Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

EFFICIENCY OF BORAGE (*Anchusa italica*) AND FRENCH JASMINE POWDERS (*Calotropis procera*) IN DETOXIFICATION OF OCHRATOXIN A AND DEOXYNIVALENOL IN POULTRY DIET

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Received – July 22, 2014; Revision – September 08, 2014, Accepted – October 07, 2014
Available Online – October 25, 2014.

KEYWORDS

Ochratoxin A

Detoxification

Poultry diet

Deoxynivalenol

Anchusa italica

Calotropis procera

ABSTRACT

The study was conducted to evaluate the efficacy of Borage (*Anchusa italica*) and French jasmine (*Calotropis procera*) powders in eliminating Ochratoxin A (Ochra A) and Deoxynivalenol (DON) contamination from poultry diet under storage conditions. These two powders were mixed separately at 5 % with a diet contaminated with 2 ppm Ochra A and 10 ppm DON. The diets were stored for 2 months and the mycotoxins concentration were evaluated by HPLC. Results of study revealed that both Borage and French jasmine powders exhibited significant reduction in Ochra A concentrations, i.e. 928.4 and 1832.4 ng/g respectively compared with chicks fed on Ochra A contaminated diet (2566.1 ng/g), and in case of DON concentrations, reduction was 1397 and 1616 ng/g respectively as compared to chicks fed on DON-contaminated diet (5062.6 ng/g) in the first month of treatment. The reduction in the concentrations of mycotoxins were continued in next month also and it reached up to zero ng/g in Ochra A for both powders, while in case of DON it reached zero and 112 ng/g respectively with these two powders. This reduction in toxic material caused gradual increase in chick's body weights. The improvement in body weights of the chicks fed on Borage and French jasmine treated diet was 225.0 and 213.5 g respectively as compared to the control group feeding on Ochra A contaminated diet (170.0g). Similar type of body weight increase was also reported in the case of DON-contaminated diet and treated with the two powders was observed, 227.5 and 215.5 g respectively compared with 174.0 g in control (contaminated with mycotoxin, non-treated with powders).

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher (www.my-vision.webs.com/horizon.html).

1 Introduction

Contamination of poultry diet with mycotoxins is one of the important reasons which can affront the poultry breeding programs in most of the developing countries (Yiannikouris & Jouany, 2002, Manafi et al., 2012). As the poultry diet is essential variable for determining the success of poultry breeding projects, several efforts were made toward the obtaining mycotoxins free diet (Devegowda & Murphy, 2005, Manafi et al., 2012). Corn seeds which are the main constituent of poultry diet, are also serve as a suitable medium for the growth of mycotoxin producing fungi (Abbas & Shier, 2009).

Several methods have been adopted to overcome the contamination of poultry diet from mycotoxins, among these, treating the diet with natural products extract including medicinal plants (leaves, seeds and roots) are more common (Gerhard & Rudolf, 2005; Scott & Turchsess, 2009). It has been reported that caffeine (0.5-1 %) with yeast extract sucrose (YES) medium exert an inhibitory effect on several strains of *A. ochraceus* growth and prevent the production of Ochra A (Tsubouchi et al., 1985).

Similarly, addition of *Liquarice Grlcyrrhiza glabra* L. extract to the diet at 450 mg/kg has reduced the toxic effect of Ochra A in rats (Malekinejad et al, 2010). Furthermore, inhibitory effect of medicinal plant like *Anchusa italic* and *Calotropis porcera* extracts on growth and sporulation of *Aspergillus* and *Fusarium* species (these fungi are actively involved in mycotoxin production) were also reported by the various researchers (Kamath & Rana, 2002; Alam et al., 2004; Akhter et al., 2006, Murthy et al., 2009). The objective of the study was to evaluate the efficacy of *Anchus italica* and *Calotropis porcera* powders in detoxification and elimination of mycotoxins Ochra A produced by *A. ochraceus* and DON produced by *F. graminarium* from contaminated poultry diet under storage conditions.

2 Materials and methods

2.1 Fungal isolates

Aspergillus ochraceus isolate OTA18MA and *Fusarium graminearum* isolate F1-DON-Fg were isolated from contaminated corn seeds collected from different localities of Iraq. It is practically well established that both the collected fungal species are contributing in the production of Ochra A and DON mycotoxins in stored poultry diet.

2.2 Mycotoxin production

A. ochraceus isolate (OTA18MA) was cultured on rice seeds for producing Ochra A. Hundred ml distilled water were added to 150 g of rice seeds in 250 ml flasks. The flasks were autoclaved twice at 121 ˚c and 1.5 kg/cm^2 for 20 minutes for two successive days. In autoclaved seeds a discs of 9 mm of OTA18MA isolate maintained on PDA were inoculated. The flasks were agitated for homogenization and maintained at 25 ± 2˚c for 21 days (Abbas et al., 1984). The contaminated seeds were transferred into paper sacks and oven dried at 50˚C. Oven dried seeds ware ground and stored at low temperature till the finishing of experiments.

The same procedure was followed for *F. graminearum* isolate F1.DON-Fg, except that after 14 days of incubation at 25 ± 2˚C, the flasks containing contaminated rice seeds were transferred to 13-16˚C for another 14 days to induce DON toxin production.

