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Corn Plant *Zea Mays L.* Involving with Fusarium Fungal Pathogen and its Main Mycotoxin Effect

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Abstract: Commonest of fungal infection involving of maize is *Fusarium* which is taken from infected maize in kut, doboni, nomania sheieksad, Samples was collected during the period of spring about 134 isolates belonging to different types of *Fusarium* that involving maize plants with determine ability of isolates to produce mycotoxin as a virulence factors. The result of our study revealed that *F.culmorum and F.gramenrium* are most common types in maize plant disease from total collected samples with percentage of 34% and 22% respectively. Depending on morphological and microscopical appearance with polymerase chain reaction study using specific primers showing that most of *Fusarium* isolates in our study produced trichothecene toxin were 76% of isolate give positive result and 24% give negative result as not producing toxin were 55% of positive isolate producing NIV while 45% producing DON toxin. Both types including *F.gramenrium* and *F.culmorum* showing highest result of toxin producing sample comparing to other types with percentage of 100% and 91% respectively indicating to significant pathogenicity on maize plant while all isolate of *F.solani* unable to producing trichothecene toxin related to lack of the Tri5 gene required for biosynthesis of trichothecene.

Keywords: *Fusarium*, mycotoxin, DON maize, virulence factor, trichothecene, root disease.

Introduction

Scientific named of corn is (*zea mays L.) were zea is Greek name meaning* "sustaining life" and mays meaning "life giver.", also called silk maka (1). It considered as very important cereals after wheats and rice in the world (2).

Maize classified according to toxoneamy to kigdome: plantaie, divesion: magnolophytae, classes: lilopsida, order: cyperals, family: poacea, genus: *zea*(3)

In the last years demand on foot in the word increased, as number of people in the world increased we need to elevate the sources of nutrition and in same time take care of human health by using food with nutritional value like maize as its rich source with phytochemical element can prevent a lot of disease. (4)

Maiz containing many vitamins like A,C,E,K, and B group vitamins including (B1,B2,B3,B5,B6), in addition to Folic acid ,presence of these vitamins and beta-carotene, selenium can improving functioning of thyroid gland and increased immunity also containing Potassium as one of the major nutrient with diuretic effect. (5).

Group B vitamenes improve skin ,hair health also important for digestive system and muscle ,joints strength(6),In many countries like India, Spain, China they using maize in treating stones and infection also in treating retentions of fliud And improving blood pressure,(7) support liver functioning regulating high cholesterol level (8), and prevent cardiovascular disease (9). Moreover one spoon per day of its oil providing the body with many fatty acid that enhance heath in chidren and adults (10).

This plants are susceptible to Fusarium fungal infection can involving seeds, stalk ,roots and ears (11)and contaminated with trichothecene toxins, early disese can occure in the stubbles and then involving ear rots during its flowering (12). Spore enter to ear of maize through wound or myclluim growing at kernel (13) maize contamination with mycotoxins (Trichothecenes) including NIV and DON, infection to animals or human can be transmitted from involved plant represent a significant risk to animal and human health as there is wide spectrum of these trichothecene (14)(15).

Affecting plant become shrunken wrinkled and lighter weight with rough appearance (16). it's important to know that isolate that producing myco-toxin(trichothecene) more pathogenic &risky than others isolate and NIV toxin is more toxic to humans and anemales than DON as reported from several countries in Asia and considered one of serous health problem due it toxic effect to human and plant (17).

MATERIAL AND METHODES

During the period of spring samples are collected were about (134) isolates belonging to *Fusarium* obtained from infected maize plants in kut, doboni, nomania sheieksad as shown in (Figure 1). Then preparing of agar media done by adding 39 gram of PDA to about 1000 ml of distilled water, strerlization done by autoclave at 121˚C for 15 minutes, then culture of isolate in petri dish, incubation for 7 days at 25 degree till examined by microscope x40 magnified and categorized *Fusarium* spp. according to morphology, shape of mycelium, conidum, conedospres shape, size and color (18).

(A) **(B)** **(C)**

Figure 1. Major diseases of maize caused by Fusarium spp.; (A) stalk rot disease and (B) ear rot, (C)kernel rot disease.

Molecular detection of trichothecene toxin by polymerase chain reaction

Depending on polymerase chain reaction with using of specific primers the ability of fusarium species in production of trichothecene toxin was examined as shown in (Table1) including Tri5 forTri5 gen detection and Tri13 for Tri13 gen detection (19).

Preparation and extraction of DNA from isolates done according to manufactures instruction and preparation of PCR master mix as in (Table 2), conditions of PCR arranged as shown in(Table 3) and (Table 4) (20).)(21) PCR product and DNA ladder loaded in well of 1.5% agarose gel by using safe view dye electrophoresis run at 100 volt for 60 min under UV light then measuring the DNA band with DNA ladder done (22)(Abdul-Hussein *et al*.,2018).

