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Isolation of some toxigenic fungi from sugarcane juice

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Abstract:

Sugarcane juice is considered the most popular fresh juice in Egypt, with cane juice shops spreading through all the Egyptian cities. Sugarcane juice contains 75 - 85% water and 10 - 21% sucrose. Sugarcane is a suitable host for many saprophytic fungi. No or little information has been reported on fungal flora and their toxins associated sugarcane juice in Egypt so, This study aimed to investigate the natural occurrence of toxigenic fungi associated sugarcane juice. Randomized of seven sugarcane juice samples were collected from seven different localities (Places) in Kalubya Governorate (Banha, Tukh and Shibin El Quanater). Fungal flora were isolated by serial dilution technique. The obtained data resulted that, isolation from seven different sugarcane juice localities yielded 322 fungal isolates. Higher total fungal count was recorded with location 6 sample. Identification indicated that, five fungal genera belonging to eight species were identified. These are *Alternaria alternata*, *Aspergillus* spp., (*Aspergillus niger*, *A. flavus* & *A. parasiticus*), *Fusarium* spp. (*Fusarium solani* & *F. oxysporum*), *Penicillium* sp. and *Rhizopus stolonifer*. *Aspergillus* species were the highest frequently present in sugarcane juice and *Aspergillus niger* was higher fungal frequency occurred. According to HPLC data, tested of mycotoxins production presented, Four isolates of *Aspergillus parasiticus* and two isolates of *A. flavus* were aflatoxins (Afs) producers. Higher aflatoxin quantity (2.91ng/mL) was produced by *A. parasiticus* (isolate No. 20) from location two of sugarcane juice samples. Whereas, four isolates of *Aspergillus niger* isolates were positive producer of Ochratoxin A (OTA). Higher Ochratoxin A (OTA) production was recorded with *A. niger*, isolate No. 25 isolated from location one which gave 2.03 ng/ml of Ochratoxin A (OTA). All *Fusarium* spp. isolates were negative producer any toxins.

Key words: Sugarcane juice, *Aspergillus*, Aflatoxin (AFs), Ochratoxin A (OTA), HPLC.

1. Introduction

Sugarcane, (*Saccharum Officinarum* L., family Poaceae), plant has a high sucrose and low fiber content. It produces over 60% of the world's total sugar requirement, while sugar beet provides the remaining 40% **(Ojo *et al.*, 2014).** Sugarcane juice is considered one of the most popular fresh juice in Egypt, with cane juice shops spreading through all the Egyptian cities **Ahmed, *et al.*, 2010 and Abbas, *et al.*, 2014.** Sugarcane juice is the first material used for the production of table sugar and other various products, such as raw sugar/brown sugar, jaggery (traditional, concentrated sugarcane juice), and molasses **(Singh, *et al.*, 2015).** Sugarcane bagasse is used as a fuel source in sugarcane mill furnaces. Other industrial purposes for bagasse includes alcohol production and papermaking. Apart from its sweet taste and being a source of energy and minerals, sugarcane juice consumption, in traditional medicine, helps in the treatment of many diseases such as jaundice, kidney stones, urogenital tract infections, and in lowering blood pressure, and healing dermal wounds; it is also reported as a natural antioxidant under various experimental conditions **(Abbas, *et al.*, 2014 and Mohamed, *et al.* 2016).** Sugarcane juice is used in holistic medicine **(Nisha, *et al.*, 2017).** Sugarcane juice is used as a diuretic, for hiccup relief, laxative, coolant, demulcent, and antiseptic. Sugarcane juice has also been recommended in ayurvedic medicine for patients suffering from low blood pressure, gastrointestinal issues, and jaundice in Indian Ayurveda. In Cambodia, sugarcane juice is an integral component of medicines used to treat ulcers of the skin and mucous membranes. Aliphatic alcohols and long chain aliphatic fatty acids, commonly isolated from sugarcane wax, are pharmacologically active substances used for their anti-inflammatory, anti-hypercholesterolemic, and anti-thrombotic effects **(Singh, *et al.*, 2015).** On the other hand, Sugarcane juice contains 75 - 85% water, 10 - 21% sucrose, 10 - 15% fiber, 0.3 - 3% reducing sugars (glucose and fructose), and other inorganic compounds **(Nisha, *et al.*, 2017).** Sugarcane is a suitable host for many saprophytic fungi, especially the aflatoxigenic ones that belong to the *Aspergillus* species **(Kumeda, *et al.*, 2003).** Sugar present in the stem of *Saccharum officinarum* represents the main source for fungal growth. **(Girei and Giroh 2012).** Sugarcane are frequently contaminated simultaneously by several moulds which are each able to produce several toxins. Microbes can adhere to surface, invade/penetrate sugarcane and multiply within the products. Mostly the sugarcane juice hawkers are situated at the road side so the number of pollutants and microorganisms in the air may add to the juice. *Aspergillus* species were also frequently present in drinking water as well as in juice samples **(Yusof *et al.*, 2000; Anaissie *et al.*, 2002 and Nazim *et al.*, 2008).** Some species of fungi are notorious for producing toxins/toxic metabolites termed as

