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Original Research Article

Detection of T-2 Toxin from Some Egyptian *Fusarium* Strains with Special Reference to its Cytotoxicity

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Abstract

In the present study some Egyptian *Fusarium* strains were isolated previously from some cereal grains, tested for production of trichothecenes mycotoxins especially T-2 toxin by thin layer chromatography method (TLC). The results revealed that nine strains gave positive results for production of trichothecenes especially T-2 toxin. These strains are *F. anthophylum, F. equiseti* (KM246255.1), *F. fujikuroi* (MG211161), *F. fujikuroi* (MG211162), *F. sporotrichioides* (MG211163), *F. solani* (MG211159), *F. solani* (MG211160), *F. subglutinans* and *F. verticillioides* (XM_018895258.1). The identity percentage of T-2 toxin produced by each strain was detected by high performance thin layer chromatography (HPTLC) where *F. solani* (MG211159), *F. fujikuroi* (MG211161) and *F. verticillioides* (XM_018895258.1) recorded the highest values (95, 98 and 98 %) respectively. Moreover, *F. solani* (MG211159) was selected for mass production of T-2 toxin on rice grains then separated and purified using column chromatography. Then, cytotoxicity of this purified T-2 toxin was examined on fibroblast cell line to detect the safe concentration of this mycotoxin when consumed by human in contaminated cereal grains. The disorder changes and toxicity of treated cells reduced by decreasing T-2 toxin concentration till certain concentration where the appearance of the treated cells resembles the normal cells. This concentration which equal 5.60 ng/ml was considered safe and nontoxic. All these results revealed that the contamination of cereal grains by the tested *Fusarium* strains will be consequently contaminated by T-2 toxin which has toxic effects on human health except at the safe concentration.

Keywords: *Fusarium*, Trichothecenes, T-2 toxin, TLC, HPTLC and Cell lines.

INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by some fungi causing toxic effects called mycotoxicosis. The adverse effects of fungal products have caused mass poisoning in both man and farm animals in many countries (Lee et al., 1994). Trichothecenes are considered family of mycotoxins comprise of closely related sesquiterpenoids and produced by various species of fungi such as *Fusarium, Myrothecium, Trichoderma, Trichothecium, Cephalosporium, Verticimonosporium* and *Stachybotrys* (Betina, 1989). They derived their name from trichothecin; the first member of this family which was discovered from a culture of *Trichothecium roseum* (Ueno, 1980). They were divided into four types (A–D) according to the characteristic functional group. There are several examples of mycotoxins belonging to trichothecenes such as aflatoxin B1, T-2 toxin, deoxynivaleno1 and lindane (Krska et al., 2001).

T-2 toxin is one of naturally occurring trichothecenes in food and feed. It is considered cytotoxic secondary

metabolite produced by several species of *Fusarium*. The importance of T-2 toxin is concentrated in causing acute and chronic toxicity and inducing apoptosis in the immune system and fetal tissues (Li et al., 2011). Also, it inhibits the protein synthesis and cell proliferation in plants (Rocha et al., 2005).Oral uptake of T-2 toxin causes several non-specific symptoms such as weight loss, feed refusal, dermatitis, vomiting (cats, dogs, pigs and ducklings), iarrhea, hemorrhages and necrosis of the epithelium of stomach and intestine, bone marrow, spleen, testis and ovary (Kretzschmann, 1991; O.W.H. and I.A.R.C, 1993; Rafai et al., 1995; Eriksen, 1998). Moreover, lot of studies recorded the haemato-toxicity of T-2 toxin on red blood cells, leukocytes and platelet progenitor cells from mice, rats and humans *in vitro* (Dugyala et al., 1994; Lautraite et al., 1995; Rio et al., 1997). Also, it affects the nervous system where exposure to it changed the levels of neurotransmitters in rat brain following dietary exposure to 2-21 mg T-2 toxin/kg/day (Kretzschmann, 1991; MacDonald et al., 1988; Wang et al., 1993). T-2 toxin produces oedema, intradermal hemorrhage and necrosis of the skin. These effects on skin have been used as a biological assay for detection of trichothecenes. T-2 toxin can be detected at 0.2 µg with a skin necrosis assay (Kretzschmann, 1991). Moreover, trichothecenes mycotoxins including T-2 toxin from *Fusarium* sp. Showed adverse effects on the immune system and spleen cells (Rosenstein et al., 1981). Recent study indicated that T-2 toxin and ochratoxin A are carcinogenic (Abassi et al., 2016).

