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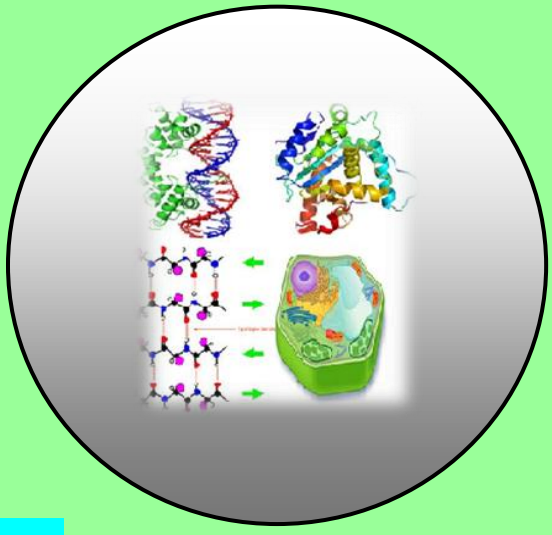

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# Save Strategies for Controlling of Fungal Growth and their Mycotoxins in Poultry Feeds

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## ABSTRACT

***Relative density (RD) and isolation frequency (Fr) of different fungal isolates from different samples of poultry feeds have been investigated. Based upon feed samples, Aspergillus isolates, the most isolation frequency (Fr) isolated fungi were A. niger and A. flavus 80% aggregates followed by A. ochraceous and A. tamarii 60 and 40% respectively. Highest RD of fungal isolates was 12.5 % for Aspergillus and Fusarium followed for Penicillium and Cladosporium 9.3%. On the other hand, lowest RD (20 %) and Fr (3.12%) were recorded for Acremonium isolate. Azadirachta indica extract was highest effectively inhibited the growth of Aspergillus flavus (37.47%), Aspergillus niger (35.90%), Aspergillus ochraceous (23.10%) and Fusarium proliferatum (37.30%), while it was lowest effectively inhibited the growth of other fungi. On the other hand, Juniperus procera was effectively inhibited the growth of Aspergillus flavus (41.62%), Aspergillus niger (44.38%), Aspergillus ochraceous (29.09 %), Fusarium proliferatum (39.07 %) and Penicillium chrysogenum (20.47 %). Avicennia marina extract was effectively on three fungal species only Aspergillus flavus (37.93%), Aspergillus niger (38.59 %) and Fusarium (32.30%). From the obtained results, inhibition % of aflatoxin B2 production was 100 % with using 250 and 500 mg of J. procera extract, while inhibition % of aflatoxin B1 was 60.47 and 100% at 250 and 500mg respectively. On the other hand, inhibition % of Cyclopiazonic acid was 14.62 and 33.93% at 250 and 500mg of J. procera respectively. Fusaric acid and dihydrofusarbin productivity decresed with increaising J. Procera extract concentration.***
***Keywords: Save Strategies, Control, Fungal Growth, Mycotoxins, Poultry Feeds.***

## INTRODUCTION

Mold occurrence and growth on poultry feeds is one of the major threats to poultry economic and health. Besides their negative impacts on nutritional and organoleptic