mycotoxins **(Brown, *et al.*, 2021).** Approximately more than 400 different types of mycotoxins have been identified worldwide **(Aydın, *et al.*, 2015).** Both *A. parasiticus* and *A. flavus* were isolated from sugarcane in connection with aflatoxin contamination of raw sugar in Japan **(Kumeda, *et al.*, 2003 and Nicholas, 2013).** The contamination of sugarcane juice with several mycoflora including *Aspergillus flavus*, *A. fumigatus*, and *A. niger* **(Takahasho *et al.*, 2004 and Ahmed *et al.* 2010),** while investigated the natural aflatoxin (B1 , B2 ,G1 ,and G2)uptake by sugarcane from contaminated soil and its persistence in sugarcane juice and jiggery (the natural sweetener made by concentrating the sugarcane juice) using thin layer chromatography and ELISA **(Mohamed, *et al.* 2016).** No or little information has been reported on fungal flora and their toxins associated sugarcane juice in Egypt so, This study aimed to investigate the natural occurrence of toxigenic fungi associated sugarcane juice which is selling at different places for human consumption in Egypt, isolate a wide variety of fungal species associated sugarcane juice samples, tested of mycotoxin production with these mold fungi by using HPLC chromatography.

Material and Methods

1-Samples collection: Randomized of seven sugarcane juice samples were collected from seven different localities (different Places) in Kalubya Governorate (Banha, Tukh and Shibin El Quanater) **Figs (1).**

Fig. (1): External and internal symptoms of healthy and natural diseased sugarcane plants before juiced

2-Mycological analysis:-

2. a- Fungal flora were isolated by serial dilution technique. For Isolation and purification the fungal flora association, one ml of each sub-sample (sugarcane juice samples) were transferred to 9 ml of sterile water in a sterilized bottle to give a dilution of 1:10. The suspension obtained was considered as the stock suspension. Subsequently, successive dilutions were made from that sample; 1 ml of suspension was transferred to a sterilized tube containing 9 ml of sterile water to dilute 1:100 (10^{-2}), (10^{-3}) and mixed well. This step was repeated to give 10^{-3}, and 10^{-4} dilutions. To

estimate the mold population, for this purpose, 0.1 mL of each dilution was transferred to sterilized Petri dishes, 3\Petri\dishes were inoculated as replicates. The Petri plates were then incubated at 26±2°C for five days. After incubated dishes, all devolving fungi were purified on plates of PDA medium (with traces of Streptomycin sulfate) using the hyphal tip or single spore techniques. The colonies were counted then the results were expressed in colony-forming units (CFU). Pure cultures of growing fungi were maintained in test tube slants containing PDA medium**.**

2. b- Fungal identification: Pure cultures seven days old were identified at the genus or species level according to the cultural and morphological characters with the help of available literature found principally in publications by **Raper and Fennell (1965)** for the genus *Aspergillus*, **Booth, (1977).** for the genus *Fusarium* and **Barnett, and Hunter, (1977)** for the genera of imperfect fungi. The frequency of identified fungal species was calculated according to the number of isolates of a genus or species/total number of fungal isolates x 100.

3-Testing of mycotoxin production: All isolates of toxigenic fungi ie (*Asperigullus spp* and *fusarium sp*) were propagated as a pure culture in 100 ml yeast extract sucrose (YES) to be tested for mycoxin production ie Aflatoxins ,and fuminsin B1 and Ochratoxin A. Each flask was inoculated with 0.1 ml spore suspension containing approximately 10^5 spores/ml. Cultures were incubated at 26±2^{o}C for 14 days. Then tested for mycoxin production by using High-Performance Liquid Chromatography (HPLC) according to the methods of **AOAC (2007).** Also, mycotoxins contents were determined by using HPLC according to the methods of **AOAC (2007).** The AOAC official method for ochratoxin A (OTA) determination by HPLC according to **Visconti *et al.* (2001) and Visconti, *et al.* (2008).**

3. Results and Discussion

1-Total fungal count: Data presented that, total fungal count isolated from seven different sugarcane juice Localities (Samples) yielded 322 fungal isolates as shown in **Table (1) and Figs (2).** Also, data indicated that, Higher fungal isolates was recorded with location three samples which record 119 fungal isolates equal 36.96% followed by location 4 sample with 88 fungal isolates equal 27.33% , location 2 and location 6 samples gives 26 fungal isolates equal 8.07% , location 5 sample record 25 fungal isolates equal 7.76% and location one sample gave 21 fungal isolates equal 6.52. Whereas, location 7 sample was less fungal isolates which record only 17 fungal isolates equal 5.28%. Similar results were obtained by **Kumeda, *et al.*, (2003)** who reported that, sugarcane is a suitable host for many saprophytic fungi**.**

Table (1): Total fungal count isolated from seven different Localities

Dilutions	T. c. /%	L = Localities (Samples)							Total
(10^{-2})		L(1)	L(2)	L(3)	L(4)	L(5)	L(6)	L(7)	
	T. c.	2	2	4	5	5	5	2	25
	%	0.62	0.62	1.24	1.55	1.55	1.55	0.62	7.76
(10^{-3})	T. c.	11	10	10	7	2	2	7	49
	%	3.42	3.11	3.11	2.17	0.62	0.62	2.17	15.22
(10^{-4})	T. c.	8	14	105	76	18	19	8	248
	%	2.48	4.35	32.61	23.60	5.59	5.90	2.48	77.02
Total	T. c.	21	26	119	88	25	26	17	322
%	%	6.52	8.07	36.96	27.33	7.76	8.07	5.28	100.00