Thin layer chromatography (TLC) is a chromato-graphic technique used to separate the components of a mixture using a thin stationary phase supported by an inert backing (Hao et al., 2015). This method was used effectively in identification and separation of trichothecenes from several *Fusarium* species (Nagaraja et al., 2016).

The high performance thin layer chromatography (HPTLC) is considered among the modern analytical tools and a powerful analytical qualitative and quantitative method (Andol and Purohit, 2010). This technique was used by (Chandratre et al., 2014) to investigate and quantify the accumulation of T-2 toxin in various organs of Wistar rats. On the other hand, some researchers (Venkataramana et al., 2013) analyzed the trichothecene from several strains of *Fusarium culmorum* which isolated from freshly harvested maize grain samples from Southern parts of India by HPTLC.

The cytotoxicity effect of *Fusarium* toxins was studied on three human epithelial cell lines (CaCo-2, Hep-2 and HeLa) by (Calvert et al., 2005) using MTT assay. This study shows that cytotoxicity of *Fusarium* toxins on epithelium cell lines is dependent on concentration and time. Also, another authors (Agrawal et al., 2015) investigated that T-2 toxin was cytotoxic at a low concentration on human neuroblastoma cells (IMR-32).

Accordingly, the aim of the present study is testing the capability of some Egyptian *Fusarium* strains, isolated from some cereal grains in producing trichothecenes especially T-2 toxin, separating and purifying it and examining its cytotoxicity on fibroblast cell line to detect the safe nontoxic concentration.

MATERIALS AND METHODS

Used reagents

All reagents used in this study were of HPLC analytical grade. T-2 toxin was purchased from Sigma –Aldrich Company and dissolved in chloroform for preparing standard solution which stored at -20°C for further analysis.

Tested *Fusarium* spp.

Some Egyptian strains belonging to different species of *Fusarium* were isolated previously by the authors from different cereal grains and their products (wheat, rice, barely, flour, corn flour and starch) (El-Rabbat et al., 2018) and identified morphologically and genetically. These species are; *F. oxysporum*, *F. solani*, *F. equiseti*, *F. fujikuroi*, *F. chlamydosporum*, *F. brachygibbosum*, *F. verticillioides*, *F. subglutinans*, *F. anthophylum*, *F. moniliforme* and *F. sporotrichioides*.

Extraction of trichothecenes from the tested *Fusarium* strains

The isolated *Fusarium* strains were further tested for trichothecenes production. Each *Fusarium* strain was cultivated separately using Potato Dextrose Agar (PDA) plates and incubated for 3 days at 20°C. After incubation period, they were sub-cultured on specific modified liquid Czapek's medium (Table 1) in 250 ml Erlenmeyer's flasks and incubated statically at 15°C for 14 days. At the end of the incubation period, trichothecenes were extracted from the tested strains as follow. Each culture flask was supplemented with twice volume of ethyl acetate, then, the mycelial mat was ruptured using ultrasonicator for 2 min. The mixture was shaked for 48 h at 120 rpm and filtrated using Whatman No.1 filter paper. The filtrate was separated using separated funnel and allowed to stand till appearance of two layers (the target products is low molecular weight compounds so centrifugation process is not recommended in this step). The lower layer was separated and filtered with Whatman filter paper No.4 containing anhydrous sodium sulfate. After that, the filtrate was evaporated in rotary evaporator to near dryness, dissolved in 1 ml ethyl acetate and stored at -20 °C until further analysis. The extract was applied to a

Table 1. Composition of modified liquid Czapek's medium

Components	Concentration (g/L)
Sucrose	30
$NaNO_3$	2
K_2HPO_4	1
$MgSO_4$	0.5
KCL	0.5
$FeSO_4$	0.005
$ZnSO_4$	0.005

Bond Elut C18 SPE cartridge (where SPE: Solid Phase Extraction) preconditioned with 3 mL methanol and 3 mL deionized water.

