**CHAPTER II**

**REVIEW OF LITERATURE**

**2.1 General Study**

**2.1.1 Aspergillosis**

Aspergillosis is mostly caused by *Aspergillus fumigatus* but *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus glaucus*, *Aspergillus nidulans* and other *Aspergillus spp.* or mixed infections can play a role in the disease (Joseph, 2000; Barton *et al*., 1992; Perelman and Kuttin, 1992). *A. fumigatus* is the predominant species of airborne fungal infections because of their spores which are much smaller than the spores of other *Aspergillus spp.* (Richard and Thurston, 1983).

**2.1.2 Epidemiology**

Active fungal proliferation and sporulation of *A. fumigatus* on organic material produce large amounts of airborne small-sized conidia that are easily dispersed in air, then potentially inhaled and deposited deep in the respiratory tract. Susceptible hosts will develop polymorphic clinical forms in relation to either localized or disseminated lesions. Acute aspergillosis generally occurs in young birds resulting in high morbidity and mortality. The chronic form is sporadic. It causes lesser mortality and generally affects older birds, especially breeders in poultry, presenting a compromised immune system due to poor husbandry conditions.

**2.1.3 Pathogenesis**

Inhalation is considered the main infection route for *A. fumigatus* in birds (Oglesbee, 1997) and because *A. fumigatus* spores are too small to be trapped completely in the nasal cavity or trachea, some are able to reach the lungs and air sacs (Fedde, 1998). The air sacs are usually the primary infection sites, since inhaled air reaches the posterior thoracic and abdominal air sacs prior to contacting epithelial surfaces in the lungs (Nardoni *et al*., 2006). In the lung parenchyma, spores get embedded in the atria and parts of the infundibulam in the para-bronchus and are engulfed by (surface) phagocytic epithelial cells (Maina, 2002). When there are too many spores or the bird has an impaired immune response, the innate defense mechanisms do not succeed in eliminating infection at the site of the air capillaries. This may lead to the development of loosely attached plaques, which may or may not become overgrown by connective tissue of the host. These plaques or necrotic debris in the respiratory tract can obstruct the trachea or bronchi and/or fill up the air sacs (Oglesbee, 1997). Occasionally, sporulation occurs in the lungs and air sacs (Nardoni *et al*., 2006; Cacciuttolo *et al*., 2009). Hyphae containing fruiting bodies can fill the lumen and may penetrate the air sacs, causing serositis and superficial necrosis in the adjacent organs (Tsai *et al*., 1992). Besides direct extension of the infection through the air sac wall, disseminated mycosis also occurs by haematogenous spread. Hyphae, which are known as tissue, are angio-invasive (Dahlhausen *et al*., 2004) and as well as host cells play a role in this spreading mechanism. Macrophages in the respiratory tract ingest spores and find their way through the interstitium into the blood and lymphatic stream and thus to other organs (Richard and Thurston, 1983).

**2.1.4 Clinical signs**

Clinical manifestations depend on the infective dose, the spore distribution, pre-existing diseases and the immune response of the host (Dahlhausen *et al*., 2004). Avian aspergillosis is often classified as acute or chronic.

Acute aspergillosis may include a variety of nonspecific clinical signs: anorexia, lethargy, ruffled feathers, respiratory signs, polydipsia, polyuria, stunting, or sudden death. In chicks, contaminated in ovo or during hatching, the disease, commonly known as **brooder pneumonia**, is highly fatal in the first 10 days of life and results in a major respiratory distress. In poultry farms, mortality rate may rise slightly or increases suddenly; peaks during a few days and then returns to initial state Respiratory signs include dyspnoea, gasping, hyperpnoea with panting, nonproductive coughing, wheezing, cyanosis and sometimes nasal discharge.

In the chronic form, dyspnoea, depression, dehydration, and emaciation are described. Nervous system involvement causes ataxia, tremor, opisthotonos, lateral recumbency, torticollis, seizures, convulsions, lameness, and hind limb paresis. Occurrence of nervous and ophthalmic troubles one week after an acute episode of aspergillosis has been reported in a turkey flock. Cloudiness of the eye with severe conjunctivitis and turbid discharge were associated with paralysis in broiler breeders.