properties, moulds can also synthesize different mycotoxins. More than 100.000 fungal species are considered as natural contaminants of agricultural and food products. However, due to genetical and ecological factors, relatively few can actually generate mycotoxins (Jemmali, 1979). According to Leibetseder (1989), 30 to 40 % of existing moulds can elaborate toxic substances under favorable conditions. The majority of the toxic species belong to the genera *Aspergillus, Penicillium, Fusarium* and *Alternaria* (Kaushal and Sinha, 1993). Quality livestock feed is necessary for the maintenance of physiological functions and animal defense systems against diseases and parasites. Traditionally, feed quality has been specified on basis of the nutritional value of every individual feed component (Fink-Gremmels, 2004). Livestock feed quality may however be affected by various microorganisms such as bacteria and fungi growing in different parts of the world. Mould contamination is wide spread in tropical countries where poultry production and processing are expanding rapidly (Mabbett, 2004). Poultry are highly susceptible to mycotoxicoses caused by aflatoxins, trichothecenes, ochratoxins and some fusariotoxins (Opara and Okoli, 2005). Poultries feeding is one of the most important branches of poultry farming. Nutritionally balanced diets are provided during phases of productive life in eggers, chicks, grower and layer stages, in broilers starter and finishing stages (Gopalakrishana and Lal, 1985). Poultry feed are prepared basically with plants materials such as maize, soybean and sorghum and these materials are known to have fungi as the commonest contaminations (Oyeka and Onochie, 1992). A mycotoxin contaminated diet may lead to substantial economic losses in livestock due to feed refusal, poor feed conversion, diminished body weight gain, immune-suppression, interference with reproductive capabilities and residues in animal products (Varga and Toth, 2005). Stoev et al. (2004) reported a significant decrease in body mass and relative weight of lymphoid organs of broiler chicken fed a mouldy diet containing Ochratoxin and Penicillic acid. They equally observe pathomorphological changes such as cloudy swelling and granular degeneration in the epi-thelium and mononuclear cell infiltration and activation of capillary endothelium in the kidney and liver; degenerative changes and depletion of lymphoid organs (bursa of fabricius, thymus and spleen). *Fusarium* species (*F. avenaceum, F. nygamai, F. proliferatum, F. semitectum, F. subglutinans, F. verticillioides*) obtained from sorghum seeds are known to produce mycotoxins deoxyninalenol (DON) zearalenone, fusaric acid and trichothecene (Benneth and Klich, 2003; Desjardins, 2006)*, Aspergillus flavus* produces aflotoxin, B1, B2, G1 and G2, *Alternaria alternata* produces alternariols.

Ogbebor et al. (2007) and Nduagu et al. (2007) confirmed the fungicidal potential of extracts of *Azadirachta indica* (neem insert common name), *Khaya senegalensis* (mahogany), *Allium cepa* (garlic) and *Zinziber officinale* (ginger) on *Alternaria solani, Colletotrichum spp., Fusarium oxysporum, Rhizoctonia solani, Penicillium corylophilim, Ceratocystis paradoxa, Drechstera heveae, Xanthomonas oryzae, and Erwinia carotovora* . Plant essential oils and their components have been known to exhibit biological activities such as antifungal (Sokovic et al., 2009). Pankaj et al. (2010) investigated the different fractions of *Juniperus* leaves and bark, it inhibit the growth of aflatoxigenic *Aspergillus flavus* and *A. niger*. Extracts from the aerial parts of *J. lucayana* were assayed against phytopathogenic fungus *Botrytis cinerea*. The hexane extract showed to have a higher antifungal activity than ethanolic extract (Ortiz-Nunez et al., 2010).

Pirzada et al. (2005) studied the effect of ethanol, methanol, ethylacetate, chloroform and aqueous extracts of *Juniperus* against the human pathogenic fungi (*Aspergillus niger* and *A. flavus*). Glisic et al. (2007) stated that the oil of *J. communis* with a high content of α-pinene, and mixture of α-pinene and sabinene showed the highest antimicrobial activity, especially against fungi. Neem leaves are efficient against pathogenic fungi, such as *Trichophyton, Epidermophyton, Microsporum, Trichosporon* and *Geotricum* (Khan & Wassilew, 1987). The activity in inhibiting the protease of *Trichophyton* (Iyer & Williamson, 1991), the production of aflatoxin of *A. parasiticus* (Allamed et al., 2001), antifeedant activity (Silva et al., 2007) and the antifungal activity against *Penicillium expansum* (Mossini et al., 2004) have been confirmed. Effect of ethanolic extracts of *Lawsonia inermis*, *Azadirachta indica*, *Vinca rosea*, *Tagetes patula*, *Ocimum sanctum*, *Colocasia antiquorum*, *Adhatoda vasica*, *Moringa oleifera*, *Datura metel* and *Curcuma longa* leaf were examined on conidial germination, mycelial growth and sporulation of certain fungi isolated from poultry feed *Aspergillus flavus*, *A. niger* and *A. fumigates* (Islam et al., 2003). This study assesses the common fungal flora of selected poultry feed raw materials in the humid tropical environment and controlling of fungal growth and their mycotoxins with save plant extracts.

