sample which compares with standard in fluorescent intensity

E.W - Effective weight which varies according to the samples

STATISTICAL ANALYSIS

All the data collected in this experiment were subjected to statistical analysis.

Results And Discussion

CHAPTER IV

RESULT AND DISCUSSION

A study is made on the estimation of aflatoxin B_1 and ochratoxin A in different feed ingredients.

The estimated values are tabulated in Table 1, Table 2, Table 3 and Table 4.

The average, minimum and maximum values are given in Table 5 and Table 6. The best fit curves for aflatoxin B_1 and ochratoxin A in each feed in different months are drawn and the results are analysed.

GENERAL AVERAGE STUDY OF AFLATOXIN B_1 IN FEED INGREDIENTS

- Among the feeds taken, aflatoxin B_1 level is maximum in DOGNC and is found to be 92.6275 ppb and soya has minimum aflatoxin level of 9.81 ppb. Aflatoxin variation in the other materials is found to be less.
- The experimental aflatoxin B_1 level of 21.2 ppb in maize agreed well with the value observed by Alp. Kocabangi *et al.* (1992).
- In the table the values zero indicates the below detectable level.

GENERAL AVERAGE STUDY OF OCHRATOXIN A IN FEED INGREDIENTS

- Ochratoxin A level is maximum in DOSFC (385.15 ppb) during the course of study.

- Ochratoxin A level in soya and layer mash were found to be 44.36 ppb and 49.55 ppb respectively. These values are in the detectable level.
- Ochratoxin A level is found to be less variable in feed (95.45 ppb), DOGNC (141.85 ppb) and maize (103.34 ppb).
- The experimental ochratoxin A level in maize (103 ppb) is more or less same as that toxin level (90 ppb) observed by Thanaboripat *et al.* (1997).

TABLE 6

MAXIMUM, MINIMUM AND AVERAGE VALUES OF AFLATOXIN B_1 (IN PPB) DURING DIFFERENT MONTHS

	DOGNC				MAIZE				SOYA		
	October	November	December	January	October	November	December	January	November	December	January
Maximum	175	145.8	145	233.3	81.6	28.5	40.8	40.8	30.6	20.4	40.8
Minimum	23	58.3	35	35	4.1	4.1	4.1	0	0	0	4.1
Average	87.39	106.04	83.83	93.25	34.14	12.37	24.08	15.10	7.76	6.13	15.55
General Average	92.6275				21.2425				9.81		

	LM		CHICKEN FEED		DOSFC	
	October	November	November	December	October	January
Maximum	40.8	20.41	30.6	51.02	61.2	40.82
Minimum	8.1	4.1	0	0	24.48	0
Average	15.77	11.84	11.43	25.70	36.73	21.51
General Average	13.805		18.56		29.1235	

TABLE 7

MAXIMUM, MINIMUM AND AVERAGE VALUES OF OCHRATOXIN A (IN PPB) DURING DIFFERENT MONTHS

	DOGNC				MAIZE			SOYA		
	October	November	December	January	November	December	January	November	December	January
Maximum	413.2	344.5	344.3	82.6	192.8	826	137.7	206.6	137.7	82.6
Minimum	0	27.5	27.5	0	27.5	27.5	0	0	0	27.5
Average	109.2467	236.92	177.2067	44.04667	68.83333	191.6333	49.56	43.14	33.04	56.9
General average	141.85				103.34			44.36		

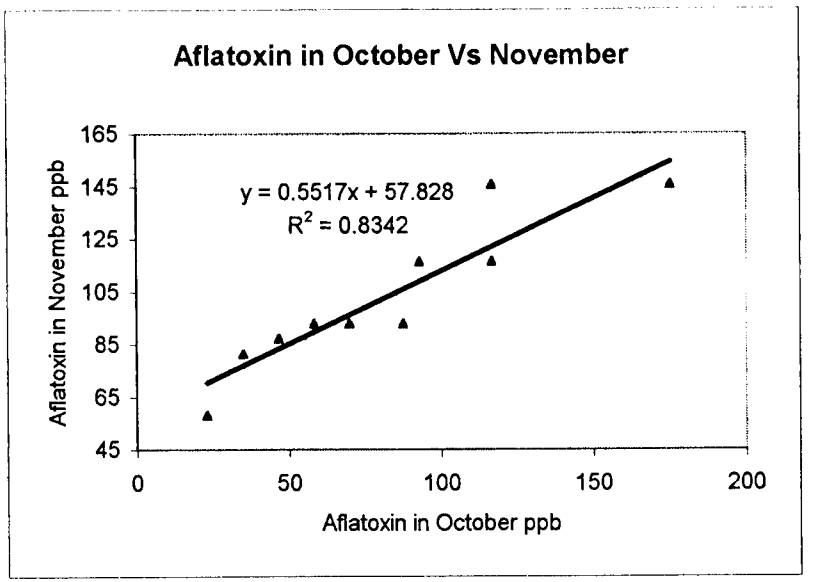
	LM		CHICKEN FEED		DOSFC	
	October	November	November	December	October	January
Maximum	82.6	82.6	137.7	344.3	440.8	275.5
Minimum	27.5	27.5	0	0	55.1	55.1
Average	51.39	47.72	48.63	142.31	440.8	275.5
General average	49.55		95.47		358.15	

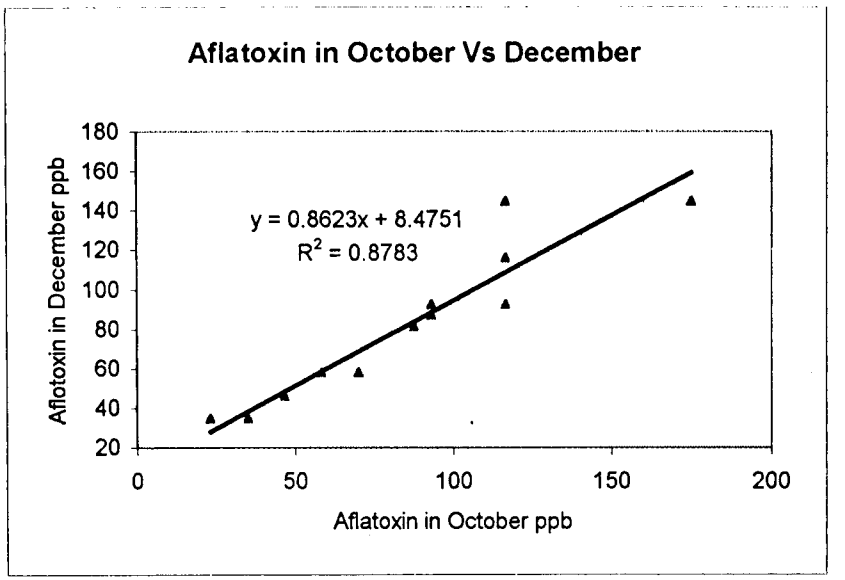
CORRELATION STUDY OF AFLATOXIN B_1

- Aflatoxin B_1 values are correlated keeping the month October as the base in DOGNC. All samples contain a maximum values of aflatoxin B_1 and they are less variable when stored. When moisture is more, the variation in aflatoxin B_1 level is found to be minimum.
- In maize during storage there is a small decrease in aflatoxin B_1 level which can be seen from the '*r*' values obtained.
- A remarkable increase in aflatoxin B_1 level can be noted in soya during storage from November to January.
- From the value of *r* (0.8152) in de-oiled sunflower cake the same effect is seen. That is during the storage period the aflatoxin B_1 level reduces.
- A very low value of *r* (0.75) is seen only in layer mash.
- In chicken feed there is notable increase in the aflatoxin B_1 level for a period of a month that is from November to December.

Figure - 1

CORRELATION OF AFLATOXIN B IN DOGNC DURING DIFFERENT MONTHS





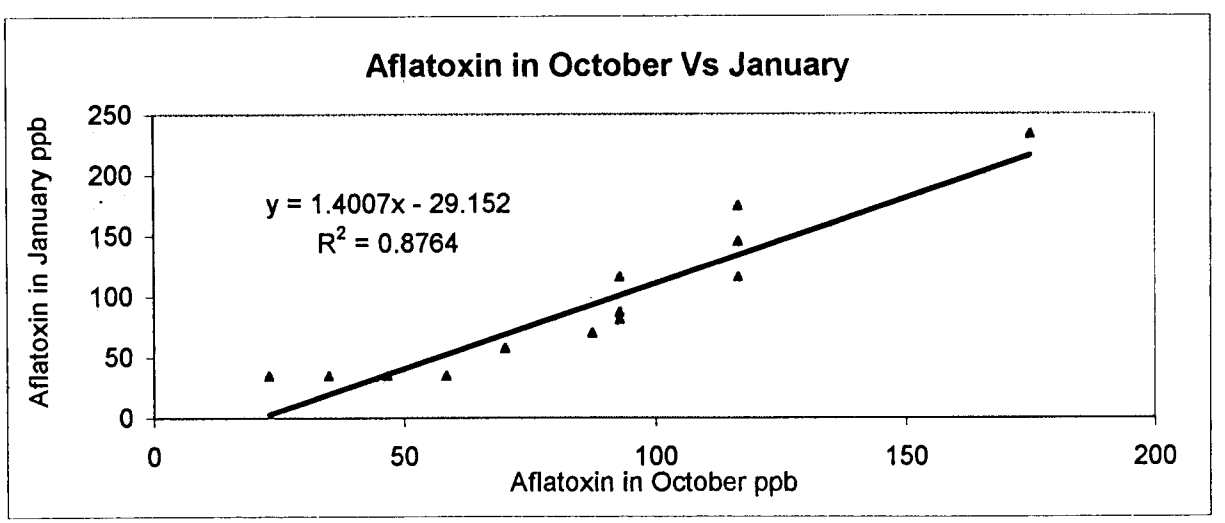
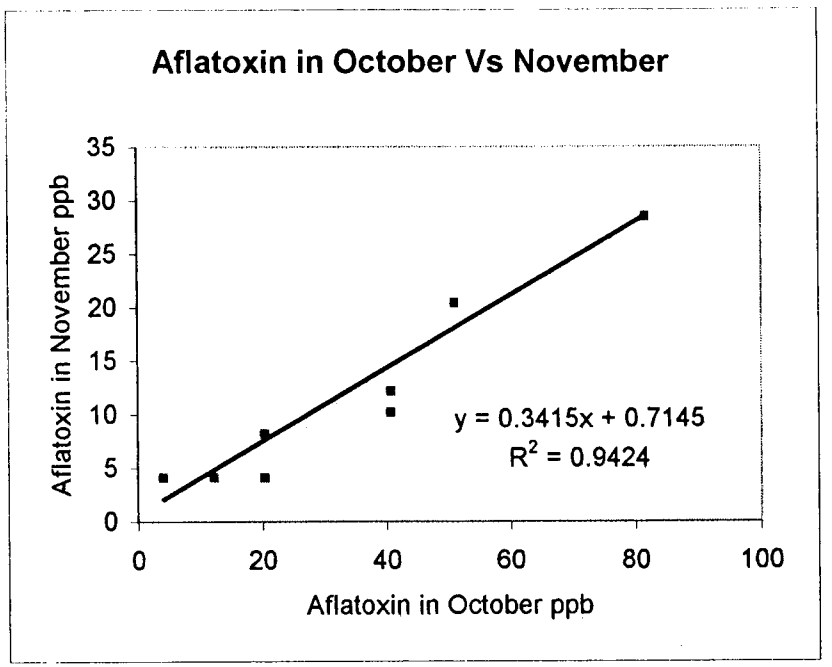
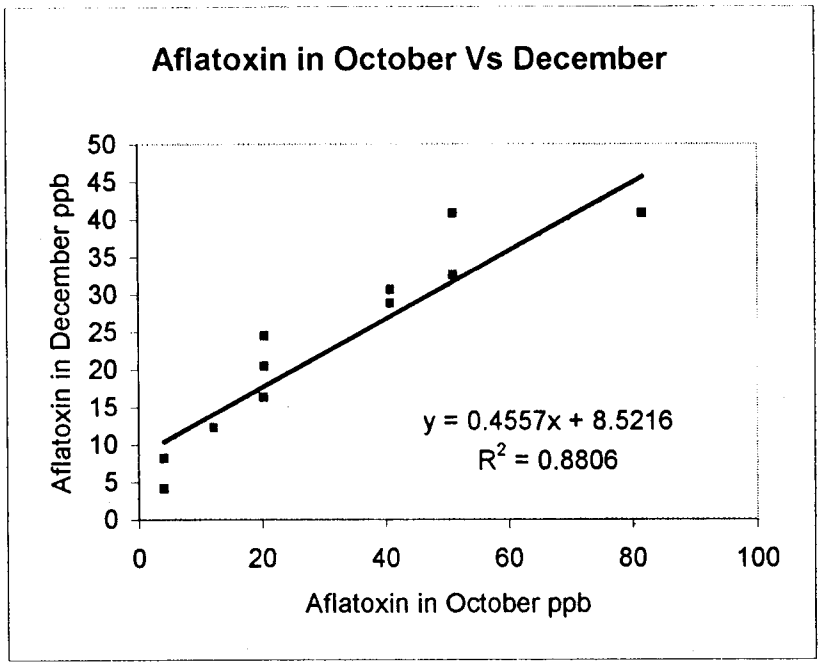