**2.1.5 Diagnosis**

The signs of aspergillosis are non-specific, making diagnosis difficult (Dahlhausen *et al*., 2004). Moreover, no single test provides certainty. Diagnosis usually relies upon an accumulation of evidence from the history, clinical presentation, hematology and biochemistry, serology, radiographic changes, endoscopy and culture of the fungus (Jones and Orosz, 2000). The clinical signs depend on which form of aspergillosis the bird develops and which organs are involved (Jones and Orosz, 2000). Hence, aspergillosis should be included in the differential diagnosis of respiratory tract and systemic diseases (Jenkins, 1991; Jones and Orosz, 2000).

Results of haematology and serum biochemistry can be considered indicative rather than diagnostic (Jones and Orosz, 2000). Leukocytosis of 20,000 to more than 100,000 white blood cells per microlitre (Jenkins, 1991; Oglesbee, 1997), heterophilia with a left shift (degenerative shift), monocytosis and lymphopenia have been described in aspergillosis cases (Forbes, 1992). In addition, non-regenerative anaemia, increased total protein and globulin fraction can be observed (Vanderheyden, 1993; Reidarson and McBain, 1995; Jones and Orosz, 2000). Acute infections often present an increase in β-globulins, while chronic infections show an increase in β-globulin and/or γ-globulin fractions. However, immune-suppressed birds may have hypo-proteinaemia (Ivey, 2000; Cray *et al*., 2009a) and white blood cells may be in the normal range (Flammer and Orosz, 2008). Overall, changes in protein electrophoresis are non-specific, but can be useful to estimate disease progression and the response to therapy (Ivey, 2000; Cray *et al*., 2009a). Serological tests have been developed to confirm an early and more definite diagnosis of aspergillosis (Peden and Rhoades, 1992).

**2.1.6 Treatment**

Treating avian aspergillosis is a challenge due to a number of factors. Both conventional and supportive treatment is required. In mild form of disease, treatment is fruitful but when lesions are moderate to severe involving lungs and air sacs, therapy is often not successful even after combination of drugs are used. Various drugs like amphotericin-B, 5-fluorocytosine, ketoconazole can be used to control the disease. Treating litter with Nystatin and Copper sulphate can reduce mold content. Copper sulphate at 60g quintal-1 of feed for 6 days is effective for treatment of aspergillosis. In outbreaks, drinking water with 1:2000 aqueous solution of copper sulphate needs to be provided. Tetracycline at 200 mg L-1 of drinking water should be given for 5 days to treat aspergillus infections in chicks. Other drugs like eniconazole and fungicidin have also been tried on experimental basis (Kuldeep Dhama et al, 2013).

Natural plant extracts may provide an alternative to chemical treatment. Over the years much effort has been devoted to the search for new antifungal materials from natural sources for food preservation *Allium* genus has over 500 members, each differing in maturing, color and taste, but with similar biochemical, phytochemical and neutraceutical content. *Alliums* were revered to possess anti-bacterial and anti-fungal activities and include the powerful antioxidants, sulfur and other numerous phenolic compounds which arouse significant interests (Karapınar, 1989; Topal, 1989).

**2.2 Effect of medicinal plants on aflatoxin producing fungi and aflatoxin production**

Various Southeast Asian medicinal plants such as Asiatic pennywort (*Centella asiatica*), betel nut (*Areca catechu*), betel vine (*Piper betle*), bitter cucumber (*Momordica charantia*), Chaa Phluu (*Piper sarmentosum*), Chinese radish (*Raphanus sativus*), clove (*Syzygium aromaticum*), eucalyptus (*Eucalyptus globules*), false coriander (*Eryngium foetidum*), hedge flower (*Lantana* *camara*), Indian mulberry (*Morinda citrifolia*), Madagascar periwinkle (*Catharanthus roseus*), mangosteen (*Garcinia mangostana*), mandarin (*Citrus reticulate*), onion (*Allium cepa*), pepper (*Piper nigrum*), pomegranate (*Punica granatum*), roselle (*Hibiscus sabdariffa*), Non Taai Yaak (*Stemona tuberosa*), tomato (*Lycopersicon esculentum*), Raang Chuet (*Thunbergia laurifolia*), Saab Sue (*Chromolaena odorata*), turmeric (*Curcuma longa*), water primrose (*Jussiaeda repens*) and wishing tree (*Cassia bakeriana*) were tested for their ability to control *A. flavus*. The results showed that the crude ethanolic extracts of some medicinal plants inhibited fungal growth to various degrees.