## MATERIAL AND METHODS

**Poultry feed sample collection:** A total of 5 poultry feed samples were collected from the feed storage rooms of different poultry markets in Jazan region. Feed material of each sample was sampled by carefully opening three randomly selected bags and collecting about 20 grams using a sterile universal bottle. The samples were transported to the laboratory for fungal analysis within 3 hours of collection.

**Isolation and identification of fungi:** Feed samples were initially inoculated onto potato dextrose agar and then sub-cultured on Czapek Dox solution agar, Czapek yeast autolysate agar (Pitt & Hocking, 1997) and yeast extract sucrose agar. The plates were incubated at 27 °C in dark for 10 days. Slide cultures were prepared for microscopic examination. Fungi were identified (Raper and Fennell, 1973; Klich & Pitt, 1988; Singh et al., 1991; Pitt & Hocking, 1997; Klich, 2002). The fungal isolates were also cultured on PDA slants, incubated at 27°C for 10 days and stored at 4°C in refrigerator for future studies. The isolation frequency (Fr) and relative density (RD) of species were calculated according to Gonzalez et al. (1995) as follows:

Fr (%) = Number of samples with a species or genus/ Total number of samples X 100.

RD (%) = Number of isolates of a species or genus/ Total number of fungi isolated X 100.

**Preparation of plant methanolic extract:** Sample (50 g) of the shade-dried powder of each plant materials (*Azadirachta indica, Avicennia marina, Juniperus procera*) was extracted separately with methanol using Soxhlet extractor until colourless extract was obtained on the top of the extractor. All the extracts were concentrated separately using rotary flash evaporator and preserved at 5°C in an air tight brown bottle until further use. All the plant extracts were subjected to antifungal activity assay.

The colony diameter was measured after 72 hrs and inhibition percentage of the fungal growth in relation to control treatment was calculated according to the given formula:

**I = C – T/ C x 100**

Where I = percentage inhibition, C = radial growth in control, T = radial growth in treatment.

**Mycotoxins analysis by High-Performance Thin-Layer Chromatography (HPTLC):**
After 10 days of incubation period, the growth medium (potato dextrose broth medium containing 200 mg of *J. procera*) of *A. flavus* and *F.oxysporum* containing extracellular metabolites was extracted twice with Chloroform/methanol (2:1v/v) and defated using n-hexane in a separating funnel, then concentrated and separated using TLC techniques. Twenty microliters of the samples, with or without treatment, were applied to HPTLC plates (10 cm x 10 cm, 0.2 mm silica gel Merck 60 F 254 precoated plate; Merck Darmstadt, Germany) using CAMMAG LINOMAT 5 application system. The TLC plates were eluted for the detection of Extracellular Metabolites in toluene/ethyl-acetate/90% formic acid 5:4:1 (TEF). Once the runs had finished, the plates were observed under visible and ultraviolet at 254 & 365 nm illumination. Griseofulvin dissolved in chloroform/methanol 2:1 was used as standard in all cases and relative Rf values to griseofulvin were calculated as $R_{fg}$. To identify the metabolites the absolute $R_f$ and the relative $R_f$ to griseofulvin ($R_{fg}$) were measured. Secondary metabolites were identified by descriptions in literature and comparison with the available standard was made (Frisvad et al., 1989; Paterson and Bridge, 1994; Thrane, 2001; Frisvad et al., 2008). Retention factor value (Rf value): The distance that the spot of a particular compound moved up on the TLC plate relative to the distance moved by the solvent front is called the retention factor or Rf value. The Rf values of individual secondary metabolites were calculated by following, Rf = Distance traveled by the compound/Distance traveled by the solvent. Quantification of secondary metabolites were done by comparing the Rf values and area % of secondary metabolite calculated using CAMMAG TLC scanner at Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University Cairo, Egypt. The percentage of Metabolite production inhibition was calculated using the following formula: (1 - Area of test sample/Area control) × 100%.

