

# AMELIORATING EFFECT OF THE PLANT EXTRACTS ON MYCOTOXINS IN POULTRY FEED

**Comment [BA1]:** The present title is somewhat ambiguous it should be reviewed. "Ameliorating the effect mycotoxins in poultry feeds using plant extracts "my suggestion

## 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 following genera, *Aspergillus*, *Penicillium* and *Fusarium*. When ingested, inhaled or absorbed through the skin, mycotoxins causes sickness or death on humans and animals. Natural substances that can prevent AFB1 toxicity would be helpful to human and animal health with minimal cost in foods and feed. (Consider recasting) Traditional medicinal plants are currently used for their antifungal, anti-aflatoxicogenic and antioxidant activity. *Aspergillus parasiticus* strain NRRL 2999 was used to produce Aflatoxin, as it is one of the highly toxigenic fungus available. Inhibition activity of ethanol extracts of plants was enhanced upon gradual increase in their concentrations. Among them, the ethanol extracts of *Trachyspermum ammi* 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 *A. lebbbeck*, *S. aromaticum* and *T. ammi* 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.

**Comment [BA2]:** Change to in

**Comment [BA3]:** "Natural substances that can prevent AFB1 toxicity to human and animal health with minimal cost will be a great advantage"

**Comment [BA4]:** Inhibitory

**Key words:** Aflatoxin, *A. lebbbeck*, *S. aromaticum*, *T. ammi*, anti-fungal, poultry feed

## INTRODUCTION

In the recent times, Poultry industry has gained paramount attention and has maximized as a phenomenal agri 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.

Comment [BA5]: Agricultural

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 as mycotoxin. The study of mycotoxin is called "Mycotoxicology". Mycotoxin is classified as *Aspergillus toxins*, *Penicilium toxin*, *Fusarium toxin* and *Ergottxin*.

Comment [BA6]: should read "called mycotoxins" or "known as mycotoxins"

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 occasionally, 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 resulting 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. 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 may reduce the performance of animal, ultimately resulting in tremendous economic loss. (Hesseltine,1996).

Comment [BA7]: "occasional"

Comment [BA8]: "varies"

Comment [BA9]: "That results in death"

Comment [BA10]: Figure what? The figure should be numbered.

Comment [BA11]: "damage, but it may reduce"

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 "SILENT KILLER" 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).

Comment [BA12]: "Silent killer, it is one"

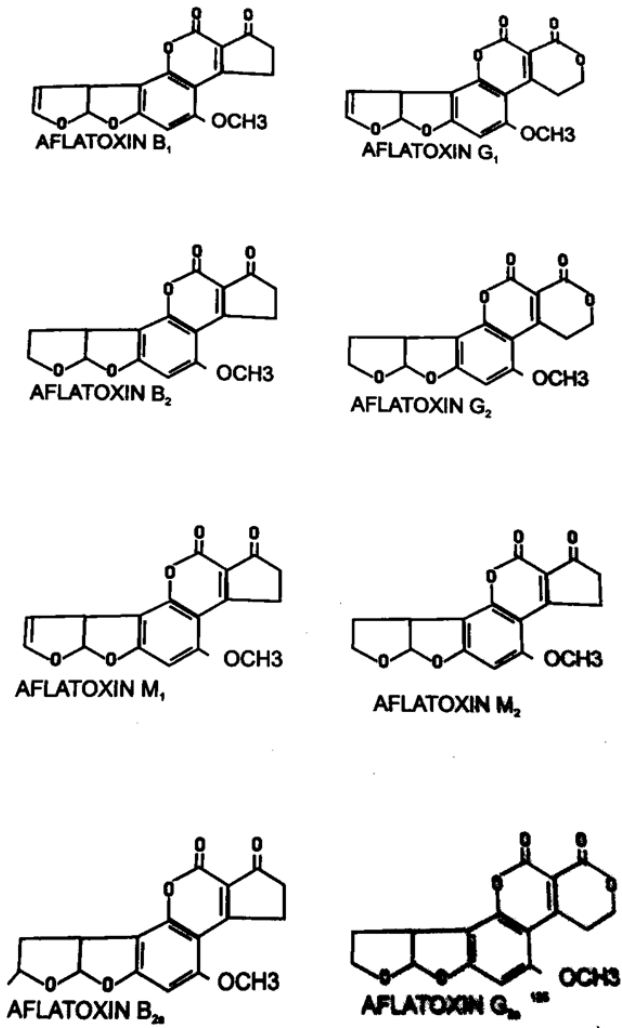


Image 1: Structure of Aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>, M<sub>1</sub>, M<sub>2</sub>, B<sub>2a</sub>, G<sub>2a</sub>