Crude aqueous extracts of garlic, carrot and clove were tested for the inhibitory effect on aflatoxin production in rice by the addition of extracts into 50g of rice to obtain the concentrations of 0, 2, 4, 6, 8 and 10% (w/v). Extracts from garlic bulbs, green garlic and green onions showed an inhibitory effect against *A. niger* and *A. flavus*. However, green garlic and green onion lost their antifungal activity against *A. niger* after being heated at 80°C and 60°C, respectively.

**2.3 Effect of essential oils on aflatoxin producing fungi and aflatoxin** **production**

Essential oils from 16 aromatic plants, i.e., safflower (*Carthamus tinctorius*), marigold (*Tagetes* *erecta*), coriander (*Coriandrum sativum*), pomelo (*Citrus maxima*), mangosteen (*Garcinia* *mangostana*), *Kaempferia parviflora*, ginger (*Zingiber officinale*), pepper (*Piper nigrum*), Boraphet (*Tinospora crispa*), aloe (*Aloe vera*), lavender (*Lavendula officinalis*), rosemary (*Rosemarinus officinalis*), cinnamon (*Cinnamomum cassia*), eucalyptus (*Eucalyptus globules*), thyme (*Thymus vulgaris*), and white wood (*Melaleuca cajuputi*) were tested for their inhibitory effect on *A. flavus*. Essential oils of cinnamon (*Cinnamomum zeylanicum*), peppermint (*Mentha* *piperita*), basil (*Ocimum basillicum*), origanum (*Origanum vulgare*), the flavoring herb *epazote* (*Teloxys ambrosioides*), clove (*Syzygium aromaticum*) and thyme (*Thymus vulgaris*) caused a total inhibition of *A. flavus* on maize kernels. The optimum dosage for protection of maize varied from 3 to 8% (v/w). The antimicrobial activity varies widely, depending on the type of spice or herb, test medium and microorganism. Contents of essential oils in different species are influenced by genetic variations between cultivars, culture conditions, environment, crop and post-crop processing.

**2.4 Medicinal plants tested in this study**

Varieties of secondary metabolites in plants are tannins, terpenoids, alkaloids,and flavonoids, which have been found *in vitro* to have fungitoxic properties, systemic in action and lack residual effect, (Naganawa *et al.*, 1996).Hence antimicrobial properties of some plant constituents are being exploited in protecting food, feed and seeds from storage moulds (Centeno *et al*., 2010).

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| Scientific name | Local name | Family | Plant part used | Medicinal use |
| *Allium cepa* | Onion oil | Amaryllidaceae | bulb | Enhance immunity,Antifungal |
| *Allium sativum* | Garlic oil | Liliaceae | bulb | Expectorant,Antibacterial,Antifungal |
| *Azadirachta indica* | Neem extract | Meliaceae | Leaves and seeds | Antibacterial, Antiviral, Antifungal |
| *Ocimum sanctum* | Tulsi extract | Lamiaceae | Leaves | Antibacterial, Antifungal |

## (Bansod and Rai, 2008)

**2.5 Review study**

Aflatoxicosis in poultry can cause disease and increased mortality (Ibrahim et al 2000, Kubena et al 1998, Oguz and Kurtoglu 2000). Concentrations of biochemical variables are used as an important tool to diagnose illness in domestic animals (Bruguere-Picoux *et al,* 1987; Kaneko, 1989). Determination of biochemical toxic effects of AFs is important for diagnosis of toxicosis in broilers (Rosa et al 2001). This serum biochemistry and enzyme activity can also help in the diagnosis of aflatoxicosis cases before major clinical symptoms appear (Oguz etal 2000a). AF toxicity in broilers may be manifested by decreased serum concentrations of total protein, albumin, total cholesterol (Kubena et al 1998, Oguz et al 2000a), uric acid (Kececi et al 1998), and increased hepatic enzyme activities such as AST and ALT (Amer et al 1998, Santurio et al 1999).

Enzyme activities in birds are variable and originate from different organs. In fowls, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gammaglutamil transferase (GGT) and lactic dehydrogenase (LDH) are synthesized in muscle, skeletal and cardiac, and in second order in the liver (Bruguere-Picoux *et al,* 1987; Campbell & Coles, 1989). The effects of aflatoxin in young broilers chickens have been studied in 1-day-old chickens. Intoxications decreased blood lipids (Tung *et al,* 1972) and caused higher activities of AST and GGT (Balachandran & Ramarkrisnan, 1988).