## RESULT AND DISCUSSION

Quality of poultry feed plays the most important role in a poultry farm (Dharumadurai et al., 2009). In the current study fungi were isolated from 5 different poultry feed samples. Among these samples, sample 1 revealed the maximum fungal population (12 fungal isolates), followed by sample 4, 3 and 2 respectively. On the other hand, the absence of fungal population was observed in sample 5 except one isolate was observed (Table 1). This result reveals that feed samples 1 and 4 are from unhygienic storage condition. Relative density (RD) of different *Aspergillus* isolates has been presented in Table 1. Based upon feed samples, *Aspergillus* isolates, the most isolation frequency (Fr) isolated fungi were *A. niger* and *A. flavus* 80% aggregates followed by *A. ochraceous* and *A. tamarii* 60 and 40% respectively. Highest RD of fungal isolates was 12.5 % for Aspergillus and Fusarium followed for Penicillium and Cladosporium 9.3%. On the other hand, lowest RD (20 %) and Fr (3.12%) were recorded for Acremonium isolate. High isolation frequencies of *Aspergillus* and *Penicillium* fungi from mixed poultry feeds have been reported in Spain (Bragualt et al., 1995), Brazil (Simas et al., 2007) and Argentina (Dalcero et al., 2002). Highest isolation frequency of *Aspergillus* in mixed poultry feed and component raw materials have also been reported in Sapin (Accensi et al., 2004). Muhammad et al. (2010) reported that the *Aspergillus* species were the most predominant followed by *Penicillium, Fusarium* and *Alternaria* of poultry feeds.

In another study, totally 40 mycotoxigenic fungi were observed in all 3 different poultry feed samples. Among the 40 isolates, 2 dominant fungal isolates were selected, characterized and identified as *Aspergillus flavus* and *Aspergillus niger* (Richard et al., 1978; Dharumadurai et al., 2009). Agricultural products including cereals and oilseeds meals constitute a major component of poultry feed ingredients. Mould contamination is wide spread in tropical countries where poultry production and processing are expanding rapidly (Van den Bergh et al., 1990; Okoli et al., 2006). Among the Aspergilli isolated from feed samples, *A. flavus, A. niger* and *Fusarium proliferatum* were the predominant species followed by, *A. ochraceous* (Table 1). These results agreement with some reports describing *A. flavus* as the predominant species followed by *A. niger* aggregates (Somashekar et al., 2004; Rosa et al., 2006).

A total number of 30 species of fungi viz., *A. candidus*, *A. flavus*, *A. fumigatus*, *A. parasiticus*, *A. niger, A. restrictus*, *A. sulphurus*, *A. sydowi*, *Alternaria alternata*, *A. brassicae*, *A. humicola*, *A. solani*, *Rhizopus oryzae*, *R. stolonifer*, *Acremonium* spp., *Geotrichum candidum*, *Mucor heimalis*, *Cochliobolus lunatus*, *Fusarium* spp., *F. culmorum*, *Rhizoctonia*, *Curvularia lunata*, *Cladosporium herbarum*, *Penicillium frequentus*, *Botrytis* spp., *Nigrospora* spp., *Humicola*, *Helminthosporium* spp. were isolated from wheat grains (Maliha, et al., 2010). According to Kpodo and Bankole (2008), the three most important genera of toxigenic fungi in the tropics are Aspergillus, Fusarium and Penicillium.