Screening for T-2 toxin producing *Fusarium* strains using thin layer chromatography (TLC)

This experiment was performed to investigate the capability of the tested *Fusarium* strains to produce T-2 toxin. For this purpose, a modified method of (Ueno et al., 1973) was conducted. Aluminum silica gel G-60 sheets (20×20) were purchased from Sigma Aldrich Company and used for separation and identification of T-2 toxin from previously prepared extracts of the tested *Fusarium* strains. Silica gel sheets were washed with methanol to remove impurities.

T-2 toxin was dissolved in chloroform to prepare standard solution. The tested extracts and standard solution of T-2 toxin, 10 µl from each sample, were spotted using automatic pipette on a line about 1.5 cm above the bottom edge of the silica sheets. The sheets were developed in the chromatographic jar containing the solvent system (chloroform - methanol) 95:5, v ⁄ v respectively until reaching the end line. After complete running of the sheets in the solvent system, gray colored bands related to T-2 toxin were detected when sprayed by 20% sulfuric acid and heated in oven at 100°C for 10-20 min. Also, these bands appeared as skylight blue color when examined by ultra violet irradiation at 360 nm. The retention factor (Rf), which is defined as the distance travelled by the compound divided by the distance traveled by the solvent system, was calculated for the resulted spots.

Quantitative analysis using HPTLC

To determine the concentration of produced T-2 toxin, a method of high-performance thin-layer chromatography (HPTLC)analysis adopted by (Hamidi et al., 2017) was utilized. The used HPTLC system was CAMAG with several mobile phase systems. Pre-coated silica gel G-60 aluminum TLC sheets (20 cm × 20 cm), layer thickness of 0.2 mm (Merck, Germany) were used as stationary phase after washing by methanol for 1h to remove any impurities. A volume of 30 µL standard T-2 toxin and extracted sample solutions were applied to the chromatographic sheets using CAMAG Linomat 5 TLC sampler. The standard solutions were prepared in different concentrations (9.8 -10.2 mg/ml) to prepare calibration curve. The sheets were developed in automatic developing chamber 2 (ADC2) containing 20 ml (chloroform-methanol) 95:5, v/v for 20 min. Then, the sheets were dried at room temperature before densitometry scanning. The HPTLC sheets were scanned by scanner UV/Vis detector at a wavelength of 254 nm. Densitogram was displayed by CAMAG winCATS computer program. Analysis and validation were identified by matching their Rf values and area under curve values, with those obtained for standard.

Mass production of T-2 toxin and its purification by column chromatography

F. solani (MG211159) was selected for mass production of T-2 toxin using rice grains. The method discussed by (Barros et al., 2012) was performed. Sixty gm of rice grains, without husks, in 250 ml Erlenmeyer's flasks were wetted to 50% moisture by dist. H_2O for few hours, with gently shaking, sterilized in autoclave for 1h then the sterilization process was repeated for 20 min in the second day. The rice grains were inoculated with 5ml spore suspension of *F. solani* (MG211159) and incubated at 20°C for 3 weeks. After the incubation period, the rice grains were dried in oven at 50°C.Then, the following steps were performed according to (Busman et al., 2011) for extraction of T-2 toxin. The rice grains were dipped in (methanol-water) 50:50, v/v, sonicated for 3 min then shaked for 48 h. The mixture was filtered and centrifuged at 2000 rpm for 15 min. The aqueous phase was taken and dried by rotary evaporator and stored at -20 °C for further analysis.

This extract of T-2 toxin was dissolved in chloroform then separated and purified according the modified methods of (Lepom and Baath, 1989; Paavola et al., 1977) using silica gel column chromatography (28 cm

×2.3 cm I.D.). A ball of cotton was deposited in the bottom of the column. Then, the column was filled with the matrix which composed of 5 gm anhydrous sodium sulphate, 15 gm silica gel (granules) and 5 gm anhydrous sodium sulphate in layers. Silica gel was activated for 1 hour before using. The matrix was saturated with 150 ml chloroform followed by 150 ml hexane. Prepared solution of T-2 toxin (about 50 mg extract dissolved in 150 ml chloroform) was poured on the top of the matrix in the column. The eluting solution composed of (chloroform-methanol) 95:5, v/v. After complete separation, T-2 toxin was recognized as specialized needle shaped crystals.

Detection of cytotoxicity of T-2 toxin

This experiment was designed to give an indication about the dose or concentration of T-2 toxin which would be safe and nontoxic using the fibroblast cell line.

