Ameliorating the effect of mycotoxins in poultry feeds using plant extracts

ABSTRACT

Mycotoxins are toxic secondary metabolites of fungal origin that tends to contaminate agricultural commodities before or under post-harvest conditions. They are mainly produced by fungi in the genera, *Aspergillus sp. (*Aspergillaceae: Eurotiales*), Penicillium sp.* (Trichocomaceae; Eurotiales) and *Fusarium sp.* (Nectriaceae: Hypocreales). When ingested, inhaled or absorbed through the skin, mycotoxins causes sickness or death in humans and animals. Natural substances that can prevent AFB1 toxicity to human and animal health with minimal cost will be a great advantage. Traditional medicinal plants are currently used for their antifungal, anti-aflatoxigenic and antioxidant activity. *Aspergillus parasiticus* strain NRRL 2999 was used to produce Aflatoxin, as it is one of the highly toxigenic fungus available. Inhibitory activity of ethanol extracts of plants was enhanced upon gradual increase in their concentrations. Among them, the ethanol extracts of *Trachyspermum ammi* (Apiaceae: Apiales) completely arrested the fungal growth and inhibited the growth significantly, even at lower concentrations (1%) in comparison with other extracts and the negative control. Hence, the present work has been carried out to find the aflatoxin antifungal activity of the plant extracts *Albizia lebbeck* (Fabaceae: Fabales), *Syzgium aromaticum* (Myrtaceae: Myrtales) and *Trachyspermum ammi* (Apiaceae: Apiales) against aflatoxin contaminated poultry feed. In conclusion, the present study serves as a base and arises a futuristic need for efficient cost effective sampling and analytical methods that can be used for detection and control of mycotoxins in poultry feed.

Key words: Aflatoxin, *Albizia lebbeck, Syzgium aromaticum, Trachyspermum ammi*, anti-fungal, poultry feed

INTRODUCTION

In the recent times, Poultry industry has gained paramount attention and has maximized as a phenomenal agricultural business with an annual turnover of Rs. 30,000 crores. In a short period, in the livestock sector, poultry is the most efficient industry for enhancing the supply of proteins, fats, minerals and vitamins. India is the third largest egg producer in the world (after China and the United States of America), and the nineteenth largest broiler producer. Undoubtedly, this impressive growth is a result of several factors, such as active developmental support from the state and central government, research and development support from research institute, (APEDA, 2006) international collaboration and private sector participation. Most disease problem in poultry is today caused by interaction of many factors where immuno-suppression plays an important role. In poultry quails are the small bird and commercially grown for their eggs and meat. In India, the commercial farming of these birds are increasing day by day.

One of the most common immune-suppression agent in poultry is mycotoxin produced by fungi. When grown on a living organism or on stored food material, the fungi may produce harmful secondary metabolites which are toxic substances and are called mycotoxins. The study of mycotoxin is called "Mycotoxicology". Mycotoxin is classified as *Aspergillus toxins, Penicilium toxin, Fusarium toxin* and *Ergottoxin*.

Aflatoxins are secondary toxic fungal metabolites produced by *Aspergillus flavus* and *A. parasiticus*. Aflatoxins not only contaminate our food stuffs but are also found in edible tissues, milk and eggs after consumption of contaminated feed by farm animals (Bennett and Klich, 2003; Fink-Gremmels, 1999). Aflatoxins are well known to be potent mutagens, carcinogenic, teratogenic, immuno suppressive and also inhibit several metabolic systems, causing liver, kidney and heart damage. These toxins have been incriminated as the cause of high mortality in livestock and some cases of death in human being (Minto and Townsend, 1997; Wogan, 1999; Bintvihok, 2002; Wangikar *et al*., 2005; Salunkhe *et al*., 1987). The contaminant occurs naturally in grain crops that are commonly feed to wild life. Aflatoxins concentration in some grains can increased dramatically during drought year. In lab and field trails Aflatoxins are known to cause liver damage, immune system suppression and liver cancer.

Although the consequences of acute Aflatoxins poisoning in wild life are occasional, the biological and economic consequence of chronic disease are likely to go unobserved. The biological effect of Aflatoxin consumption is similar in all groups of domestic livestock and wild life. The individual animal susceptibility to Aflatoxins varies with respect to species, age and individual variation (Pier, 1992). One of the primary complication in detecting the effect of Aflatoxins is that is rarely the acute poisoning that results in death or injury. An animal increasing susceptibility to infectious disease is a primary concern. In other words, a suppression of the immune system may be a major consequence of Aflatoxin consumption. This makes it a difficult task to detect in any wild life population. There are many types of Aflatoxin B1, B 2, G1, G2, M1, M2, B2a, G2a, as displayed in the Figure 1. The hydroxylated metabolites of Aflatoxin B1, B2 are found in milk or milk product obtained from the livestock that have been ingested with Aflatoxin contaminated feed. Aflatoxin in a poultry feed are a source of significant economic loss to the poultry producers. Aflatoxin adversely influence performance of animal by altering by nutrient composition of feed ingredients, decreasing efficiency of nutrient, utilization and by producing toxic secondary metabolites. Though low level of mold metabolites might not cause apparent physiological or pathological damage but it may reduce the performance of animal, ultimately resulting in tremendous economic loss. (Hesseltine,1996).

Aflatoxin in low concentration in the feed has been reported to cause weakness, decrease resistance to disease and has induced carcinogenesis in many species like quails, bobwhite, ducks, turkeys, fowls, goose and pheasant. Aflatoxin consumption can cause severe aflatoxicosis. It may cause vaccines to fail, increase the susceptibility of bird disease and result in suppression of natural immunity to infection. Aflatoxin is a "SILENTKILLER" is one of the most common toxins that threaten the human life. Turkey "X" disease was reported the human origin (Allcroft and Carnaghan, 1963) due to the incorporation of Brazilian peanut meal (Blount, 1961) which was one of the common ingredients of the feed stock for the turkeys and a chloroform extract of the meal yielded toxic components which were responsible for the occurrence of the disease in duckling. (Allocraft et al., *1961*).

