European Journal of Scientific Research
ISSN 1450-216X / 1450-202X Vol. 115 No 4 December, 2013, pp.607-613
http://www.europeanjournalofscientificresearch.com

Detection of Mycotoxin (Deoxynivalenol) in Wheat Seeds Infected with *Fusarium Graminearum*

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Abstract

This study was conducted in Mycotoxin laboratory/College of Agriculture /University of Baghdad to evaluate seeds contamination of local and imported wheat with Deoxynivalenol(DON),and to test the ability of sodium sulfate and Fylax for detoxification of DON toxin . Results of fungal isolation showed that several fungi such as *Alternaria. Aspergillus .Penicillium* and *Fusarium* were associated with wheat samples for both local and imported wheat.Thin layer chromatography showed that 54.16%of *Fusarium graminearum* isolates were able to produce DON toxin on rice media .Results also showed that Fylax and Sodium sulfate inhibited the growth of the fungus *F.graminearum* and reduce toxin level from 45.6mg/g to0.27and 1.14 respectivily.

Keywords: Deoxynivalenol ; wheat seeds ; *Fusarium graminearum*

Introduction

Wheat crop consist main source of food to millions of human around the world .This crop was affecting by many pathogens like *Fusarium graminearum* the causal agent of Fusarium head blight which reduce the quantity and quality of the yield[23].This fungus are known by its ability to produce group of compound known Trichothecens which effect many biological activity in human and animals[3].One of the Trichothcenes are Deoxynivalenol(DON) which cause divers effects to human and animals when they consume foods contaminated with it ,it cause many symptoms like food refuse, vomiting, diarrhea , skin inflammation in addition to inhibiting protein synthesis and immune system [5].It is also found that this mycotoxin make living organism more sensitive to Viral and Bacterial infection [24].It is also cause liver and stomach cancer [34]and effect biosynthesis of nucleic acid and enzymatic activity [11;21].Previous studies indicate that wheat seeds were contaminated with DON in many countries like India, Canada, Germany in level over tolerance [26;7;25].The tolerance of DON on wheat seeds and flour were determined as 0.0-2 and 0.75 mg/kg respectively [14].So because wheat

plant are infecting always by *F.graminearum* the main producer of DON and because the lack of information about contamination of wheat seeds with this toxin this study were done in aim to;- Determine the level of the DON toxin in local and imported wheat and evaluate the efficacy of Fylax and sodium sulfate in inhibition fungal growth and detoxification of DON toxin.

Material and Methodes

1. Isolation and Identification of Fungi Associated with Wheat Seeds

Thirty samples were collected from local and imported wheat seeds from Al-Basrah saylo,10 samples were taken from imported wheat and 20 samples from local wheat .each sample weight about three to five kg .four hundered seeds were taken randomly, surface sterilized by sodium hypochloride (2%),after that seeds were washed by distilled and sterilized water and dried,10 seeds were transfered to each petri dish contain sterilized PDA , petri dishes were incubated at 25*C*° for 4-5 days ,after incubation period the fungus colonies were identified according to[4;6]*Fusarium graminearum* were identified according to [32]

Occurrence of each genus was calculated according to following formula:-

$$\%Occurrance = \frac{Number\ of\ the\ genus\ identified}{Total\ Number\ of\ samples} \times 100$$

2. Study the Ability of *F.Graminearum* Isolate to Produce Deoxynivalenol on Rice Seeds

Two hundred gm of rice seeds were placed in glass plates (20Cm) diameter, each Plates were moist with distilled water for two hours ,after that plates containing rice seeds were autoclaved .After sterilization plates were inoculated with disc(1cm) of *F.graminearum* colony grown on PSA ,Plates were incubated at 25 *C*° for 7 days after that temperature were reduced to 13-16 *C*° to enhance toxin production .After the incubation period rice seeds were dried ,grinded and packaged in paper bag for next experiment [1].Twenty four isolates were used in three Replicate for each.

