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Efficiency of Borage and French Jasmine Powders in Detoxification of Ochratoxin A and Deoxynivalenol in Poultry Diet

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Abstract The study was conducted to evaluate the activity of Borage (*Anchura italic*) and French jasmine (*Calotropis procera*) powders in destroying and eliminating Ochratoxin A (Ochra A) and Deoxynivalenol (DON) in contaminated poultry diet under storage conditions. The two powders were mixed separately at 5 % with a diet contaminated with 2 ppm Ochra A and other contaminated with 10 ppm DON. The two diets were stored for 2 months and the mycotoxins concentration were followed by HPLC. Chiks weight of *Coturnix japonica*, feeded on the diets were also determined. Results showed that both Borage and French jasmine powders exhibited significant reduction in Ochra A concentrations, 928.4 and 1832.4 ng/g respectively compared with 2566.1 ng/g of chiks feeded on Ochra A- contaminated diet (control), and in DON concentrations, 1397 and 1616 ng/g respectively compared with 5062.6 ng/g of chiks feeded on DON-contaminated diet (control) in the first month of treatment. The reduction in mycotoxins concentrations were continued to attain zero ng/g in Ochra A, zero and 112 ng/g in DON with the two powders respectively in the second month of treatment. Gradual increase in chiks body weights feeded on mycotoxins contaminated diet treated with borage and French jasmine powders were observed. The weights of the chiks feeded on Ochra A-contaminated diet were attained to 225.0 and 213.5 g respectively compared with 170.0 g in control. Similar increase in chiks weight feed on 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.

Keywords: Ochratoxin A, detoxification, poultry

Introduction

The contamination of poultry diet with mycotoxins is one o the most important problem affronting poultry breeding development in underdeveloped countries (Yiannikouris and Jouany, 2002, Manafi *et al*., 2012). As the poultry diet is the essential variable determining success or failure the poultry breeding projects, efforts were oriented toward obtaining mycotoxins free diet (Devegowda and Murphy, 2005, Manafi *et al*., 2012). Corn seeds, main

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constituent of poultry diet and suitable medium for fungi producing mycotoxins growth, were considered the main source of diet contamination with mycotoxins (Abbas and Shier, 2009).

Several means were adopted to overcome the contamination of poultry diet with mycotoxins, among those treating the diet with natural products extracted from medicinal plants. Extracts and powders of medicinal plants (leaves, seeds, roots) were used to inhibit the growth of fungi producing mycotoxins, detoxification and elimination of mycotoxins from poultry and animal diet (Gerhard and Rudolf, 2005, Scott and Turchsess, 2009).

It has been found that the caffeine in yeast extract sucrose (YES) medium at 0.5-1 % exert an inhibition effect on several strains of *A. ochraceus* growth and prevented ochra A production. 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).

Several studies reported that alcoholic extracts of *Anchusa italic* and *Calotropis porcera* were highly effective in inhibition of mycelia growth and sporulation of several *Aspergillus* and *Fusarium* species (Murthy *et al.*, 2009, Kamath and Rana, 2002, Alam *et al.*, 2004, Akhter *et al.*, 2006).

The objective of the study was to evaluate the activity of *Anchus italica* and *Calotropis porcera* powders in detoxification and elimination of Ochra A produced by *A. ochraceus* and DON produced by *F. graminarium* mycotoxins from contaminated poultry diet under storage conditions.

Materials and methods

Fungal isolates

Aspergillus ochraceus isolate OTA18MA and *Fusarium graminearum* isolate F1. DON-Fg previously proved to produce Ochra A and DON mycotoxins respectively were used in this study.

Mycotoxin production

A. ochraceus isolate (OTA18MA) was grown on rice seeds for producing Ochra A. Hundred ml distilled water were added to 150 g of rice seeds in each of 20x5 cm petri plates. The plates were autoclaved twice at 121 ˚c and 1.5 kg/cm^2 for 20 min. in two successive days. The seeds were contaminated with two discs of 9 mm/plate of OTA18MA isolate growth on PDA. The plates were agitated for homogenization and maintained at 25 ± 2˚c for 21 days. The contaminated seeds were transferred into paper sacks, oven dried at 50 ˚c, ground and conserved for the storage experiments.