Previous studies performed with high levels of AF (2.5–5 mg/kg diet) showed significant decreases in serum total protein, albumin, total cholesterol and uric acid levels (Kubena et al 1998, Oguz et al 2000a, Rosa et al., 2001). No significant changes were reported in serum biochemistry for the lower dietary AF, such as 50 ppb (Abdelhamid et al 1994) and 200 ppb (Johri et al 1990), in broiler food. However, when AF levels increased in food up to 300 ppb and more (Jindal et al 1994, Raju and Devegowda 2000) the serum biochemistry was significantly affected and total protein, albumin and cholesterol levels were decreased. Serum AST and ALT activities are considered sensitive indicators of hepatocellular damage/dysfunction, indicating liver inflammation, lesions or obstruction of the biliary tract (Kubena et al 1998).

Experimental aflatoxicosis was produced in 'Cobb' broiler chickens by feeding them with dietary aflatoxin at concentrations of 1 and 3 ppm for a period of four weeks from 0 to 28 days. Twelve birds in triplicate were used for each treatment of which three from each replicate were sacrificed at weekly intervals for enzymatic estimations of SGPT, SGOT, and Serum amylase and serum lipase. Thus each treatment contained nine weekly observations and 36 observations in total. The SGPT activity was not found in appreciable amounts either in control or in toxin treated birds. However, the SGOT levels were in measurable amounts and elevated levels were observed in toxin treated birds from first week onwards at both levels of toxin and were dose related. The treatment means of SGOT differed significantly (P less than 0.05). The serum lipase levels increased from first week onwards and the treatment means showed significant difference (P less than 0.01). Serum lipase changes in toxin treated birds occurred to about the same extent. But the serum amylase levels decreased from first week onwards and their treatment means differed significantly (P less than 0.01) at both levels of toxin treated birds. Serum amylase concentrations decreased as the aflatoxin level increased in the diet(Balachandran and Ramarkrishnan, 1988).

This study investigated the effects on growth and composition of the blood of dietary aflatoxin fed at levels of 0, 2.5, or 5.0 μg/g of diet for a three week period beginning at 1, 7, 14, and 21 days of age in commercial broilers. Packed cell volume (PCV), erythrocyte counts, mean corpuscular volume, hemoglobin content and mean corpuscular hemoglobin concentration, body weight, and mortality were measured weekly. Plasma cholesterol and total plasma protein levels were determined weekly. Dietary aflatoxin at levels of 2.5 and 5.0 μg of aflatoxin per gram of diet when fed to young chicks for three weeks, beginning at either one or seven days, depressed body weight and PCV in a dose related fashion. Body weight and PCV continued to be depressed by the 5.0 μg/g diets in chicks treated from 2 to 5 weeks of age but not at the lower dosages. The feeding of aflatoxin at levels of up to 5.0 μg aflatoxin per gram of diet from 3 to 6 weeks of age did not significantly depress body weight or PCV from control levels. Plasma cholesterol and total protein were found to be more sensitive to aflatoxin treatment at the later ages than body weight and other blood values. Plasma cholesterol was significantly depressed by the 5.0 μg/g level of aflatoxin in all treatment periods, but the 2.5 μg/g level of aflatoxin did not significantly reduce cholesterol during the 3 to 6 week treatment period. Plasma proteins were found to be the most sensitive criteria for detecting broiler susceptibility to aflatoxin, being depressed by all levels of aflatoxin for all age groups (Lanza et al., 1980b).

Two-week-old ducks and chickens were fed for a 14-day period diets containing either groundnut meal (GNM) or fish meal (FM) contaminated with the following aflatoxin (AF) levels: 0, 50, 100, 200 and 400 micrograms AF B1 equivalent per kg ration; nitrogen and energy balances were measured, liver lesions assessed, and various biochemical analyses in blood, livers and muscles were made. Both ducks and chickens fed diets containing GNM were more affected by dietary AF than those fed diets with FM. In ducks, in addition to the reduction in growth and utilization of protein, dietary AF caused liver damage and significantly affected most of the blood constituents; chickens were either not affected or affected to a lesser degree, but no liver damage was recorded. Individual blood tests or enzyme ratios did not provide a sufficiently precise diagnosis of aflatoxicosis. However, blood clotting time and De Riti's ratio, when used in a multivariate regression allowed projection of a degree of liver damage caused by AF in ducks fed GNM diet with 83.6% of variance being accounted for. (Ostrowski, 1984).

