Feed is the single most important input in increasing chicken and fish culture production and profits. Feeds and feed stuffs are an excellent media for the growth of fungi. Fungi are major spoilage agents of foods and feedstuffs. During manufacturing, feeds can be contaminated with mould spores. The major causing sever feed deterioration (Aspergillus flavus Fungus) was isolated from chicken and fish feed contaminants. So that, the aim of this study was focuses on prevent growth of Aspergillus flavus fungus by using some physical and biological methods. The obtained data presented that, A. flavus was found to reduce significant (P<0.001 and P<0.05) protein and fibers contents and increase fats and moisture percent of contaminated fish feed sample. In in vivo, data show that, sunlight was found to decrease significant (P<0.001 and P<0.05) the total fungal population (count) of tested chicken and fish feed samples association, as well as increase significantly (P<0.001 and P<0.05) the decreasing percent with increasing the time period to sunlight exposure. In in vitro, data indicated that, data indicated that, propolis extract was found to decrease significant (P<0.001) the linear growth of A. flavus fungus when cultured with 2, 5, 10 and 20% of propolis extract. Increase decreasing percent with increasing the concentration of propolis extract. It was found to effectively suppress mycelial dry weight, significantly (P<0.001) inhibited spore germination (viability) and effectively suppress morphological characteristics causing several changes and damages of mycelia.

Keywords: Feed Deterioration, Aspergillus flavus, Sunlight, Propolis Extract
Introduction

Feeds and feedstuffs are excellent media for the growth of fungi and so, very high standard of hygiene is necessary to avoid feed contamination. Mold contamination has a potential to produce fungal toxins (mycotoxins) that are harmful to chickens, turkeys, ducks, fish etc... One of the best ways to control feed contamination and mycotoxin problem is to investigate potentially toxigenic fungal population in feeds (Dalceró, et al., 1998). Fungi belonging to Aspergillus, Penicillium and Fusarium species are responsible for causing mycotoxins of agro-economic importance (Vasanthi and Bhat, 1998). Commercial feedstuffs are an important component in modern animal husbandry, but there is no information available about fungal contaminations (Krnjaja, et al., 2008). These fungi can grow on a wide range of agricultural food and feed products. They are of worldwide importance, causing problems in public health, agriculture, and high economic losses (Herzallah, et al., 2008). The presence of molds in foods and feeds, generally has been recognized as undesirable. Aflatoxins chiefly produced by fungus Aspergillus flavus and Aspergillus parasiticus have been found as a natural contaminants in food and feed stuffs cause sickness and death in farm animals and even in human beings (Spring and Fegan, 2010; Probst, et al., 2011 and Bedi, and Agarwal 2014). Information about fungi associated with food and feeds is important in assessing risk of mycotoxin contamination (Almeida, et al., 2011). This type of raw materials has been associated with contaminants produced by molds during the initial stages of the crop production. During manufacturing, feeds can be contaminated with mould spores, especially when the cereal grains are ground and the feeds are pelleted. Mould contamination not only causes deterioration of food and feeds, but also can adversely affect the health of humans and animals, once they may produce toxic metabolites (Mehrim and Salem 2013). The presence of microscopic fungi affects the quality of feeds, their organoleptic attributes, and nutritional quality. Molds are capable of reducing the nutritional value of feedstuffs as well as elaborating several mycotoxins. Mycotoxin-contaminated feed has adverse effects on animal health and productivity (Greco, et al., 2014). For quality control the identification of the contaminating mycobiota is essential because it provides data on the potential production of its mycotoxins and is a helpful indicator to determine feed hygienic quality (Greco, et al., 2014). One of the most effective ways to control the problems caused by aflatoxins is to prevent the growth of fungi in the substrate to suppress the spore germination of the fungi, as well as the development of the fungal mycelium, in the substrate susceptible to contamination by these toxins (Upadhaya, et al., 2010). Bee products (propolis) are natural substances (collected by worker bees Apismellifera) with beneficial effects on human health and body with strong antimicrobial characteristics (Quirogaet al., 2006 and Giovanelli, 2008). It provides protection against harmful fungi, bacteria and viruses (Masood, et al., 2006). Propolis has been widely used for many years for its antimicrobial activity against many bacteria, yeasts and fungi. However, its use as an agricultural antimicrobial agent has only recently been assessed (Sánchez, et al., 2016). Propolis has a complex composition with a wide range of effects, including antibacterial, antiviral, antifungal, antiflogistic, antioxidant, hepatoprotective, carminative and immunomodulatory properties. Propolis has been shown to exhibit antifungal action against C. albicans, C. tropicalis and C. krusei. Propolis acts on the aflatoxicogenic types of Aspergillus such as Aspergillus flavus by inhibiting the condom germination Šabanović, et al., 2019.

The aim of the present study was to determine the hygienic and nutritional quality and the occurrence of Aspergillus flavus fungus in poultry feeds, and the evaluation of some variables related to nutritional quality (crude proteins, fats, humidity, and total ashes), focuses on prevent A. flavus by using some physical and biological methods. So, it is possible to use sunlight treatment. Also, this study was focused to see the effect of propolis as a natural product on the linear growth, mycelial dry weight, spore germination (viability) and decontamination of A. flavus.