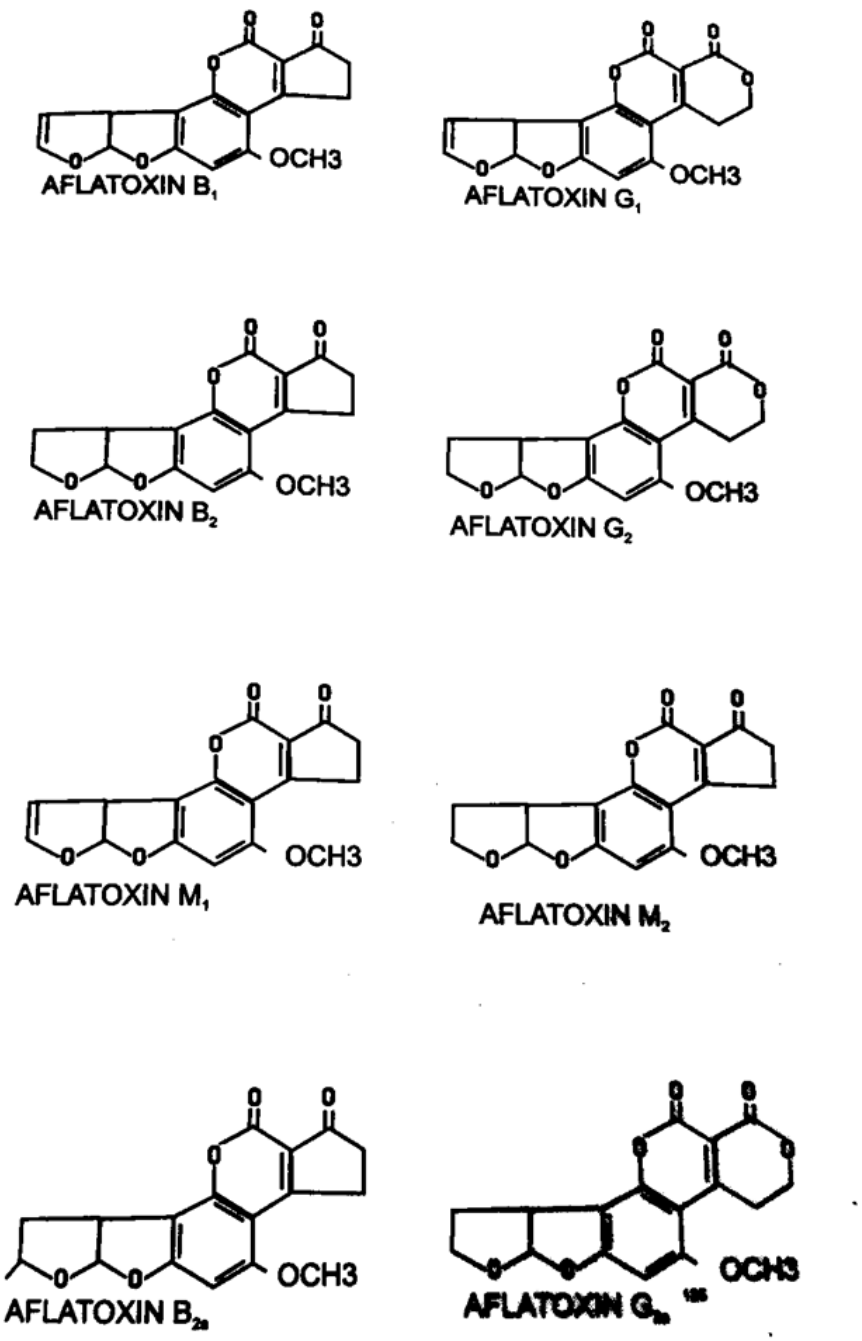

Image 1: Structure of Aflatoxin B1, B 2, G1, G2, M1, M2, B2a, G2a

Herbal plants have become the main source of drug preparations. They are widely accepted in developing countries for primary health care because of better compatibility and fewer side effects. In recent years, usage of herbal drugs in veterinary medicine has tremendously increased. Consequently, the demand for the herbal formulation is increasing day by day (Yadav and Singh, 2011). Naturally available compounds find greater application in human and animal health with minimal cost in foods and feed. Traditional medicinal plants were explored by some investigators for their antifungal, anti-aflatoxigenic and antioxidant activity (Joseph *et al*., 2005; Kumar *et al*., 2007). *Albizia lebbeck* is native to deciduous and semi-deciduous forests in Asia from eastern Pakistan through India and Sri Lanka to Burma. *Albizia lebbeck* is a fast-growing, medium-sized deciduous tree with a spreading umbrella-shaped crown of thin foliage and smoothish, finely fissured, greyish-brown bar. The, annual height growth ranges from 0.5 to 2.0 m depending on the geographical location; on good sites, individual trees attain an average maximum height of 18 to 25 m and 50 to 80 cm d.b.h. (Parrotta 1988a, Troup 1921). Its leaves, seeds, bark, and roots are all used in traditional Indian medicine (Chopra and others 1956, Kirtikar and others 1935). Phytochemical screening of successive extracts of *Albizia lebbeck* shows presence of alkaloids, glycosides, tannins, saponins, flavonoids, carbohydrates, proteins, and amino acids. (Rahul *et al*., 2010). Shahid and Firdous (2012) reported that crude methanolic extract of *A. lebbeck* were tested in vitro for their antifugal activities againt six fungal strains viz., *Aspergillus parasiticus, Aspergillus niger, Candida albicans, Aspergillus effusus, Fusarium solani* and *Saccharomyces cerevisiae* and the extracts showed significant activity against all fungal strains.