3. Detection of DON Toxin in Rice Media and Determination of Toxin Producer Isolates

Standard DON toxin was obtained from sigm-Aldrich,chemie,Gmbh Munich,Germany. as crystallized powder in small bottle containing 1mg . Series of concentrations 5,10,25,50 µg/g were obtained from toxin stander by dissolving 1mg of the stander toxin in 1ml of chlorophorm. Extraction DON toxin was done according to Trucksess *et al*[33]. Acetonitral : water (84:16) were used as extraction buffer solvent

Purification of toxin was done by column chromatography according to Scott *et al*[31]. TLC was carried out on silica gel (TLC silica gel 20 X 20 X 0.23) with chlorophorm : aceton : isopropanol 8:1:1. 30ml from sample (extract) were spotted on TLC plates and the solvent front was allowed to run up 16cm. Plates were dried and observed under u.v light(220nm).Conformation detection were done by spread the plates with alcoholic phosphoric acid and dehydrated Almunium Chlorid (AlCl2-6H2O) as regent solvent [26;10]

4. Quantitative Determination of DON

Quantitative determination of DON extracted from different isolates grown on rice media were done by High performance liquid Chromatography(HPLC) model LC-2010 A(Shimazada Japan).The HPLC analytical column was Reverse phase C1BDB and the mobile phase consisted of potassium phosphate 0.0l with pH6 at a flow rate of 1.0 ml/min and detected at a wave length of 220 nm. The concentration of DON produced by each isolate were calculated as below.

$$Concentration\ of\ DON = \frac{Area\ of\ sample\ curve \times com\ of\ standard\ solution \times NO\ of\ dilution}{Area\ of\ standard\ curve}$$

5. Effect of Sodium Sulfate and Fylax on Growth of *F.Graminearum*

Sodium sulfate and Fylax solution was added to 250 ml of sterilized PDA to obtain final concentration as 1,2,3% from sodium sulfate and 0.1,0.2,0.3% from Fylax. PDA contain each concentration was poured in petri dishes (9cm) after the PDA became soild Petridish were inoculated with 0.5 cm disc of 7 days old culture of *F.graminearum* grown on PDA. Plates were incubated at 25*C*° until the fungus in control treatment reach the margin of the plate .The percentage of inhibition were calculated as below.

$$\%\ inhibition = \frac{Growth\ in\ control - Growth\ in\ treatment}{Growth\ in\ control} \times 100$$

6. Evaluation Efficiency of Sodium Slulfate and Fylax to Detoxification of DON Toxin in Wheat Seeds

The best concentration of sodium sulfate and Fylax which gave best inhibition of the fungus growth were used in this expirement.DON toxin was added to rice media in concentration of 5mg/kg seeds, samples were mixed by glass rod, after that sodium sulfate and Fylax were added at concentration of 3and 0.3% respectively. Samples were kept in Disscator for one month .Two control treatment were used ,wheat seeds only and wheat seeds contaminated with DON toxin. Toxin extraction and quantitative determination by HPLC were done as in paragraph 3 and 4.

Results and Discussion

1. Isolation and Identification of Fungi Associated with Wheat Seeds

Results of isolation and identification of fungi associated with local and imported wheat revealed that *Alternaria , Aspergillus* and *Penicillium* were the most fungi associated with wheat seeds as occurrence percentage reached 47,40,32% respectively in local seeds, while occurrence of these fungi reached 55,30,and 21% in imported wheat. Results also revealed that *Fusarium* occurrence reached 19 and 35% in local and imported wheat respectively. Previous studies indicate that *Alternareia, Penicillium, Aspergillus* and *Fusarium* were the most fungi associated with wheat seeds in different area of the world[2;9;19].The species *F.graminearum* was identified according to Booth[6]and Seifert[32]. Isolates of this fungus were kept in slant at 4*C*° to be used in next studies.

Table 1: Percentage Occurrence of fungi associated with local and imported wheat

Fungi	% of occurrence the imported wheat	% of Occurrence the local wheat
Alternaria spp.	55	47
Aspergillus spp	30	40
Penicillium spp	21	32
Fusarium spp.	35	19
F.graminearum	16	11
Rhizopus spp.	6	11
Mucor spp	1	-

2. The Ability of *F.Graminearum* Isolates to Produce the DON Toxin

TLC results showed that 54.1% from *F.graminearum* isolates were able to produce DON toxin (Table2).It is also found that 57.14 and 50% from fungal isolate which were isolated from imported and local wheat respectively were able to produce DON toxin .Isolates ability for toxin production was differ greatly, as highest concentration of DON toxin determined by HPLC reached 1.52 µg/gm for

DON-Fg isolate .This result was in agreement with previous studies indicated that rice seeds media was the best media suitable for fungus to produce DON toxin[12;27]

Table 2: Ability of *F.graminearum* isolates for DON toxin production

Samples	No of isolates tested	No of isolates produced toxin	% of isolates produce DON
Imported wheat	14	8	57.4
Local wheat	10	5	50.00
Total No	**24**	**13**	**54.16**

3. Detection of DON Toxin in Local and Imported Wheat

Results of this study showed in table (3)revealed that 50% of wheat samples were contaminated with DON toxin. The percent of contaminated sample reached 60 and 45% for imported and local wheat respectively . Quantitative determination of toxin by HPLC indicate that highest concentration of toxin reached 1.58 µg/g in imported wheat while it 0.2-0.8 µg/g in local wheat(table 4).Many previous studies indicate the presence of DON toxin in wheat sample in concentration reached 1.3-6.4 µg/g [35;22].Hochsteirer and Schuh[17] showed that 49.6% from wheat and Corn seeds were contaminated with DON.