Materials and Methods

Microorganism

Aspergillus flavus (No. 10/59) was isolated from chicken and fish feed samples by Embaby, et al., (2015).

Changes in Chemical Composition (Feed Deterioration)

Ground state of chicken and fish feed composition (provided by the company) were listed in Table (1). Effect of A. flavus, on the chemical composition of fish feed samples was studied. A. flavus, isolate was inoculated (at 10^3) in flasks (500 ml) each containing 100 g of sterilized fish feed and water (1: 1 v/v) then incubated at 26°C ± 2 for 14 days. Protein, fat, fibers, ash (g) and moisture percent were determined as described by AOAC (2007) in the National Research Centre (NRC). Loss and reduction percent of the infected fish feed sample with A. flavus were calculated and compared to the ground state of fish feed (control) as follow:

\[
\text{Loss(L)} = \text{U - T}, \text{U = Untreated control; T = Treated; Reduction percent (R%) = U - T / U x 100}
\]

Physical method (Sunlight Treatment)

About 10 g each from chicken and fish feed samples were placed in polyethylene page under direct bright sunlight at noon during the summer season for 0, 12 and 24 hours of exposure time per day (the max temperature of sun was 38°C) according to Herzallah, et al., (2008). Added 10g of each sample were suspended in 90ml sterilized water then
shocked for 3-5 min and filtered to give 1:100 of dilution and routinely diluted to 10^3. Added 1ml of each dilution in sterilized Petri dishes 9cm then mixed with 10ml of sterilized PDA medium with Streptomycin sulfate. Plates were incubated at 26±2 °C for 7 days. After incubation period, Total Fungal Count (TFC) in fish and chicken feeds were calculated and recorded (NWabueze and NWabueze, 2011).

| Type of Feed | Company                  | Protein | Fats  | Fibers | Mineral | kcal/kg |
|--------------|--------------------------|---------|-------|--------|---------|---------|
| Chicken      | Masoud group             | 17.0    | 3.70  | 4.20   | 3.21    | 2800    |
| Fish         | Elmorshedyekhwan         | 25.0    | 5.56  | 5.7    | -       | 3800    |

Kcal/kg = energy available feed (calories)

**Biological Method (Propolis treatment)**

**Extraction**

Propolis was prepared by adding 100 g of the collected propolis to 900 mL of 70% methanol to give 10% Methanol Extract of Propolis (MEP), then heating for evaporating methanol at 50 °C for 5 hours till methanol was evaporated completely and agitating. Sterilized distilled water was then added then centrifuged for 25 minutes at 3000 rpm. The supernatant was further filtered through filter paper (Whatman No. 1) and stored at ambient temperatures in a bottle according to Ngoepe and Straker (2004) and Özdemir, et al., (2010). The final solution was termed ‘methanol extract of propolis’ (MEP) to produce a final solution from various propolis samples Giovanelli, (2008). kept at 4°C in dark storage until use. The propolis extract was diluted at 2, 5, 10 and 20 ml per 100 ml of PDA (Oadi, et al., 2014).

**Effect of Propolis Extract on the Linear Growth in Vitro**

The ‘Methanol Extract of Propolis’ (MEP) was dissolved in sterilized PDA at 2, 5, 10 and 20% concentration per 100ml PDA, then poured it into 9 cm diameter of sterilized Petri dishes. One disc 0.5 cm diameter of *A. flavus* was cut using a sterile cork borer 5 mm from 1-week-old cultures on the PDA plates then placed on the centre of the dish based on methodology of Ngoepe and Straker (2004). Other Petri dishes free of propolis extract were used as control. Three Petri dishes were used as replicates per treatment then incubated at 25± 2°C. Colony diameter was measured after incubation period. The average mean growth was calculated and compared statistically between each concentration of propolis extract (Giovanelli, 2008 and Özdemir, et al., 2010). Growth inhibition (%) was calculated by the formula: (C-T)/C×100

Where; C = growth in control, T = growth in treatment

**Effect of Propolis Extract on the Mycelial Weight in Vitro**

Propolis extract was dissolved in Yeast Extract Sucrose (YES) broth at rates 2, 5, 10 and 20% concentration per 100ml (YES) medium in vitro. One disc from *A. flavus* fungus (0.5cm) 1-week-old cultures was cut by using sterilized cork borer from PDA plates (containing mycelial growth) then placed in the liquid media and incubated at 26±2°C for 15 day. YES liquid medium free of propolis extract was used as a control. After incubation period, cultures were filtered through pre-weighed Whatman filter paper No. 1. One disc from *A. flavus* fungus was placed in the YES liquid medium (0.5cm) 1-week-old cultures was cut by using sterilized cork borer then incubated at 25± 2°C (3 replication for each concentration). The sporulations of tested fungus were studied separately by collecting spore suspension of the respective fungus after passing the culture filtrate through muslin cloth. To harvest spores, 10 mL of sterile water were poured over the plate and the concentration was adjusted to 1x10^3 conidia/mL. Each plate was inoculated with 0.1 mL of spore suspension that was spread evenly over the plate. Each treatment had three replicate plates that were incubated at 26°C. After 48 h of incubation, the proportion of spores that had germinated was calculated (Meena and Mariappan 1993).

**Morphological Changes**

Propolis extract was studied to evaluate their effects on morphological characteristics of *Aspergillus flavus* fungus. Data obtained from microscopic observations due to mycelial damages were recorded according to Abyaneh, et al., (2005).