Phytochemicals present in [medicinal plant](http://www.scialert.net/asci/result.php?searchin=Keywords&cat=&ascicat=ALL&Submit=Search&keyword=medicinal+plant)s have health benefits and [antimicrobial activity](http://www.scialert.net/asci/result.php?searchin=Keywords&cat=&ascicat=ALL&Submit=Search&keyword=antimicrobial+activity) against some [pathogenic bacteria](http://www.scialert.net/asci/result.php?searchin=Keywords&cat=&ascicat=ALL&Submit=Search&keyword=pathogenic+bacteria). However, little research has been undertaken on the antifungal activity of these extracts. This research aim at testing the antifungal activity of organic ethanol extracts of onion (Allium cepa), ginger (Zingiber officinale) and garlic (Allium sativum) against three fungal isolates (A. flavus, A. niger and C. herbarium) in Potato Dextrose Agar (PDA). Filtered plant extracts were obtained using [ethanolic extract](http://www.scialert.net/asci/result.php?searchin=Keywords&cat=&ascicat=ALL&Submit=Search&keyword=ethanolic+extract)ion method. Antifungal sensitivity testing was undertaking using the pour plate technique and results obtained by measuring diameter of fungal growth over a 7 day incubation period. All organic plant extracts inhibited growth resulting in a marked significant difference (p<0.01) in growth diameter of fungi on media with extracts compared with same fungi on Potato Dextrose Agar without extracts. Ginger had the highest antifungal activity on all test fungi with a mean diameter of 1.40 cm followed by garlic (1.70 cm) and onion (1.80 cm) respectively whilst A. niger (2.54 cm) showed the highest resistance to the plant extracts followed by A. flavus (2.50 cm) and C. herbarum (1.18 cm). All plant extracts inhibited any observable growth pattern in C. herbarum for a 2 day period and <1 cm growth diameter in A. Flavus and A. Niger whilst the least growth measurement after day one of incubation in PDA only was >2.0 cm. This study confirms the antifungal potential of these plant extracts on the test fungi and suggests the possibility of employing them in food preservation were spoilage is mainly caused by fungi. (D.N.A Tagoe, H.D. Nyarko and R. Akpaka, 2011.)

Tulsi is known as “Queen of plants” “The mother medicine of nature”. Tulsi i.e. Ocimum sanctum is a plant with enormous properties for curing and preventing diseases. Essential oil present in most of the Ocimum species is responsible for its antifungal, antibacterial and antiviral properties. Microorganisms develop resistance against various antibiotics and due to this an immense clinical problem develops in treatment of infectious diseases. Medicinal plants can be used to overcome this problem. Tulsi leaves have been reported to show strong antifungal activities against the Aspergillus species. Essential oil from Ocimum sp which contain eugenol, carvacrol, methyl eugenol, caryophyllene are considered mainly to be responsible for various antimicrobial properties (Singh V, amdekar s, Verma O. Ocimum, 2010).

Neem plant is a known inhibitor of aflatoxin production. We studied the effects of different concentrations of aqueous neem leaf extract on fungal growth and aflatoxin production by Aspergillus parasiticus at different incubation times. The toxigenic fungus was cultured on sucrose low salts medium in the presence of various concentrations of extracts (0.2, 0.8, 3.12, 12.5 and 50% v/v). After shaking incubation of cultures for 2, 4, 6, 8, 10 and 12 days at 28 °C, the fungal mycelia was collected and processed for determination of dry weight. Mycelia and culture media were assayed by TLC method to detect aflatoxin B1 (AFB1). The extracts did not have any obvious effect on fungal growth. AFB1 production in the control samples increased to the maximum level on the 8th day. The inhibition of aflatoxin synthesis by plant extracts was found to be time and dose dependent. The maximum inhibitory effect was 80–90% in the presence of 50% concentration that when compared with control samples were significant (P < 0.05). AFB1 secretion/production ratio in all of control and treated samples, other than 2nd day, approximately stayed and neem had no effect on it (Ghorbanian et al).