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 lebbek* is native to deciduous and semi-deciduous forests in Asia from eastern Pakistan through India and Sri Lanka to Burma. *Albizia lebbek* is a fast-growing, medium-sized deciduous tree with a spreading umbrella-shaped crown of thin foliage and smoothish, finely fissured, greyish-brown bark. 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 *A. lebbek* 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. lebbek* were tested in vitro for their antifungal activities against six fungal strains viz., *A. parasiticus*, *A. niger*, *Candida albicans*, *A. 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, anticarcinogenic 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 herb, 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). From this current investigation ethanol extract of *A. Lebbeck*, *S.aromaticum* and *T.ammiis* checked against the Aflatoxin present in poultry feed by *in vitro*.

**Comment [BA13]:** Should read “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 AND METHODS

In the present investigations, anti-toxicology efficacy of *Albizia lebbek*, *Syzygium aromaticum* and *Trachyspermum 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 *Aspergillus 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 tube 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<sup>th</sup> 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 12<sup>th</sup> 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°C overnight, which was then ground to fine powder for experimental analysis (Figure 2).

### 2.2.3. EXTRACTION OF AFLATOXIN

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. Thus the crude extract of Aflatoxin was prepared. To avail the contaminated feed source.

**Comment [BA14]:** The collected liquid was evaporated to dryness to obtain the crude extract of Aflatoxin

**Comment [BA15]:** This sentence does not seem necessary here.

### 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 were 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 to 0.45. Aflatoxin B1

**Comment [BA16]:** Should be "was"

content was calculating according to AOAC, (1980) specification (Pons *et al.*,1966).

Comment [BA17]: should be "calculated"

$$\text{Aflatoxin (mg/g or ppm)} = S \times Y_x / V = X \times W$$

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 lebeck*, 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. lebeck*, 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 the ethanol extracts of plants viz. *A. lebeck*, *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). And the same was repeated for 3 weeks consequently with replicate.

**Comment [BA18]:** should read the same process was replicated and repeated for 3 consecutive weeks

### 3. Results and Discussion

The present study was aimed to analyze the beneficial therapeutic ability of few traditional medicinal plant extracts against Aflatoxin contaminated feeds. The results of this study indicated that all the plants extracts significantly inhibit at higher concentration and found effective with repeated treatments. Aflatoxin growth level is compared with each other and with the control. The control had a severe malnutritions value of mean (23.00±1.22) in the 1<sup>st</sup> week with Aflatoxin contamination. With reference to mean value the 3<sup>rd</sup> plant that is *T. ammi* is found to be significant when compared to other plants, *A. lebeck* and *S. aromaticum*. It is evident from the observations that *T. ammi* acted as a significant anti-fungal agent against the aflatoxin infestation.

**Comment [BA19]:** the word "effect" will be more appropriate here

**Comment [BA20]:** this sentence is not clear perhaps you mean to say "the results indicated that all the plant extracts were found effective at higher concentrations even with repeated treatments"

**Comment [BA21]:** does the word "malnutrition" here mean the feed was fed to animals?

**Comment [BA22]:** Not necessary here

**Comment [BA23]:** remove

**Comment [BA24]:** These two words are not necessary here.

The concentration chart of various plant extract indicates that there is a highly significance difference with the increasing concentration levels. Mean (±S.E) value aflatoxin levels with *A. lebeck* in 1<sup>st</sup> 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. Among them, 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.

**Comment [BA25]:** Please provide write full meaning

**Comment [BA26]:** Among them could be removed from here

A substantial body of evidence has studied the antifungal activity of various plant oils, such as essential oils of *T. vulgaris*, *Z. multiflora*, *M. piperita*, *M. pulegium* and *O. basilicum* (Table 6). They extended the inhibitory activity against growth of *flavus* and other microorganisms. However, in this investigation, ethanol extracts of *T. ammi* and *S. aromaticum*

**Comment [BA27]:** "Oils from various plants" may be more appropriate here

**Comment [BA28]:** "A.flavus"

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* 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 *S. obtusifolia* 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 *S. alata* 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. lebbbeck*, *S. aromaticum* and *T. ammi* against Aflatoxin contaminated poultry feed.