Figure - 2

CORRELATION OF AFLATOXIN B IN MAIZE DURING DIFFERENT MONTHS





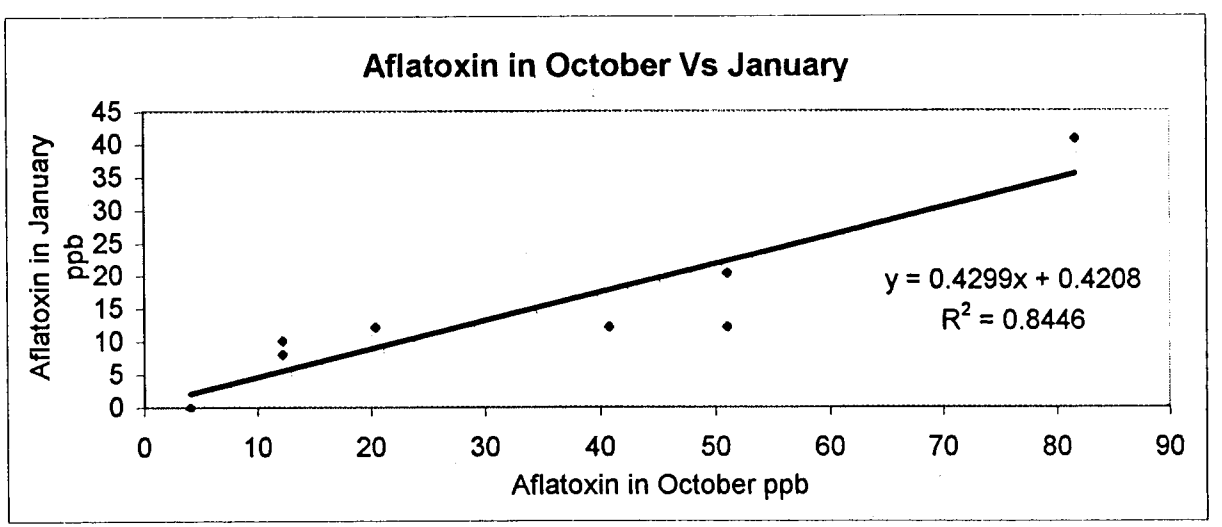
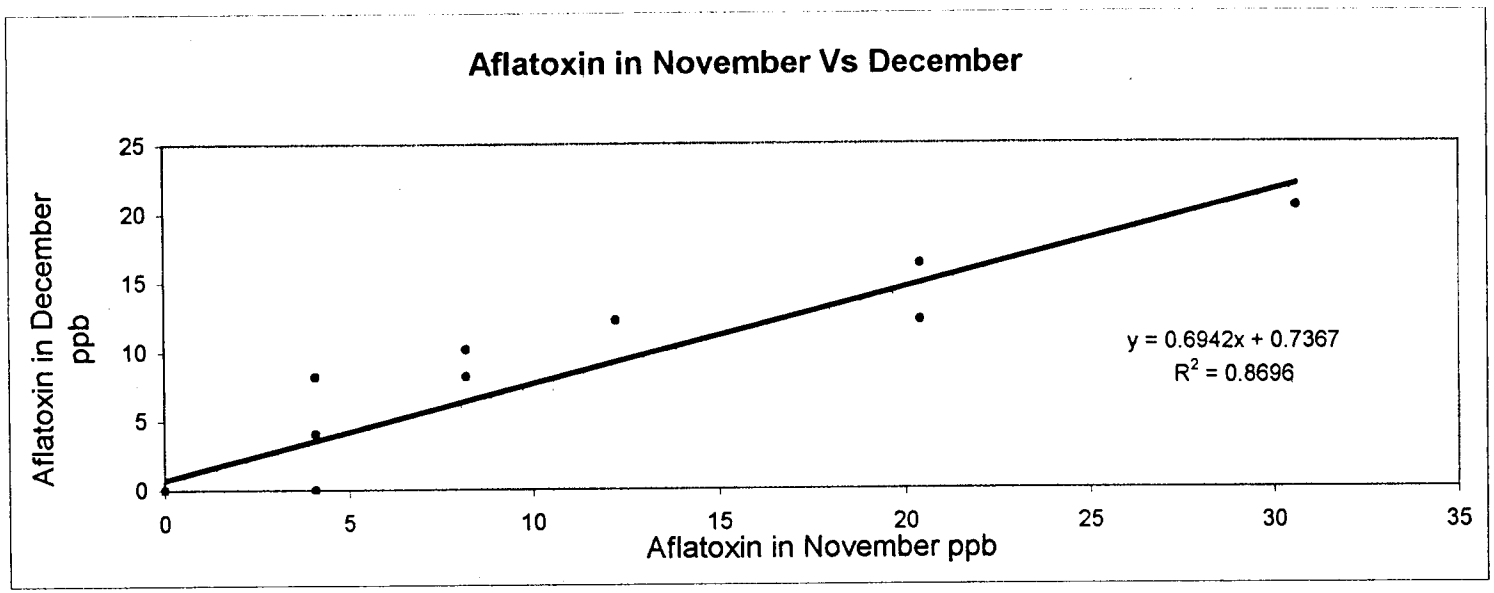