**Table 1. Isolation frequency and relative density of fungal genera isolated from Poultry feed samples.**

| Isolate No. | Fungal isolate | Poultry feed samples | | | | | Isolation frequency (%) | Relative density (%) |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | | |
| 1 | *Aspergillus flavus* | + | - | + | + | + | 80 | 12.50 |
| 2 | *A. niger* | + | + | + | + | - | 80 | 12.50 |
| 3 | *A. ochraceous* | + | + | - | + | - | 60 | 9.30 |
| 4 | *A. tamarii* | + | - | - | + | - | 40 | 6.25 |
| 5 | *Acremonium sp* | + | - | - | - | - | 20 | 3.12 |
| 6 | *Fusarium proliferatum* | + | + | + | + | - | 80 | 12.50 |
| 7 | *F. moniliforme* | + | + | - | + | - | 60 | 9.30 |
| 8 | *F. solani* | + | + | - | + | - | 60 | 9.30 |
| 9 | *Alternaria alternata* | + | - | - | + | - | 40 | 6.25 |
| 10 | *Penicillium chrysogenum* | + | + | - | + | - | 60 | 9.30 |
| 11 | *Cladosporium herbarum* | + | + | - | + | - | 60 | 9.30 |
| 12 | *Rhizopus stolonifer* | + | - | - | - | - | 20 | 3.12 |

**+, detected ; -, undetected**

Regular monitoring of toxigenic mycoflora of the agricultural based feeds and foods is an essential pre-requisite for development of strategies to control or prevent mycotoxins exposure of feed animal and human population. In the present study, *A. indica* extract was highest effectively inhibited the growth of *Aspergillus flavus* (37.47%), *Aspergillus niger* (35.90%), *Aspergillus ochraceous* (23.10%) and *Fusarium proliferatum* (37.30%) (Table 2), while it was lowest effectively inhibited the growth of other fungi.

**Table 2. Antifungal activity of methanolic extract of different plants extracts on growth of fungal isolates.**

| Fungal isolates | Colony radius (cm) Control | *A. indica* | | *J. procera* | | *A. marina* | |
|---|---|---|---|---|---|---|---|
| | | Colony radius (cm) | Inhibition % | Colony radius (cm) | Inhibition % | Colony radius (cm) | Inhibition % |
| *A. flavus* | 5.43±0.40 | 3.40±0.36 | 37.47 | 3.17±0.29 | 41.62 | 3.37±0.15 | 37.93 |
| *A. niger* | 5.70±0.12 | 3.70±0.35 | 35.90 | 3.17±0.29 | 44.38 | 3.50±0.50 | 38.59 |
| *A.ochraceous* | 4.33±0.29 | 3.33±0.29 | 23.10 | 3.07±0.12 | 29.09 | 4.77±0.25 | 0.00 |
| *A. tamarii* | 4.97±0.25 | 4.30±0.26 | 13.48 | 4.70±0.17 | 5.43 | 4.60±0.17 | 7.44 |
| *Acremonium sp.* | 3.33±0.29 | 3.40±0.01 | 0.00 | 3.27±0.25 | 1.80 | 3.70±0.17 | 0.00 |
| *F. proliferatum* | 5.63±0.23 | 3.53±0.06 | 37.30 | 3.43±0.40 | 39.07 | 4.53±0.06 | 19.53 |
| *F. moniliforme* | 5.17±0.19 | 3.50±0.01 | 32.30 | 4.10±0.26 | 20.69 | 3.50±0.50 | 32.30 |
| *F. solani* | 5.33±0.29 | 4.17±0.29 | 21.76 | 4.67±0.29 | 12.38 | 4.67±0.29 | 12.38 |
| *A. alternata* | 3.90±0.14 | 3.65±0.21 | 6.41 | 3.40±0.17 | 12.82 | 3.73±0.25 | 4.35 |
| *P. chrysogenum* | 2.93±0.40 | 2.83±0.29 | 3.41 | 2.33±0.29 | 20.47 | 3.00±0.50 | 0.00 |
| *C. herbarum* | 3.33±0.29 | 3.07±0.12 | 7.80 | 3.40±0.17 | 0.00 | 3.47±0.06 | 0.00 |
| *R. stolonifer* | 4.67±0.29 | 4.40±0.17 | 5.78 | 4.17±0.29 | 10.76 | 4.33±0.29 | 7.28 |

