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DETECTION OF TRICHOTHECENE OF *Fusarium solani* ISOLATES BY USING HPLC IN MELON PLANTS

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ABSTRACT

Fusarium solani is one of the important phytopathogenic causing serious losses on cucurbit plant in Iraq, which are responsible for crown, and Root rots of melon. Ability of Fusarium solani isolates to produce T2-toxen and DON analyzed by using HPLC. The results indicated that the F. *solani* has potential capacity to produce trichothecene. Thirty-nine isolates investigated with the HPLC method were identified to produceT2 toxin. Also, F. *solani* isolated that demonstrated a potential capacity for the synthesis of trichthecence DON.

Keywords – *Fusarium solani* f. sp. *cucurbitae*, mycotoxins, HPLC

INTRODUCTION

Mycotoxins are defined as low molecular weight fungal secondary metabolites that are toxic to vertebrates (Desjardins and Hohn, 1997). All trichothecene producing *Fusarium* species are destructive pathogens that can attack a wide range of plant species. The accurate identification of toxin producer *Fusarium* species is very important because each of them possesses a specific toxigenic profile and it is important to know the potential toxigenic risk of the contaminated plant or food products (Mulé *et al.*, 2004). Trichothecenes are produced by a number of *Fusarium* spp., including *F. acuminatum*, *F. crookwellense*, *F. culmorum*, *F. equiseti*, *F. graminearum* (*G. zeae*), *F. lateritium*, *F. poae*, *F sambucinum* (*G. pulicaris*), *F. solani*, and *F. sporotrichioides*(Marasas et al. 1984; El-Banna et al. 1984; Clark et al. 1995).The objective of this study was to Detection of Trichothecene of *F. solani* isolates by using HPLC in melon plants

MATERIALS AND METHODS

Isolation and Identification of the Pathogen: Forty isolates were obtained from (Abdul Hasan 2017) single spore technique was made for each isolate through serial dilutions in sterile water on PDA in petri-plates. Isolates were identified to the species level according to their cultural and morphological characteristic (Leslie and Summerell, 2006).

Fusarium solani were grown on rice seeds. One hundred twenty-five ml distilled water were added to 100 g of rice seeds in petri-plates of 20 cm diameter and 5 cm depth. The plates were autoclaved twice at 121°C and 1.5kg/ cm2 for 20 min. during 24 hours. The sterilized seeds were inoculated with 2 disks of 0.5 cm diameter of fungal growth and homogenized. The plate was maintained at 25 ± 2°C for 14 days, then transferred to 13 additional 14 days to induce the production of DON. The contaminated seeds were dried and ground in electrical grinder

DON and T2 extraction: Fifty gm of rice seeds powder were added to 200 ml of Acetonitrile: water mixture (84:16 v:v) in 500 ml flask. The flask was agitated for 30 min. and the extract was filtered through Whatman No.2 filter paper. One hundred twenty through What-man No.2 filter paper. One hundred twenty-five ml of the filtrate were added to 50 ml of Hexane in separating funnel, subjected to agitation for 20 sec. and let to settle. Fifteen gm of ammonium sulfate were added to the lower layer and passed through filter paper (WhNo.2). The filtrate was passed through 10 gm of anhydrous Na_2SO_4 and the filtrate dried and conserved in dark vial.

DON detection: DON, T2 toxin detection by the mixture of chromatography used was Chloroforme: Acetone: Isopropanol (8:1:1 V:V:V). At the end of chromatography, the plate was sprayed with 20 % ammonium chloride in methanol. The plate was maintained at 120spots were detected by UV light.

RESULTS

The results showed that *Fusarium solani* forms white to cream color mycelium on the PDA. Microscopic examination was showed three type of spores, macroconidia were spindle to cylindrical shape containing between three to five septa with distinctly or barely notched basal cells, microconidia formed on false head on long monophilids, growth laterally on the aerial mycelium. Chlamydospores terminal and intercalars single or in chains.

Fusarium solani to produce mycotoxin in the fungal culture filrate was analyzed by HPLC. It was observed that 39 of 40 isolates *Fusarium solani*

were able to produceT2 toxin, while 37 isolates produced DON toxin Fig -1.