**Statistical Analysis**

Obtained data were subjected to statistical analysis of variance according to Gomez and Gomez, 1984.

**Result and Discussion**

**Fish Feed Deterioration Caused by A. flavus**

Data in Table (2) presented that, *A. flavus* fungus was found to reduce significant (P<0.001 and P<0.05) both protein...
and fibers (g) contents while fats (g) and moisture percent were increased. It was found to decrease protein content from 25.00(g) with ground state as a control to 3.76(g) which loss 21.24(g) equal 84.96 % decreasing and reduce fibers (g) content from 5.7(g) with ground state (control) to 2.62(g) and loosed 3.08(g) equal 54.04% decreasing. On the other hand data show that, A. flavus fungus was found to be increasing fat content from 5.56(g) to 44.18 g which record 87.42% increasing. Moisture content was increased from 4.2% to 21.18% and gives 80.17% hike. Okoli, et al., (2006) reported that, molds like other microorganisms will assimilate and utilize the most readily available nutrients in the materials they grow upon and spoilage may result in the loss of 5 to 100% of the nutrients in the feed. Mariana, et al., 2014 stated that, molds are capable of reducing the nutritional values of feedstuff. When conditions are optimal for molds, their first effect is utilization of nutrients for their metabolism and propagation. This results in decreased nutritional value of feeds.

Table 2.Fish feed deterioration caused by A. flavus fungus

| Composite    | G     | C     | L.S.D@1% | L.S.D@5% |
|--------------|-------|-------|----------|----------|
| Proteins(g)  | 25.00 | 3.76  | 1.219    | 0.841    |
| L            | 21.24 |       |          |          |
| D%           | 84.96 |       |          |          |
| Fibers(g)    | 5.7   | 2.62  | 1.219    | 0.841    |
| L            | 3.08  |       |          |          |
| D%           | 54.04 |       |          |          |
| Fats(g)      | 5.56  | 44.18 | 1.219    | 0.841    |
| L            | 38.62 |       |          |          |
| %I           | 87.42 |       |          |          |
| %Moisture    | 4.2   | 21.18 | 1.219    | 0.841    |
| I            | 16.98 |       |          |          |
| %I           | 80.17 |       |          |          |

G = Ground state (control), C = contaminant, L = Loss,%D = Decrease percent, I = Increase, %I = Increase percent

Physical Method (Sunlight Exposure)

This study revealed that exposure of contaminated feed to direct sunlight is helpful in reducing total forming colonies of fungal associated feed samples (Table 3). Data show that, sunlight was found to decrease significantly (P<0.01 and P<0.05) the total forming colonies of fungal associated both tested chicken and fish feed samples. Increase significantly (P<0.01 and P<0.05) the loss percent with increasing the time period of exposure to sunlight. Total colonies forming were found to decreased from 17 colonies with ground state(untreated control) of chicken feed sample at zero time to 5 and one colonies when exposed for 12 h and 24 h respectively, with 29.4% and 6.00% percentage of total colonies forming which loosed 12 and 16 colonies equal 70.6 and 94.00 loss % respectively. Also data showed that, total forming colonies of fungal associated fish feed sample were decreased from 3 colonies with ground state to 2 and 1 colonies when exposed at 12 and 24 h with 66.7 and 33.3% of total colonies forming and 33.3 and 66.7% loss percent. Peraica, et al., (2002) reviewed that the physical methods should be avoided as much as possible for feed decontamination because they may reduce the nutritional value of feed, and leave residues of mycotoxins or their toxic metabolites. Mani et al., (1997) and Gowda et al., (2007) reported that, hot air oven drying of the diet resulted in an average reduction of 57.6% in aflatoxin content in sheep fed, where the sun drying reduced the aflatoxin content by 83.7%. It can be concluded that drying of feed either in hot air oven (80°C / 6 h) or in sunlight (14 h) is effective in reducing the aflatoxin level. Herzallah, et al., (2008) investigate the efficiency of decontamination of aflatoxin residues in poultry feeds through exposure to sunlight (solar radiation), γ-radiation (60Co), and microwave heating in artificially contaminated feed samples. They reported that, photo degradation of aflatoxin by sunlight has been found to cause a significant (P<0.05) decrease in both B1 and the total aflatoxins. Moreover, the degrees of aflatoxins degradation were dependent on the exposure time. Both aflatoxin B1 and total aflatoxins were decreased by 42.3, 39.9, 75.5, and 65.9% when feed samples exposed to direct sunlight for 3 h and 30 h. Therefore, the solar radiation was more effective in aflatoxin B1 reduction when compared with γ irradiation and microwave heating. Santha and Sreenivasamurthy, (1980) and Choudhary, and Kumari, (2010) found that, sunlight destroyed 99% of the aflatoxin present in 15 min, whereas, tungsten lamp light and ultraviolet light destroyed 82% to 85% of aflatoxin in 18 hours and 30% to 40% of aflatoxin in 2 hours, respectively.
Complete destruction of aflatoxin was also obtained when aflatoxin contaminated peanut oil kept in glass containers was exposed to direct sun light (50,000 Lux) for one hour. Bedi and Agarwal, (2014) shows that sunlight exposure (temperature varying from 37°C to 42°C in the month of June for 5 hour) of the contaminated feed materials may reduce the aflatoxin level upto 10-17%.