Figure - 3
CORRELATION OF AFLATOXIN B IN SOYA DURING DIFFERENT MONTHS



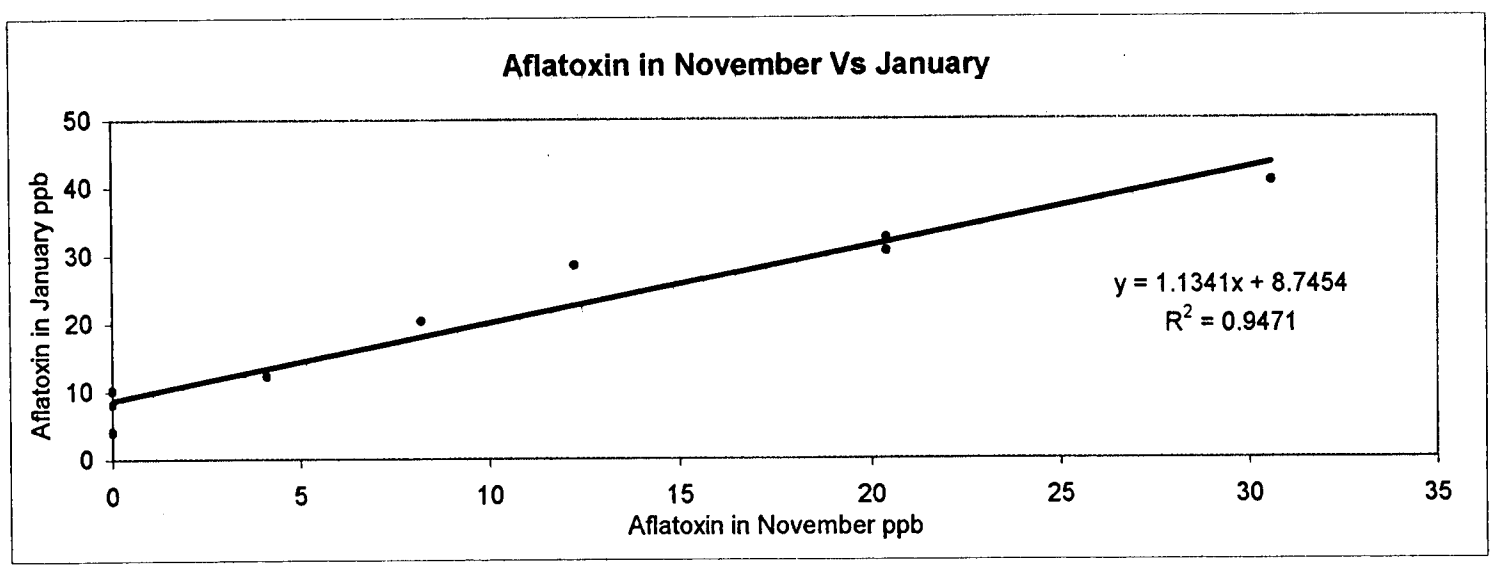
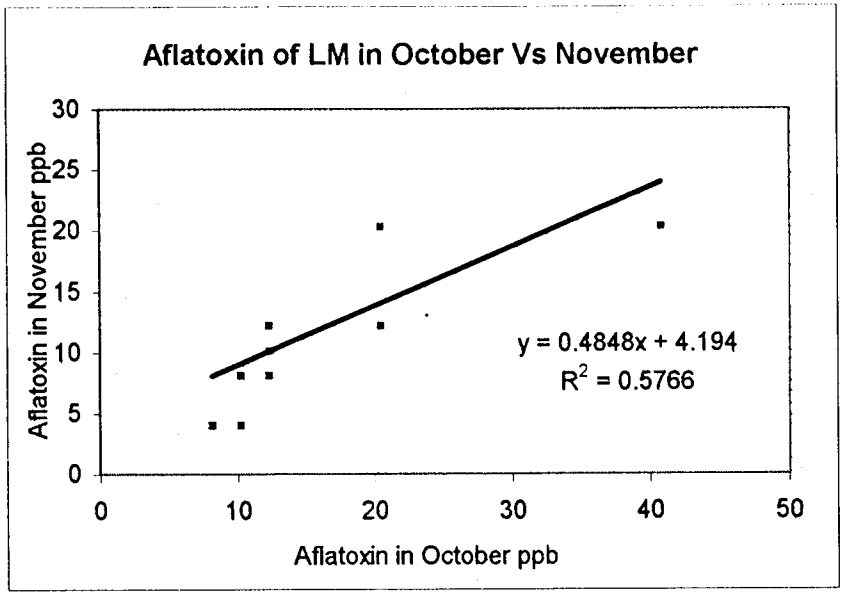
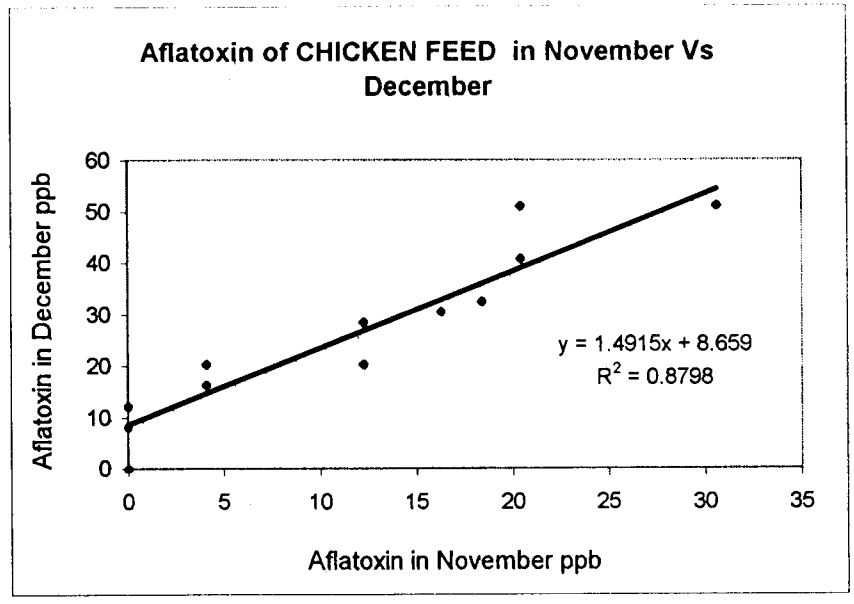
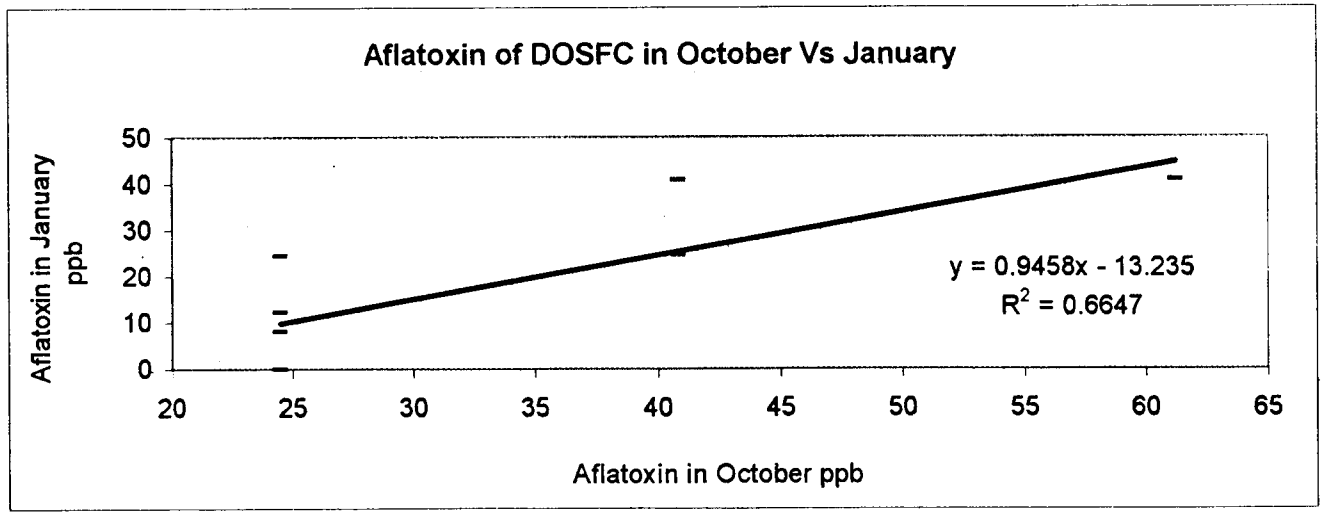


Figure - 4

CORRELATION OF AFLATOXIN B IN LM, CHICKEN FEED AND DOSFC DURING DIFFERENT MONTHS





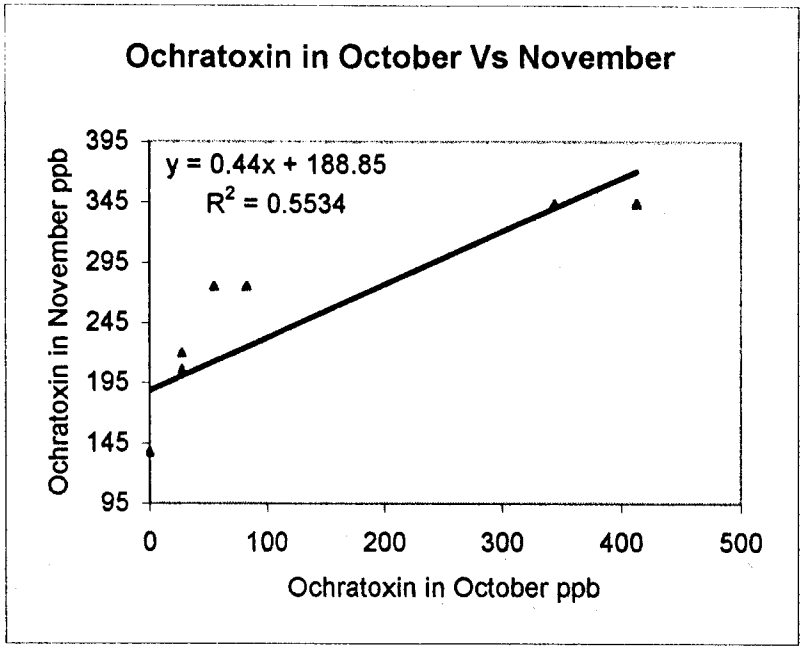


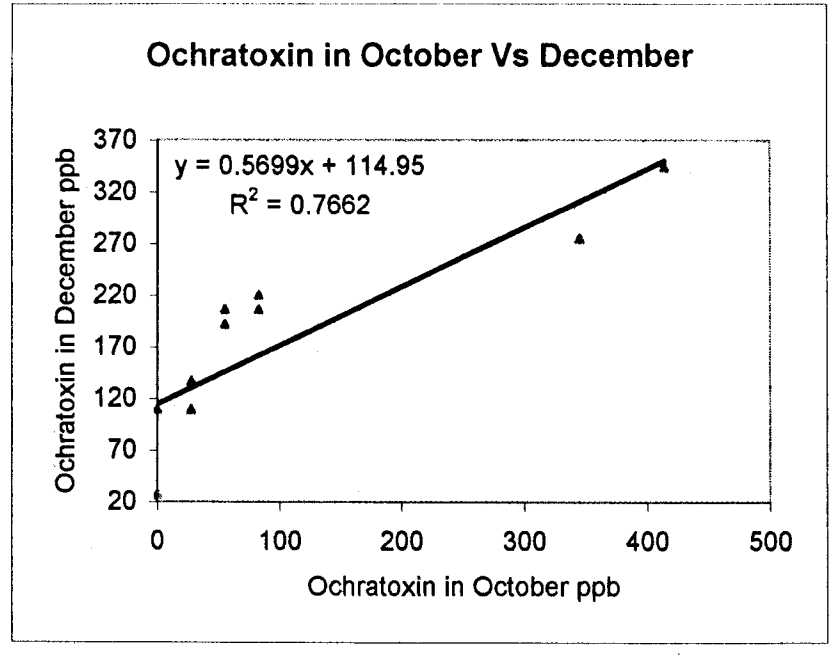
CORRELATION STUDY OF OCHRATOXIN A

- The following information can be obtained when the correlation graphs October vs November, October versus December and October versus January are analysed. A gradual increase in *r* value is seen in all graphs of DOGNC.
- A high value of *r* (0.9689) is noted in layer mash which indicates that the ochratoxin A level increases during the rainy season (ie) when the moisture in the atmosphere is high.
- Ochratoxin A values are correlated keeping November as base month in maize. From the observation it is noted that *r* value increases. Hence toxicity due to ochratoxin A increases in the course of time.
- In soya during storage there is a remarkable decrease in ochratoxin A level which can be seen from the '*r*' values obtained.
- In chicken feed the toxicity level is more during the month November and December. From this it can be inferred that where there is fall in temp in the atmosphere, toxic level of the feed goes high.
- Ochratoxin A value is maximum in the case of DOGNC during the period of study that is from October to January. The correlation coefficient value *r* is found to be 0.9540.