Comment [BA29]: Highlighted "in" the

## 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 lebbbeck* (Fabaceae). Based on the results of this work, it can be proposed that ethanol extract of *T. ammi*, *S. aromaticum* and *A. lebbbeck* 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* is effectively inhibit the Aflatoxin content to fully at its lower concentration level (1%) after 8 hours of

Comment [BA30]: remove

Comment [BA31]: remoe

Comment [BA32]: remove

exposure. Evaluation of synergistic activity of the examined plants in prevention of the fungus growth for longer periods of time followed by isolation and identification of their active compounds are recommended for the further studies.

#### **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.

#### **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.

#### **REFERENCES**

- Abayneh Unasho, 2005. Investigation of antimicrobial activities of *Albizia gummifera* and *Ferula communis* on *Streptococcus pneumoniae* and *streptococcus pyogenes* causing upper respiratory tract infections in children. Thesis Biology, Faculty of Science, Addis Ababa University.
- Allen, E.H. and Thomas, C.A., 1971. Trans-trans-3, 11-tridecadiene-5, 7, 9-triene-1, 2-diol, an antifungal polyacetylene from diseased safflower (*Carthamus tinctorius*). *Phytochemistry*, 10 (7), 1579-1582.
- Ana Elena Aguilar-Gonzalez, Enrique Palou, Aurelio Lopez-malo., 2015. Antifungal activity of essential oils of clove (*Syzygium aromaticum* and mustard (*Brassica nigra*) in vapor phase against gray mold (*Botrytis cinerea*) in strawberries. *Biochemistry*: 40, 983-995.
- Pinto M.M, Gonzalez E, Rossi M.H, Felício J.D, Medina C.S, Fernandes M.C.B, Simoni I.C. , 2001. Activity of the aqueous extract from *Polymnia sonchifolia* leaves on Growth and production of aflatoxin

b1 by *aspergillus flavus*. *Brazilian Journal of Microbiology*, 32:127-129.

APEDA. 2006. Residue monitoring plan for 2006, for drugs and pesticides for exported egg products to the European Union. New Delhi, Agricultural and Processed Food Products Export Development Authority.

Atanda, O., Oguntubo, A., Adejumo, O., Ikeorah, J. and Akpan, I. (2007). *Chemosphere*. 68, 8, 1455-1458.

Bais, H. P., Walker, T. S., Schweizer, H. P. and Vivanco, J. M. (2002). *Plant Physiology*

Bennett, J.W. and Klich, M., 2003. Mycotoxins. *Clin. Microbiol. Rev.* 16, 497.

Bintvihok, A., 2002. New insights to controlling mycotoxin danger in ducks. *Feed Technol.* 6 (1), 28.

Bulent K, Alan DW, IS ILV (2006). Strategies to Prevent Mycotoxin Contamination of Food and Animal Feed: A Review. *Critical Review of Food Science and Nutrition*, 46: 593–619.

Chulet Rahul., Pradhan Pankaj, Sarwan, S.K., Mahesh. J. K., (2010). Phytochemical screening and antimicrobial activity of *Albizia lebbek*. *Journal of chemical and pharmaceutical research*. Vol. 2. No. 5. Pp 476-484.

Dan Bensky, Steven Clavey, Erich Stoger and Andrew Gamble, 2004. *Chinese Herbal Medicine, Materia Medica*, Third Edition.

Doughari, J. H., El-mahmood, A. M. and Tyoyina, I. (2008). *African Journal of Pharmacy*

Eman, E. A. and Craker, L. E. (2010). *Journal of Herbs, Spices & Medicinal Plants*. 15,

Eugenia Pinto, Luis Vale-Silva, Carlos Cavaleiro and Ligia Salgueiro. (2009). *Antifungal*

activity of the clove essential oil from *Syzygium aromaticum* on *Candida*, *Aspergillus* and dermatophyte species. *Journal of Medical Microbiology*. 58, 1454–1462.

Fink-Gremmels, J., 1999. Mycotoxins: their implications for human and animal health. *Vet. Q* 21, 115.