| Temperature | Type of feed | Chicken feed | Fish feed |
|-------------|--------------|--------------|-----------|
|             | T.F.C. x 10³ | %T.F.C. | L. | %L | T.F.C. x 10³ | % T.F.C. | L. | % L. |
| 12          | 5            | 29.4       | 12.00 | 70.6 | 2            | 66.7       | 1.00 | 33.3 |
| 24          | 1            | 6.00       | 16.00 | 94.00 | 1            | 33.3       | 2.00 | 66.7 |
| Control     | 17           |            |       |     | 3            |            |      |      |
| L.S.D@1%    | 0.660        |            |       |     | 0.362        |            |      |      |
| L.S.D@5%    | 0.454        |            |       |     | 0.248        |            |      |      |

Table 3. Effect of sunlight exposure on the total fungal count (forming colonies)

T.F.C. = Total fungal colonies, % T.F.C. = percentage of total fungal colonies
L = Loss of fungal colonies, %L = Loss percent

**Biological Method using a Natural Product (Propolis Treatment)**

- Effect of propolis extract on the linear growth in vitro

Data in Table (4) and Figure (1) presented that, propolis extract was found to decrease significantly (P<0.01) the linear growth of *A. flavus* with all concentration used. Also data show that increase significantly (P<0.01) the decreasing percent with increasing the concentration used. The linear growth of *A. flavus* was found to reduce from 88 mm with untreated control to 60 mm and loosed 28 mm equal 68.2% and 31.8% decrease when cultured with 2% of propolis extract, followed by 49 mm of linear growth which loosed 39 mm equal 56.0% and 44.3% decreasing when treated with 5% conc., of propolis extract. Moderate percent was recorded with 10% conc., of propolis extract which reduced the linear growth of *A. flavus* from 88 mm with untreated control to 46 mm which loosed 42 mm equal 52.3% and gave 47.7% decreasing. While 20% of propolis extract was found to enhanced concentration which reduce the linear growth of *A. flavus* from 88 mm to 31 mm which loosed 57 mm equal 35.2 and 64.8% decrease. The result of the present study are in agreement with the observations of Hegazi and El-Hady (2002) they determined the antifungal activities of Egyptian propolis, in terms of Minimum Inhibitory Concentrations (MICs), against nine fungal genera namely *Cladosporium, Mucor, Scopulariopsis, Penicillium, Rhizopus, Fusarium, Aspergillus, Alternaria* and *Rhodotorula*. MIC values were determined by the agar dilution assay at different concentrations. The MIC values obtained were between 1.20 and 3.60 mg ml-1 with the former for *Alternaria* and *Fusarium* and latter for *Aspergillus* and *Penicillium*. Vallabh and Straker (2005) used a concentration gradient of 1, 2, 5, 7, and 10 mg ml-1 against *P. guipinii* and *Glomerellacingulata* (a sexual stage is *Collerotrichum gloeosporioides*) with the agar dilution method. According to Masood, et al., (2006) propolis (EEP) was effective on controlling the growth of fungal mycelia of *Aspergillus flavus* and *Aspergillus parasiticus*, the most important fungi producing aflatoxin in pistachio nuts. Giovanelli, (2008) found that, hyphal radial growth of *Colletotrichum* sp. and *P. guipinii* was inhibited by 81.7% at the low concentration of 1.16 mg ml-1 EEP. All the assessed fungi within this study were sensitive to all the concentrations of EEP tested, with high inhibition occurring at 5, 7, and 10 mg ml-1. However, the two fungi were significantly different (P<0.01) when incubated on MEA with EEP. Soylu, et al. (2004 & 2008) they reported that, the application of 1%, 5%, and 10% concentrations of Ethanol-Extracted Propolis (EEP) inhibited *Penicillium digitatum* growth *in vitro* and limited the growth of *Botrytiscincerea* on strawberry. Grigoryan et al. (2010), who proved that ethanol propolis extract and mix with hydrogen peroxide showed a stable antifungal effect. The PrEE inhibition percentage of *A. flavus* mycelial growth was 40%-60%. According to Amna, Saddiq and Enas Danial (2014) the effect of propolis extract on fungi was more activity against *Aspergillus flavus* than *Aspergillus niger*. The inhibition zones 30mm against *Aspergillus flavus* while 20mm against *Aspergillus niger*. Manjula and Ramachandra (2014) Tested the temperature of open yard sun drying around 37°C and solar tunnel drying around 60°C to prevent fungal proliferation. They found that, solar tunnel dried chilli samples were less aflatoxin content as compared to the open yard sun drying but, the aflatoxin found in both the drying methods was not harmful. Oadi, et al. (2014) in their study showed the effect of propolis and *Boswellia* sp. extract as antifungal on *Aspergillus flavus* on CMA culture media. Ezaizi and Davari, (2018) placed mycelial disks of fresh fungi cultures in the center of treated medium and the growth rate of fungi was measured to determine the inhibition percent.
Results showed that there are significantly different (P≤0.01) among the fungal species (Aspergillus flavus, Botrytis cinerea, A. tubingensis and Cladosporium cladosporioides), concentrations and interaction between fungal species and concentration. The sensitivity of fungal species was different in contrast to specified concentration of the extract, generally the highest inhibition was observed at the highest used concentration. The results of this study showed propolis possesses high antifungal potential and it may be used for biological control of plants fungal diseases after further researches. According to Embaby, et al., (2019) ‘Ethanol Extract of Propolis’ (EEP) showing, a significant antifungal activity against linear growth of A.alternata, A. niger, Fusarium sp. and P . expansum. Percentage of inhibited growth was found to increase with increase in concentration of extracts.