Figure - 5

CORRELATION OF OCHRATOXIN A IN DOGNC DURING DIFFERENT MONTHS





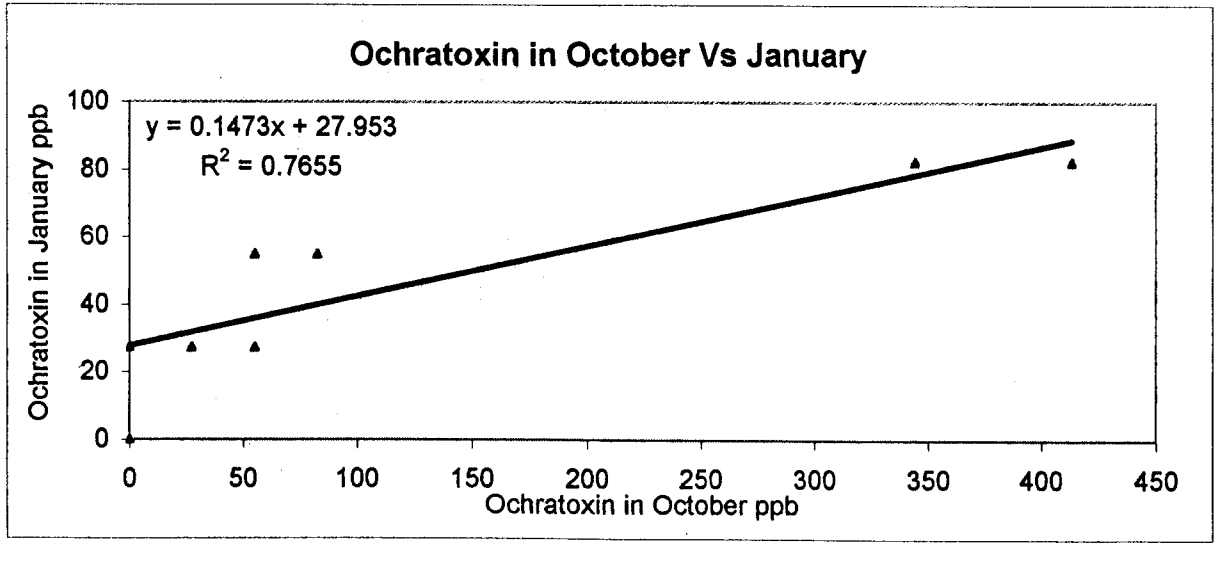
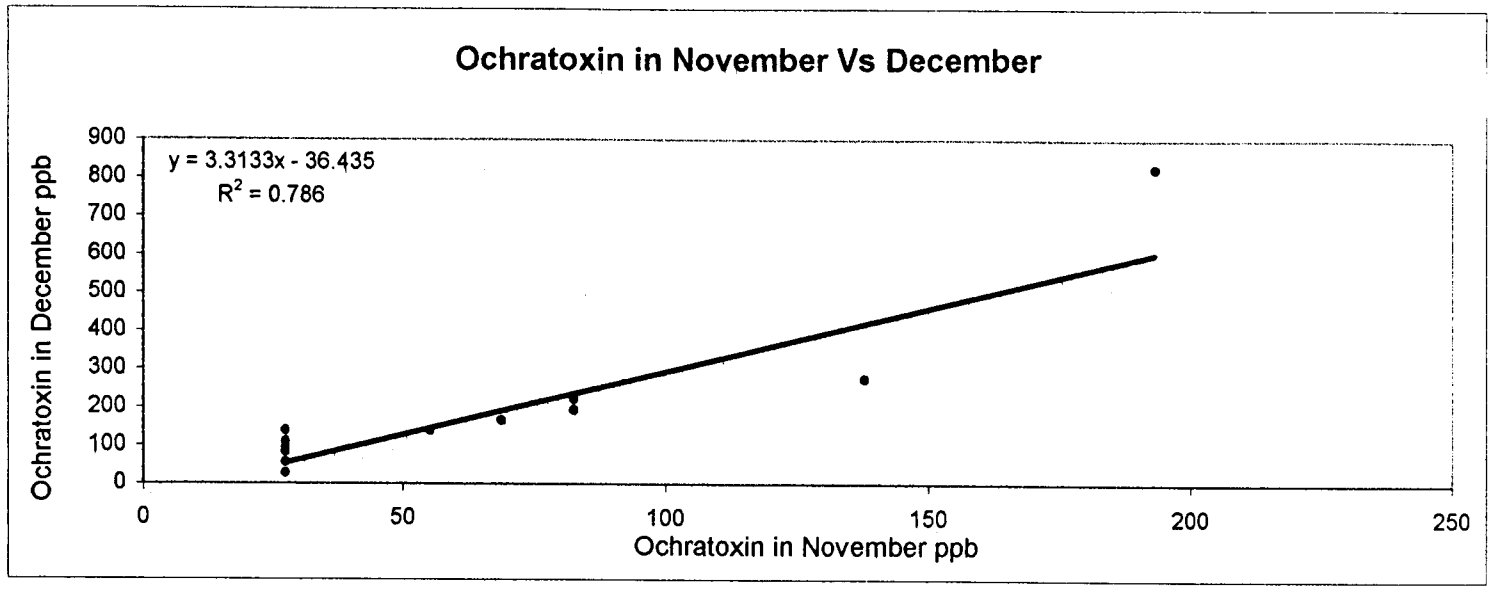
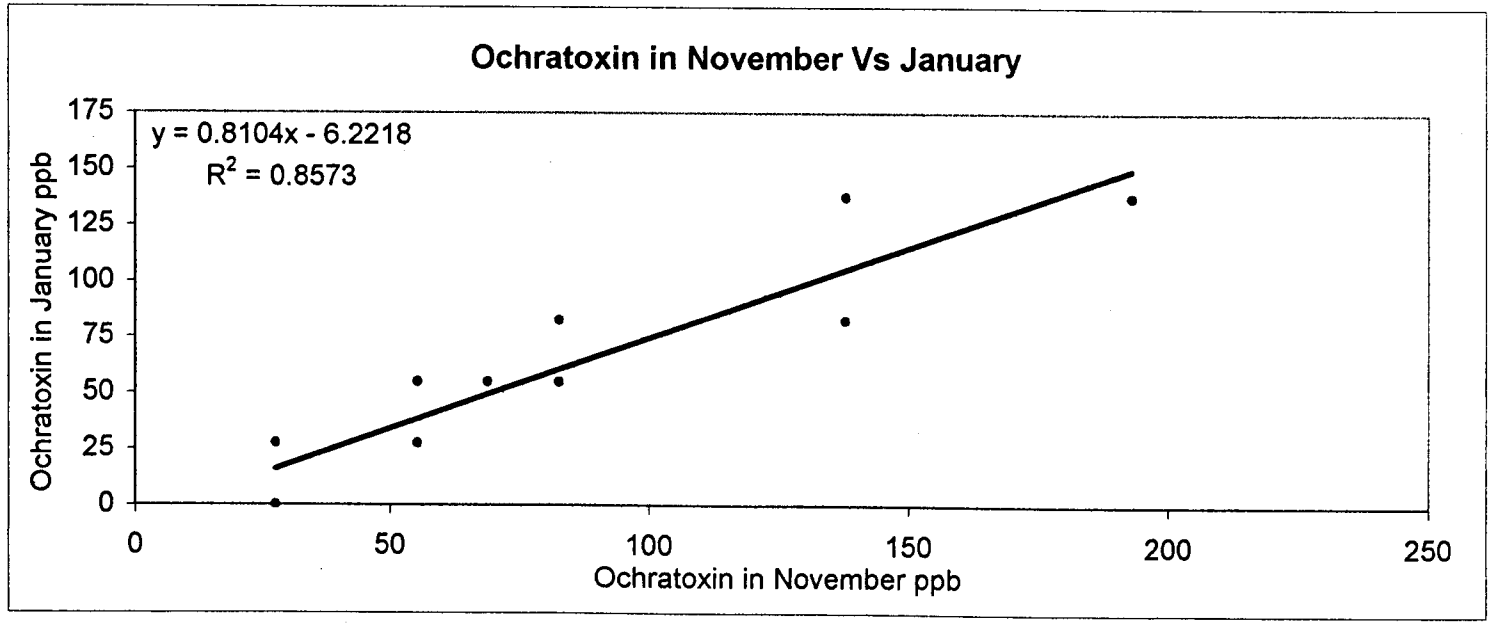