Chemicals

Dimethyl sulfoxide (DMSO) and 3(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazoliumbromide (MTT) were used for cytotoxic assay in addition to sterile phosphate buffer saline (PBS) (BIO BASIC CANADA INC). Different dilutions of T-2 toxin (5.6, 11.21, 22.43, 44.87, 89.75 and 179.5 ng/ml) were used.

Preparation of fibroblast cell culture

Sample of human foreskin was cut into small pieces and applied to maintenance medium (5 ml trypsin EDTA) in 50 ml falcon tube and incubated for 30 min with shaking. After that, the tube was centrifuged for 2 min at 800 rpm then the supernatant was discarded. Another 10 ml trypsin EDTA was added to the pellet and incubated for 20 min with shaking then centrifuged at 1200 rpm for 5 min and the supernatant was discarded. Then, the pellet was cultured in tissue culture flask with complete minimum essential medium (MEM) 10% serum. After the complete sheet appearance, the flask was trypsinized and the cells are now ready for the methyl thiazol tetrazolium (MTT) assay.

Methyl Thiazol Tetrazolium (MTT) assay

The 96 well tissue culture plates were inoculated with 1 X 10^5 cells / ml (100 µl / well) and incubated at 37°C for 24 hours to develop a complete monolayer sheet. After the incubation period, the growth medium was decanted from 96 well micro titer plates after confluent sheet of cells were formed. The cell monolayer was washed twice with wash medium. Two-fold dilutions of tested sample were made in Roswell park memorial institute medium (RPMI) with 2% serum (maintenance medium). 0.1 ml of each dilution was tested in different wells leaving 3 wells as control, receiving only maintenance medium. The plate was incubated at 37°C and examined. Cells were checked for any physical signs of toxicity, e.g. partial or complete loss of the monolayer, rounding, shrinkage, or cell granulation. MTT solution was prepared (5mg/ml in PBS) (BIO BASIC CANADA INC). 20 µl MTT solutions were added to each well. Place on a shaking table, 150 rpm for 5 minutes, to thoroughly mix the MTT into the medium, incubate at 37°C and 5% CO_2 for 1-5 hours to allow the MTT to be metabolized. Dump off the media (dry plate on paper towels to remove residue if necessary). Resuspend formazan (MTT metabolic product) in 200 µl DMSO. Place on a shaking table, 150 rpm for 5 minutes, to thoroughly mix the formazan into the solvent. An ELISA reader (mindray mR-096A) set at a wavelength of 560 nm was used to determine survival ability of cell lines through MTT cleavage.

The optical density values were measured at 560 nm and used for calculation of the percentage cell death/survival. The percentage cell death/survival was calculated using the formula:

cell survival (%) = OD of treated cells/ OD of negative control cells ×100

The percentage cell death was calculated as 100 % cell survival.

Experiments were carried out in triplicates and the mean of the results was expressed with standard error (SE) using Microsoft Office Excel program 2016.

RESULTS AND DISCUSSION

Extraction of trichothecenes from the tested *Fusarium* strains

The extraction process of trichothecenes from the tested *Fusarium* strains resulted in different colored filtrates which varied in color among the strains (Table 2). After dryness of these filtrates, the trichothecenes were appeared in the form of white needle shaped or pink crystals in the bottom of the bakers. These dried crystals were solubilized in warm ethyl acetate at 37°C and separated to their components by TLC.

Screening for T-2 toxin producing *Fusarium* strain using thin layer chromatography (TLC)

Results of TLC revealed the ability of nine strains from the tested *Fusarium* strains to produce trichothecenes especially T-2 toxin. Figure 1 showed that *F. anthophylum, F. equiesti* (KM246255.1), *F. fujikuroi* (MG211161), *F. fujikuroi* (MG211162), *F. sporo-trichioides* (MG211163), *F. solani* (MG211159), *F. solani*

Table 2. Colors of tested *Fusarium* strains filtrates containing trichothecenes

Tested *Fusarium* strains	Filtrate color
F. anthophylum	colorless
F. brachygibbosum	white yellow
F. brachygibbosum	colorless
F. chlamydosporum	pale yellow
F.equiseti	colorless
F.equiseti	colorless
F. fujikuroi (MG211162)	Pink
F. fujikuroi (MG211161)	colorless
F. oxysporum	colorless
F. oxysporum	colorless
F. oxysporum	colorless
F. solani(MG211160)	colorless
F. solani(MG211159)	colorless
F. sporotrichioides(MG211163)	Brownish Yellow
F. subglutinans	colorless
F. verticillioides (XM_ 018895258.1)	Bluish pink
Fusarium sp.	Pale yellow