On the other hand*, J. procera* was effectively inhibited the growth of *Aspergillus flavus* (41.62%), *Aspergillus niger* (44.38%), *Aspergillus ochraceous* (29.09 %), *Fusarium proliferatum* (39.07 %) and *Penicillium chrysogenum* (20.47 %). *A. marina* extract was effectively on three fungal species only *Aspergillus flavus* (37.93%), *Aspergillus niger* (38.59 %) and *Fusarium* (32.30%). From the current study, certain fungi were not sensitive to plant extracts (Table 2).

Our study was supported with previous studies, where Pankaj et al. (2010) investigated the different fractions of Juniperus leaves and bark extract, it inhibit the growth of aflatoxigenic *Aspergillus flavus* and *A. niger*. Also extracts from the aerial parts of *J. lucayana* were assayed against phytopathogenic fungus *Botrytis cinerea*. Mariana and Camelia (2012) reported that Juniper oil has inhibition action against *Aspergillus niger, Fusarium oxysporum, Monascus purpureus* and *Penicillium hirsutum.* Recently, Abdelghany (2014) reported that the radial growth *Aspergillus flavus* and *Fusarium oxysporum* was determined on medium amended with *Juniperus procera* methanolic extract where ,the precentage of reduction was 48.54% and 59.86% for *A. flavus* and *F. oxysporum*, respectively. *A. marina* and *A. officinalis* were used as test plants due to the presence of much evidence that prove their therapeutic value against microbial infections (Abeysinghe and Wanigatunge, 2006). Also preliminary studies have been demonstrated that the mangrove plant extracts have antibacterial activity against pathogenic bacterial strains; *Staphylococcus sp., Pseudomonas sp*. and *E. coli* (Abeysinghe et al., 2002).

**Table 3. Mycotoxins of *Aspergillus flavus* and *Fusarium proliferatum* cultivated on medium supplemented with *J. procera* extract.**

| *A. flavus* Mycotoxins | Mycotoxins of *A. flavus* cultivated on medium | | | | | | Inhibition % of mycotoxin synthesis at | |
|---|---|---|---|---|---|---|---|---|
| | Without plant extract | | With 250 mg plant extract | | With 500 mg plant extract | | | |
| | Area (AU) | Area% | Area (AU) | Area% | Area (AU) | Area% | 250 mg | 500 mg |
| Aflatoxin B1 | 6494.6 | 15.5 | 2567.5 | 13.9 | 0.0 | 0.0 | 60.47 | 100 |
| Aflatoxin B2 | 6453.6 | 25.6 | 0.0 | 0.0 | 0.0 | 0.0 | 100 | 100 |
| Sterigmatocystin | 2367.3 | 20.4 | 2132.0 | 26.5 | 2100.5 | 26.7 | 11.28 | 9.40 |
| Cyclopiazonic acid | 4672.9 | 10.7 | 3989.9 | 23.8 | 3087.8 | 25.8 | 14.62 | 33.93 |
| Aspergillic acid | 2382.5 | 27.8 | 2231.7 | 35.8 | 2230.9 | 47.5 | 6.33 | 6.37 |
| *F. proliferatum* Mycotoxins | Mycotoxins of *F. proliferatum* cultivated on medium | | | | | | Inhibition % of mycotoxin synthesis at | |
| | Without plant extract | | With 250 mg plant extract | | With 500 mg plant extract | | | |
| | Area (AU) | Area% | Area (AU) | Area% | Area (AU) | Area% | 250 mg | 500 mg |
| Fusaric acid | 7267.7 | 35.5 | 6456.7 | 39.5 | 4356.4 | 53.4 | 11.16 | 40.06 |
| Dihydrofusarbin | 3876.5 | 30.4 | 2987.6 | 30.5 | 1987.6 | 46.6 | 22.93 | 48.72 |
| T2-Toxin | 1786.3 | 34.1 | 1213.0 | 30.0 | 0.0 | 0.0 | 32.09 | 0.0 |