Table 4. Effect of propolis extract on the linear growth (P<0.01)

| Conc. | Growth (mm) | %D | L (mm) | % L |
|-------|-------------|----|--------|-----|
| 2     | 60          | 31.8 | 28     | 68.2 |
| 5     | 49          | 44.0 | 39     | 56.0 |
| 10    | 46          | 47.7 | 42     | 52.3 |
| 20    | 31          | 64.8 | 57     | 35.2 |
| Control | 88          |     |        |      |
| L.S.D@1% | 0.287     |    |        |      |

%D=%decrease, L=Loss of the linear growth, %L=Loss percent

Figure 1. Effect of propolis extract on the linear growth rate of A. flavus at different concentration (2, 5, 10 & 20 respectively)

- Effect of propolis extract on the mycelial dry weight in vitro

Estimation of mycelial biomass resulted that, propolis extract was found to significant (P<0.01) effectively suppressed mycelial biomass weight of Aspergillus flavus fungus and have inhibitory effect at different levels with all tested concentration as shown in Table 5. Higher significantly (P<0.01) of inhibitory effect was recorded with A. flavus fungus which reduced mycelial biomass of dry weight from 0.75g with untreated control to 0.63 g and loosed 0.12 g equal 16.0% loss percent and 84.0 decreasing percent when treated with 20% proplis, followed by 10% conc. which reduce the mycelial biomass of dry weight to 0.61g and lose 0.14 g equal 18.7% loss with 81.3% decrease. Followed by 5% concentration it is showed that reducing in the mycelial biomass of dry weight to 0.44 and loosed 0.31g equal 41.3% loss percent and 58.7% decreasing. Less inhibitory effect was recorded with 2 % conc. of Methanolic Extract of Propolis (MEP) which record 0.36 g and lose 0.39 g equal 52.0% loss with 48.0% decrease. It was previously stated by several investigators and research, Farre, et al., (2004) reported that, on comparison of anti-fungal activity of ethanolic extracts of propolis with those of griseofulvin, against two varieties of Aspergillus flavus, it can be seen that both substances reduce dry mycelial mass, the growth and production of aflatoxin B1. Similarly, Ghaly et al. (1998) and Giovanelli (2008) demonstrated inhibition properties of an ethanol extract of propolis (termed PEE) against conidia of Aspergillus flavus at a concentration gradient of 1 to 4 mg ml⁻¹ and mycelial dry mass was weighed to determine percentage of inhibition. The EEP probably diffused into the agar once the spore-EEP mixture was spread onto the MEA which would dilute the concentration of EEP. According to Embaby, et al., (2019) ‘Ethanol Extract of Propolis’ (EEP) showing effective in inhibiting dry weight of A. alternata, A. niger, Fusarium sp. and P. expansum mycelia at all the tested concentration used compared with control.

Table 5. Effect of propolis extract on the mycelia dry weight of A. flavus

| % Conc. | Dry weight(g) | % D | L (g) | % L |
|---------|---------------|-----|-------|-----|
| 2       | 0.36          | 48.0 | 0.39  | 52.0 |
| 5       | 0.44          | 58.7 | 0.31  | 41.3 |
| 10      | 0.61          | 81.3 | 0.14  | 18.7 |
| 20      | 0.63          | 84.0 | 0.12  | 16.0 |
| Control | 0.75          |     |       |      |
| L.S.D@1% | 0.020       |     |       |      |