Identification: Identification indicated that, eight species belonging to five fungal genera were identified. These are *Alternaria alternata*, *Aspergillus* spp., (*Aspergillus niger*, *A. flavus* & *A. parasiticus*), *Fusarium* spp. (*Fusarium solani* & *F. oxysporum*), *Penicillium* sp. and *Rhizopus stolonifer* as shown in **Figs. (2)& (3).** These results were confirmed by **Ahmed, *et al.*, (2010) who** isolated 18 different species belonging to 11 different genera of fungi which isolated by direct plating method of sugarcane juice were *Absidia corymbifera, Acremonium* sp., *Aspergillus flavus, A. fumigatus, A. niger, A. sulphureus, A. terreus, A. wentii, Fusarium semitectum, F. sporotrichoides, Humicola grisea, Gilmanieila humicola, Curvularia lunata, Monilia* sp., *Rhizopus stolonifer*, *R. oryzae, Penicillium* sp., and yeast (*Saccharomyces* spp.) whereas *Aspergillus candidus, A. subolivaceous, A. erythrocephalus* and *A. tamarii* were isolated in addition to these by serial dilution techniques. The highest number of fungi were isolated by serial dilution technique and *A. niger* appeared as a dominant fungus of sugarcane juice with and without lemon by both of the techniques. The addition of lemon juice reduced the occurrence of *A. corymbifera, C. lunata* and *A. erythrocephalus* by serial dilution technique.

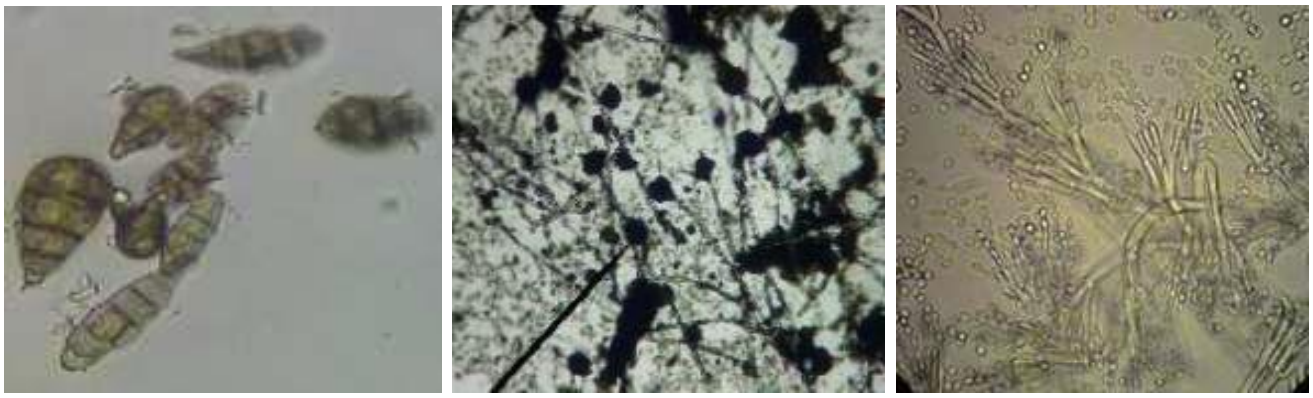

Fig. (2). *Alternaria alternata*, *Aspergillus niger* and *Penicillium* sp. (40x)

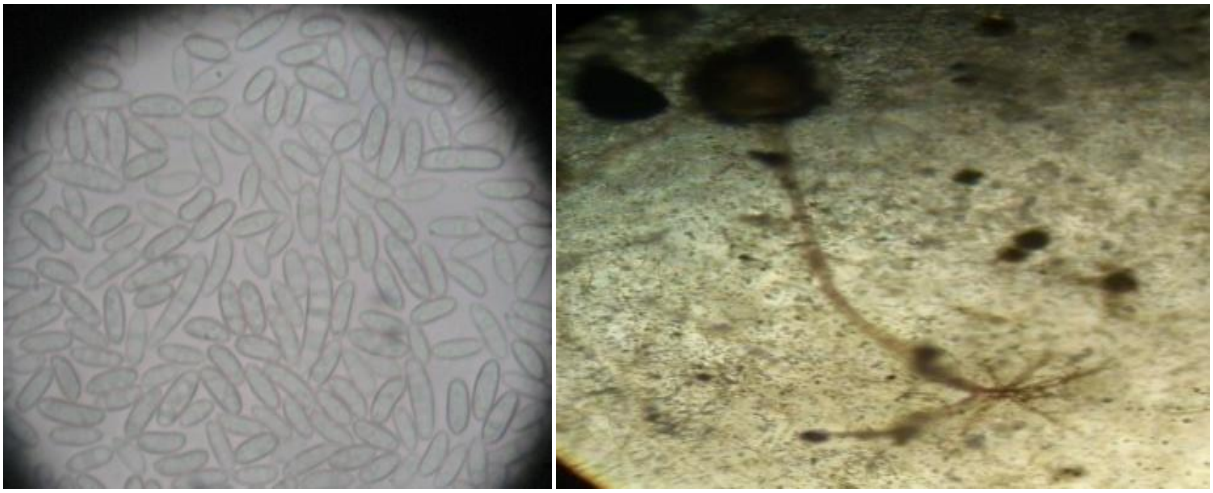

Fig. (3). *Fusarium* sp. (40x) *Rhizopus stolonifer* (20x)