Our study conducted *in vivo* found that the plant extracts exhibit remarkable effects on mycotoxin production. Poultry feed is frequently contaminated by mycotoxins. Mycotoxin potential residues may greatly influence meat and eggs production. Presence of mycotoxins in animal feed may also be a threat for human health. (Oliveira et al., 2006). The intake of very low levels of mycotoxins causes overt mycotoxicosis but also leads to the impairment of immune and acquired resistance to infections causing health problems which lead to economic losses in the form of decreased productivity (Dalcero *et al*., 1998).

From the obtained results, inhibition % of aflatoxin B2 production was 100 % with using 250 and 500 mg of *J. procera* extract (Table 3), while inhibition % of aflatoxin B1 was 60.47 and 100% at 250 and 500mg respectively. On the other hand, inhibition % of Cyclopiazonic acid was 14.62 and 33.93% at 250 and 500mg of *J. procera* respectively. Fusaric acid and dihydrofusarbin productivity decresed with increaising *J. Procera* .Surprisingly T2-Toxin productivty was not inhibited at 500mg of *J. Procera* (Table 3). These results are partially supported by reports indicating that extracts of certain plants were able to inhibit production of aflatoxin, sterigmatocystin and cyclopiazonic acid (Sánchez et al., 2005; Kocić-Tanackov et al., 2012; Swami and Alane, 2013). Recently, Abdelghany (2014) reported that the addition of the *J. procera* extract, the productivity percentage of aflatoxins B2, aflatoxins B1, sterigmatocystin, cyclopiazonic acid and fusaric acid was reduced by100, 67.44, 96.28, 60.33 and 8.36%, respectively as a result of applied *J. procera* extract.

**Table 4. Mycotoxins of *Aspergillus flavus* and *Fusarium proliferatum* cultivated on medium supplemented *A. indica* extract.**

| *A. flavus* Mycotoxins | Mycotoxins of *A. flavus* cultivated on medium | | | | | | Inhibition % of mycotoxin synthesis at | |
|---|---|---|---|---|---|---|---|---|
| | Without plant extract | | With 250 mg plant extract | | With 500 mg plant extract | | | |
| | Area (AU) | Area% | Area (AU) | Area% | Area (AU) | Area% | 250 mg | 500 mg |
| Aflatoxin B1 | 6494.6 | 15.5 | 5765.8 | 21.5 | 3765.5 | 17.3 | 11.22 | 42.02 |
| Aflatoxin B2 | 6453.6 | 25.6 | 4987.0 | 16.8 | 3989.8 | 16.5 | 22.72 | 38.17 |
| Sterigmatocystin | 2367.3 | 20.4 | 2569.0 | 25.9 | 2576.5 | 26.3 | 0.0 | 0.0 |
| Cyclopiazonic acid | 4672.9 | 10.7 | 3987.1 | 18.5 | 2987.2 | 25.4 | 14.67 | 36.07 |
| Aspergillic acid | 2382.5 | 27.8 | 3298.0 | 17.3 | 2987.5 | 14.5 | 0.0 | 0.0 |
| *F. proliferatum* Mycotoxins | Mycotoxins of *F. proliferatum* cultivated on medium | | | | | | Inhibition % of mycotoxin synthesis at | |
| | Without plant extract | | With 250 mg plant extract | | With 500 mg plant extract | | | |
| | Area (AU) | Area% | Area (AU) | Area% | Area (AU) | Area% | 250 mg | 500 mg |
| Fusaric acid | 7267.7 | 35.5 | 5765.4 | 34.9 | 0.00 | 0.00 | 20.00 | 100 |
| Dihydrofusarbin | 3876.5 | 30.4 | 3098.6 | 32.5 | 2987.6 | 45.4 | 20.06 | 22.93 |
| T2-Toxin | 1786.3 | 34.1 | 2123.9 | 32.6 | 1987.8 | 54.6 | 0.00 | 0.00 |