% D = %decreasing, L(g) = Loose, % L = Loose percent

- Effect of propolis extract on spore viability

Data resulted that, propolis extract have significantly (P<0.01) inhibited spore viability of A. flavus fungus. Spore viability was found to be decrease significantly (P<0.01) with increasing propolis concentration (Table 6). On the other hand, propolis extract was found to decrease spore germination of A. flavus fungus from 517 x 10² spores with untreated (control group) to 366, 250, 140 and 53 x 10² germinated spores (viability) which caused 29, 51.64, 72.92 and 89.75% decreasing percent when treated at 2,
5, 10 and 20% conc. respectively. The decrease in conidial production and their germination recorded in the present study is similar with the reports of Ghaly et al. (1998) and Giovanelli (2008) demonstrated the germination inhibition properties of an ethanol extract of propolis (termed PEE) against conidia of Aspergillus flavus at a concentration gradient of 1 to 4 mg ml\(^{-1}\). At 1 g l\(^{-1}\) germination was inhibited by 11% as compared to 80% at 4 g l\(^{-1}\). Resulted from a concentration of 5 mg ml\(^{-1}\)EEP to 10 mg ml\(^{-1}\), and 1 mg ml\(^{-1}\) EEP results in very low inhibition, as well as to the high germination inhibition. The EEP probably diffused into the agar once the spore-EEP mixture was spread onto the MEA which would dilute the concentration of EEP. Farre et al., (2004) reported that, on comparison of anti-fungal activity of ethanolic extracts of propolis with those of griseofulvin, against Aspergillus flavus, it can be seen that both substances reduce dry mycelial mass, the germination of conidia, the growth and production of aflatoxin B\(_{1}\) the greater the concentration gave greater effect. Giovanelli, (2008) found that, Colletotrichum sp. conidial germination was inhibited by 98.95% when incubated on the medium (MEA) containing 5 mg ml\(^{-1}\) EEP. P. guipinii conidial germination was inhibited by 40.41% when incubated on the medium MEA containing 5 mg ml\(^{-1}\) EEP. The assessed fungi were sensitive to all the concentrations of EEP tested, with high inhibition occurring at 5, 7, and 10 mg ml\(^{-1}\). Abeer, Hashem et al., (2012) reported that propolis caused significant decrease in conidial production and conidial germination as well as mycelial growth (both radial and dry weight) of A. parasiticus. According to Lorini, et al., (2018) propolis produced by Apismellifera - red was effective in inhibiting mycelial growth and spore germination of A. flavus. Embaby, et al., (2019) reported that, ‘ethanol extract of propolis’ (EEP) was found to reduce all spore germination (viability) as well as increase reduction percent with all tested fungi i.e. Alternaria alternata, Aspergillus niger, Fusarium sp., and Penicillium expansum. Percentage of inhibition was also found to increase with increase in concentration of extracts.

| Table 6. Effect of propolis extract on spore germination (viability) |
|------------------|------------------|------------------|------------------|
| Conc.       | No. S.G. | % S. G. | No. L. S | % L     |
| 2           | 366.00   | 70.8   | 151.00   | 29.20 |
| 5           | 250.00   | 48.36  | 267.00   | 51.64 |
| 10          | 140.00   | 27.08  | 377.00   | 72.92 |
| 20          | 53.00    | 10.25  | 464.00   | 89.75 |
| Control     | 517.00   | 0.00   | 0.00     | 100.00 |
| L.S.D@1%    | 19.410   |        |          |        |

No. S.G. = Number of spore germination (viability), % S. G. = Percentage of spore germination
No. L. s = Loss of spores, %L = Loss percent

- Morphological changes of tested fungi

Morphological changes of Aspergillus flavus fungus cultured in the presence of propolis extract were examined. Results show that, propolis extract was found to effectively suppress morphological characteristics, causing several changes and damages of mycelia. Direct examined of this fungus with propolis extract on PDA surface resulted that there are differences between colonies features. Colonies grown on PDA plus propolis extract were not only small in size than control colonies but their appearance differed too, such as white mycelia occurred (colorless) in comparisons with the morphological features of PDA without propolis extract. Aerial mycelial were appeared and conidiophores’ appeared with less or free spores. While PDA free propolis extract were in normal conidiophores and sporulation. Cushnie and Lamb, (2005) stated that, upon the presence of propolis with the ethanol, the fungi were unable to inhibit the formation of the pores; or (ii) the propolis itself had a similar effect as ethanol on the fungi, but on a greater scale. The latter is the more likely as flavonoids are chemically defined as a 2-phenyl benzol[a] pyrane ring, which are two benzene rings linked together through a heterocyclic pyrane ring. Giovanelli, (2008) found that, micrographs of Pestalotiopsis sigirunii, Colletotrichum sp. and Colletotrichum gloeosporioides/ Pseudocercospora sp. (CgPcomplex) incubated on agar media containing EEP clearly indicated signs of cell wall damage with large pores within the hyphae. Surface conidial chains collapsed followed by death of the fungi. Severe damage of the Colletotrichum sp. hyphae was observed in the form of large pores when incubated on 5 mg ml\(^{-1}\) EEP. Most of the hyphae which were not in contact with the agar, i.e. growing over other hyphae, were undamaged and resembled that of the control. A few hyphae were extensively damaged as though the cell wall structure had been degraded. Diba et al., 2018 evaluate effects of the alcoholic extract of Propolis on 18 isolated Aspergillus including: Aspergillus fumigatus, Aspergillus flavus and Aspergillus niger, in addition to quantifying the effect of the extract on colony growth, morphological changes in colony color, colony diameter and sporulation rates were observed, which depended on the dilution of the extract. Morphological evolutions of Aspergillus flavus (color colonies and spore formation) diluted from 1/20 to 1/320 of Propolis extract. As can be observed, the size of the colonies and the colorimetric and spore formation were decreased with increasing concentration. The experimental findings showed significant macroscopic changes in the colony production of Aspergillus, including color and diameter, sporulation rate depending on dilution titers of the Propolis alcoholic extract. Observable differences in the inhibition capacity of Propolis alcoholic extract in the same laboratory conditions may indicate the differences between physical and cellular
structures.

**Conclusion**

Molds are capable of reducing the nutritional value of feedstuff as well as elaborating several mycotoxins. There are no known alternate fungicides that are environmentally friendly and with no adverse health risks, on the market to date. Physical treatment with sunlight exposure decontamination appears to be an efficient and cheaper method to use. Also, this study is one of the first where an antifungal property of propolis against *A. flavus* fungus which contaminant chicken and fish feeds. Besides biological control, there has been very little recent research in the development of alternate fungicides for controlling *A. flavus* fungus which contaminant chicken and fish feeds. These results suggest that propolis extract is an excellent antifungal activities.