Hema, R., Kumaravel, S. and Sivasubramanian, C., 2010. GC-MS study on the potentials of *Syzygium aromaticum*. 2(12):1-4.

Huang, Y., Ho, S. H., Lee, H. C. and Yap, Y. L., 2002. Insecticidal properties of eugenol, isoeugenol and methyleugenol and their effects on nutrition of *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *J. Stored Prod.*, 38:403–412.

Joseph, G.S., Jayaprakasha, G.K., Selvi, A.T., Jena, B.S., Sakariah, K.K., 2005. Antiaflatoxic and antioxidant activities of *Garcinia* extracts. *Int. J. Food Microbiol.* 101, 153–160.

Kamal, H., Hassan, M., Parvin, M. N., Hasan, M., Islam, S. and Haque, A. (2012). *Journal of applied pharmaceutical science*. 2, 6, 186-190.

Kumar, R., Mishra, A.K., Dubey, N.K., Tripathi, Y.B., 2007. Evaluation of *Chenopodium ambrosioides* oil as a potential source of antifungal, anti-aflatoxic and antioxidant activity. *Int. J. Food Microbiol.* 115, 159.

Kumar, V., Basu, M. S., & Rajendran, T. P. (2008). Mycotoxin research and mycoflora in some commercially important agricultural commodities. *Crop Protection*, Vol. 27, Jun, pp. 891-905, 2061-2194.

Lagow, B., 2004. PDR for herbal medicines. In *Clove Syzygium aromaticum*, (3rd ed.), pp. 204–8, Thomson PDR, USA.

Lee, S. J., Umanob, K., Shibamoto, T., Lee, K. G. (2005). *Food Chemistry*. 91, 131-137.

Minto, R.E., and Townsend, C.A., 1997. Enzymology and molecular biology of Aflatoxin biosynthesis. *Chem. Rev.* 97, 2537.

Montes-Belmont, R and Carvajal, M. (1998). *Journal of Food Protection*. 5, 513-648.

Nadia Ameen Abdulmajeed, (2011). Therapeutic ability of some plant extracts on Aflatoxin B1 induced renal and cardiac damage. *Arabian Journal of Chemistry* 4, 1–10.

Omidbeygi, M. Barzegar, M., Hamidi, Z. and Naghdibadi, H. (2007). *Food Control*. 18, 12, 1518-1523.

Owoyale, J. A., Olantunji, G. A. and Oguntoye, S. O. (2005). *Journal of Applied Sciences*  
Pandey RK, Bhardwaj SP, Mahajan VK, Nirman KPS. (1996). Economic Study of Poultry Production in India. *Proceedings of the 20th World Poultry Congress*. New Delhi, India. v.3,p.527-34.

Pandey RK, Bhardwaj SP, Nirman KPS, Mahajan VK, (1996b). Economic Analysis of Price Behaviour and Marketing of Eggs. *Indian Journal of Agricultural Marketing*. 3:343-357.

Pinto, E., Vale-silva, L., Cavaleiro, C. and Salgueiro, L., 2009. Antifungal activity of the clove essential oil from *Syzygium aromaticum* on *Candida aspergillus* and dermatophyte Species. *J. Med. Microbiol.*, 58: 1454-1462.

Pinto, M. M.,Gonçalez, E., Rossi, M. H., Felício, J. D., Medina, C. S., Fernandes, M. J. A. and Simoni, I. C. (2001). *Brazilian Journal of Microbiology*. 32,127-129.

Pons, W. A., Cucullu, A.P., Lee, L.S., Robertson, J.A. and Goldblatt, L.A. 1966. Determination Aflatoxin in agricultural products. Use of aqueous acetone for extraction. *Journal of official*

*analytical chemists*, 49: 554-562.

Rasooli, I. and Abyaneh, M. R., (2004). *Food Control*. 15, 6, 479-483.

Reddy, K. R. N., Reddy, C. S., Abbas, H. K., Abel, C. A., & Muralidharan, K. (2008). Mycotoxigenic Fungi, Mycotoxins, and Management of Rice Grains. *Toxin Reviews*, Vol. 27, pp. 287-317,1556-9543.

Sahar Omidpanah, Abolfazl Haseli and Azadeh Manayi, (2015). Evaluation of antifungal properties of some medicinal plants against *Aspergillus flavus* isolated from contaminated Corn *in vitro*. *Mediterranean Journal of Chemistry*. 3(6), 1093-1099.

