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Detection the effectiveness of antagonism and its role in production of fungal toxin

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Abstract:

Thirty-five samples of maize were collected from the local markets of Diwaniyah city , after the isolation, the results showed 16 isolated of fungi , including 7 isolates of F. moniliform .The susceptibility of these isolated was detected on the production of trichothecenes showed that 4 isolated have the ability to produced fungal toxin and varying concentrations (18 , 21 , 24 , 31)µg/kg. Trichoderma harzianum was showed the ability of inhibited the production of fungal toxin from F. moniliform, through the antagonism between it and F. moniliform.

Keywords: *Fusarium oxysporum , antagonism , fungal toxin .*

I. Introduction:

Mycotoxins are important fungal secondary metabolites with low molecular weight; they are representing a potential threat to human and animal [1,2]. Mycotoxins present a challenge for scientists working on a wide range of disciplines such as microbiology, biochemistry, structural chemistry, toxicology, pharmacology and genetics [3]. Trichothecenes are about 148 type of closely related mycotoxins which are widely distributed in nature such as T-2 toxin, Diacetoxyscirpenol (DAS), Deoxynivalenol(DON) and HT-2 toxin [4]. Trichothecenes are group of metabolites produced by species of the genus Fusarium, in addition to other genera including Mythecium, Trichothecium, Verticimonosporium, Cefalosporium, Trichoderma, Gibberella, and Stachybotrys [5]. Many diseases reported after ingestion of moldy feed [6], these diseases characterized by feed refusal, depression, diarrhea, vomiting, and hemorrhage in intestine and muscles which lead to death of animals [7].

II. Materials and Methods:

1-Isolation and Identification of *Fusarium oxysporum* :

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The fungi isolated according to [8] about 30 randomly selected corn seeds were surface sterilized then seeds were cultured on Potato Dextrose Agar (PDA), 2 seeds for each plate then incubated at 25ºC for 7 days, then the grown Fusarium (Which was morphologically identified) purified by new sub culturing (each one in single plate of Potato Sucrose Agar (PSA)) this purified isolates were used for identification. Pure isolates were identified by direct examination with light microscope on glass slide and according to taxonomic system of Snyder and Hansen [9].

2-Production, Extraction and Identification of trichothecenes to produce trichothecenes, spore suspension prepared according to [8,10], PDA inoculated with spore suspension(8.1×10 spore\ml) and incubated at 25ºC for 1 week and at 27 ºC for 1 week and then at 8 ºC for 1 week. And according to [11, 12], toxin extracted and kept in small vials in dry and cold place. By using TLC method trichothecenes have been detected and identified in comparison with standard T-2 toxin using benzene: acetone in 3:2 ratios as developing system for separation of trichothecenes and examined under U.V. light at 366(nm) Wave length (W.L.) [11], with determination of Rf value [13,14], the amount of toxin was estimated in comparison with standard toxin using scanning densitometer [15].

III. Resultes and Discussion:

1-Isolation and Identification of *F. Oxysporum* Maize has been selected as a natural source for isolation of *F. oxysporum*, when maize put on surface of PDA media gave a good result for isolation of F. oxysporum [8]. The average growth rate of cultures was about 5 ± 0.5 cm, the fungus is represented by white mycelium on the surface of the dish, but with purple to brownish tinge[14,16]. Microconidia born on simple phialides arising laterally on hyphae or from short branched conidiophores. Generally microconidia abundant, cylindrical, straight and 1-2 septate. Macroconidia are born on more branched conidiophores, they were thin walled, 3-5 septate and pointed at both ends, this results obtained according to [9].

2- Production and extraction of trichothecenes

Table (1), shows the isolated fungi proved to be efficient producer for trichothecenes (750 ppb) in comparison with other isolates used for production of trichothecenes [17]. According to our experimental work, the method used was a suitable method for recovery of the toxin from the culture, which is characterized by clear red to pink fluorescence of trichothecene derivatives under U.V. light (366 nm) with Rf value 0.2 on silica gel plate after developing the chromatogram. The solvent system is efficient in separation the components of fungal culture extract.