This approach is considered as environmental friendly approach in contrast to physical and biological techniques. The cost of the decontamination process is very important in choosing the cheapest and the most effective method in *A. flavus* removal from the contaminated feed products. Propolis can be termed as a “natural antibiotic” as it shows inhibitory effect on *A. flavus* fungus. The results of this investigation further indicate the potential use of propolis as an alternative to chemical feed preservative agents.

**References**

1. Hashem A, Abd-ALLAH EF, Hend A et al. Effect of Propolis on growth, aflatoxins production and lipid metabolism in Aspergillusparasiticus Spear. *Pak J Bot* 2012; 44(3): 1153-1158.
2. Abyaneh MR, Alameh A, Al-Tiralhi T et al. Studies on the mode of action of neem (*Azadirachta indica*) leaf and seed extracts on morphology and aflatoxin production ability of *Aspergillus Parasiticus*. Bioprospecting & Ethnopharmacology 2005; 1: 123-127.
3. Almeida I, Martins HM, Santos S et al. Mycobiota in feed for farmed sea bass (*Dicentrarchuslabrax*). *Biotechnology in Animal Husbandry* 2011; 27(1): 93-100.
4. Saddiq AA, Danial EE. Effect of Propolis as a food additive on the growth rate of the beneficial bacteria. Main Group Chemistry 2014; 13; 223-232
5. A.O.A.C. Of Official Analytical Chemists. Official Methods of Analysis of AOAC.International 17th ed., Nature Toxins. AOAC International, Arlington, Virginia, USA, chapter 49. 2007.
6. Bedi PS, Agarwal RK. Detoxification of aflatoxin B1 by phisical and chemical methods. *World J. of Pharmacy and Pharmaceutical Scinces* 2014; 3(12): 995-1002.
7. Choudhary AK, Kumari P. Management of mycotoxin contamination in pre-harvest and post-harvest: Present status and future prospects. *Journal of Phytoology* 2010; 2(7): 37-52.
8. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. *Int J Antimicrob Agents* 2005; 26: 343-356.
9. Dalcero A, Magnoli C, Luna M et al. Mycoflora and naturally occurring mycotoxins in poultry feeds in Argentina. *Mycoopathologia* 1998; 141: 37-43.
10. Diba K, Mahmoodi M, Hashemi J. In vitro activity of Propolis alcoholic extract on opportunistic pathogenic fungi. *International Journal of Research in Applied and Basic Medical Sciences* 2018; 4(2): 68-73.
11. Embaby EM, Ayaat NM, El-Gailil MMA et al. Mycoflora and mycotoxin contaminated chicken and fish feeds. *Middle East Journal of Applied Sciences* 2015; 5(4): 1044-1054.
12. Embaby EM, Hazaa MM, El-Dougdoug KH et al. Control Apple Fruit Decay by Using ‘Ethanol Extract of Propolis’ (EEP). *International Journal of Advances in Medical Sciences* 2019; 4(3): 01-11.
13. Ezazi R, Davari M. Antifungal activity of Ethanolic Extract of Propolis (EEP) against some postharvest fungi. *Biological Control of Pests & Plant Diseases* 2018; 103-107. DOI: 10.22059/jbioc.2018.226807.184.
14. Farré R, Frasquet I, Sánchez A. Propolis and human health. *Ars Pharmaceutica* 2004; 45: 1: 21-43.
15. Giovanelli LC. Evaluation of an Ethanolic Extract of Propolis as a Potential Pre- and Post-Harvest Fungicide for ‘Fuerte’ Avocado (*Persea americana* Mill.) Fruits and Orchards. M.Sc. degree, Faculty of Science, University of the Witwatersrand, Johannesburg, 2008; 113.
16. Gomez KA, Gomez AA. Statistical procedures for agricultural research, 2nd Ed. John Wiley and Sons Ltd., New York, 1984; 680.
17. Gowda N, Suganthi R, Malathi V et al. Efficacy of heat treatment and sun drying of aflatoxin-contaminated feed for reducing the harmful biological effects in sheep. *Anim Feed Sci Tech* 2007; 133(1): 167-175.
18. Greco, (Mariana) V, Franchi (Maria) L, Golba (Silvia) L et al. Research Article, Mycotoxins and Mycotoxigenic Fungi in Poultry Feed for Food-Producing Animals. *The Scientific World Journal* 2014. Article ID 968215, 9 pages, http://dx.doi.org/10.1155/2014/968215.
19. Grigoryan K, Sargsyan M, Hakobyan L et al. Inhibition activity of Ethanol Extract of Propolis (EEP) relation mycotoxigenic fungi. In Proceedings of IHC meeting, International Symposium on Authenticity and Quality of Bee Products and 2nd World Symposium on Honeyed Honey, Chania, Greece, 7-10 April, 22.
20. Hegazi AG, El-Hady FKA. Influence of Egyptian propolis as antifungal agent. *Egypt J Vet Sci* 2002; 36: 45-49.
21. Herzallah S, Alshawabkeh K, AL Fatah A. Aflatoxin Decontamination of Artificially Contaminated Feeds by Sunlight, γ-Radiation, and Microwave Heating. *J Appl Poult Res* 2008; 17: 515-521.
22. Krnjaja V, Stojanović Lj, Cmiljanić R et al. The Presence of Potentially Toxigenic Fungi in Poultry Feed. Biotechnology in Animal Husbandry 2008; 24(5-6): 87-93.

