Extraction and identification of trichothecene (T-2) toxin from *Fusarium solni*

Bagher Hussein Abd Alhamza[1] , Qassim Haleem Kshash[1] , Sadyia Yasser Offi[2]
University of Al-Qadissiya- Veterinary Medicine College[1], Biotechnilogy College[2]

Abstract

The present study was undertaken with on objective to appraisal the effectiveness of trichothecene (T-2 toxin) on goats, which is mycotoxin produce by *Fusarium solni* by in plantation of corn grain on malachite green agar and the fungal growth was sub cultured on potato dextrose agar to isolate *F. solani* that inoculated in to specific media (Medium A) to produce T-2 toxin that finally purified and estimated their concentration by ELISA technique .

Fusarium solni is a mould appear macroscopically as pale-brown to brown cottony mycelium and microscopically had (3-5) septate hyphae that are borne on short conidiophores which simple branched monophialides . Macroconidia are moderately curved, stout, thick-walled .Long monophialides are bearing microconidia .

The toxin production with specific detectable exam by Elisa which was revealed colorless, crystalline substrate had dissolvability in the solvent of moderate polarity such as chloroform and acetone and insoluble in water ,had a peppery odor and melting point (151-152°C), and this could be turned into a powder by lypholization .

Keywords: Trichothecene ,T2,Toxin , *Fusarium solni*

استخلاص وتوصيف ذيفان الترايكوثيسين (T2) من عفن *Fusarium solani*

باقر عبدالحسين حمزة[١] ، قاسم حليم كشاش[١] ، سعدية ياسر عوفي [٢]

جامعة القادسية –كلية الطب البيطري[١] ،كلية التقانات الاحيائية[٢]

الخلاصة:

إجريت الدراسة بهدف عزل وتنقية وتوصيف ذيفان الترايكوثيسين (T2) والذي يفرزه فطر *Fusarium solani* بواسطة زراعة حبوب الذرة على وسط المليكايت الأخضر وبعد نموالفطر اخذت منة عزلة وزرعت على (PDA)ثم اخذت عزلة السولاني ووضعت في وسط خاص (Medium A) لإنتاج السم (T2) الذي تمت تنقيته وبعد ذلك قدرتركيزة من خلال تقنية الاليزا.

والفيوزيرم سولاني هو عفن يمتلك تشعبات قطنية بنية اللون الى بنية شاحبة على الاوساط الزرعية الانتخابية ومجهريا ذو خيوط مقطعة ب (٣-٥) حواجز . Macroconidia موجودة ومقوسة بشكل معتدل، قوي البنية، سميكة الجدران، وتحمل على monophialides

يتميز الذيفان بكونه مادة كريستلانية عديمة اللون شفافة ولاتذوب بالماء ولكنها سريعة الذوبان بالكلوروفورم والاسيتون ،يمتلك رائحة قوية تشبه رائحة الفلفل الحار، درجة انصهاره تتروح مابين (١٥١ - ١٥٢) درجة مئوية.

Introduction:

Trichothecenes are chemically belonged to very large family of mycotoxins produced by diffrents species of *Fusarium, Myrothecium*, *Cephalosporium, Verticimonosporium*, *Trichoderma, Trichothecium,* and *Stachybotrys.* Trichothecenes are produced on many grains like wheat, oats or maize by various Fusarium species such as *F. solani , F. sporotrichioides, F. poae and F. equiseti. Trichothecenes* belong to sesquiterpene compounds. The structural features causing the biological activities of trichothecenes are: the 12,13-epoxy ring, the presence of

hydroxyl or acetyl groups at appropriate positions on the trichothecene nucleus and the structure and position of the side-chain (1).

These substances could be present as contaminants from moulds to foodstuffs or in livestock feeds and the symptoms may appear among exposed humans or animals. The developing effects of toxin usually following exposure depends on such variables as: toxin type ,purity, dose, and duration of exposure (2).