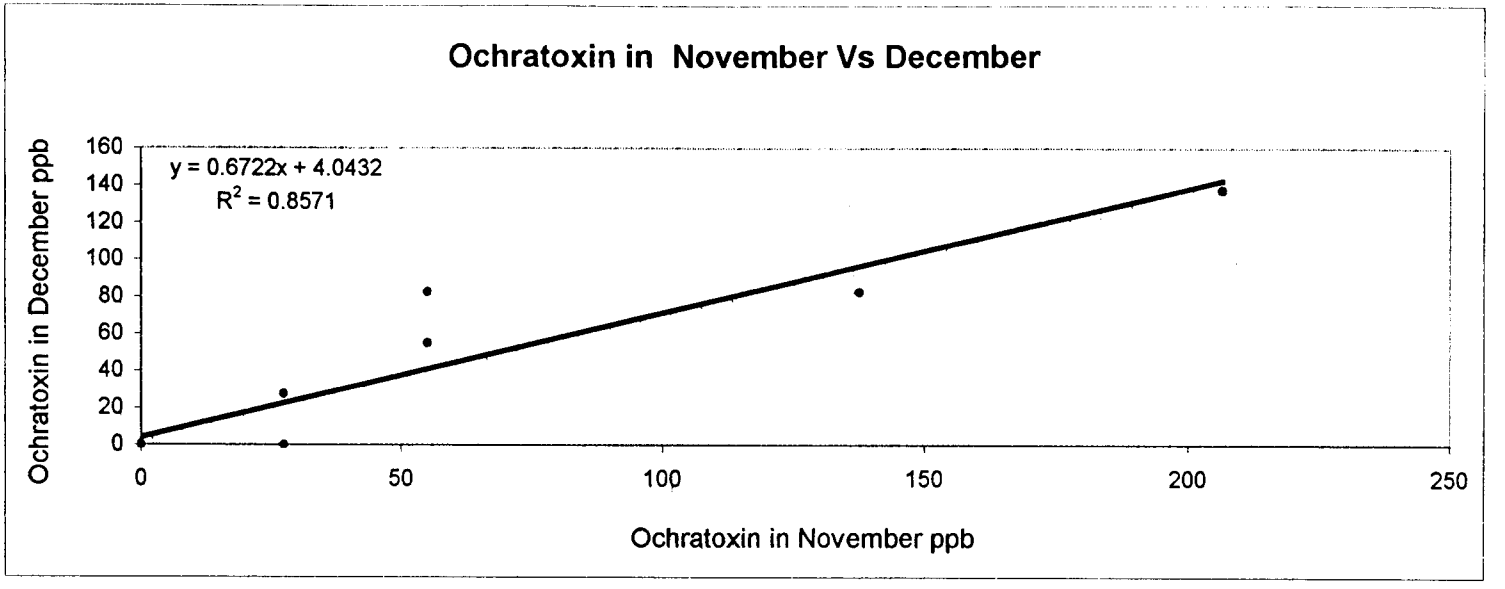
Figure - 6

CORRELATION OF OCHRATOXIN A IN MAIZE DURING DIFFERENT MONTHS





CORRELATION OF OCHRATOXIN A IN SOYA DURING DIFFERENT MONTHS



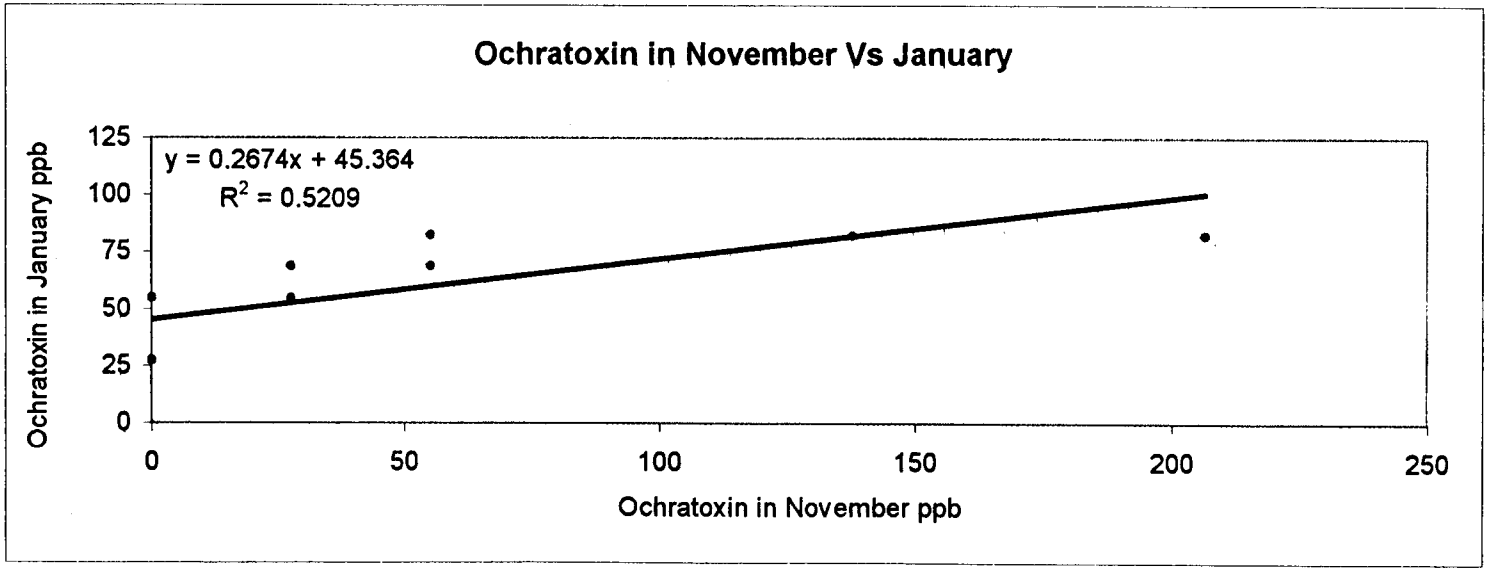
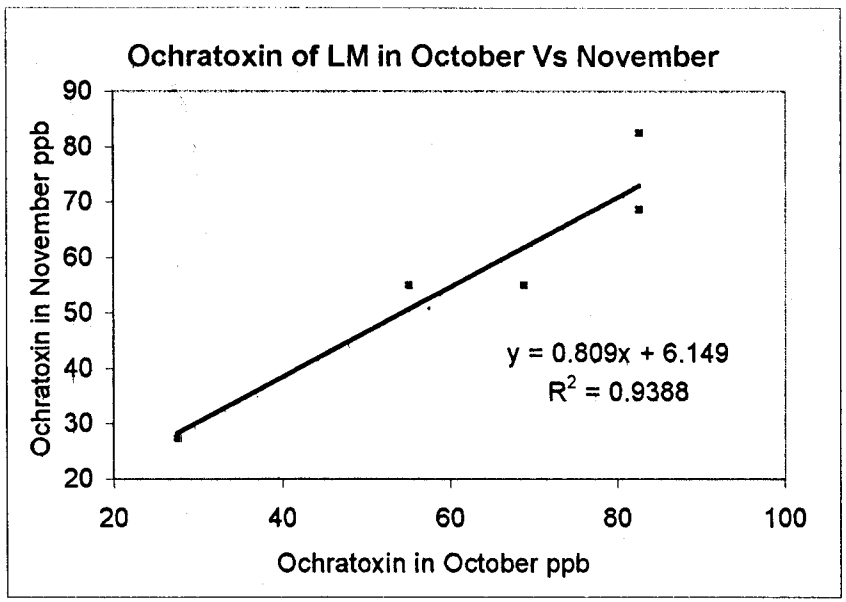
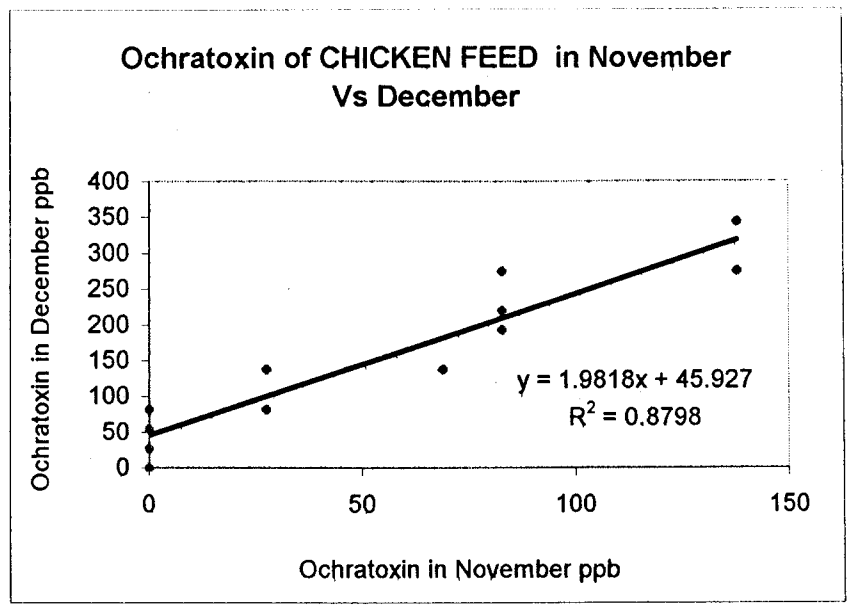
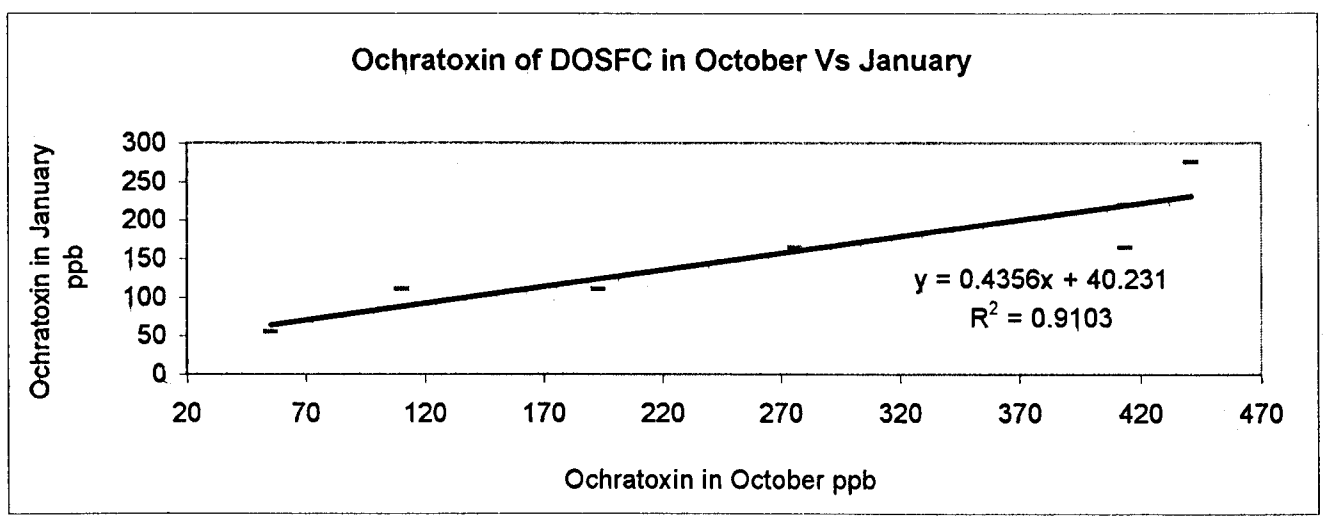


Figure - 8

CORRELATION OF OCHRATOXIN A IN LM, CHICKEN FEED, DOSFC DURING DIFFERENT MONTHS





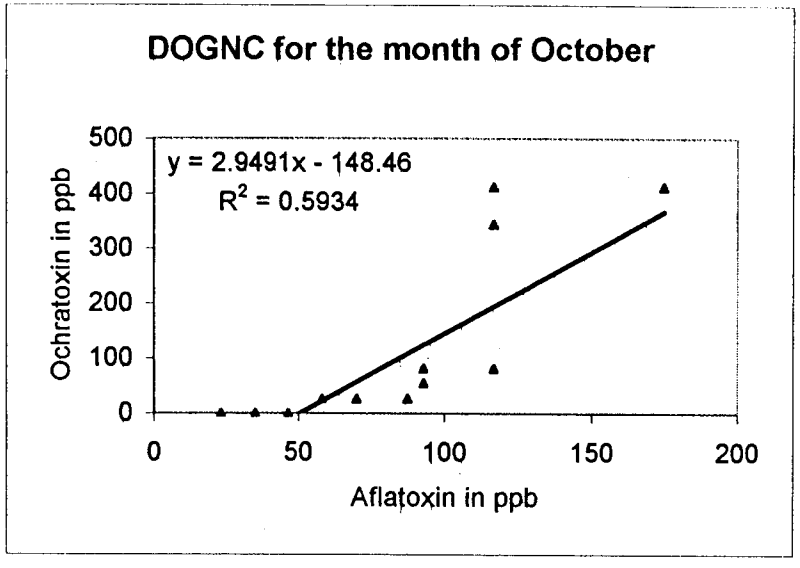


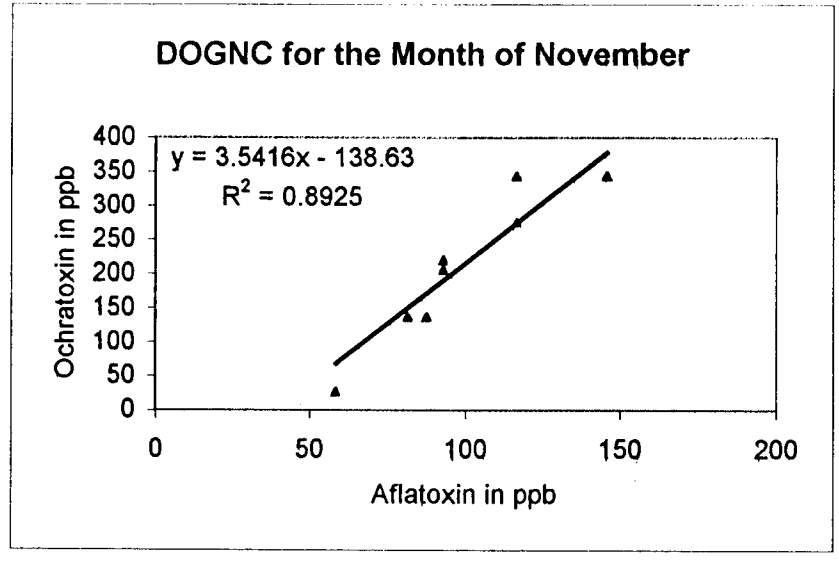
CORRELATION OF AFLATOXIN B_1 VERSUS OCHRATOXIN A IN DIFFERENT TYPES OF FEED INGREDIENTS