The presence of DON toxin in wheat sample poses a risk to human and animals health as this toxin characterized by its chemical stability(Cole and cox,1981).This toxin cause diverse harmful effects to human and animals like inhibition of protein synthesis, reduction of white blood cell and decay of borne marrows [29] .This toxin also cause food refuse, vomiting and diarrhea[35]

Table 3: Samples of local and imported wheat contaminated with Don toxin Determined by TLC

Imported wheat (Australin)	Result of test	Local wheat	Result of test	Local wheat	Result of test
1	-	11	+	21	+
2	+	12	+	22	-
3	+	13	-	23	+
4	-	14	-	24	+
5	+	15	+	25	-
6	-	16	-	26	-
7	-	17	-	27	+
8	+	18	-	28	+
9	+	19	+	29	-
10	+	20	-	30	-

No of samples contaminated(imported)=6
No of samples contaminated(local) =9

Table 4: Quantitative determination of DON toxin on wheat samples determined by HPLC

Imported wheat/No of samples	Concentration of DON µg/g	Local wheat/No of samples	Concentration of DON µg/g	Local wheat/No of samples	Concentrati of DON µg/g
1	-	11	0.37	21	0.20
2	0.14	12	0.53	22	-
3	0.12	13	-	23	0.8
4	-	14	-	24	0.75
5	1.58	15	1.58	25	-
6	-	16	-	26	-
7	-	17	-	27	0.23
8	0.50	18	0.50	28	0.5
9	0.17	19	0.17	29	-
10	0.14	20	0.14	30	-

4. Ability of Sodium Sulfate and Fylax in Inhibition the Growth of *F.Graminearum*

Results of this study showen in table(5) indicated that sodium sulfate and Fylax inhibited the growth of *F.graminearum* on PSA. The percentage of growth inhibition differ with concentration. The highest inhibition percentage reached 79% when sodium sulfate used in concentration of 3% while the highest percentage of growth inhibition obtained when 0.3% of Fyalx were used. The percentage of inhibition reached 37.5-66.4% for other concentrations. Previous studes indicate that sodium Sulfate and Fylax solution had the ability to inhibit the growth of different fungi such as *Aspergillus flavus ;A.ochraceous*; *Penicillium palitans* and *F.graminearum*[20;18;16].Fylax solution consisted of mixure of organic acid such as Propnic acid Sorbic acid, Formic acid Acetic acid et.. and its used in many studies to prevent contamination of wheat seeds with mycotoxin such as Ochratoxin[16] and Zearalenon [30]

Table 5: Effect of sodium sulfate and Fylax on growth of *F.graminearum*

Compound tested	%of growth inhibition
Fylax 0.1%	37.5
Fylax0.2%	55.25
Fylax0.3%	73.0
Sodium sulfate1%	55.29
Sodium sulfate2%	66.12
Sodium sulfate3%	79.00
Control	0.00
L.S.D	8.2

5. The Ability of Sodium Sulfate and Fylax Solution in Detoxification of DON Toxin

Results of quantitative determination of DON toxin by HPLC table (6), indicate that DON toxin level were reduced from 45.6µg/gm in control treatment to0.27 and 1.14 µ/gm in wheat samples treated with Fylax and sodium sulfate respectively .Result of this study are in agreement with previous study which indicate that sodium sulfate and Fylax solution were able to reduce the contamination level of Don toxin[13;36;16]

Results of this study indicate that more than 50% of local and imported wheat samples were contaminated with DON toxin and that need more studies to determine the level of this toxin in different agricultural products. Fig(1,2)showed the graph of standred DON toxin and the graph of DON toxin in control treatment

Table 6: Ability of Fylax and sodium sulfate compound to detoxification of DON toxin

Treatments	Concentration of DONµ/gm
Wheat seed only (negative control)	0.00
Wheat seeds+DON	45.6
Fylax 0.3%	0.29
Sodium sulfate 3%	1.14

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