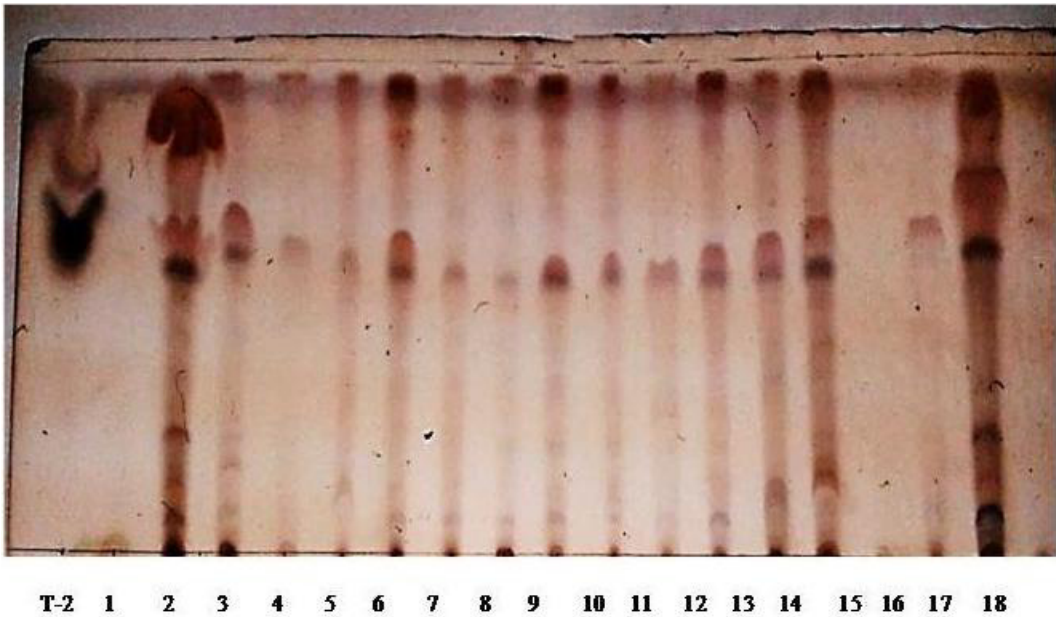

Figure 1. TLC of the extracts of tested *Fusarium* strains. Gray color bands indicated presence of T-2 toxin.

1-F. chlamydosporum; 2-F. fujikuroi (MG211162); 3-F. equiseti; 4-F.brachygibbosum; 5-F. oxysporum; 6-F. equiseti; 7-F. equiseti; 8-F. fujikuroi(MG211161); 9-F.subglutinans; 10- F. anthophylum; 11- F. oxysporum; 12- F. solani(MG211160); 13- F. sporotrichioides(MG211163); 14- F. verticillioides (XM_ 018895258.1); 15- F. oxysporum; 16- F. solani; 17- F. solani (MG211159); 18- F. brachygibbosum

(MG211160), *F. subglutinans* and *F. verticillioides* (XM_ 018895258.1) exhibited gray bands on TLC after spraying with 20 % H_2SO_4 which indicated the presence of T-2 toxin. Using of the TLC method in detection of T-2 toxin as a member of trichothecenes from some *Fusarium* species was also used and agreed studies of other researchers such as (Abbas et al., 1984; Nagaraja et al., 2016).

Quantitative analysis using HPTLC

The concentration of T-2 toxin in the resulted bands of TLC was detected by HPTLC as a quantification step. According to TLC results, nine *Fusarium* strains were selected to analyzed by HPTLC scanner, which characterized by both qualitative and quantitative detection by two factors; Rf and UV absorbance.