Materials and methods:

Isolates of fusarium:

Corn grains collection:

The local corn grains randomly collected from different places of grain stores in the province of Najaf and the equivalent of 500 grams of corn from each stores was collected and after mixing ,the corn grains used in fungal isolation.

Fungus isolation :

Corn placed in a sterile flask contain the 1% sodium chloride solution for two minutes after it has been cleaned and impurities from the dust and washed several time, after that washed with distill water to get rid of chlorine and placed on filter paper until then dried, then planted on the malachite green agar in table (3-4) as 5 pills of corn on each Petri dish and incubated (28 C°) and monitoring to (7-10) days (3).

Purification of fungi:

After the growth of fungus on the center of the malachite green agar made sub culture on PDA (potato dextrose agar) to obtain pure isolates and then placed in the incubator for 7 day in (27-28) C° with light for 6 hours\ day for the purpose of the appearance of pigments for each type of *Fuasirum spp*.(3) .

Diagnosis of fungi:

After sub culturing, the fungal growth diagnosed depending on the colony characteristics and microcharacteristics in microscopic and then took *F.solani* isolated and neglected the rest according to(4).

Production of toxin:

The fungus*(F.solani)* was developing in a beaker containing medium A Its components as in the table (3-6) and put the mixture in a Shaker incubator in 27 C◦ for five days and then the resulting liquid placed in test tubes and storage to identify poison (5).

Extraction of T2-toxin:

The sample was placed in a high speed blander for two minutes and then the mixture was filtering for extracting the crude toxin with Filter paper (Whatman No.1) and get the filtrate, which has become ready for testing (6)

Estimate the amount of trichothecene by ELISA:

Examination was conducted according to(7) and the company instructions.

Results:

***Fusarium solani* Isolation:**

Fusarium solani was identified based on macroscopic and microscopic characterization of the isolates:

Macroscopic morphological result:

After made sub culture on PDA for 7 days at (27-28) °C with light for 6 hours \day , colonies growing rapidly forming white aerial mycelium that generally take light-dark cottony appearance, as well as dark brown center of colonies which them gave the agar variant pigmentation from pale brown –yellowish brown in color .

Microscopic results:

Hyalinic septate hyphae beared on short conidiophores phae are septate and hyaline. Conidiophores are simple branched monophialides . Macroconidia are moderately curved, stout, thick-walled, 3-5 septate,

and are borne on short conidiophores that soon form sporodochia. Microconidia are borne from long monophialides

T2-toxin extraction:

After the processing of toxin production with specific detectable exam by Elisa which was revealed colorless, crystalline substrate had dissolvability in the solvent of moderate polarity such as chloroform and acetone and insoluble in water ,had a peppery odor and their chemical structure is $C_{24}H_{34}O_9$, melting point (151-152°C), and this could be turned into a powder by lypholization .

Discussion:

Isolate and extract of T2 toxin:

The results show that the macroscopic morphology of *Fusarium solani* that growth on PDA appeared as white woolly-cottony colonies with white aerial mycelium which in agreement with (8) and (9) , and the microscopic observation results were agreed to (10) as feature of conidiogenous cell with long branched monophialides were observed in end single long monophialides as also mention by(11) , Macroconidia usually moderately curved, with short, blunt apical and indistinctly pedicel late basal cells, mostly 3-5 septate which agreement with (5).

The production of T2-toxin by using the media A as similar technique that applicator by (12) that liquid way is one of the important ways in the production of T2-toxin and also agreed with (13) that placed

isolation in liquid culture were grown in Erlenmeyer flask volume of medium equal to one half the volume of the flask for 5-7 day at 28 C° on shaker operating at 180-200pm and thus is the easiest and velocities to production trichothecene (T2-toxin) in addition to remind (7) , the enzyme-linked immunosorbent assay (ELISA) was described by (14) and also confirmed by (15) and confirmed by (16) as the most sensitive methods had been developed for rapid screening purposes and could be used for quantification.

The features of extracted T2-toxin in the present study is in similar that described by (17) as it a mycotoxin that resultant from a secondary metabolite of filamentous fungi, which is low-molecular-weight and highly stable in natural conditions.