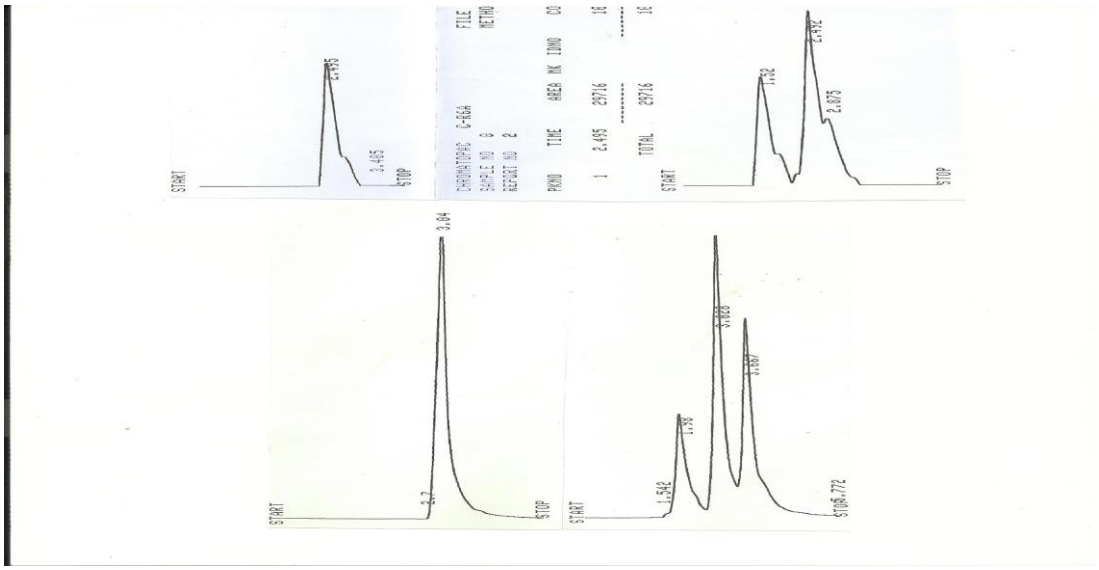

Figure -1: Chromatography HPLC data generated of *F. solani* culture extracts. (a) representingT-2 toxin (b*) F. solani, 1* representing DON.

Table 1 Fusarium *solani* isolates to produce T2-toxen, DON analyzed by using HPLC

NO	Code	Samples set	Concentration T2 (ng/ml)	concentration DON (ng/ml)
•	B1 Fs3	Babylon	1245.2	289.9
•	B1Fs2	Babylon	1361.3	376.3
•	B1Fs1	Babylon	295.9	401.5
•	B2Fs6	Babylon	756.9	556.6
•	B2Fs5	Babylon	2376.7	2228.7
•	B2Fs4	Babylon	564.6	523,3
•	B3Fs9	Babylon	751.5	1975.4
•	B3Fs7	Babylon	2791.8	579.8
•	B3Fs8	Babylon	765.7	476.3
•	D4Fs10	Diwaniyah	642.7	1478.9
•	D4Fs12	Diwaniyah	1314.8`	1100.8
•	D4fs11	Diwaniyah	1213.8	312.6
•	D5Fs13	Diwaniyah	698.5	419.6
•	D5Fs14	Diwaniyah	731.1	205.6
•	D5fs15	Diwaniyah	654.9	1756.8
•	D6Fs16	Diwaniyah	2317.9	2324.5
•	D6Fs18	Diwaniyah	398.7	343.9
•	D6Fs17	Diwaniyah	651.9	219.9
•	BG7Fs19	Baghdad	1265.8	0.00
•	BG7Fs20	Baghdad	114.6	312.9
•	BG7Fs21	Baghdad	213.7	403.9
•	BG8Fs24	Baghdad	1261.	2890.9
•	BG8Fs22	Baghdad	342.7	540.3
•	BG8Fs23	Baghdad	254.1	1340.4
•	BG9Fs25	Baghdad	2231.3	600.1
•	BG9Fs27	Baghdad	524.8	607.8
•	BG9Fs26	Baghdad	1650.8	1509.9
•	N10Fs29	Najaf	1986.6	989.9
•	N10Fs28	Najaf	2130.0	0.00
•	N 10Fs30	Najaf	980.6	612.6
•	N11Fs32	Najaf	1870.8	660.0
•	N11Fs31	Najaf	1923.9	1260.6
•	N11Fs33	Najaf	132.9	603.4

•	N12Fs36	Najaf	345.6	543.4
•	N12Fs34	Najaf	1967.8	554.4
•	N12Fs35	Najaf	0.0	0,00
•	M13Fs39	Ministry of science	2156.5	543.4
•	K14Fs39	Collage of Agriculture Kufa	128.5	889.6
•	K15Fs37	Collage of Agriculture Kufa	242.2	299.9
•	C16Fs40	Collage of science Babylon	1980.9	671.6

DISCUSSION AND CONCLUSION

Fusarium solani f. sp. *cucurbitae* produces T2, DON. capable of inducing symptoms as root rot and crown rot. The quantitative detection on T2, DON. Toxin showed that deference of Fusarium solani isolates to production toxin according to site of isolates, Diwaniyah (D6Fs16) isolate was the more active in T2, DON toxin.

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