Syzygium aromaticum are used in Indian ayurvedic medicine. It is commonly called clove, belongs to the family Myrtaceae. Clove bud oil has biological activities, such as antibacterial, antifungal, antiinflammatory, chemopreventive, hepatoprotective, neuroprotective, insecticidal, analgesic, antispasmodic, anticarminative and antioxidant properties (Huang *et al.*, 2002; Lagow, 2004). Clove oil include acetyl eugenol, beta- caryophyllene, vanillin, crategolic acid, tannins, gallotannic acid, methyl salicylate, the flavonoids like eugenin, kaempferol, rhamnetin, and eugenitin; triterpenoids like oleanolic acid, stigmasterol and campesterol and several sesquiterpenes (Yang and Lee, 2003; Dan Bensky *et al*., 2004; Hema *et al*., 2010). Antifungal activity of *S. aromaticum* in vapor phase is tested against gray mold (*Botrytis cinerea*) in strawberries (Aguilar- Gonzalez *et al*., 2015). Pinto *et al*., (2009) concluded that *S.*

aromaticum acts as an ideal candidate as anti-fungal that are pathogenic to humans. Eugenol is a broad-spectrum agent which inhibited not only dermatophytes, Aspergillus and Candida species (such as *C. albicans*, *C. tropicalis* and *C. parapsilosis*), but also fluconazole- resistant *C. albicans* isolates, *C. krusei*, which is intrinsically resistant to fluconazole and *C. glabrata*, whose resistance is easily inducible.

Trachyspermum ammi is a traditional potential herbs, is widely used for curing various diseases in humans and animals. In India, it is cultivated in Madhya Pradesh, Uttar Pradesh, Gujarat, Rajasthan, Maharashtra, Bihar and West Bengal. *Trachyspermum ammi* L. belonging to family Apiaceae a highly valued medicinally important seed spice. The roots are diuretic in nature and the seeds possess excellent aphrodisiac properties. The seeds contain 2 to 4.4% brown coloured oil known as ajwain oil. Thymol, the main component of this oil is used against gastrointestinal ailments, lack of appetite and bronchial problems. The oil exhibits fungicidal (Singh and Singh, 2000) antimicrobial (Sivropoulou *et al*., 1996) and anti-aggregatory effects on humans (Srivastava, 1988). This current study investigates the action of the ethanolic extract of *A. Lebbeck, S.aromaticum* and *T.ammi* against the aflatoxins present in poultry feeds in-vitro.

2. MATERIALS ANDMETHODS

In the present investigations, anti-toxicology efficacy of *A. lebbeck*, *S. aromaticum* and *T. ammi* against Aflatoxin present in the natural feed diet of poultry was studied *in vitro*.

2.1. CULTURE

Aspergillus parasiticus strain NRRL 2999 was used to produce Aflatoxin because it is one of the highly toxigenic fungus available. This strain is very stable and yield high level of Aflatoxin especially B1 even after many transfers (Shotwell *et al*., 1966).

2.2. METHODS

2.2.1. SUBCULTURE

Inoculum was prepared by inoculating the tubes of potato-dextrose agar slant with spores of *A. parasiticus* NRRL 2999. The potato dextrose agar was prepared as follows: 4.1 grams of potato dextrose agar powder was mixed with 100ml of distilled water in a conical flask. The conical flask was then kept in the microwave-oven for few minutes so that it was mixed properly. The melted solution was distributed into the test tubes and closed with cotton plug. The test tubes were placed into the autoclave under 15 lb pressure for about 15 minutes for sterilization. The test tubes were kept in a slanting position and allowed to cool. The tubes were then ready for the subculture of *A. parasiticus*.

A. parasiticus were scraped with a sterilized inoculating wire and the spores were spread on the slant of the agar medium. The inoculated test tube was placed undisturbed for about 7-11 days. On the 11^{th} day, a velvety growth of green spores of *A. parasiticus* was observed (Figure 1).

2.2.2. PRODUCTION OF AFLATOXIN ON RICE

Aflatoxin was produced on rice (Shotwell *et al*., 1966). Fifty grams of cleaned polished rice was taken in a 250ml washed and dried Erlen mayer flask and plugged with the cotton. Rice was soaked in 25 ml of distilled water for about 2 hours with frequent shaking. The flask was

autoclaved and cooled, and the inoculated flask was kept at room temperature. The flasks were shaken around 10-15 times a day. After 48 hours of inoculation, mold growth was seen as white spots on the surface of rice later turning to bright yellow in colour. On the 12th day, these flasks were autoclaved for 5 minutes to kill the spores and then the culture rice was transferred to a tray and kept in hot air oven at 60^{o} C over night, which was then ground to fine powder for experimental analysis (Figure 2).

2.2.3. EXTRACTION OF AFLATIOXIN

The extraction of Aflatoxin was done as per the procedure of (Pons et al., 1966). A total of 50 gm of sample of the material was taken in a 500ml. Erlen mayer flask and extracted with 70% aqueous acetone for 1 hour with the help of a horizontal shaker. The contents were filtered and the filtrate was cooled. The volume of the filtrate was reduced to 140ml on a hot water bath. 20ml of lead acetate and a bowl of distilled water were added after cooling. The contents were filtered through Whatman No.1 filter paper and the filtrate was centrifuge at 10,000 rpm for 10 minutes. The obtained supernatant was extracted with 50 ml of chloroform in a separating funnel. The chloroform layer was collected passed through anhydrous sodium sulphate. The collected liquid was evaporated to dryness to obtain the crude extract of Aflatoxin.

2.2.4.Estimation of Aflatoxin by Thin Layer Chromatography

Thin layer chromatography (TLC) plates were prepared (0.25mm thickness) using silica gel G and distilled water (2:1). Slurry applicator was used to spreading the gel on the plates. The sample and the Aflatoxin standard were spotted on the plates using micropipettes. The chromatography was developed in a chromatography tank containing toluene, ethyl acetate and formic acid (60:30:10) to the depth of less than 1cm. The solvent was allowed to run for 10 to 12cm. The plates were dried in horizontal position and viewed under ultraviolet lamp. The Rf value were checked with standard Aflatoxin with the sample. The presence of blue fluorescent spot was corresponding to Aflatoxin B at Rf 0.05 to Aflatoxin G at Rf 0.04 to0.45.Aflatoxin B_1 content was calculated according to AOAC, (1980) specification (Pons *et al.*,1966).