Percentage of fungal frequency occurred: Percentage of fungal frequency occurred were tabulated in **Table (2).** Data in this table indicated that, *Aspergillus* species were highest frequently present in sugarcane juice and *Aspergillus niger* was the most fungal frequency occurred which record 99 fungal isolates equal 30.80% followed by *Fusarium solani* which gave 67 fungal isolates equal 20.80%, *Penicillium* sp. with 58 isolates equal 18.00%, *Rhizopus stolonifer* 50 fungal isolates (15.60%), *Aspergillus flavus* 20 isolates (6.20%), and each of *Alternaria alternate* and *Aspergillus parasiticus* which gives 12 fungal isolates equal 3.70%. *Fusarium oxysporium* was less fungal frequency occurred which record only four fungal isolates equal 1.20%. Similar results were obtained by **Yusof *et al.*, (2000); Anaissie *et al.*, (2001) and Nazim *et al.*, (2008)** found that, *Aspergillus* species were frequently present in sugarcane juice as well as in drinking water samples. **Takahasho *et al.*, (2004)** screened the contamination of sugarcane juice sold in Pakistan with several mycoflora including *Aspergillus flavus*, *A. fumigatus*, and *A. niger*. These results were nearly similar to results obtained by **Ahmed *et al.* (2010)** who isolated 18 different species belonging to 11 different genera of fungi (*Aspergillus flavus, A. niger, A. terreus, A. fumigatus, A. wentii, A. sulphureus, Absidia corymbifera, Acremonium* sp., *F. sporotrichoides, Fusarium semitectum, Curvularia lunata, Monilia* sp., *Rhizopus stolonifer, R. oryzae, Penicillium* sp., and *Saccharomyces* spp.) from sugarcane juice**.** *Aspergillus niger* was the highest number of fungi isolated by serial dilution technique from sugarcane juice.

Garber, (2013) reported that the infection of sugarcane stems by *Aspergillus parasiticus* ranged from 95% in billets prepared for commercial planting to 52% in hand-collected sugarcane stems**. Romao-Dumaresq *et al.* (2016)** isolated *Aspergillus, Alternaria, Acremonium, Penicillium, Fusarium,*

Chaetomium, Curvularia, and *Mucor* from root and rhizosphere of sugarcane plant**. Silva *et al*. (2019)** identified *Aspergillus parasiticus* as the main species isolated from the sugarcane system. **Youssef, *et al.*, (2021)** reported that twenty-five species and four species varieties belonging to 8 genera were isolated from 30 sugarcane bagasse samples. Aspergillus was the most common genus, occurring in (100% of the samples, 88.3% of the total count of fungi), in which, *Aspergillus flavus, A. niger, A. tubengensis,* and *A. phoenicis* were the most dominant species and collected in moderate frequencies of occurrence, while *Acremonium, Fusarium, Curvularia, Penicillium, Mucor* and *Verticillium* were collected and identified in rare frequencies of occurrence**.** Also, **Younos and Embaby (2023)** isolated 219 fungal isolates belonging to 6 fungal species from sugarcane juicebelonging to *A. alternate, A. flavus, A. niger, A. parasiticus, Fusarium* spp., and *Penicillium* spp. *Penicillium* spp. had the most fungal frequency contaminated sugarcane juice which recorded 37.90% followed by *Fusarium* spp. (29.51%), *A. parasiticus* (22.83%), *A. niger* (12.79%), *Fusarium* spp. (10.50%) and *A. flavus* (8.22%). A. alternate has less fungal frequency (7.76%).

Table (2): Percentage of fungal frequency associated of tested sugarcane juice samples

Fungi	T. c. /%	Localities (Samples)							Total
		L(1)	L(2)	L(3)	L(4)	L(5)	L(6)	L(7)	
Alternaria alternate	T. c.	0	0	0	2	3	6	1	12
	% Fr.	0.00	0.00	0.00	0.62	0.93	0.06	0.31	3.70
Aspergillus niger	T. c.	10	10	2	20	15	17	25	99
	% Fr.	3.10	3.10	0.62	6.2	4.65	5.27	7.76	30.80
Aspergillus flavus	T. c.	4	3	0	4	3	2	4	20
	% Fr.	1.2	0.93	0.00	1.2	0.93	0.62	1.2	6.20
A. parasiticus	T. c.	3	1	1	2	1	3	1	12
	% Fr.	0.93	0.31	0.31	0.62	0.31	0.93	0.31	3.70
Fusarium solani	T. c.	0	7	11	18	13	12	6	67
	% Fr.	0.00	2.17	3.41	5.59	4.03	3.7	0.06	20.80
F. oxysporium	T. c.	0	0	4	0	0	0	0	4
	% Fr.	0.00	0.00	1.2	0.00	0.00	0.00	0.00	1.20
Penicillium sp.	T. c.	0	4	12	2	24	10	6	58
	% Fr.	0.00	1.2	3.7	0.62	7.45	3.10	0.06	18.00
Rhizopus stolonifer	T. c.	0	18	1	10	0	10	11	50
	% Fr.	0.00	5.59	0.31	3.10	0.00	3.10	3.41	15.60
Total	T. c.	14	47	30	58	59	60	54	322
	% Fr.	4.30	14.60	9.30	18.10	18.30	18.60	16.80	100.0

T. c = Total count Fr.% = Frequency percent

Mycotoxins production: Mycotoxins are secondary metabolites produced by a variety of fungal species that colonize different crops around the world. More than 400 mycotoxins have been described, but only a few have relevance as food contaminants **(Buszewska-Forajta, 2020).** The International Agency for Research on Cancer (IARC) has classified aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2, AFM_1, and AFM_2) in Group 1 as carcinogenic to humans **(IARC. 2012).** Ochratoxin is a mycotoxin produced by several species of the genera *Aspergillus* and *Penicillium*. The International Agency for Research on Cancer (IARC) classified OTA as possibly carcinogenic to humans, in group 2B (**Varga, *et al.*, 2015).**