Table 1. showing the primers used in study .

Primer	Gene	Primer sequence	Product size	Reference
Tri5	Tri5	F 5′-AGCGACTACAGGCTTCCCTC-3′	544	Doohan *et al*., 1999
		R 5′-AAACCATCCAGTTCTCCATCTG-3′		
Tri13	Tri 13	F 5′-TACGTGAAACATTGTTGGC-3′	200-300 DON	Waalwijk *et al*., 2003
		R 5′-GGTGTCCCAGGATCTGCG-3′	400-450 NIV	

Table 2. showing the content of master mix conventional PCR .

PCR master mix	Volume
DNA template	5 µl
Forward Primer	1.5 µl
Reverse Primer	1.5 µl
Free -Nuclease Water	1.2 µl

Tables 3. showing the PCR program used for Tri5 gene .

PCR step	Repeat cycle	Temperature	Time
initial denaturation	1	94 ºC	2Min
Denaturation	30	95 ºC	35sec
Annealing	30	62 ºC	30 sec
Extension	30	72 ºC	45sec
final extension	1	72 ºC	7min
Hold		4 ºC	Forever

Tables 4. showing the PCR program used for Tri 13 gene **.**

PCR step	Repeat cycle	Temperature	Time
initial denaturation	1	95 ºC	3Min
Denaturation	30	95 ºC	30sec
Annealing	30	60 ºC	30 sec
Extension	30	72 ºC	45sec
final extension	1	72 ºC	7min
Hold		4 ºC	Forever

RESULTS

Depending on morphological microscopical appearance and classification keys mentioned by Summerell *etal*.(2003) (23), from the total number(134) there is 46 isolate belong to *F.culmoruim, 30 for F. graminruim ,26 for F.pseudogramenrium ,17 F.solani and 12 for F.equiseta ,and just 3 for F. oxysporuim a*s shown in as shown in (Table 5) .

Table 5. Show numbers and parentage of *Fusarium spp.*

Fusarium spp.	Number of isolates	Percentage %
F. culmorum	46	34
F. gramenrium	30	22
F. pseudogramerium	26	20
F.solani	17	13
F.equiseti	12	9
F.oxysprum	3	2
Total	134	100%

Results of PCR detection for trichothecene toxin showing that the fungi containing TRI 5 gene are able to produce trichothecene toxin while those that do not contain this gen are unable to produce trichothecene toxin. This primer TRI 5 especial for product of 544bp for samples producing trichothecene toxin as in (Figure 2) A were (102)sample from the (134)total number give positive result as 76% while 24% given negative result this agreed with (24)(Covarelli *et al.*, 2015) .

While the Tri13 primers for TRI 13 amplifying frgments in 200-300bp range for DON producing samples and 400 -450bp for NIV product samples of fusarium species as shown in figure (2B and C). The result of study showing that the highest percentage of toxin production seen in *F.graminrum* by 100% followed by *F.culomrum* with 91% and *F.pseudogramerium* 84% , *F.equiseti* 42% and *F.oxysporum* 33% as shown in (Table 6) .

Table 6. samples producing toxin and types with percentage **.**

Fusarium isolate	DON toxin %	NIV toxin %	Trichothecene producing sample %
F.graminearum	25	75	100
F.culmorum	46	54	91
F.pseudogramineaum	52	48	84
F.equiseti	60	40	42
F.oxysporum	100	0	33
F.solani	0	0	0