2.3 Extraction of Mycotoxin from selected fungal species

2.3.1 Extraction of Ochratoxin A

100 ml of Acitonitril : water (90:10) mixture were added to 25 g of contaminated rice seeds powder in 300 ml flask. The flask was tightly covered and agitated on flask shaker for 30 minutes. The extract was passed through filter paper (Whatman No.2) and 25 ml of hexane were added to the filtrate in 250 ml separating funnel to eliminate fat. The flask was gently agitated for 30 seconds and taken for separation. The lower layer was mixed with 25 ml distilled water, 8 ml saturated sodium bicarbonate solution, and 25 ml chloroform. The mixture was left 3 minutes for separation and the upper layer was mixed with 15 ml of HCl 1N and 20 ml of chloroform in separating funnel. The lower layer which was passed through filter paper containing anhydrous sodium sulfate and the filtrate was evaporated in Rotary evaporator at 70˚C. The precipitate was dissolved in 1 ml of Acitonitril:Benzen (2:98) mixture and conserved in small vials under freezing (Balzer et al., 1978).

2.3.2 Deoxynivalenol (DON) extraction

Two hundred of Acitonitril: water (84:16) mixture were added to 50 g of contaminated rice seeds powder in volumetric flask of 500 ml, after this flask was agitated on shaker for 30 minutes and passed through filter (Whatman No.2). Fifty ml of Hexane were added to 125 ml of the filtrate in separating funnel with agitation for 20 seconds. Later on 50g of ammonium sulfate were added to the extract in 250 ml flask and passed through filter paper (Whatman No. 2). The filtrate was passed through 10g of anhydrous sodium sulfate, dried, and conserved in dark vials under freezing (Trucksess et al., 1984).

2.4 Mycotoxin purification

The mycotoxins were purified by column chromatography using silica gel 60g as described by Scott et al. (1981). The silica was activated at 110-130˚C in electrical oven for 1 hour. A piece of cotton glass with 0.5g of anhydrous sodium sulfate were introduced in the base of 75 cm x 15 mm column and a suspension of 2 g activated silica in chloroform was gently added into the column, then 100 ml of benzene-ethylcitrate

(80:20) mixture were added and the filtrate was collected. The filtrate was evaporated at 50˚C in water bath until dryness and conserved under freezing.

2.5 Evaluation of mycotoxin concentration

The mycotoxin concentration was determined by High performance liquid chromatography (HPLC) system, model LC 2010 A, Schimadzu co. Koyoto, Japan, in reverse phase column C18DB (50 x 4.6 mm) with 3 mm particle size with mobile phase 0.01 N potassium phosphate solution (KH_2PO_4) pH 6.0 at flue rate 1 ml/min. The absorption values were followed by spectrophotometer at 220 nm. The concentration was evaluated by comparison the absorption curve obtained with mycotoxin standard curve according to the following equation as described by Caprita et al. (2007).

Mycotoxin concentration =

[Area of sample curve * Standard solution conc. * Dilution factor]/ Area of mycotoxin standard curve

2.6 Storage experiment

Powders of rice seeds contaminated with OTA18MA isolate producing Ochra A and F1-DON0Fg producing DON toxin were mixed separately with 500 g of mycotoxin free poultry diet in a desiccators for obtaining 2 mg/kg and 5 mg/kg of Ochra A and DON in the diet respectively. The two diets were homogenized with 5 ml water to obtain 14.7 % relative humidity. Each diet was divided into two part, one of each was amended with 5 % Borage powder and the other with 5% French jasmine powder. The concentrations of mycotoxins in the diet were followed by HPLC system for two months. *Coturnix japonica* chicks at 4 weeks age were fed on the treated diet and the chick weights were taken for 4 weeks.

3 Results

Significant reduction in Ochra A was obtained when *A. italica* and *C. procera* powders were mixed at 5% with toxin contaminated poultry diet with superior effect of *A. italica* powder as proved by HPLC system. The concentrations of Ochra A in the diet were found to be 928.4 and 1832.4 ng/g for the two powders respectively compared to control (2566.1 ng/g) after one month of treatment, this reduction percentages of 63.8 and 28.6% respectively (Table 1). This reduction in Ochra A concentration was continued to attain zero ng/g for these two powders in the second month also.

Similar results were obtained when the powders were mixed at 5% with poultry diet contaminated with DON toxin produced by *F. graminearum*. High significant reduction in DON concentration was obtained, 1397 and 1626 ng/g for these two powders respectively compared to 5062.6 ng/g of control, and this reduction percentage was of 72.4 and 67% respectively and in this manner reduction Borage powder was slight higher than the *C. procera* after one month of treatment (Table 2). DON concentrations have reached to 0 and 112 ng/g for the two powders respectively compared with 5752 ng/g in control in the second month of treatments.

Feeding of *Coturnix japonica* chicks on diet contaminated with 2 ppm Ochra A, 10 ppm of DON mycotoxins separately has induced significant reduction in chick's weight, and the weight was reported 170 & 174 g respectively in treated chicks and it was comparatively lower (237.0 g) than the control (fed on contamination free diets). This reduction in the weight was associated with death percentages (28.6, 21.5% respectively) compared with 0.0% death in control (Table 2).