In this study, all toxigenic fungi i. e. *Aspergillus* spp., *Fusarium* sp., which isolated from sugarcane juice samples were tested for mycotoxins production. Data in **Table (3)** indicated that, positive reaction of aflatoxin producer were recorded with some samples collected from 1, 2, 3, 4, 5 and 7 localities whereas, only samples collected from location 6 were negative of aflatoxin producer. All *Fusarium* sp isolated from sugarcane juice samples were negative reaction when tested for Fumonisin (FB_1) production. On the other hand, positive reaction for Ochratoxin A (OTA) production were recorded with 1, 3, 4 and 5 Localities (Samples) whereas, samples collected from localities 2, 6 and 7 were negative reaction. According to **Kumeda, *et al.*, (2003)** who reported that, sugarcane is a suitable host for many saprophytic fungi, especially the aflatoxigenic ones that belong to the *Aspergillus* species**.** Also, **Kumeda, *et al.*, (2003); Takahashi, *et al.*, (1999) and Nicholas, (2013)** isolated both *A. parasiticus* and *A. flavus* from sugarcane in connection with aflatoxin contamination of raw sugar**. Takahashi *et al.*, (2004)** reported that, the occurrence of mold from sugarcane and sugarcane juice are also common. The distribution of *Aspergillus flavus* and *A. parasiticus* in sugarcane field soils and on harvested sugarcane stems. It was found that aflatoxin production were 89% in 146 of 164 and of all the isolates 69% were *A. flavus* isolates. Aflatoxin G was produced by 40 % of *A. flavus* isolates. **Garber, (2013)** found that, aflatoxin-producing fungi infecting sugarcane stems ranged from 52 - 95% *A. parasiticus* in hand-collected samples and billets for commercial planting, respectively. **Nicholas, (2013)** reported that, aflatoxin-producing fungi infecting sugarcane stems ranged from 52 - 95% *A. parasiticus* in hand-collected samples and billets for commercial planting, respectively**.** Whereas, **Visconti, *et al.* (2008)** reported that, Ochratoxin A (OTA) producing black aspergilli include principally *Aspergillus carbonarius*, followed by *A. niger* and possibly *A. tubingensis***.** Also, **Nielsen, *et al.*, (2009) and Noorabadi, *et al.*, (2020)** reported that, Some species of the section Nigri have been reported as producers of mycotoxins, such as ochratoxin (OTA) and fumonisin (FB2), which can thus affect the safety of sugarcane and its related biotechnological products.

Table (3): Reaction of mycotoxins production i. e. Aflatoxin (AFs), and Ochratoxin A(OTA) production by representative strains of toxigenic species

Mycotoxins tested	Location (Samples)						
	L(1)	L(2)	L(3)	L(4)	L(5)	L(6)	L(7)
Aflatoxin (AFs)	15	20	2	51	57	ND	89
Fumonisin (FB_1)	ND	ND	ND	ND	ND	ND	ND
Ochratoxin A(OTA)	25	ND	31	35	39	ND	ND

L = Location ND =Not detected

4- Quantities of Aflatoxin (Afs) production: According to HPLC data, Determination of Aflatoxin (Afs) production were tabulated in **Table (4) and Figs. (4-10).** Data presented that, *Aspergillus flavus*, isolate No. 57 which isolated from location 5 was found to produce 0.30ng/ml of Aflatoxin (Afs) belonging to 0.01, 0.02 and 0.27 of AFB_1, AFG_1 and AFG_2 respectively, whereas, *Aspergillus flavus*, isolate No.89 which isolated from location 7 was found to produce 0.15 ng/ml of Aflatoxin (Afs) belonging to 0.05 and 0.10 of $AFB_1$and AFG_1respectively. On the other hand, *A. parasiticus* isolate No.51 which isolated from location 4 was found to produce 0.76ng/ml of Aflatoxin (Afs) belonging to0.02 , 0.09 , 0.53 and 0.12 of AFB_1, AFB_2 , AFG_1 and AFG_2 respectively, whereas, *A. parasiticus*, isolate No.2 which isolated from location 3 was found to produce 1.15ng/ml of Aflatoxin (Afs) belonging to 0.08 , 0.12 , 0.60 and 0.35 of AFB_1, AFB2 , AFG_1 and AFG_2 respectively, *A. parasiticus*, isolate No.20 which isolated from location 20 was found to produce 2.91ng/ml of Aflatoxin (Afs) belonging to 0.31 , 0.84 , 0.52 and 1.24 of AFB_1, AFB_2 ,AFG_1 and AFG_2 respectively and *A. parasiticus*, isolate No.15 which isolated from location 1 was found to produce 1.02 ng/ml of Aflatoxin (Afs) belonging to 0.10 , 0.18 , 0.57 and 0.17 of AFB_1, AFB_2, AFG_1 and AFG_2 respectively.