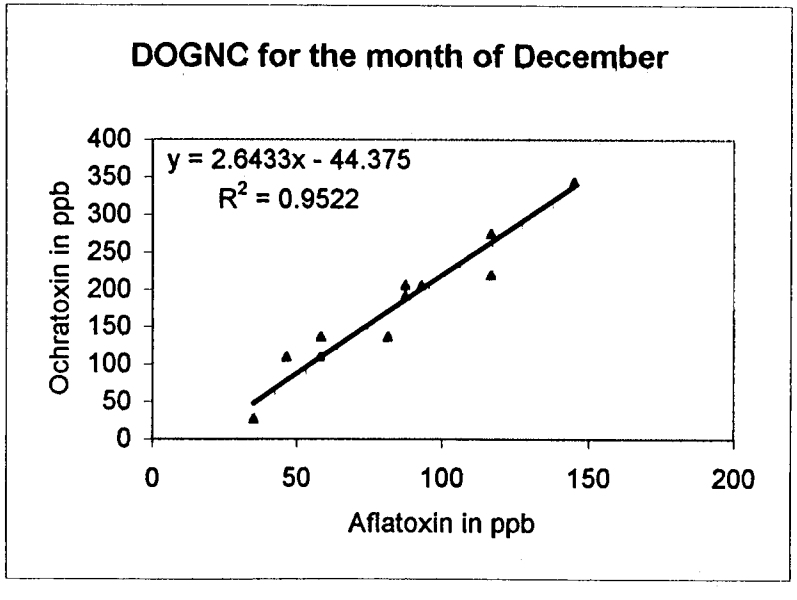
- As we see from the graph there is no much correlation between ochratoxin A and aflatoxin B_1 during the month of October.
- During the months of November and December the presence of the toxins affects the other. That is both have influence on each other. The correlation coeffeciet value is high in DOGNC during this period.
- There is a sudden decrease in r value(0.9042)in the month of January. From the above r value we can conclude that the toxicity r value is greatly enhanced by the presence of moisture in the atmosphere.
- For maize the correlation value r is maximum in the month of December and before and after this month it is not high.
- The correlation value r of layer mash is comparable during the months October and November.But in November the value is high. This may be due to the increase in moisture content.
- In feed, the ochratoxin A level is more, in the month of December and the correlation value r of ochratoxin A and aflatoxin B_1 also goes high which shows that ochratoxin A level is maximum in the winter season.
- As we see from the graph there is no much correlation between aflatoxin B_1 and ochratoxin A in DOSFC from the month of October to January.
- In soya the correlation is high in the month of November and December. But in the month of January it decreases. We also infer that ochratoxin A

level is minimum in soya during the month January compared to all the other feed and feed ingredients.

Figure - 9
CORRELATION OF AFLATOXIN Vs OCHRATOXIN IN DOGNC







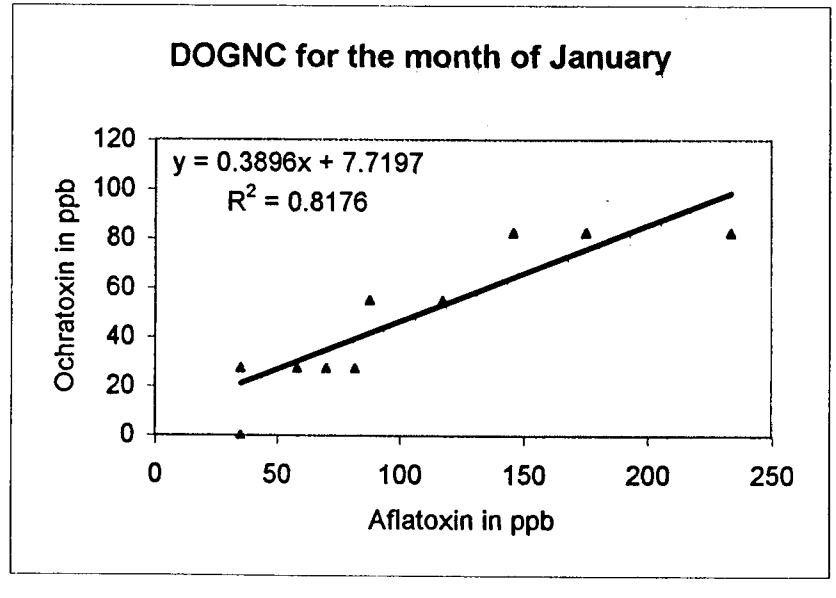
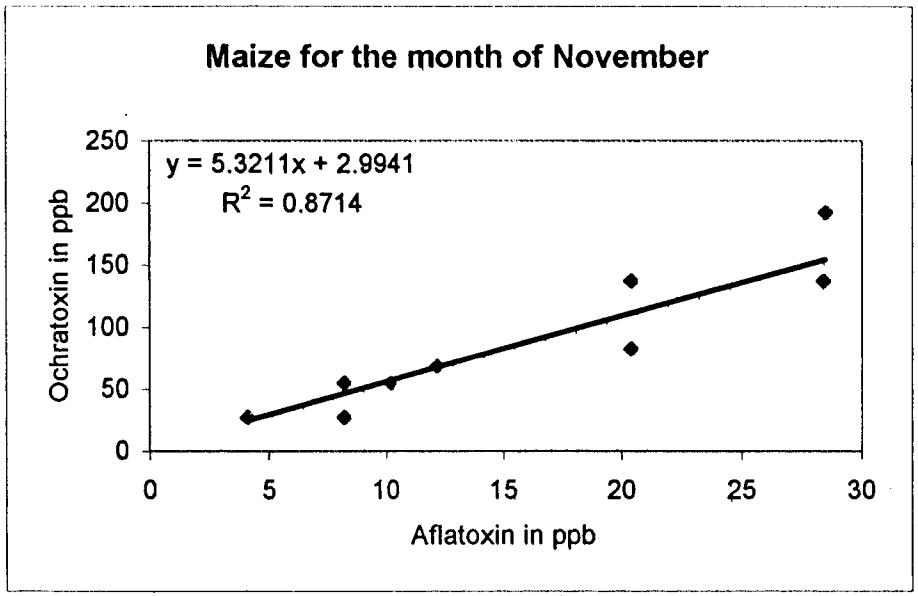
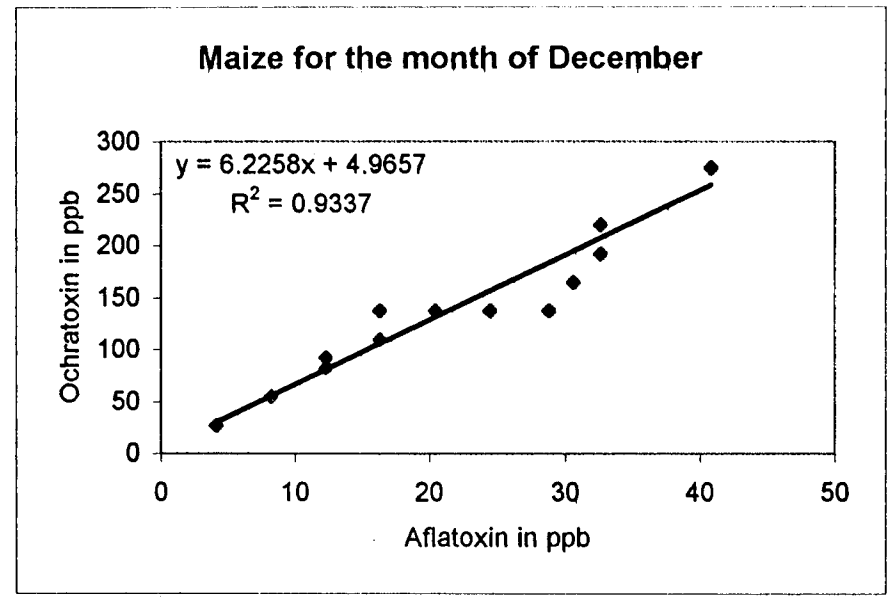