The addition of Borage and French jasmine powders into the contaminated diet separately induced significant increases in chick's weight. It has been found that the weights attained were 225.0 and 213.5 g for chicks fed on Ochra contaminated diet and treated with the two powders respectively compared with 170 g for chicks fed on Ochra A contaminated diet. The weights of chicks fed on DON contaminated diet and amended with 5% of Borage and French jasmine powders were found to be 227.5 and 215.0 g respectively compared with 174.0 g for chicks fed on DON contaminated diet.

4 Discussions

This study was aimed to search an effective harmless natural products alternative to synthetic chemicals for destroying and eliminating mycotoxins from contaminated poultry diet. The results obtained from the study demonstrated that feeding of *Coturnix japonica* chicks on diet contaminated with ochre A produced by *Aspergillus ochraceus* and Deoxynivalenol (DON) produced by *Fusarium graminearum* caused high reduction in body weight associated with high percentage of chicks death compared with control. On the other hand the amendment of the mycotoxin contaminated diet with 5% of the medicinal plant powders (Borage and French jasmine) induced highly significant reduction in mycotoxin concentration as determined by HPLC and significant reduction in death percentage of *Coturnix japonica* chicks fed on this diet associated with significant increase in chicks body weights.

The activity of Borage and French jasmine powders in destroying and eliminating mycotoxin from the contaminated poultry diet can be attributed to their contents of active compounds such as organic acids, salicylic acid, potassium nitrate, and volatile oils in Borage (Bandoniene et al., 2002; Sridhar et al., 2003), cardenolides, steroids and triterpenes in French jasmine (Akhter et al., 2006). These compounds may interact with the active groups of the mycotoxin, mainly hydroxyl (OH), causing breaks of the active rings in the mycotoxins leading finally to destroy or convert the toxin to non-toxic compounds (Huwig et al., 2001; Bandoniene et al., 2002, Velazhahan et al., 2010).

It has been also reported that extracts from peppermint *Mentha piperita* L., Thyme *Thymus capitalus* (L.) Link, Aniseed *Pimpinella anisum* L., and Cinnamon *Cinnamomum zelanicum* exhibit high activity against many fungi producing mycotoxins and reduced the mycotoxin concentrations that produced (Soliman & Badeaa, 2002). Increasing in chicks weight fed on the mycotoxins contaminated diet and treated with powders was observed in the first week of feeding compared with control, this may attributed to the ability of certain compounds in the powder to act as anion scavenger through combination with the mycotoxin rendering the complex non-absorbable by the cell membrane in chicks and get it out of the body. Many investigators have reported that some antioxidant phenolic compounds found in the medicinal plants exhibit antagonistic activity against pathogens and acts as anion scavengers of mycotoxins (Atroshi et al, 2002, Mhamdi et al, 2010).

In conclusion the activity of Borage and French jasmine powders in reduction and elimination of mycotoxins from contaminated poultry diet indicated to the possibility of using natural products with the poultry diet as a simple harmless mean for inhibiting the contamination with fungi producing toxins and cleaning the diet from the mycotoxins that may produced.

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Table 1 Activity of Borage and French jasmine powders at 5% in reduction of Ochra A and DON mycotoxins in poultry diet.

Treatments	1st month		2nd month	
	Mycotoxin concentration ng/g	% reduction	Mycotoxin concentration ng/g	% reduction
Diet contaminated with Ochra A	2566.1	0	2907	0
Diet contaminated with Ochra A + 5% Borage powder	928.4	63.8	0	100
Diet contaminated with Ochra A + 5% French jasmine powder	1832.4	28.6	0	100
Diet contaminated with DON	5062.6	0	5752	0
Diet contaminated with DON + 5% Borage powder	1397	72.4	0	100
Diet contaminated with DON + 5% French jasmine powder	1626	67.79	112	98.1
Diet only	0	100	0	100

LSD p = 0.05

Table 2 Effect of Borage and French jasmine powders at 5% in diet contaminated with 2 ppm Ochra A and 10 ppm DON mycotoxin on *Coturnix japonica* body weight and death percentage

Treatments	Body weight / g				
	1st week	2nd week	3rd week	4th week	% death
Diet contaminated with 2 ppm Ochra A	162.0	182.0	177.5	170.0	28.6
Diet contaminated with 2 ppm Ochra A + 5% Borage powder	181.5	210.0	215.0	225.0	0.0
Diet contaminated with 2 ppm Ochra A + 5% French jasmine powder	175.0	201.0	205.5	213.5	7.1
Diet contaminated with 10 ppm DON	164.5	185.0	180.0	174.0	21.5
Diet contaminated with 10 ppm DON + 5% Borage powder	182.0	210.0	216.0	227.5	0.0
Diet contaminated with 10 ppm DON + 5% French jasmine powder	169.0	191.5	193.5	215.5	7.1
Diet only (control)	188.5	216.0	222.5	237.0	0.0

LSD p = 0.05

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