Sajed, H., Sahebkar, A. and Iranshahi, M. (2013). *Journal of Ethnopharmacology*. 145,

Salunkhe, D.K., Adsule, R.N., Padule, D.N. (Eds.), 1987. Occurrence of Aflatoxin. Aflatoxin in Foods and Feeds. p. 44.

Shakir Ahmad Shahid and Najma Firdous., 2012. Antimicrobial screening of *Albizia lebbek* (L.) Benth. and *Acacia leucophloea* (Roxb.) *African Journal of Pharmacy and Pharmacology*. Vol. 6(46), pp. 3180-3183.

Shotwell, O.L., Hesseltine, C.W., Stubblefield, R.D. and Sorenson, W.G. 1966. Production of Aflatoxin on rice. *Applied microbiology*, 14: 425-428.

Singh I, Singh VP (2000). Antifungal properties of aqueous and organic extracts of seed plants against *Aspergillus flavus* and *A. niger*. *Phytomorphology*. 20: 151-157.

Sinha KK (1998). Detoxification of mycotoxin and food safety. In: Sinha KK and Batnagar D Eds., *Mycotoxins in Agriculture and food safety*. Merceel Dekker, Inc., (New York) 381- 405 p.

Sivropoulou A, Papanikolaou E, Nilolaou C, Kokkini S, Lanaras T, Arsenakis (1996).

M. Antimicrobial and cytotoxic activities of origanum essential oils. *J Agric Food Chem.* 44: 1202-1205.

Sokovic, M. D., Vukojevic, J., Marin, P. D., Brkic, D. D., Vajs, V. and Van Griensven, L. J. (2009). *Molecules.* 14, 1,238-249.

Srivastava KC (1988). Extract of a spice Omum (*Trachyspermum ammi*) shows anti- aggregatory effects and alters arachidonic acid metabolism in human platelets. Prostaglandins Leukot. *Essent Fatty Acids.* 33: 1-6.

Wagacha, J. M., & Muthomi, J. W. (2008). Mycotoxin problem in Africa: Current status, implications to food safety and health and possible management strategies. *International Journal of Food Microbiology*, Vol. 124, May 10, pp. 1-12,0168-1605.

Wangikar, P.B., Dwivedi, P., Sinha, N., Sharma, A.K., and Telang, A.G., 2005. Teratogenic effects in rabbits of simultaneous exposure to ochratoxin A and Aflatoxin B1 with special reference to microscopic effects. *Toxicology* 215, 37.

Wogan, G.N., 1999. Aflatoxin as a human carcinogen. *Hepatology* 30, 573.

Xu, Z. R., Han, X. Y., Huang, Q. C., Li, W. F., & Jiang, J. F. (2008). Changes in growth performance, digestive enzyme activities and nutrient digestibility of cherry valley ducks in response to Aflatoxin B(1) levels. *Livestock Science*, Vol. 119, Dec, pp. 216- 220, 1871-1413.

Yadav, P. and Singh, R., 2011. A review on anthelmintic drugs and their future scope. *Int. J. Pharm. Pharma. Sci.*, 3(3): 17–21.

Yahya Abadi, S., ZibaNejad, E. and Doodi, M. (2011). *Iranian Journal of Herbal Drugs.* 2, 1,

Yang, Y. C. and Lee, S. H., 2003. Ovicidal and adulticidal effects of *Eugenia caryophyllata* bud and leaf oil compounds on *Pediculus capitis*. *J. of agri. and Food Chem.*, 51: 4884- 4888.