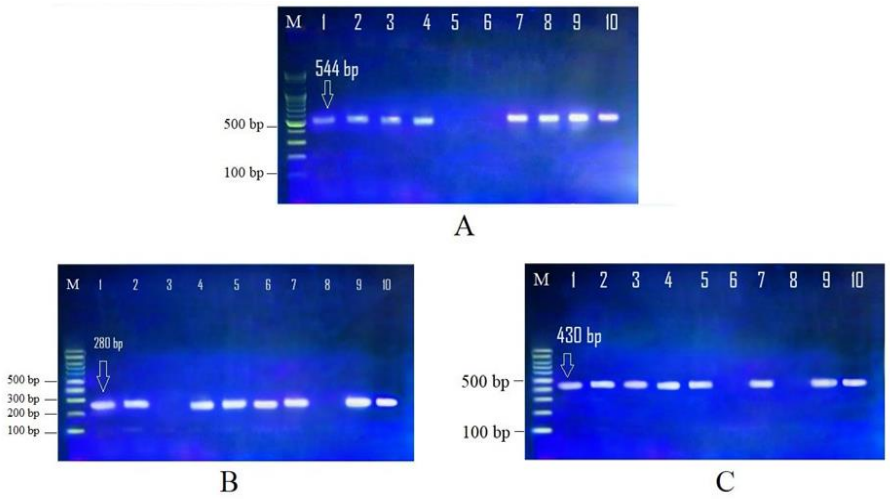

Figure 2. show amplification product in agarose gel 1.5% (A) using primer Tri 5F/R produced PCR product with size 544bpfor isolate containing Tri 5 gene , (B)Tri 13F/R primer for isolate with product size 200-300 bp from DON producing *Fusarium* isolate , (C) 400-450 bp for NIV producing isolate

Our results showed that 57% of isolates producing NIV toxin where 43% of isolates produced DON and all samples of *F.solani* give negative result indicating its inability to producing toxin due to lack of Tri 5 gene responsible to producing trichothecene toxin this agreed with finding of (Mui-Keng and Niessen ,2003). [25]

DISCUSION

mycotoxin including DON and NIV can be produced by fungal pathogen that affect maize , fusarium considered from main fungal infection in this plants with dominance of *F.culmorum and F.graminearum* in number of samples 46 and 30 sample respectively comparing to other types of *Fusarium* with percentage of 34% and 22%.*F.gramirum ,F.culmorum* showing the highest result of toxin producing sample comparing to other types with percentage of 100% and 91% respectively suggesting of its effective toxic activity for maize plant disease caused by *Fusarium* agreed *with* Manty *et al.(*2018) (26) he found that *F.culmoruim* affecting of wheat as main agent of wheat fungal disease in Iraq . and agrred with (Gale *et al.*,2011). In dominance of NIV toxin in some Asian country as in korai ,Japan and Australia (27)

our study showing that nearly most of *Fusarium* isolate producing toxin except of *F.solani* isolate which given negative result in all its sample as its unable to producing toxin this in agreement with (Mui-Keng and Niessen ,2003) were showed that *F. solani* unable to producing trichothecenes Related to deficiency of this gene (TRI 5) needed for formation of trichothecene (Lee *et al.*,2002)(28). In Germany Schodlenberger *et al.*(2002) pointed out the contamination of stored grains taken from grain stores with DON and NIV toxins (29).

A study by Camphell *et al.*(2002) (30)the concentrations of 13 fungal toxins were detected in four groups of grains 673 yellow corn samples 99 wheat samples 116 barley samples and 73 rye samples collected from regions of western Canada from 1991-1998, they were found the most important toxins and predominant substance in all samples of the four groups of crops is the toxin DON with the highest percentage in yellow corn followed by wheat barley and then rye. Hestbjerg *et al.*(2002) indicated that DON toxin was found in barley grains19 days after treating barley seeds contaminated with isolates of *F.culmorum* this speed in the production of DON toxin gives a clear picture of the danger of this toxin which necessitates searching for strategies to prevent the production of such toxins(31)

In survey of all animal feeds in the Kingdom of Saudi Arabia to detect trichothecenes the percentage of DON toxin was higher than the rest of the mycotoxins as it was found in all samples it was at

high concentrations (32).that ranged between 2-40 µg/g (Al-Julaifi and AL-Falihi ,2001) Saemar *et al.*(2001) showed that presence of DON toxin in bread made from contaminated cereal by this toxin as French bread and Vienna bread with a high percentage reaching to 56% and 41%, respectively. (33)

Our result showing that most of *Fusarium* isolates contain both type of trichothecene toxin including DON,NIV with mild dominance of NIV types as (55%)for NIV producing sample and (45%)DON producing samples this agree with Miedaner *et al.*(2000) which confirm presence of both types of toxin in Asian , Europe ,Africa only DON type has been detected in north America ,both types of toxin seen in south American century with dominance of DON at these areas .

In previous study Park *et al.* (1991) revealed the contamination of rice barley and corn crops with trichothecene toxins DON and NIV at concentrations of 605-861 µg/kg and 426-981 µg/kg for two seasons in South Korea respectively among 190 wheat barley and oat samples that were tested it was found that 78% of the samples wheat was contaminated with DON and NIV (34)

In a study conducted in Iraq the genus *Fusarium* was isolated among the fungi showed their ability to produce DON toxin agreed with Snijders and Perkowsk. (1990) which confirmed the direct relationship between infection with *Fusarium* fungal disease and contamination with these toxin (35)

CONCLUSIONS

mycotoxin metabolite had crucial role in the processes of fungal disease pathogenicity on maize plant affected by *Fusarium spp* ,required more effort and studies to trying decreased its effect on plant first and on general heath of living creatures.

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