These results were in agreement with **Suman, *et al*. (2000)** who stated that, the outer fiber layer of the sugarcane stem may be attacked by fungi, especially following insect invasion or other parasites pre- or post-harvest, resulting in the contamination of sugarcane juice with AFs. **Garber, (2013)** found that, aflatoxin-producing fungi that contaminated sugarcane stems ranged from 52 to 95% *A. parasiticus* in hand-collected samples and billets for commercial planting, respectively. **Ojo, *et al*., (2014)** detected of mycotoxins including aflatoxins B_1, B_2, G_1 and G_2, zearalenone and diacetoxyscirpenol

mycotoxins in the examined samples of *Saccharrum officinarum***.**

Hariprasad *et al*. (2015) found that 57 samples of sugarcane juice were taken from Indian local markets, and of those, 22.2% and 19%, respectively, came from Mysore and Mandya. The levels of contamination ranged from 0.5 to 6.5 mg/kg**. Mohamed *et al.*, (2016)** reported that, only aflatoxin B_1 (AFB_1) and aflatoxin G_1 (AFG_1) were detected in sugarcane grass and juice intended for human consumption in Upper Egypt. The prevalence of AFB_1 was in 48% of grass samples and in 58% of juice with a maximum concentration of 30.6 ng/kg and 2.10 ng/kg, respectively. AFG_1 was detected in 10% of grass samples (7.76 ng/kg) and 18% of juice samples (34 ng/kg). **Iamanaka *et al*. (2019)** reported that *A. parasiticus* was identified as the main aflatoxigenic species isolated from sugarcane and sugarcane soil, and the majority of samples of sugarcane juice (68.5%) were contaminated by aflatoxins, which ranged from 0.4 to 10.2 mg/kg**. Silva *et al*. (2019)** found that the main aflatoxigenic species found in sugarcane and its by products is *A. parasiticus***. Younos and Embaby (2023)** found that, four isolates of *A. parasiticus* were found to produce aflatoxins. On the other hand, a higher aflatoxin quantity was produced by *A. parasiticus* isolates from the sugarcane stem, in which isolate No. 21 from location B samples produced 1434.92 ng/ mL)1229.38 AFB_1, 98.38 AFG_1, 96.61 AFB_2 and 10.55 ng/mL AFG_2), followed by isolate No. 7 from location C samples which produced 1159.7 ng/mL) 964.74, 73.59, 111.25 and 10.12 ng/mL of AFB_1, AFG_1, AFB_2, and AFG_2 respectively, while A. *parasiticus* isolates from sugarcane juice produced less aflatoxins quantity, whereas isolate No. 13 from location C samples produced 609.55 ng/mL (510.34 AFB_1, 23.06 AFG_1, 54.87 AFB_2, and 21.28 ng /mL AFG_2). while isolate No. 5 from location.

Table (4): Quantities of Aflatoxin (Afs) (ng/ml) production

Localities (Samples)	Type of fungi	Isolate No.	Aflatoxin (Afs) conc. (ng/ml)				
			AFB_1	AFB_2	AFG_1	AFG_2	Total
Standard (STD)	-	(STD)	40.00	12.00	40.00	12.00	104.00
L(1)	*Aspergillus parasiticus*	15	0.10	0.18	0.57	0.17	1.02
L(2)	*A. parasiticus*	20	0.31	0.84	0.52	1.24	2.91
L(3)	*A. parasiticus*	2	0.08	0.12	0.60	0.35	1.15
L(4)	*A. parasiticus*	51	0.02	0.09	0.53	0.12	0.76
L(5)	*Aspergillus flavus*	57	0.01	0.00	0.02	0.27	0.30
L(7)	*A. flavus*	89	0.05	0.00	0.10	0.00	0.15

L = Location

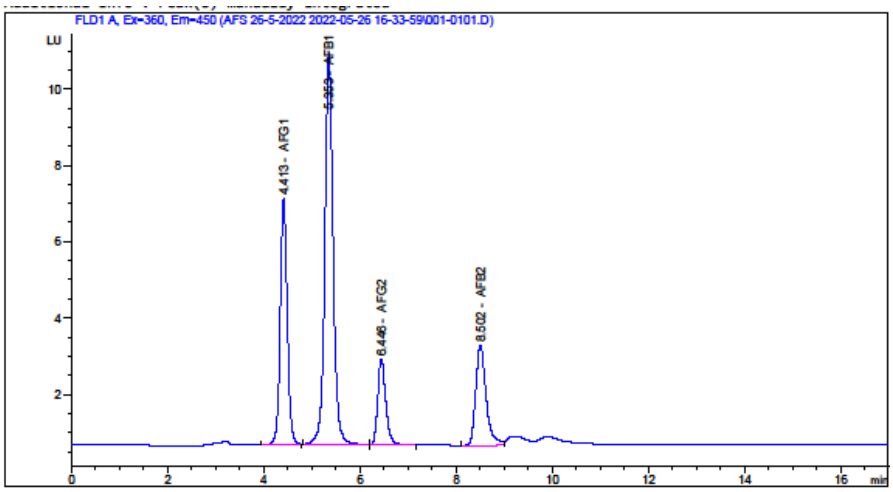

Fig. (4): Standard (STD) spiked in the HPLC chromatogram of aflatoxins AFG_1, B_1, G_2& B_2