The same procedure was followed with *F. graminearum* isolate F1.DON-Fg except that after 14 days of incubation at 25 ± 2˚c, the petri dishes containing contaminated rice seeds were transferred to 13-16 ˚c for another 14 days to induce DON toxin production. The seeds were dried, ground, and conserved in paper sack for the storage experiments as before.

Mycotoxin extraction

Ochratoxin A

Hundred 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 submitted to agitation in flask shaker for 30 min. 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 for defating. The flask was gently agitated for 30 sec. and let for separation. The lower layer was mixed with 25 ml distilled water, 8 ml of saturated sodium bicarbonate solution, and 25 ml chloroforme. The mixture was left 3 min. for separation and the upper layer was mixed with 15 ml of Hcl 1N and 20 ml of chloroforme in separating funnel. The lower layer 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).

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, agitated in flask shaker for 30 min. and passed through filter paper (Whatman No.2). Fifty ml of Hexane were added to 125 ml of the filtrate in separating funnel with agitation for 20 sec. Fifteen g 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 filter paper (Whatman No. 2), 10 g of anhydrous sodium sulfate, dried, and conserved in dark vials under freezing (Trucksess *et al*., 1984).

Mycotoxin purification

The mycotoxins were purified by column chromatography using silica gel 60 g 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 0.5 g of anhydrous sodium sulfate were introduced in the base of 75 cm x 15 mm column and a

suspension of 2 g avtivated 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.

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) 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:

$$\text{Mycotoxin concentration} = \frac{\text{area of sample curve}}{\text{area of mycotoxin standard curve}} \times \text{standard solution conc.} \times \text{dilution factor}$$

(Caprita *et al*., 2007).

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 dessicator for obtaining 2 mg/kg and 5 mg/kg of Ochra A and DON in the diet respectively. The two diet were homogenized with 5 ml water to obtain 14.7 % relative humidity. Each diet was divided into two parts, 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* chiks at 4 weeks age were feeded on the treated diet and the chik weights were followed for 4 weeks.

Results

High significant reduction in Ochra A was obtained when Borage *Anchusa italica* and French jasmine *calotropis procera* powders were mixed at 5% with toxin contaminated poultry diet with superior effect of Borage 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 with 2566.1 ng/g in control in the first month of treatment, at reduction

percentages of 63.8 and 28.6% respectively (Table 1). The reduction of Ochra A was continued to attain zero ng/g for the two powders compared with 2907.1 ng/g in control in the second month.

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 concentrate was obtained, 1397 and 1626 ng/g for the two powders respectively compared with 5062.6 ng/g in control, at reduction percentage of 72.4 and 67% respectively with slight superiority of Borage powder in the first 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 treatment.

Feeding of *Coturnix japonica* chiks on diet contaminated with 2 ppm Ochra A, 10 ppm of DON mycotoxins separately has induced significant reduction in chiks weight, 170, 174 g respectively compared with 237.0 g in control associated with death percentages of 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 chiks weight. It has been found that the weights attained to 225.0 and 213.5 g for chiks feeded on Ochra contaminated diet and treated with the two powders respectively compared with 170 g for chiks feeded on Ochra A contaminated diet. The weights of chiks feeded 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 chiks feeded on DON contaminated diet.

Discussions

This study was aimed to search for effective harmless natural products alternative to synthetic chemicals for destroying and eliminating mycotoxins from contaminated poultry diet. The results obtained demonstrated that feeding of *Coturnix japonica* chiks 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 chiks 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* chiks feeded on this diet associated with significant increase in chiks 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.

It has been 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 and Badeaa, 2002).

Increasing in chiks weight feeded 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 chiks 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.

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	56.3	5.2	48.9	0.84

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	5.53	17.3	22.4	9.47	7.34

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