Aflatoxin (mg/g or ppm) = SxYx / V = XxW

Where,

S = ml Aflatoxin standard which matched the unknown

Y = concentration of standard Aflatoxin in mg/ml extract

V = ml of solvent required for final dilution of sample extract

X = ml of sample extract spotting giving fluorescent intensity equivalent to S (Standard)

W = Weight in grams of original sample contained in the final extract

2.3. Collection of Plant material

The Bark of *Albizia lebbeck*, the buds of *Syzygium aromaticum* and seeds of *Trachyspermum ammi* (Figure 3), were purchased from Lakshmi stores, Chennai - 600 003. The plant materials obtained were identified and authenticated by a botanist in the Department of Botany.

2.3.1. Extraction of Plant materials

The Bark of *A. lebbeck*, the buds of *S. aromaticum* and seeds of *T. ammi* were cleaned, shade dried and coarsely powdered. Successive solvent extraction was done by cold percolation method (Harborne, 1998) by soaking in ethanol, in an aspirator bottle for 48 hours. After 48 hours, the extracts were filtered by Whatman Filter paper No.1. The solvent was removed by distillation using Rotary Evaporator and the extracts were concentrated and dried in Freeze Dryer (Figure 4).

2.3.2. Preparation of feed source

Estimation was done for aflatoxin inoculated feed sample and kept as control. From the same 25gms were taken in container and theethanol extracts of plants viz. *A. lebbeck, S. aromaticum* and *T. ammi* at increasing concentration of (1, 3 and 5 mg/ml) were mixed with the help of sonicator, to form a thorough mixture (Figure 5). The same process was replicated and repeated for 3 consecutive weeks.

3. Results and Discussion

The present study was aimed to analyze the beneficial effect of few traditional medicinal plant extracts against Aflatoxin contaminated feeds. The results indicated that all the plant extracts were found effective at higher concentrations even with repeated treatments Aflatoxin growth level is compared with each other and with the control. The control had a severe mal nutritions value of mean (23.00±1.22) in the 1stweek with Aflatoxin contamination, as the animals do not feel to consume the feed due to aflatoxin contamination. With reference to mean value the 3rd plant, *T. ammi* is found to be significant when compared to *A. lebbeck* and *S. aromaticum*. It is evident from the observations that *T. ammi* acted as a significant anti-fungal agent.

The concentration chart of various plant extract indicates that there is a highly significance difference with the increasing concentration levels. Mean (± Standard error (S.E)) value aflatoxin levels with *A. lebbeck* in 1st concentration is 10.00±1.22 which is reduced to 6.33±1.58 in the last week of experiment (Table. 1-5). Inhibition activity of ethanol extracts of plants was enhanced with an increase in their concentrations. The ethanol extracts of *T. ammi* completely arrested the fungus growth and effectively prohibited the fungus growth even at lower concentrations (1%) in comparison with other extracts and the negative control.

A substantial body of evidence has studied the antifungal activity of oils from various plants, such as essential oils of *Thymus vulgaris (Lamiaceae: Lamiales), Zataria multiflora (Lamiaceae: Lamiales), Mentha piperita (Lamiaceae: Lamiales), Mentha pulegium (Lamiaceae: Lamiales), Ocimum basilicum ((Lamiaceae: Lamiales)* (Table 6). They extended the inhibitory activity against growth of *A. flavus* and other microorganisms. However, in this investigation, ethanol extracts of *T. ammi* and *S. aromaticum* arrested growth of the fungus related to their polar chemical constituents, which are water soluble. The results of our study are in line with the earlier findings, which suggested that aqueous extracts of thyme and coriander mostly inhibit the isolated strain of *A. flavus* followed by dill and rose extracts (Yahya Abadi *et al*., 2011). Result of an experiment showed that safflower, which was wound inoculated with *Phytophthora drechsleri* (Peronosporaceae: Peronosporales) produces a polyacetylene compound that inhibits the growth of the mentioned fungus *in vitro* (Allen and Thomas, 1971). Both polar and non-polar

extracts of *C. senna* did not exhibit antifungal activity toward *A. niger* (Kamal *et al*., 2012). On the other hand, antimicrobial activity of some *Senna* spp. against different microorganisms in the previous studies was demonstrated. For instance, aqueous extract of *Senna obtusifolia* (Fabaceae: Fabales) containing alkaloids and flavonoids prevented *A. niger* more than other extract of the plant (Doughari *et al*., 2008).

Moreover, an unidentified flavonoid glycoside isolated from leaves of *Senna alata* (Fabaceae: Fabales) prohibited growth of *A. niger* with MIC value of 70 µg/mL (Owoyale, *et al*., 2005). Hairy root culture of *O. basilicum* produced rosmarinic acid that induced cytoskeleton damages with broken interseptas and convoluted cell surfaces in *A. niger* (Bais *et al*., 2002). Overall, the antifungal activity of the plants attributed to the various kinds of secondary metabolites like flavonoids, alkaloids, phenolic acids and the essential oils (Owoyale *et al*., 2005; Bais *et al*.,2002) were highlighted in the current study. Hence, the present work has been carried out to find the antifungal activity of the plant extracts, *A. lebbeck, S. aromaticum* and *T. ammi* against Aflatoxin contaminated poultry feed.

Conclusion

Taking together in this study, the plants *Trachyspermum ammi* belong to Apiaceae family, along with *Syzygium aromaticum* (Myrtaceae) were more active against the Aflatoxin than the plant *Albizia lebbeck* (Fabaceae). Based on the results of this work, it can be proposed that ethanol extract of *T. ammi*, *S. aromaticum* and *A. lebbeck* effectively inhibit *A. parasiticus* growth attributed to their polar secondary metabolites and are suitable as natural antifungal agents to prevent the fungus activity of Aflatoxin. Hence, these three extracts inhibited fungus growth most effectively with concentration of 1, 3 and 5% with repeated treatment in comparison with each other. This current investigation revealed that ethanol extract of *T. ammi* effectively inhibit the Aflatoxin content fully at lower concentration level (1%) after 8 hours of exposure.

Ethical Approval:

As per international standard or university standard ethical approval has been collected and preserved by the authors.