23. Lorini A, Wobeto C, Bonaldo SM et al. Chemical composition and antifungal activity of propolis on Aspergillus flavus. Biosci. J., Uberlândia, 2018; 34(5): 1298-1307.

24. Mani K, Narahari D, Kumararaj R et al. Effect of sun light exposed aflatoxin B, contaminated feed on the performance of broiler chicks. Indian Vet J 1997; 74(9): 768-771.

25. Manjula B, Ramachandra CT. Effect of drying methods on physical and chemical characteristics of dried Byadagichilli. J Inno Agri 2014; 1(1): 22-30.

26. Mariana VG, María LF, Silvia LRG et al. Mycotoxins and Mycotoxicogenic Fungi in Poultry Feed for Food-Producing Animals. The Scientific World Journal 2014. Article ID 968215, 9 pages; http://dx.doi.org/10.1155/2014/968215.

27. Masood K, Rostami S, Saberi RR et al. Effect of Propolis and Clotrimazole on Controlling Aflatoxin in Pistachio (Pistacia vera L.). Int J Agri Biol 2006; 8(5): 606-608.

28. Meena SS, Mariappan V. Effect of plant products on seed borne mycoflora of sorghum. Madras J Agric 1993; 80(7): 383-387.

29. Mehrim AI, Salem MF. Medicinal herbs against aflatoxiosis in Nile tilapia (Oreochromis niloticus): Clinical signs, postmortem lesions and liver histopathological changes. Egy J aquac 2013; 3(1): 13-25, ISSN: 2090-7877.

30. Ngoepe EC, Straker C. Propolis as a natural antimicrobial agent for control of fungal pathogens of plants. Ph. D. Honours dissertation submitted to the University of the Witwatersrand, South Africa. 2004.

31. Nwabueze AA, Nwabueze EO. Microbial Flora of Fish Feeds Sold in Asaba, Southern Nigeria. American Journal of Experimental Agriculture 2011; 1(2): 27-32.

32. Oadi NM, Abdul-Karim EM, Naemah RB et al. Activity of Propolis and Boswellia sp. resins extract agent of white rot disease of Sclerotiniasesclerotiorum causal agent of white rot disease of Phaseolus vulgaris and Daucuscarota. Under storage conditions. Journal of Experimental Biology and Agricultural Sciences 2014; 2(1): 65-71.

33. Okoli IC, Nweke CU, Okoli CG et al. Assessment of the mycoflora of commercial poultry feeds sold in the humid tropical environment of Imo State, Nigeria. International Journal of Environmental Science and Technology 2006; 3(1): 9-14.

34. Özdemir AE, Çandir EE, Kaplankiran M et al. The effects of ethanol-dissolved propolis on the storage of grapefruit cv. Star Ruby. Turkish Journal of Agriculture and Forestry 2010; 34: 155-162.

35. Peraica M, Ana-M D, Jurjevic Z et al. Prevention of exposure to mycotoxins from food and feed. Arh Hig Rada Toksikol 2002; 53: 229-237.

36. Probst C, Bandhopadhyay R, Price Lee LE et al. Identification of a toxigenic Aspergillus flavus isolates to reduce aflatoxin contamination of maize in Kenya. Plant Disease 2011; 95(2): 212-218.

37. Quiroga EN, Sampietro DA, Soberon JR et al. Propolis from the northwest of Argentina as a source of antifungal principles. J Appl Microbiol 2006; 101: 103-110.

38. Šabanović M, Saltović S, Mujkić A et al. Impact of Propolis on the Oral Health. Balk J Dent Med 2019; 23: 1-9.

39. Sánchez C, Duarte P, Vasilenko P et al. Potential application of Portuguese propolis to control blue mould disease in ‘Rocha’ pear. Acta Hortic 2016; 1144(53): 359-364

40. Santha T, Sreenivasamurthy V. Storage of groundnut oil detoxified by exposure to sunlight. Indian J Technol 18: 346.

41. Soylu EM, Ozdemir AE, Ertürk E et al. Antifungal activity of propolis against Penicillium Digitatum, causal agent of green mold of citrus fruits. Proceedings of the First European Conference of Apidology ‘EurBee’, Udine, Italy, 2004; 160.

42. Soylu EM, Özdemir AE, Ertürk E et al. Chemical composition and antifungal activity of propolis against Penicilliumdigitatum. Asian Journal of Chemistry 2008; 20: 4823-4830.

43. Spring P, Fegan DF. Mycotoxins- A rising threat to aquaculture, Alltech Inc.: Brentwood, TN, USA. 2010.

44. Upadhaya SD, Park MA, Jong KH. A Review: Mycotoxins and Their Bio transformation in the Rumen. Asian-Aust J Anim Sci 2010; 23(9): 1250-1260.

45. Vallabh S, Straker C. The efficacy of propolis as a natural control agent of fungal plant pathogens and insect pests. Honours dissertation.University of the Witwatersrand. South Africa. 2005.

46. Vasanthi S, Bhat RV. Mycotoxins in foods-occurrence, health & economic significance and food control measures. Ind J Med Res 1998; 108: 212-224.