Table 3. HPTLC results including Rf, concentration of T-2 toxin (µg/100 mL filtrate) and identity (%) for the tested *Fusarium* strains

***Fusarium* strains**	**Rf**	**Concentration µg/100 mL filtrate**	**Identity (%)**
F. anthophylum	0.81	137.4	73
F. equiseti(KM246255.1)	0.94	27.7	85
F.fujikuroi (MG211161)	0.91	76.5	98
F.fujikuroi (MG211162)	0.92	18.3	97
F. sporotrichioides(MG211163)	0.94	29.8	93
F.solani (MG211159)	0.81	49.8	95
F.solani (MG211160)	0.78	136.3	72
F.subglutinans	0.95	17.8	67
F. verticillioides (XM_018895258.1)	0.87	10.8	98

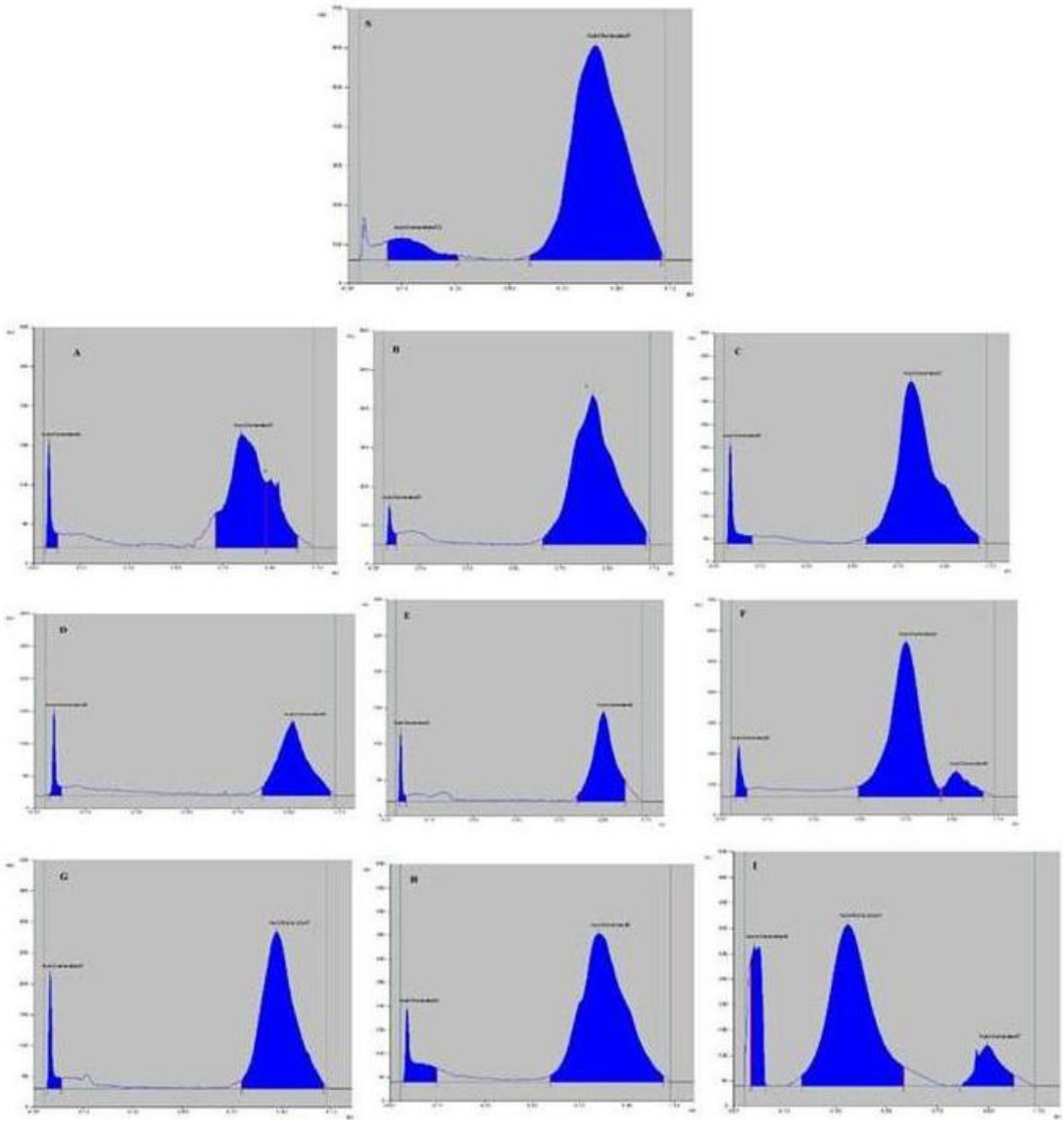

Figure 2. HPTLC chromatogram obtained using densitometry, of S: T-2 toxin standard, A-*F. solani* **(**MG211159**)**, B- *F. verticillioides* **(**XM_018895258.1**)**, C- *F.solani* (MG211160**)**, D - *F. sporotrichioides***(**MG211163**)**, E- *F. equiseti* (KM246255.1), F- *F. subglutinans*, G - *F. fujikuroi* **(**MG211161), H - *F. anthophylum* and I- *F. fujikuroi* **(**MG211162).

Figure 3. The growth of *F. solani* (MG211159) on rice grains at 20°C for 3 weeks.

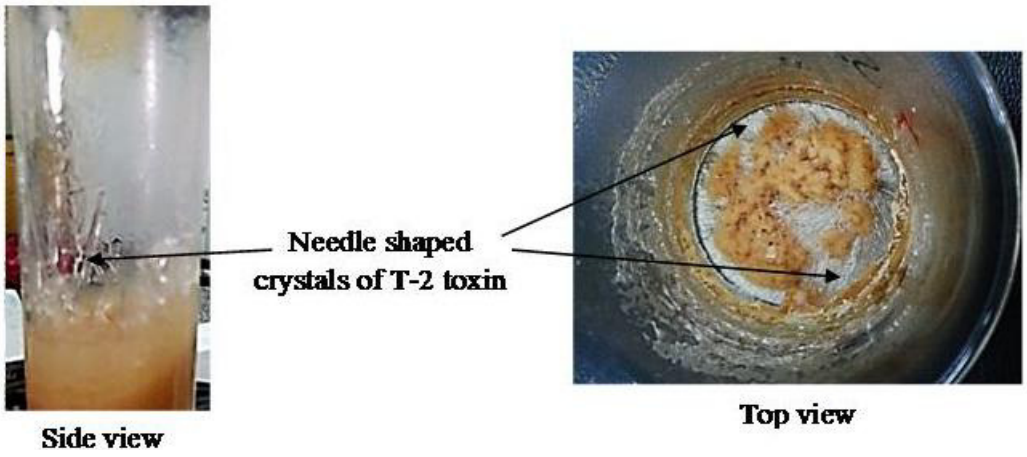

Figure 4. Appearance of needle shaped crystals of T-2 toxin in column chromatography.

The results of HPTLC were recorded in Table 3. The Rf value of standard T-2 toxin was 0.93 and UV absorbance was 600. Different values of Rf and UV absorbance were recorded by the samples extracted from the tested *Fusarium* strains. The high concentration of T-2 toxin was 137.4 and 136.3µ/100 mL filtrate of *F. anthophylum* and *F. solani* (MG211160) respectively. On the other hand, *F. verticillioides* (XM_018895258.1) was found to be the lowest producer of T-2 toxin where the concentration was 10.8µ/100 mL