NOTE:

The study highlights the efficacy of "herbal formulation" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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Tables

Table. 1. Anova Table

Source	Type III Sum of Squares	Mean Square	F	Significance
Corrected Model	8328.667a	237.962	356.943	0.000
Intercept	7301.333	7301.333	10952.000	0.000
Plant	8090.667	2896.889	4045.333	0.000
Week	51.167	25.583	38.375	0.000
Concentration	8.667	4.333	6.500	0.003
Plant* Week	102.833	17.139	25.708	0.000
Plant* Conc	37.333	6.222	9.333	0.000
Week* Conc	9.833	2.458	3.687	0.009
Plant* Week* Conc.	28.167	2.347	3.521	0.000
Error	48.000	667		
Total	15678.000			
Corrected Total	8375.667			

R Squared = .994 (Adjusted R Squared = .991)

Table 2. Estimated Aflatoxin in the feed source (Control)

Week	Conc.	Mean	Std. Deviation
1st	1	20.0000	1.00000
	3	23.0000	1.00000
	5	22.0000	1.00000
	Total	23.0000	1.22474
2nd	1	24.0000	1.00000
	3	23.0000	1.00000
	5	22.0000	1.00000
	Total	23.0000	1.22474
3rd	1	20.0000	1.00000
	3	24.0000	1.00000
	5	24.0000	1.00000
	Total	22.6667	2.17945
Total	1	21.3333	2.17945
	3	23.0000	1.22474
	5	22.3333	1.58114
	Total	22.2222	1.78311

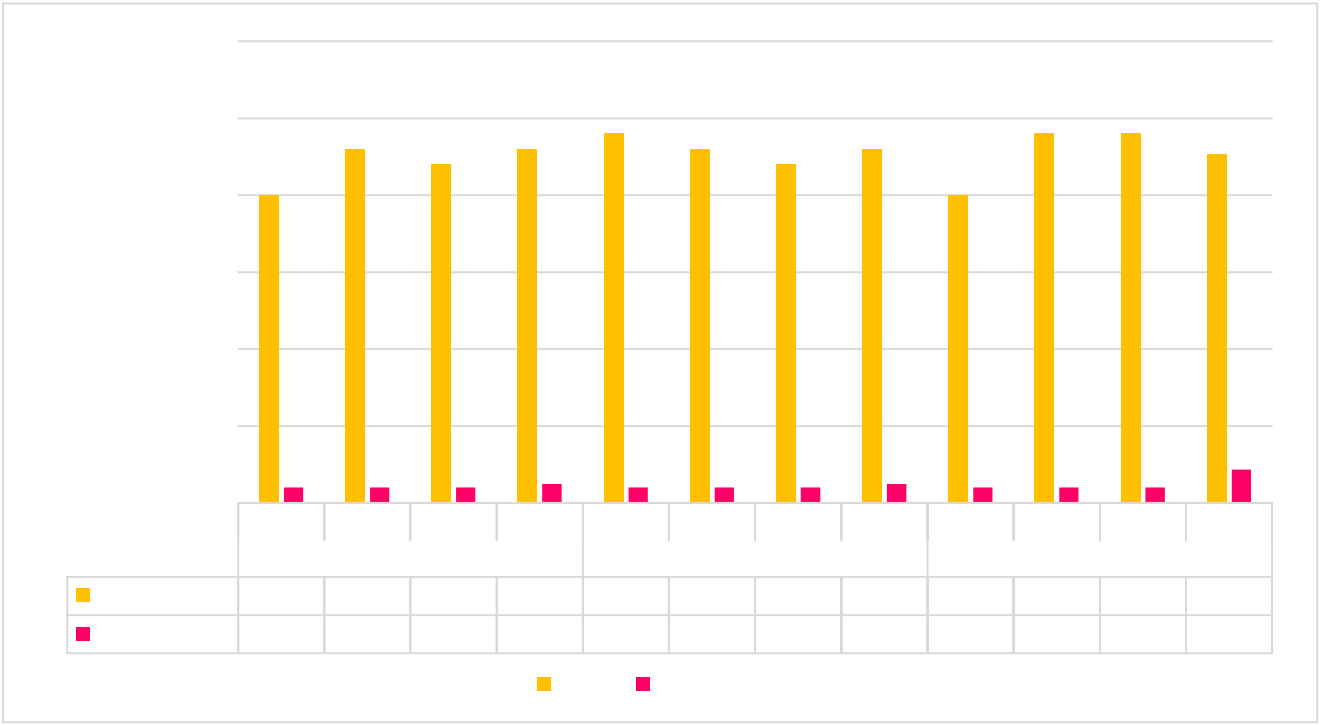


Table 3. Effect of *Albizia lebbeck* on Aflatoxin contaminated feed source

Effect	Conc.	Mean	Std. Deviation
1st	1	11.0000	1.00000
	3	10.0000	1.00000
	5	9.0000	1.00000
	Total	10.0000	1.22474
2nd	1	10.0000	1.00000
	3	9.0000	1.00000
	5	8.0000	1.00000
	Total	9.0000	1.22474
3rd	1	8.0000	1.00000
	3	6.0000	1.00000
	5	5.0000	1.00000
	Total	6.3333	1.58114
Total	1	9.6667	1.58114
	3	8.3333	2.00000
	5	7.3333	2.00000
	Total	8.4444	2.04438