Table (1): Trichothecene concentrations that produced of fungal isolated.

No.	Fungal isolates	Concentration of trichothecene µg
1	*F. moniliform1*	18
2	*F. moniliform2*	21
3	*F. moniliform3*	24
4	*F. moniliform4*	31

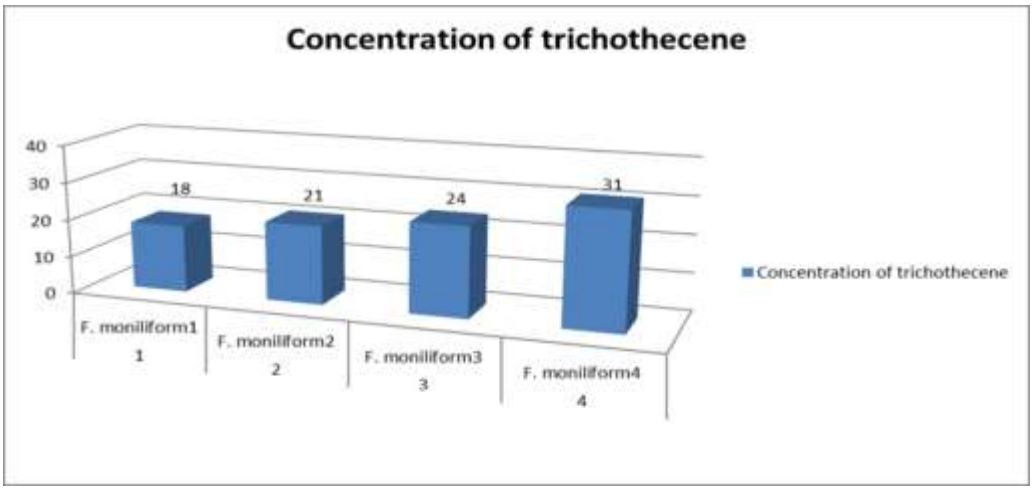

Figure (1): Trichothecene concentrations that produced of fungal isolated.

Antagonistic capability of *T. harzianum* isolate against *F. oxysporum* and in vitro:

Fifteen ml of MEA medium in 9 cm Petri-dishes were inoculated with two disks (7 mm in diameter) of five day old antagonistic fungi (*T. harzianum* isolate). Two disks of the tested pathogen obtained from four-day old cultures were then placed at the periphery of each plate at the same distance. The inoculated plates in addition to plates inoculated with the pathogen only (control treatment) were kept at 25°C. Three replicates were used for each treatment. Antagonistic effect was evaluated by scoring the width of the inhibition zone (clear area) [18,19], where: 0 : no inhibition 1 : 20 mm (high antagonism). 4 :over growth (mycoparasytism) *T. harzianum* isolate that showed a high antagonistic capability screened for subsequent studies. Antagonistic capability of *Trichoderma* sp. mutants was also determined in the same procedure (table-2).

Table (2): Inhibition zones and concentration of toxins produced by Fungal isolated

No.	Antagonism		Inhibition zone mm	Concentration of toxin µg/kg
	Fungal isolated	Antagonist fungal		
1	*F. moniliform*	*Trichoderma harzianum*	2.7	1.6
2	*F. moniliform*	*Trichoderma harzianum*	3.2	2.1
3	*F. moniliform*	*Trichoderma harzianum*	1	1.1
4	*F. moniliform*	*Trichoderma harzianum*	2.2	1.8
LSD			0.2	0.5

IV. Conclusion:

Antagonistic capability of *T. harzianum* against *F. oxysporum* and in vitro :

Trichoderma harzianum was showed the ability of inhibited the production of toxin from *F. moniliform*, by antagonism between it and *F. moniliform.* Antagonistic effect was evaluated by scoring the width of the inhibition zone (clear area) , where: 0 : no inhibition 1 : 20 mm (high antagonism), over growth (mycoparasytism) *T. harzianum* isolate that showed a high antagonistic capability screened for subsequent studies. Antagonistic capability of *Trichoderma* sp. mutants was also determined in the same procedure.

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