Tables

Table. 1. Anova Table

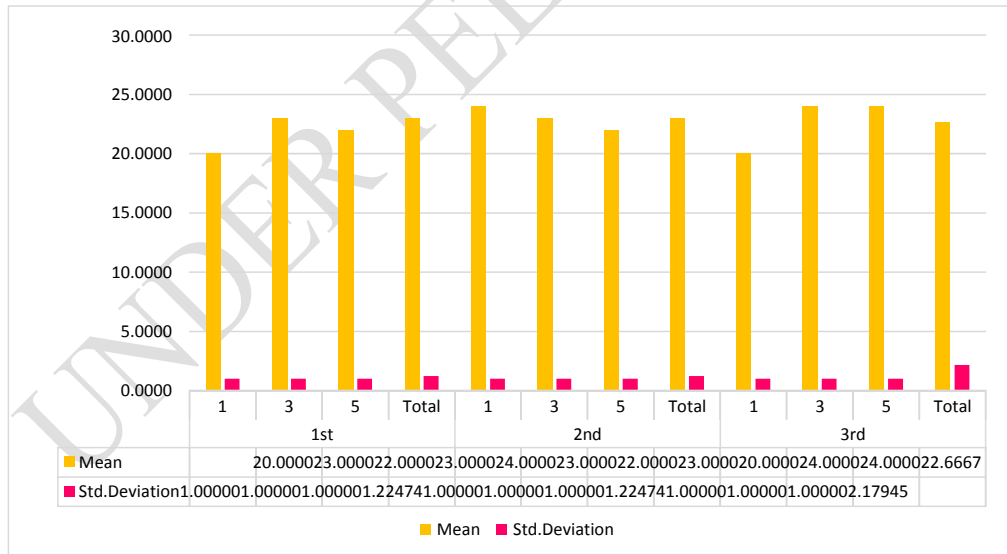
Source	Type III Sum of Squares	Mean Square	F	Significance
Corrected Model	8328.667a	237.962	356.943	0.000
Intercept	7301.333	7301.333	10952.000	0.000
Plant	8090.667	2896.889	4045.333	0.000
Week	51.167	25.583	38.375	0.000
Concentration	8.667	4.333	6.500	0.003
Plant* Week	102.833	17.139	25.708	0.000
Plant* 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

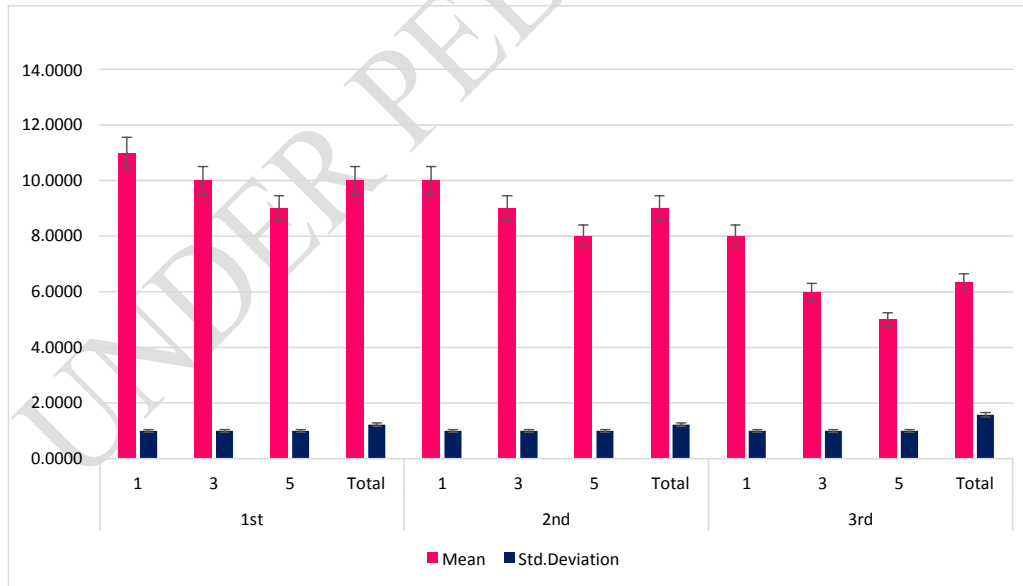
**Graph 1: Estimated Aflatoxin in the feed source (Control)**