Figure - 10
CORRELATION OF OCHRATOXIN Vs AFLATOXIN IN MAIZE





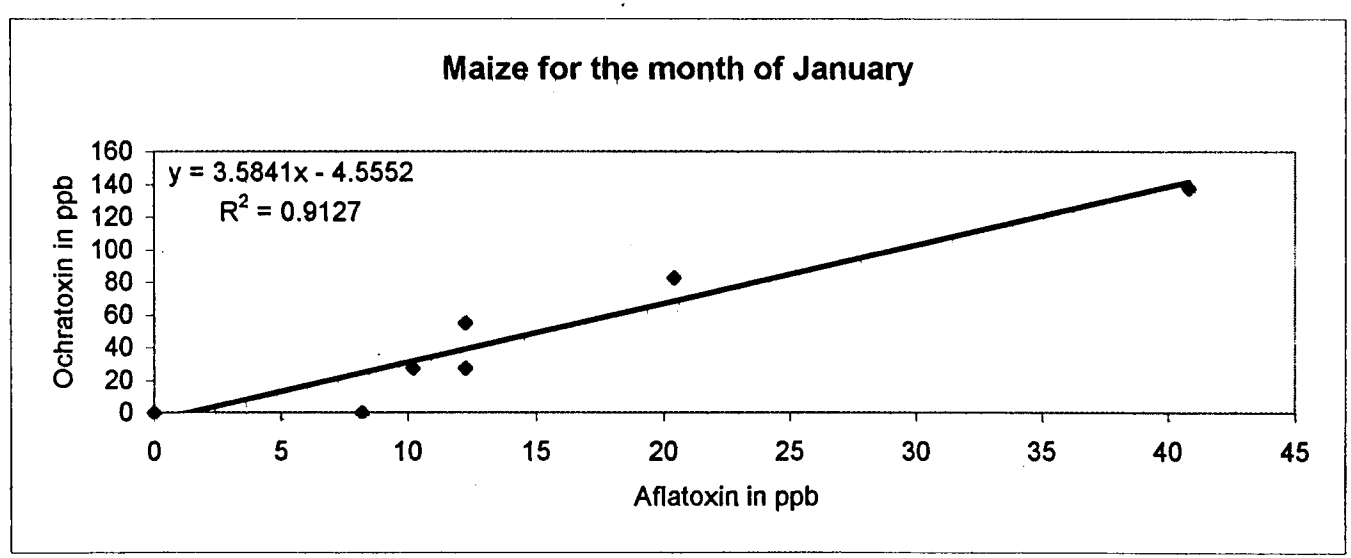
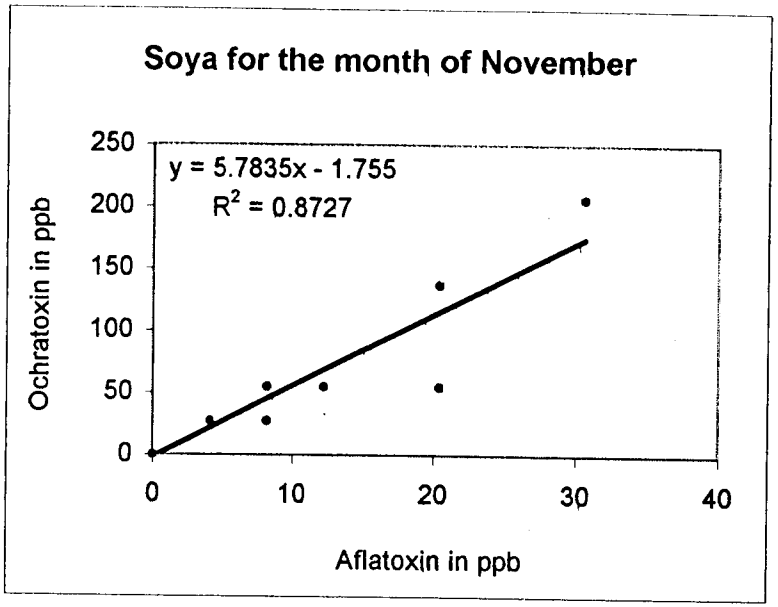
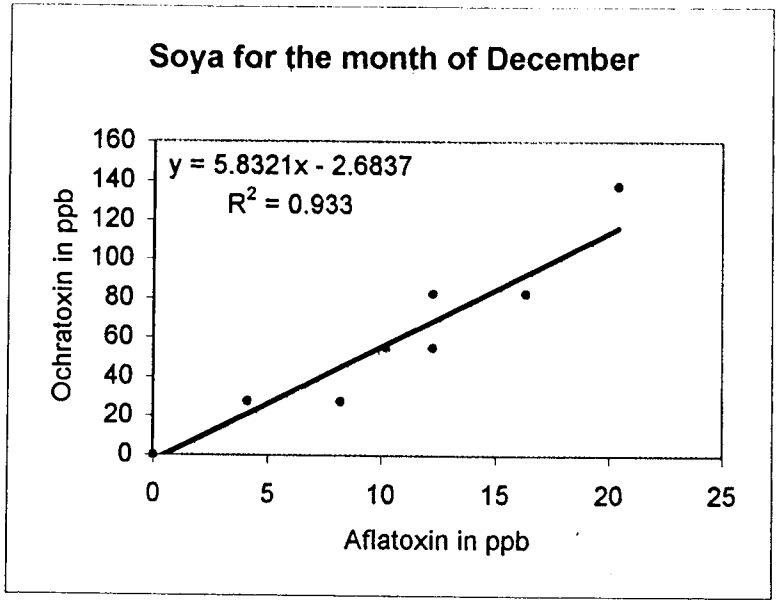


Figure - 11
CORRELATION OF AFLATOXIN Vs. OCHRATOXIN IN SOYA





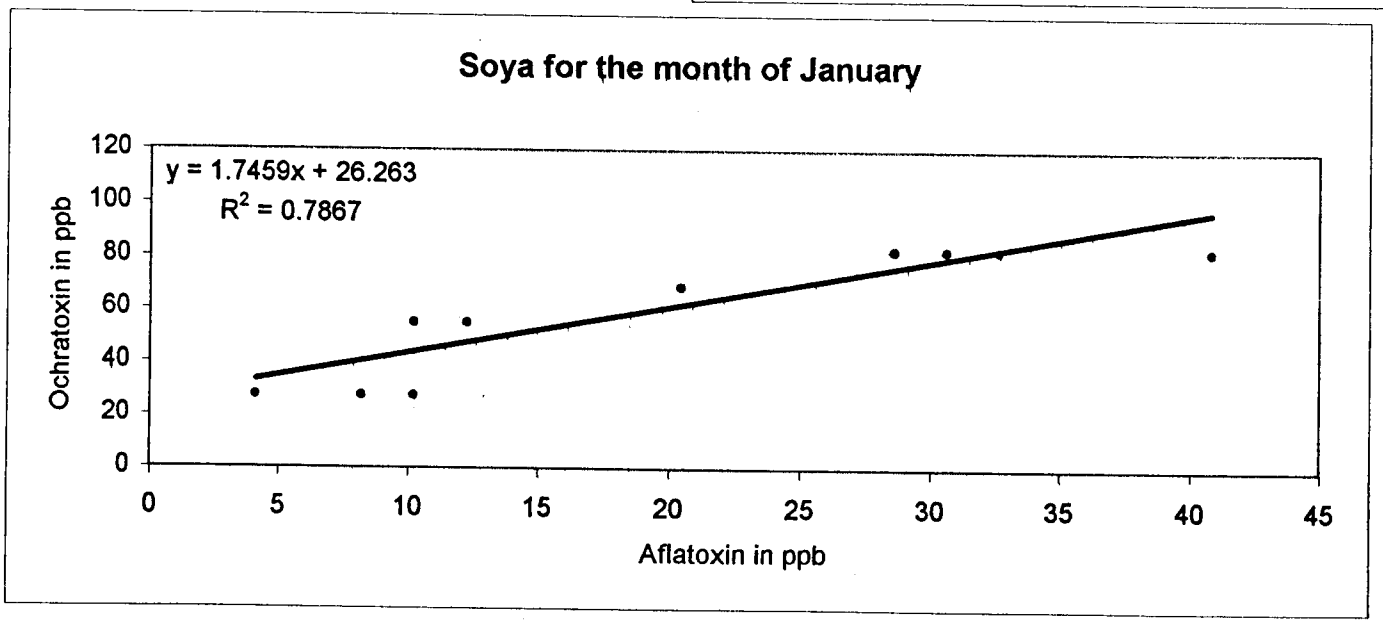
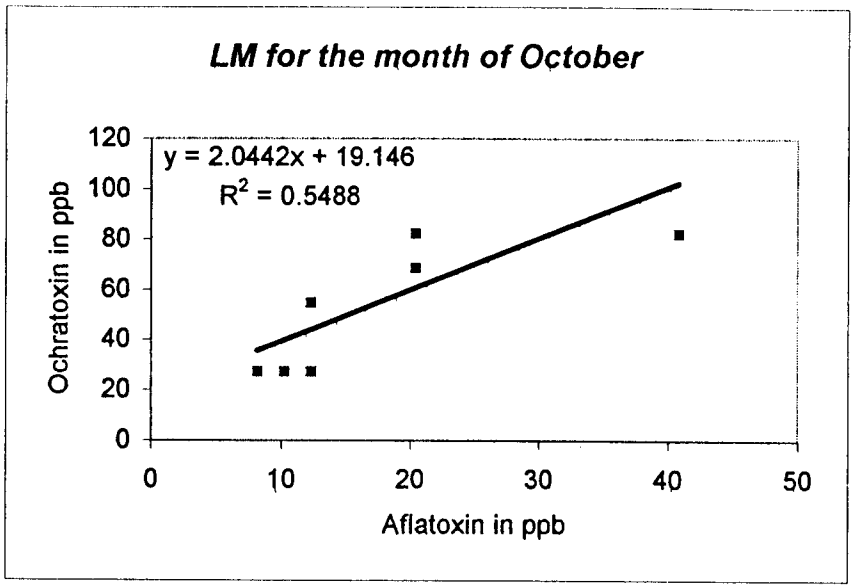
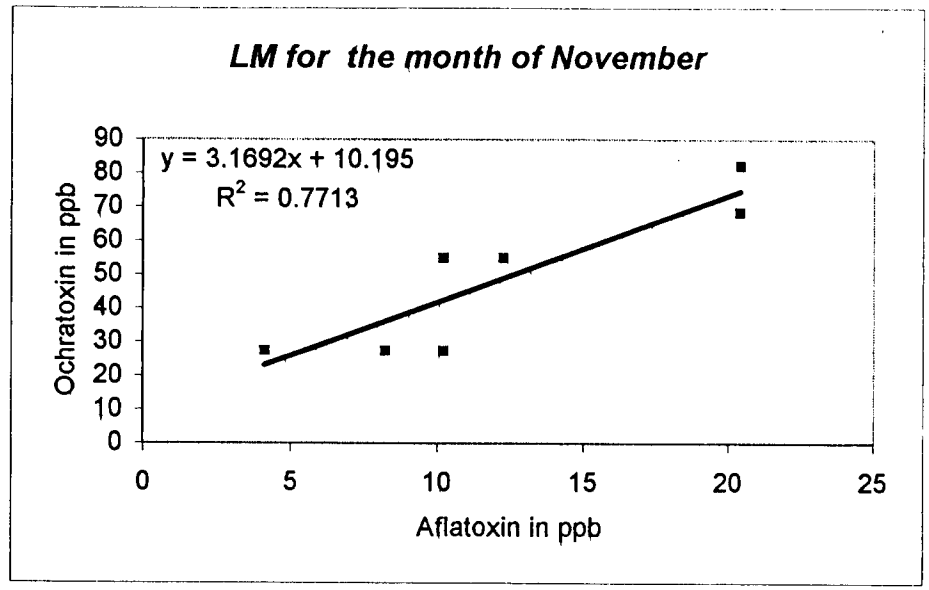
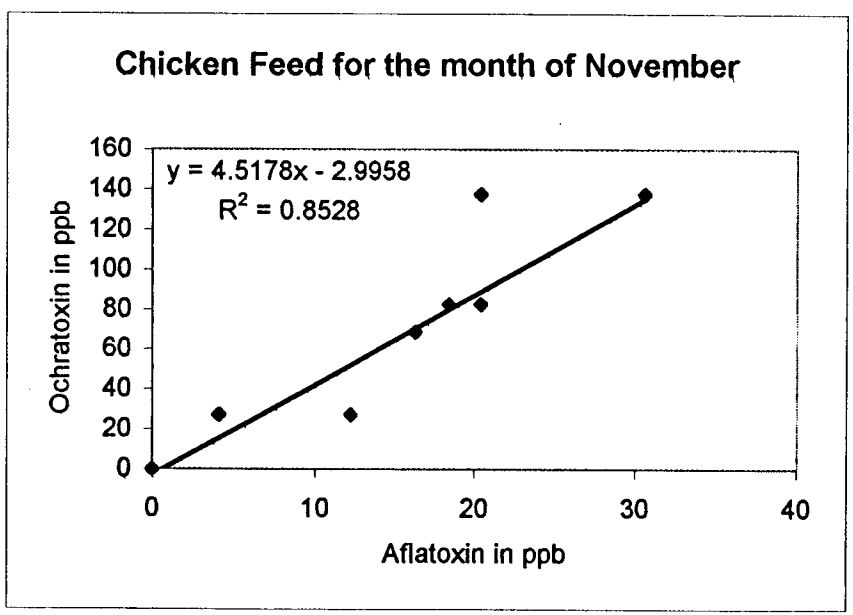