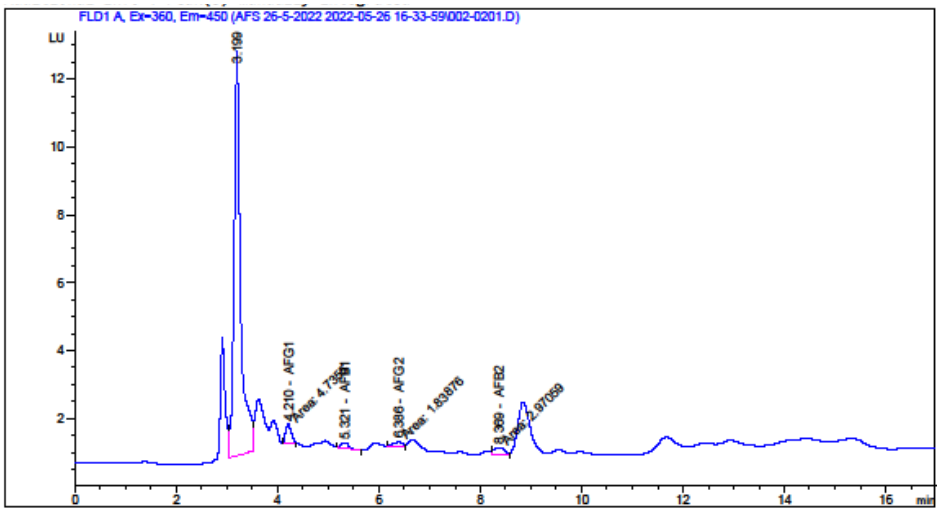

Fig. (5): HPLC chromatogram of aflatoxin produced by *A. parasiticus* isolate No. 15 from Location No. 1.

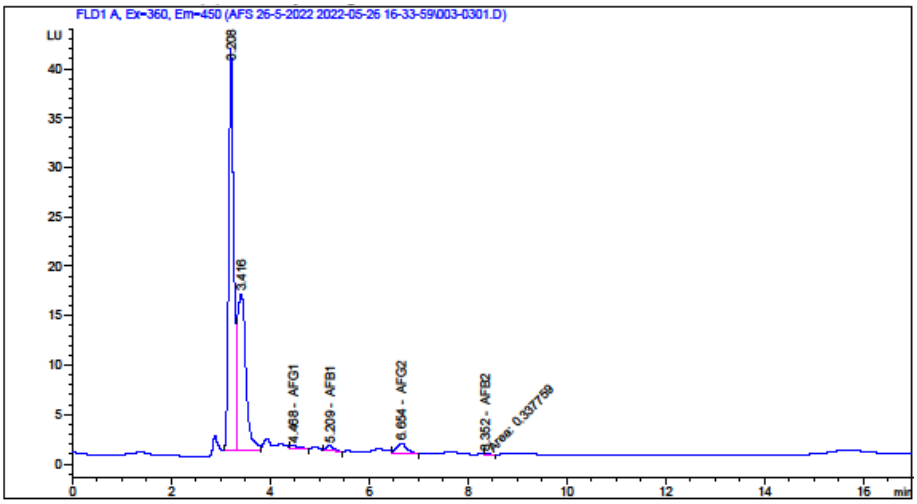

Fig. (6): HPLC chromatogram of aflatoxin produced by *A. parasiticus* isolate No. 20 from Location No. 2.

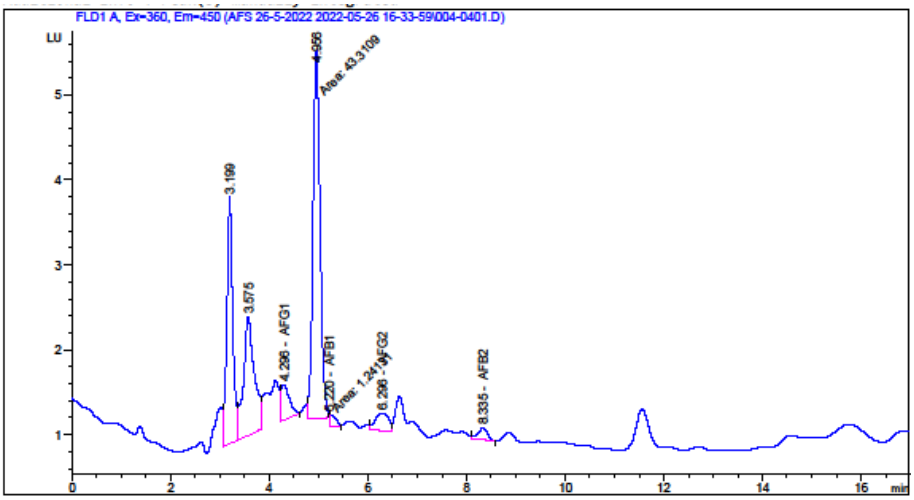

Fig. (7): HPLC chromatogram of aflatoxin produced by *A. parasiticus* isolate No. 2 from Location No. 3.

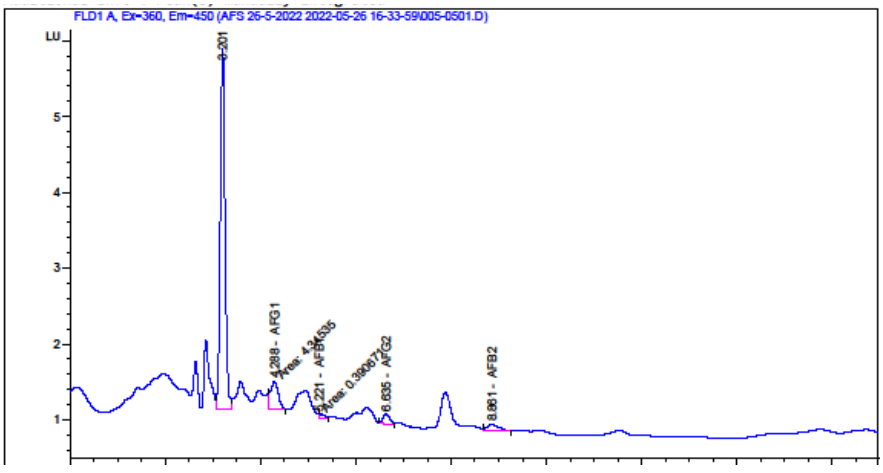

Fig. (8): HPLC chromatogram of aflatoxin produced by *A. parasiticus* isolate No. 51 from Location No. 4.