With using *A. indica* extract all mycotoxin productivity decreased with increasing plant extract concentration (Table 4). Inhibition % of Aflatoxin B1, Aflatoxin B2 , Cyclopiazonic acid, Fusaric acid and Dihydrofusarbin was 42.02, 38.17, 36.07,100 and 22.93% respectively at 500mg compared with inhibition % at 250mg of *A. indica* extract. Similar results were found by Zeringue and Bhatnagar (1994), where the application of aqueous extract from *A. indica* leaves in cotton balls infected with *Aspergillus flavus*, was able to inhibit up to 98% the Aflatoxin B1 production. Recently Rao et al. (2015) reported that *A. indica* was effective inhibitor of okratoxin production. Inhibitory effect of *A. marina* on mycotoxins productivity by *A. flavus* and *F. proliferatum* was studied (Table 5).

It has been shown that extracts of *A. marina* exhibit remarkable effect on inhibition T2-Toxin productivity only while other mycotoxins synthesis not inhibited with using *A. marina* extract except negligale inhibtion of Aflatoxin B1 . Generally, plant metabolites and plant based pesticides appear to be one of the better alternatives as they known to have minimal environmental impact and danger to consumers in contrast to the synthetic pesticides (Varma and Dubey 1999; Kuri et al., 2010; Abdel Ghany and Hakamy, 2014, Abdelghany et al., 2015a & b), but according to Cavaleiro et al. (2006) and Rosca- Casian et al. (2007), the variation in the efficacies of the tested plant extracts against the toxigenic fungi may be due to the considerable variation in their constituents.

**Table 5. Mycotoxins of Aspergillus flavus and Fusarium proliferatum cultivated on medium supplemented *A. marina* extract.**

| *A. flavus* Mycotoxins | Mycotoxins of *A. flavus* cultivated on medium | | | | | | Inhibition % of mycotoxin synthesis at | |
|---|---|---|---|---|---|---|---|---|
| | Without plant extract | | With 250 mg plant extract | | With 500 mg plant extract | | | |
| | Area (AU) | Area% | Area (AU) | Area% | Area (AU) | Area% | 250 mg | 500 mg |
| Aflatoxin B1 | 6494.6 | 15.5 | 7123.5 | 18.5 | 6391.8 | 15.6 | 0.0 | 1.58 |
| Aflatoxin B2 | 6453.6 | 25.6 | 6459.6 | 27.6 | 6897.5 | 27.7 | 0.0 | 0.00 |
| Sterigmatocystin | 2367.3 | 20.4 | 3243.4 | 24.1 | 3234.8 | 21.1 | 0.0 | 0.00 |
| Cyclopiazonic acid | 4672.9 | 10.7 | 4699.5 | 13.8 | 4687.2 | 18.5 | 0.0 | 0.00 |
| Aspergillic acid | 2382.5 | 27.8 | 1987.5 | 16.0 | 1992.7 | 17.1 | 16.57 | 16.36 |
| *F. proliferatum* Mycotoxins | Mycotoxins of *F. proliferatum* cultivated on medium | | | | | | Inhibition % of mycotoxin synthesis at | |
| | Without plant extract | | With 250 mg plant extract | | With 500 mg plant extract | | | |
| | Area (AU) | Area% | Area (AU) | Area% | Area (AU) | Area% | 250 mg | 500 mg |
| Fusaric acid | 7267.7 | 35.5 | 7345.0 | 39.1 | 7299.4 | 35.7 | 0.00 | 0.00 |
| Dihydrofusarbin | 3876.5 | 30.4 | 3907.7 | 34.7 | 3587.6 | 38.5 | 0.00 | 7.45 |
| T2-Toxin | 1786.3 | 34.1 | 1608.9 | 26.2 | 1287.8 | 25.8 | 9.93 | 27.90 |

## CONCLUSION

The results of the present work indicate that plant extracts may be promising safe alternatives to harmful fungicides for controlling fungi and their mycotoxins contamination in poultry feeds.

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