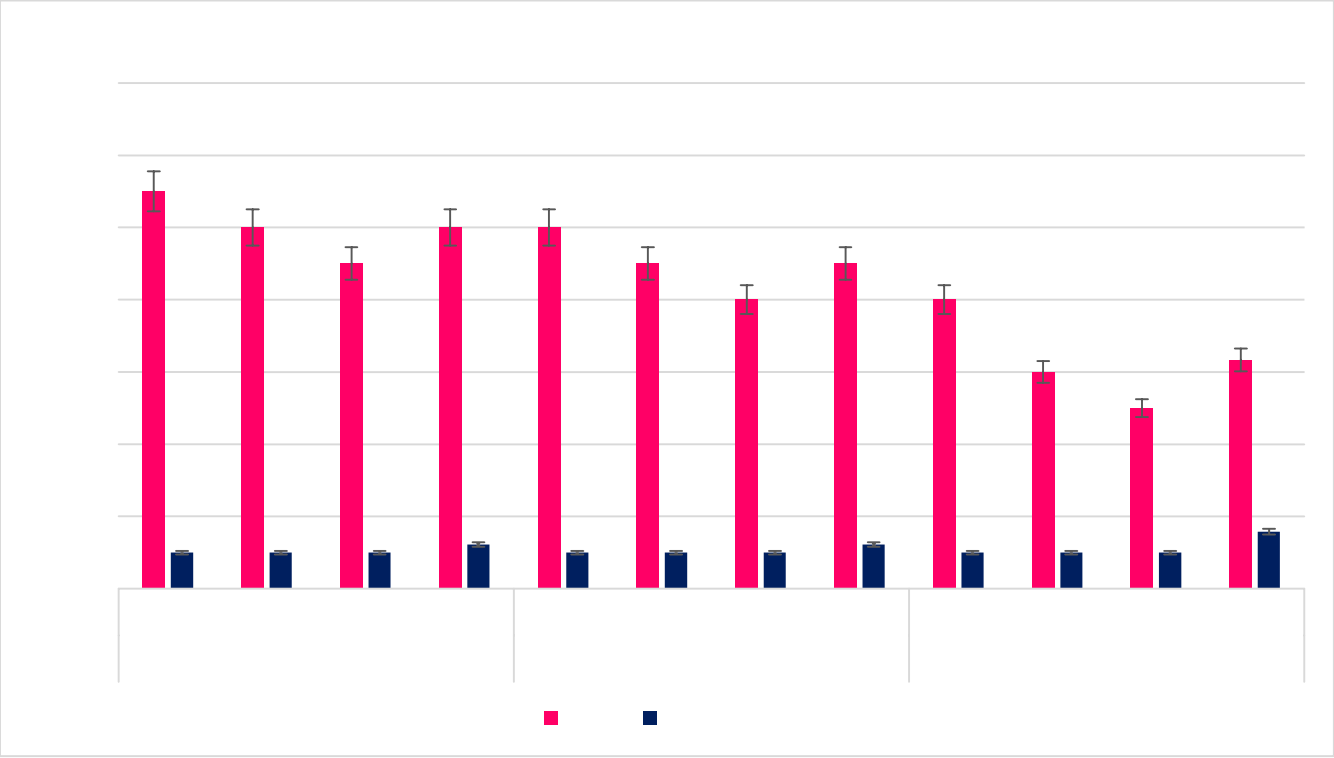
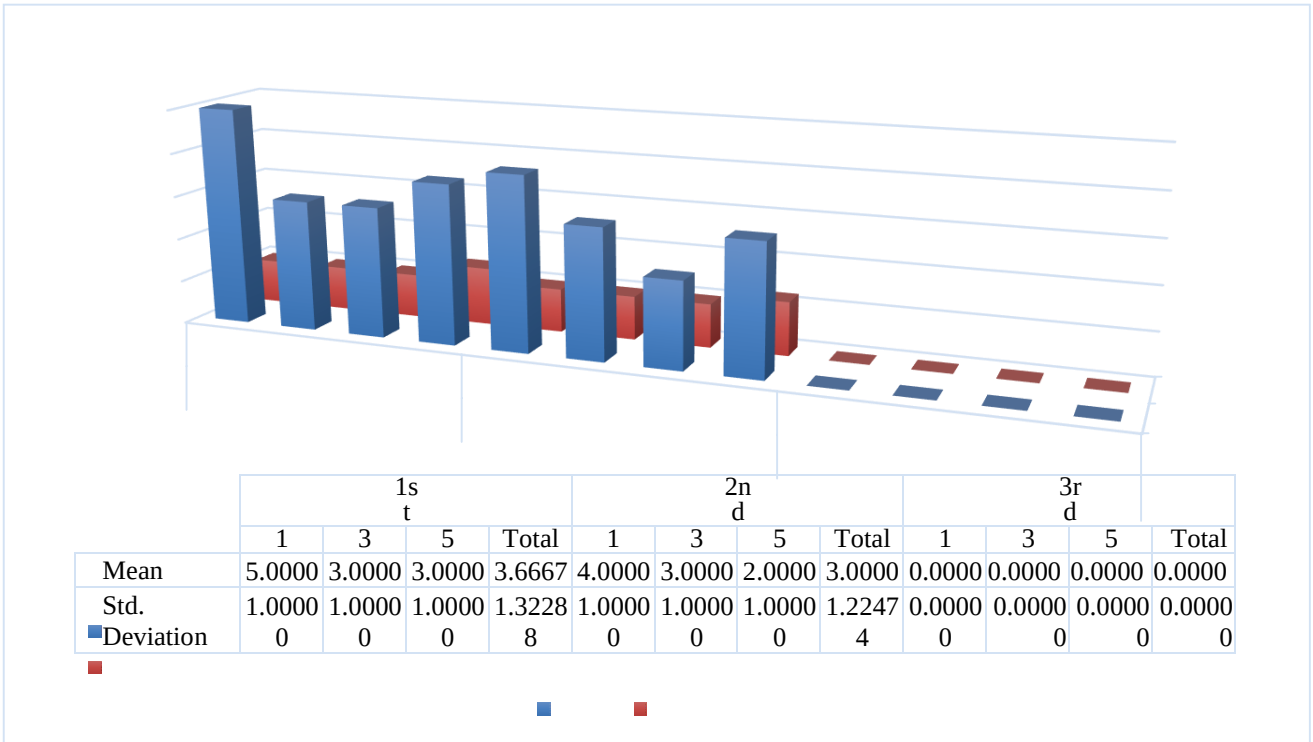