filtrate. Moreover, Table 3 and Figure 2 represented the identity between the T-2 toxin standard solution and the tested extracts. The HPTLC chromatogram (Figure 2) revealed that, the identity between the standard and extracts of *F. fujikuroi* (MG211161) and *F. verticillioides* (XM_018895258.1) recorded the highest value; 98(%) followed by *F. fujikuroi* (MG211162), *F. solani* (MG211159) and *F. sporotrichioides* (MG211163) which recorded 97, 95 and 93(%) respectively. The lower identity was investigated by *F. subglutinans* which recorded 67(%).

Mass production of trichothecenes including T-2 toxin

The massive growth of *F. solani* (MG211159) on rice grains for 3 weeks was represented in Figure 3. Trichothecenes were extracted from these cultures by (methanol-water) 50:50, v/v, then the T-2 toxin was separated by column chromatography. After completing the separation process, different fractions were appeared where the fraction containing the T-2 toxin was yellow in color and T-2 toxin was recognized as needle shaped colorless crystals (Figure 4). These results were compatible with (Bosch and Mirocha, 1992) who detected some mycotoxins including T-2 toxin when cultivated

Table 4. Optical density as a measure of cell death by T-2 toxin.

concentration	Fibroblast cell line			
	O.D ± SE	**Viability (%)**	**Toxicity (%)**	**IC_{50}%**
ng/ml	0.296 ± 0.006	100	0	ng/ml
5.60	0.297 ± 0.003	100.225	0	28.66
11.21	0.276 ± 0.004	93.356	6.644	
22.43	0.155 ± 0.008	52.477	47.523	
44.87	0.057 ± 0.004	19.144	80.856	
89.75	0.028 ± 0.003	9.572	90.428	
179.5	0.022 ± 0.002	7.545	92.455	

O.D ± SE: Optical density ± Standard Error

some *Fusarium* species isolated from stored sugar beets on rice grains.