**Table 3. Effect of *A. lebbek* 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

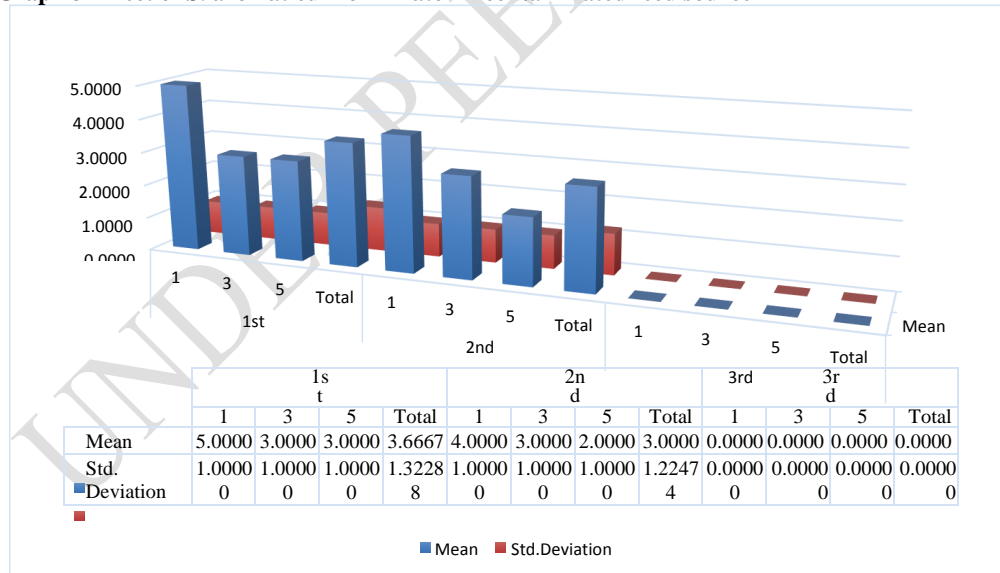
**Graph 2: Effect of *A. lebbek* on Aflatoxin contaminated feed source**



**Table 4. Effect of *S. 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

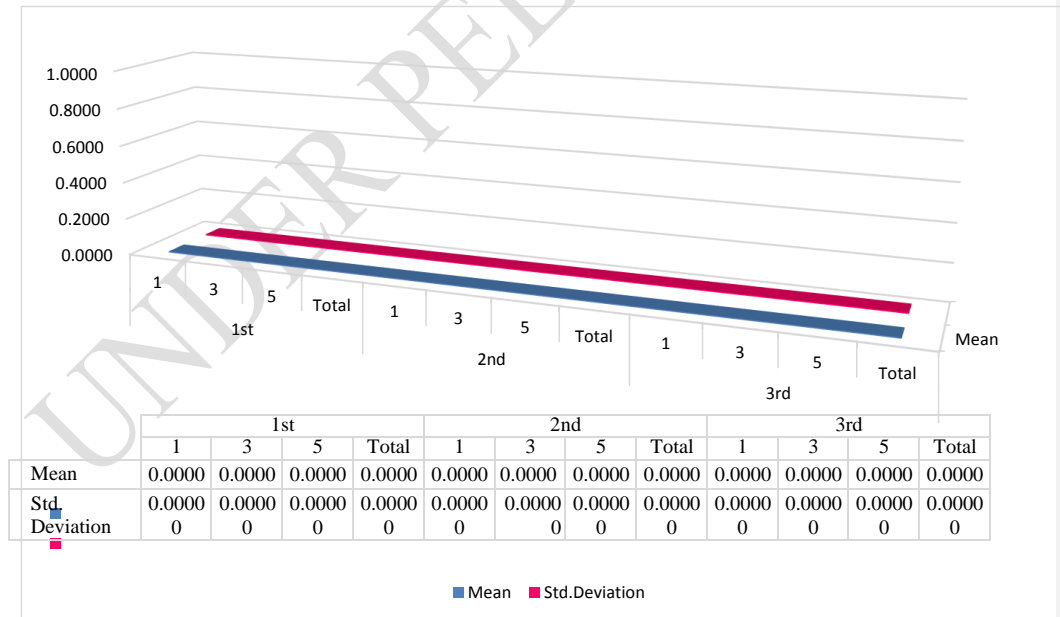
**Graph 3 Effect of *S. aromaticum* on Aflatoxin contaminated feed source**