Table 4. Effect of *Syzygium aromaticum* on Aflatoxin contaminated feed source

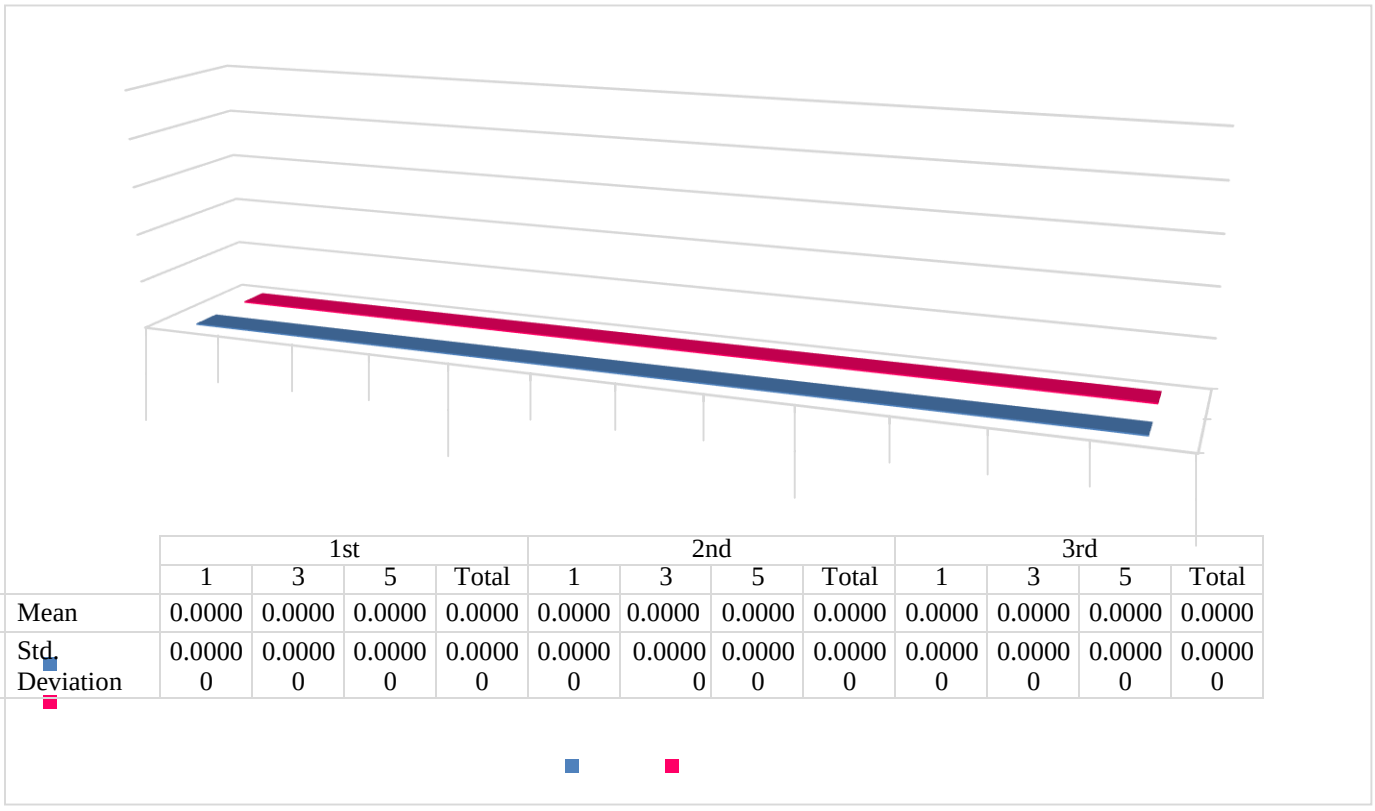
Week	Conc.	Mean	Std. Deviation
1st	1	5.0000	1.00000
	3	3.0000	1.00000
	5	3.0000	1.00000
	Total	3.6667	1.32288
2nd	1	4.0000	1.00000
	3	3.0000	1.00000
	5	2.0000	1.00000
	Total	3.0000	1.22474
3rd	1	0.0000	0.00000
	3	0.0000	0.00000
	5	0.0000	0.00000
	Total	0.0000	0.00000
Total	1	3.0000	2.39792
	3	2.0000	1.65831
	5	1.6667	1.50000
	Total	2.2222	1.90815



	1st				2nd				3rd			
	1	3	5	Total	1	3	5	Total	1	3	5	Total
Mean	5.0000	3.0000	3.0000	3.6667	4.0000	3.0000	2.0000	3.0000	0.0000	0.0000	0.0000	0.0000
Std. Deviation	1.00000	1.00000	1.00000	1.32288	1.00000	1.00000	1.00000	1.22474	0.00000	0.00000	0.00000	0.00000

Table 5. Effect of *Trachyspermum ammi* on Aflatoxin contaminated feed source

Week	Conc.	Mean	Std. Deviation
1st	1	0.0000	0.00000
	3	0.0000	0.00000
	5	0.0000	0.00000
	Total	0.0000	0.00000
2nd	1	0.0000	0.00000
	3	0.0000	0.00000
	5	0.0000	0.00000
	Total	0.0000	0.00000
3rd	1	0.0000	0.00000
	3	0.0000	0.00000
	5	0.0000	0.00000
	Total	0.0000	0.00000
Total	1	0.0000	0.00000
	3	0.0000	0.00000
	5	0.0000	0.00000
	Total	0.0000	0.00000



	1st				2nd				3rd			
	1	3	5	Total	1	3	5	Total	1	3	5	Total
Mean	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Std. Deviation	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000