Detection of cytotoxicity of T-2 toxin by MTT assay

The untreated culture of fibroblast cell line was adherent, normal, showing little variation in optical density values between 5.6 and 179.5 ng/ml. When fibroblast cell line was treated with different concentrations of the previously purified T-2 toxin (179.5, 89.5, 44.87, 22.3, 11.21 and 5.60 ng/ml) for 24h, the cell death percentage was 92.455, 90.428, 80.856, 47.523, 6.644 and 0% respectively. The percentage of cell viability percentage was 7.545, 9.572, 19.144, 52.477, 93.356, and 100.225 % respectively. These findings indicated that the safe nontoxic concentration on the tested cell line is 5.60ng/ml. The half maximal inhibitory concentration (IC_{50} %), which defined as the inhibitor concentration that decreases the biotransformation of a substrate at a single, specified concentration by 50%, was recorded 28.66 ng/ml of T-2 toxin.

Table 4 shows the optical density and cytotoxicity when the fibroblast cell line was treated with different concentrations of T-2 toxin. This experiment has shown that the degree of cell survival is dependent on concentration of toxin exposure for 24 h.T-2 toxin exhibited the most cytotoxic response against the cell line tested, resulting in a lack of cell survival at 24h exposure.

Many authors have recommended the usage of MTT assay as an accurate and a rapid tool for assaying the viability of various cell lines when exposed to mycotoxins (Hanelt et al., 1994; Kitabatake et al., 1993; Visconti et al., 1991; Widestrand et al., 2003). Also, our results are in accordance with (Berek et al., 2001) who demonstrated the immunosuppressive effects of T-2 toxin on blood mononuclear cells.

Apoptosis (Cell necrosis) was investigated microscopically (Figure 5) in fibroblast cell line. Control at Figure 5 appears as confluent monolayer cells which considered 0% cell death. The rate of cells apoptosis decreased with decreasing T-2 toxin concentration where it recorded 92.455% cell death at 179.5 ng/ml and 90.428% at 89.75 ng/ml. A distinct morphological feature

Figure 5. Fibroblast cell line exposed to T-2 toxin at different concentration. Magnified 100 times.

of apoptosis; the condensation and fragmentation of nuclear chromatin was observed under microscope. Some cell ultrastructure changes were also observed compared to the untreated control cells. These changes were summarized in the electron density in nucleus and cytoplasm of the cell line decreased to a different extent, swelling, vacuolar degeneration and increased density of mitochondria was also observed after exposure cell line to T-2 toxin. These results agreed (Zhuang et al., 2013) who reported that,T-2 toxin induces the apoptosis in mammalian cells.

CONCLUSIONS

In the present study some Egyptian *Fusarium* strains were tested for production of trichothecenes mycotoxins especially T-2 toxin. Nine *Fusarium* strains namely *F. anthophylum, F. equiseti* (KM246255.1), *F. fujikuroi* (MG211161), *F. fujikuroi* (MG211162), *F. sporotrichioides* (MG211163), *F. solani* (MG211159), *F. solani* (MG211160), *F. subglutinans* and *F. verticillioides* (XM_018895258.1)were found to be producers of T-2 toxin using TLC method. Then, the quantity of the produced T-2 toxin from each strain was detected by HPTLC where *F. fujikuroi* (MG211161), *F. fujikuroi* (MG211162) and *F. solani* (MG211159) recorded the highest identity compared with the standard T-2 toxin. Thereafter, pure T-2 toxin was separated from extract of *F. solani* (MG211159) strain as needle shaped crystals using column chromatography. After that, the cytotoxicity of pure T-2 toxin on fibroblast cell line revealed that, the safe nontoxic concentration was 5.60 ng/ml.

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