**Table 5. Effect of *T. 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

**Graph 4: Effect of *T. ammi* on Aflatoxin contaminated feed source**



**Table 6. Antifungal activity of Medicinal plants**

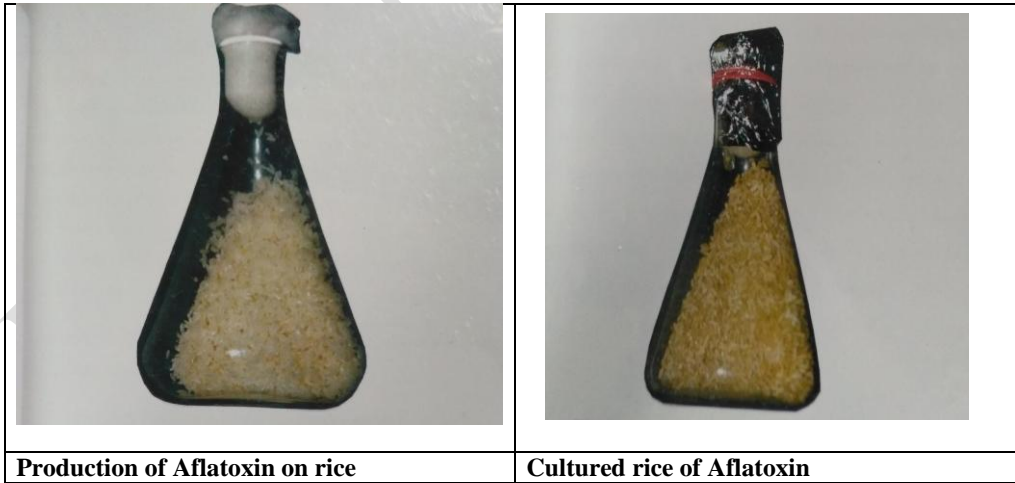
Plant sample	Extract	Microorganism	References
<i>T. vulgaris</i> , <i>Satureja hortensis</i> , <i>Syzygium aromaticum</i>	essential oil	<i>A. flavus</i>	Omidbeygi <i>et al.</i> , 2007
<i>Polymnia sonchifolia</i>	aqueous extract	<i>A. flavus</i>	Pinto <i>et al.</i> , 2001
<i>T. vulgaris</i>	essential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

**Figures**

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



**Figure 2. Production of Aflatoxin**



**Figure 3. Plant materials**




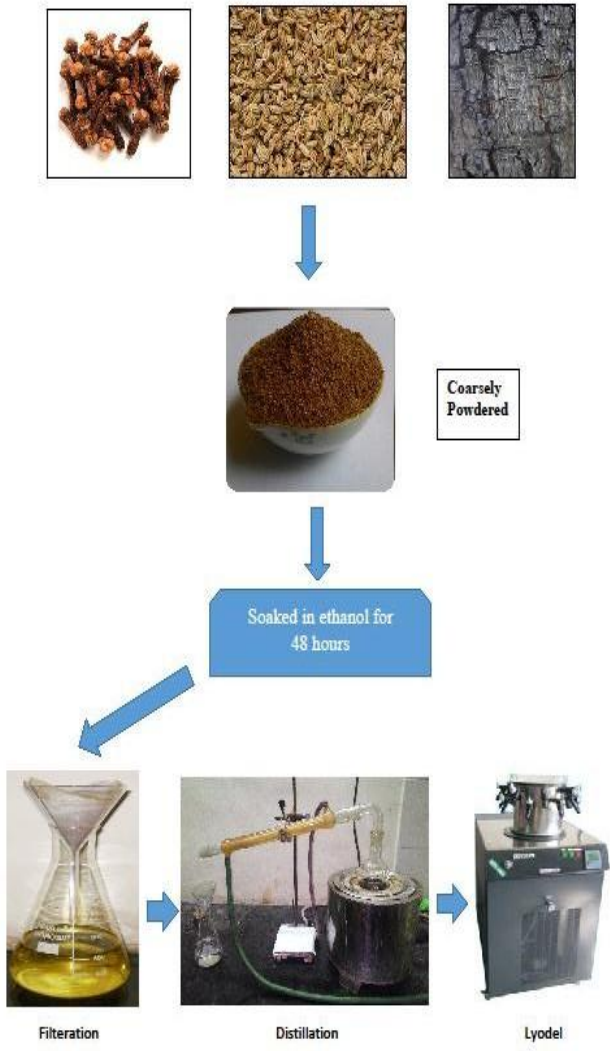



		
<i>Albizia lebeck</i>	<i>Syzgium aromaticum</i>	<i>Trachysper mumammi</i>

Figure 4. Preparation of plant extract



**Figure 5. Preparation of feed source**

		
<p><b>Contaminated feed source</b></p>	<p><b>Mixing of various plant extract in formulated feed</b></p>	<p><b>Formulated feed sealed in airtight containers</b></p>

UNDER PEER REVIEW