Table 6. Antifungal activity of Medicinal plants

Plant sample	Extract	Microorganism	References
T. vulgaris, Satureja hortensis, Syzygium aromaticum	essential oil	*A. flavus*	Omidbeygi *et al*., 2007
Polymnia sonchifolia	aqueous extract	*A. flavus*	Pinto *et al*., 2001
T. vulgaris	essential oil	*A. parasiticus, A. flavus*	Rasooli and Abyaneh, 2004; Eman and Craker, 2010
T. vulgaris, T. tosevii, M. spicata, M. piperita	essential oil	*A. niger, A.ochraceus, A.versicolor, A.flavus, A.terreus*	Sokovic *et al*., 2009
Cinnamomum zeylanicum, M. piperita, O. basilicum, Origanum vulgare, Teloxys ambrosioides, Syzygium aromaticum, T. vulgaris	essential oil	*A. flavus*	Sajed *et al*., 2013
Z. multiflora	essential oil	*A. parasiticus, A. flavus*	Nimbkar, 2002; Montes-Belmont and Carvajal, 2008
T. vulgaris	essential oil	*Aspergillus spp.*	Lee *et al*., 2005
M. piperita	essential oil	*A. fumigatus, A. flavus, A. ochraceus*	Zomorodian *et al*., 2011
M. pulegium	essential oil	*A.niger, A.flavus*	Lo´pez-Malo *et al*., 2005; Daferera *et al*., 2000
T. vulgaris, Coriandrum sativum, Anethum graveoles, Rosa damascena	aqueous extract	*A. flavus*	Kamal *et al*., 2012
S. obtusifolia	aqueous extract	*A. niger*	Moghtader, 2013

Figures

Figure 1. Showing green spores of *Aspergillus parasiticus* culture on potato dextrose agar

Figure 2. Production of Aflatoxin

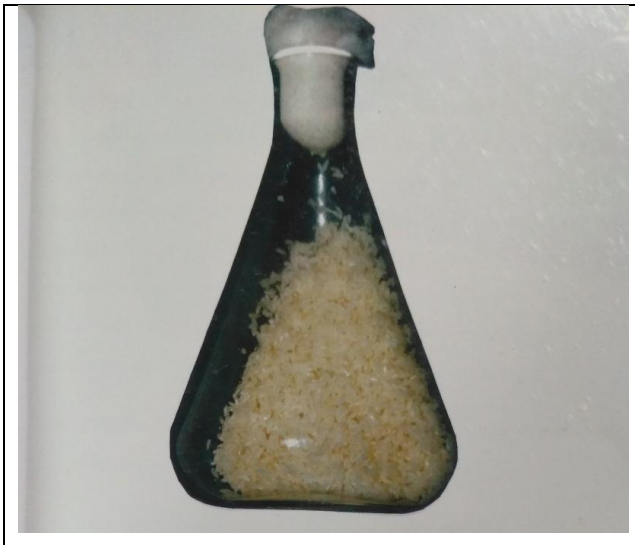
	
Production of Aflatoxin on rice	**Cultured rice of Aflatoxin**

Figure 3. Plant materials

		
Albizia lebbeck	***Syzgium aromaticum***	***Trachysper mumammi***

Figure 4. Preparation of plant extract

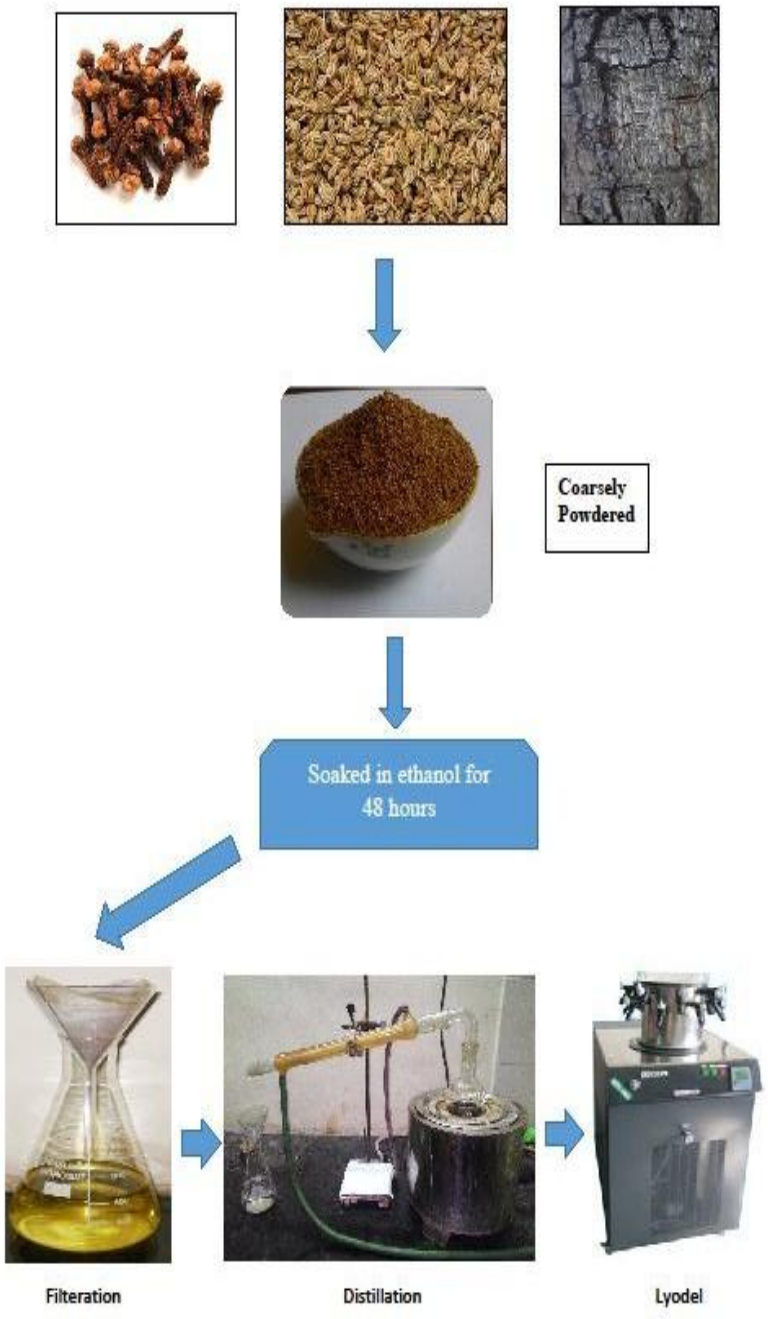


Figure 5. Preparation of feed source

		
Contaminated feed source	**Mixing of various plant extract in formulated feed**	**Formulated feed sealed in airtight containers**