References:

1-Etzel, RA. (2002). Mycotoxins. J.American Medical Association 287 (4): 425–427.

2-Dohnal, V.; Jezkova, A.; Jun, D.and Kuca, K. (2006).Metabolic pathways of T-2 toxin. Current Drug Metabolism, 9 (1): 77–82.

3-Lenc , PK.; Vairamuthu, S.; Balachandran, C.; Manohar, BM. and Raj, GD.(2008).Induction of apoptosis by fungal culture materials containing cyclopiazonic acid and T-2 toxin .Mycopathologia ,159:393-400.

4-Leslie , S. and Summerell , C. (2006).Recent advances in mycotoxin determination in food and feed by hyphenated chromatographic techniques mass spectrometry. Mass Spectrum Rev. 25:54–76.

5-Ueno, Y.; Ishii,K.; Sakai, K.; Kanaeda,S. and Tsunoda, H. (1972). Toxicological approaches to the metabolites of Fusaria. IV. Microbial survey on "bean-hulls poisoning of horses" with the isolation of toxic trichothecenes, neosolaniol and T-2 toxin of *Fusariumsolani*M-1-1. Japanese J. Exp. Med., 42: 187-203.

6-Ueno, Y.; Sato,N.; Ishii,N.; Sakai,K.;Tsunoda,H. and Enomoto,M.(1970). Biological and chemical detection of trichothecenemycotoxins of *Fusarium* species. Appl.Microbiol J., 25:699-704.

7-Simth ,RP. and Moss , YW.(1985).Trichothecenes. In: Sharma,RP.and Salunkhe, DK.(Eds.). Mycotoxins and Phytoalexins. Boca Raton, Fla: CRC Press ,P:339–359.

8-Kornerup, A.andWancher, JH.(1978). Handbook ofMycotic Atlas Colour. 3rd ed. Eyre Methuen Ltd, London.Methuen.

9-Nelson, P. E.; Toussoun, T. A. and Marasas, W. F. O. (1983). *Fusarium* species. An illustrated manual for identification. Pennsylvania State University Press, University Park, PA.

10-Sutton, D A.; Fothergill, A W. and Rinaldi M. G.(1998). Guide to Clinically Significant Fungi, 1st ed. Williams and Wilkins, Baltimore.P:989-93.

11-Burgess,LW.;Summerell, BA.; Bullock, S. ;Gott,KP.and Backhouse, D.(1994).Laboratory Manual for *Fusarium* Research. 3rded. University of Sydney and Botanic Garden; Sydney, Australia: P:432-455.

12-Marian, N.and Beremand ,AS.(1987).Beremand microbiology isolation and characterization of mutants blocked in toxin biosynthesis and Applied Environm. Microbial. American Society Journal,53(8):12-19.

13-Beremand,M.N.;Black,P.J.and Platlner,S .(1988).Isolation and characterization of mutants fungus in T2 toxin biosynthesis. J.Cell Biochem. 12:261

14-Chung, Y.J.; Jarvis, B.;Heekyung, T. and Pestka. J.(2003). Immunochemical assay for satratoxin G and other macrocyclic trichothecenes associated with indoor air contamination by *Stachybotryschartarum*. Toxicol. Mech. Methods, (13):247-252.

15-Chu, FS.(1991).Development and use of immunoassays in detection of the ecologically important mycotoxins. In Bhatnagar, D., Lillehoj,EB., Arora, DK. (Eds), Handbook of applied mycology, New York/Basel: Marcel Dekker, 87–136.

16-Shim, W.; Kolosova, A.Y.; Kim, Y.; Yang, Z.; Park, S.; Eremin, S.A.; Lee, I. and Chung, D.(2004). Fluorescence polarization immunoassay based on a monoclonal antibody for the detection of ochratoxin A. International Journal of Food Science and Technology, 39: 829-837.

17-Fink , HP.(1999). Mycotoxins: Regulations, quality assurance and reference .Nature J.,12: 321-330.