Figure - 12
CORRELATION OF AFLATOXIN Vs OCHRATOXIN IN LM AND CHICKEN FEED







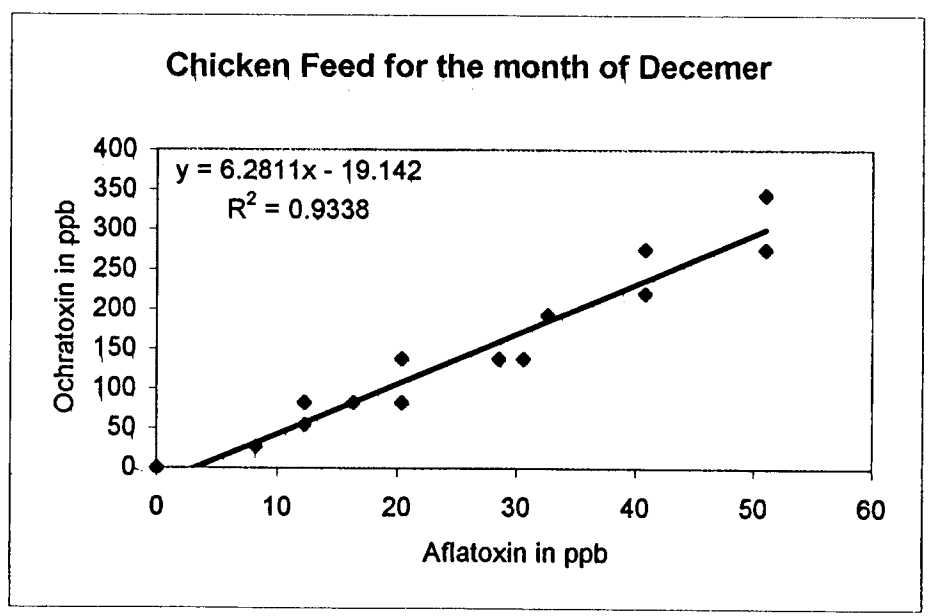
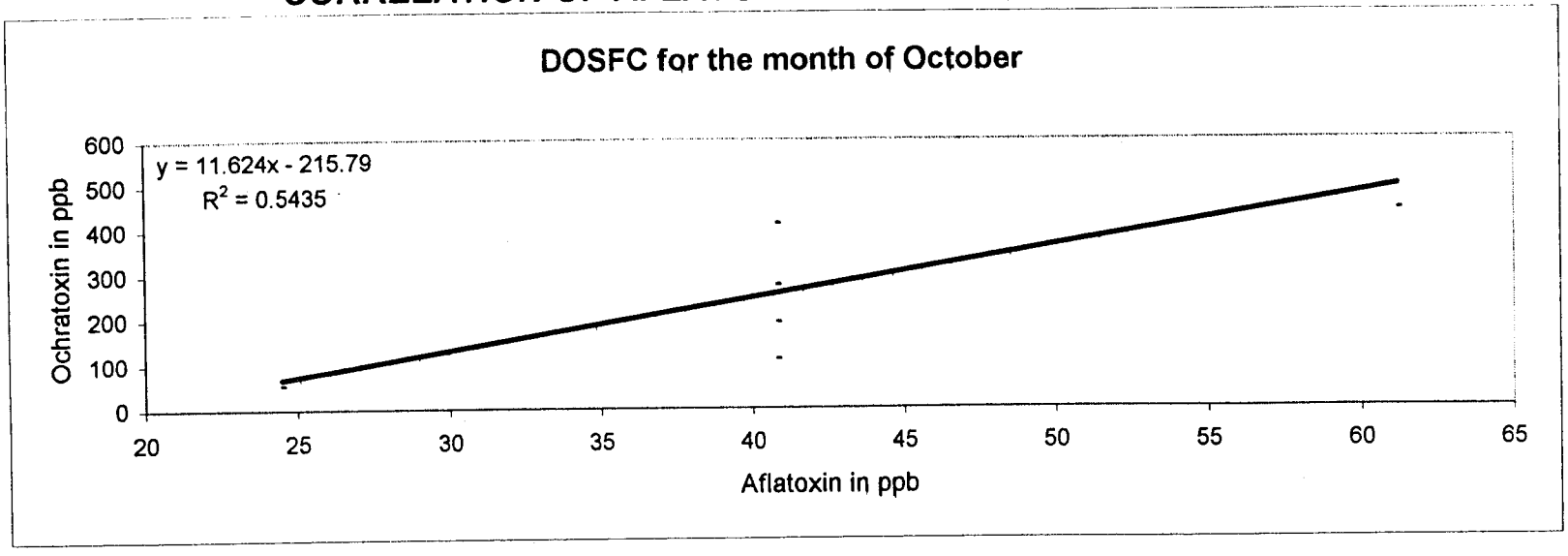
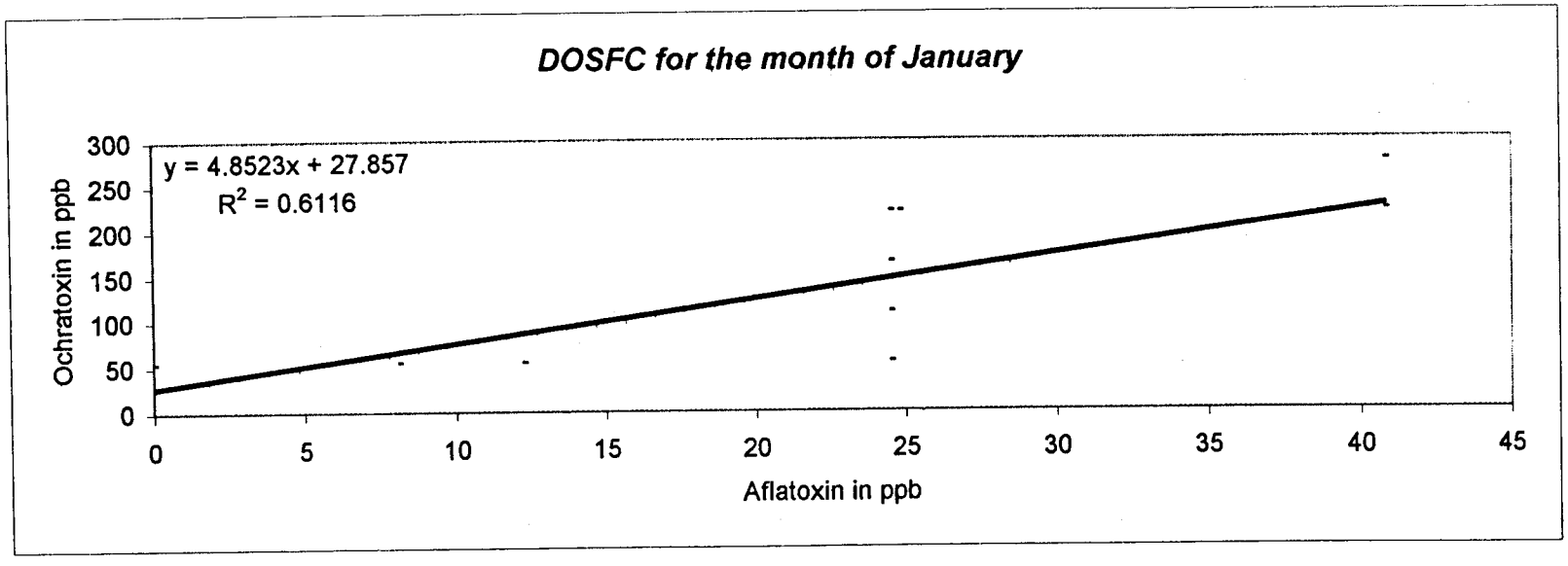


Figure - 13
CORRELATION OF AFLATOXIN Vs OCHRATOXIN IN DOSFC





Summary and Conclusion

CHAPTER V

SUMMARY AND CONCLUSION

Six experiments were conducted to study the level of Aflatoxin B_1 Ochratoxin A in chicken feed , layer mash and also in the following feed ingredients.

Maize

Soya

DOGNC

DOSFC.

The experimental study was carried out by two dimensional semi quantitative TLC method which involved the following steps.

- Grinding.
- Filtration.
- Separation.
- Evaporation.
- Spotting.
- Developing.
- Comparing the intensity under U.V cabinet.

Aflatoxin B_1 levels were correlated between different feeds and feed ingredients during different months. Similarly ochratoxin A levels were also correlated. A correlation study was also done between the two toxin.

For the samples in each month the maximum, minimum and average values determined. The general average value of all the months for all the samples were also determined.

From the general average value of aflatoxin B_1 ,ochratoxin A in DOGNC, soya, layer mash, chicken feed and DOSFC . We can concluded that DOGNC has a high level of aflatoxin B_1 while DOSFC has a high level of ochratoxin A .

Soya has a low level of ochratoxin A and aflatoxin B_1 in all the months compared to all the other feed and feed ingredients.

The ochratoxin A level in layer mash is also low. Depending up on the moisture content and storage, the toxicity level varies.

The supply of large quantity of soya will minimise the effect of toxins in the feeds. Hence DOSFC and DOGNC can be replaced by soya during the period from October to January.

Toxins cause damaging effects on all living beings. Hence the contamination in eggs and chicken must be reduced. Selecting a proper feed and feed ingredients can do this.

The most important conclusion is that all those concerned in the production, processing and marketing of feed stuffs, as well as those concerned poultry feeds, should make every effort so that the poultry producer will be provided with feeds that not only are complete from a nutrient requirements standpoint, but also have minimal levels of mycotoxin in them.

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Appendices

APPENDIX 1

PREPARATION OF SOLUTIONS FOR TWO DIMENSIONAL METHOD OF ANALYSIS OF MYCOTOXINS IN FEED AND FEED INGREDIENTS

DEVELOPING SOLTION I

Chemical required

Acetone : 88 ml

Chloroform : 12 ml

Developing solution I is the mixture of 12 ml of chloroform and 88 ml of acetone. In this solution the TLC plates were developed horizontally.

DEVELOPING SOLUTION II

Chemicals required

Toluene : 100 ml

Ethyl acetate : 80 ml

Formic acid : 20 ml

Developing solution II is the mixture of 100 ml of toluene, 80 ml of ethyl acetate and 20 ml of formic acid. Vertically the TLC plates were developed after horizontal development.

APPENDIX 2

PREPARATION OF REFERENCE STANDARD SOLUTIONS

1. Standard Aflatoxin B_1 Preparation

Prepared solution of 10 mg / ml of aflatoxin B_1 in benzene and aceto nitrile (98+2) combined portions to give 1 mg / ml of aflatoxin B_1.

2. Standard ochratoxin A Preparation

5 mg/ml of ochratoxin A and B in 40 mg/ml of (1:99) glacial acetic acid and benzene were prepared.

Limit for Detection

Aflatoxin - 20 ppb

Ochratoxin - 45 ppb