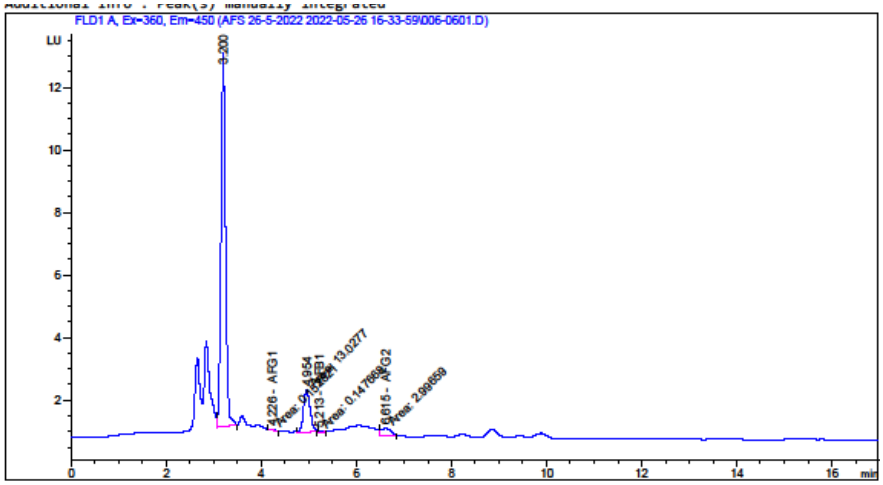

Fig. (9): HPLC chromatogram of aflatoxin produced by *A. flavus* isolate No. 57 from Location No. 5.

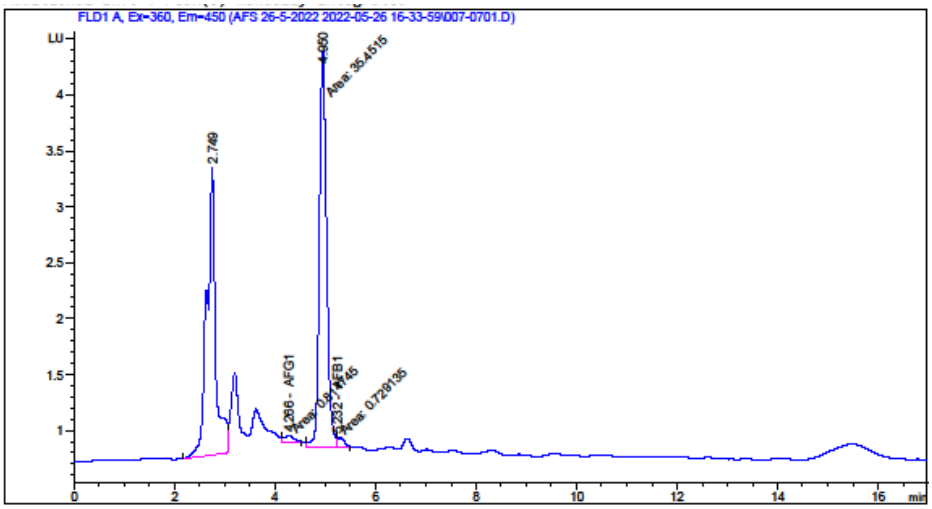

Fig. (10): HPLC chromatogram of aflatoxin produced by *A. flavus* isolate No. 89 from Location No. 7.

Quantities of OAT (ng/ml) production: According to HPLC data, determination of Ochratoxin A (OTA) indicated that, all *Penicillium* sp., isolates were negative producer. On the other hand, four isolates of *Aspergillus niger* isolates were positive producer of Ochratoxin A (OTA) as shown in **Table (5) and Figs (11 - 17)**. Higher Ochratoxin A (OTA) production was recorded with *Aspergillus niger*, isolate No. 25 which isolated from location 1 was found to produce 2.03ng/ml of Ochratoxin A (OTA) followed by *A. niger*, isolate No. 31 isolated from location 3 was found to produce 1.19ng/ml and *A. niger*, isolate No. 35 isolated from location 4 was found to produce 1.09ng/ml, respectively. *Aspergillus niger* isolate No. 39 isolated from location 5 was less producer which record 1.07 ng/ml.

JECFA (2001), Esteban *et al.* (2004) and Visconti, *et al.* (2008) reported that,Ochratoxin A (OTA) is a major mycotoxin, produced by several species of *Aspergillus* and *Penicillium*, naturally

occurring in a variety of food commodities prior to harvest or more commonly during storage. Ochratoxin A (OTA) producing black aspergilli include principally *Aspergillus carbonarius*, followed by *A. niger* and possibly *A. tubingensis*. OTA production by *A. niger* ‘‘aggregate’’ normally occurs at 20–25^{o}C .

Table (5): Quantities of OAT (ng/ml) production

Localities (Samples)	Type of fungi	Isolate No.	OAT conc. (ng/ml)
Standard	-	-	5.00
1	*Aspergillus niger*	25	2.03
2	*Aspergillus niger*	12	-
3	*Aspergillus niger*	31	1.19
4	*Aspergillus niger*	35	1.09
5	*Aspergillus niger*	39	1.07
6	*Penicillium* sp.	58	-
7	*Penicillium* sp.	41	-

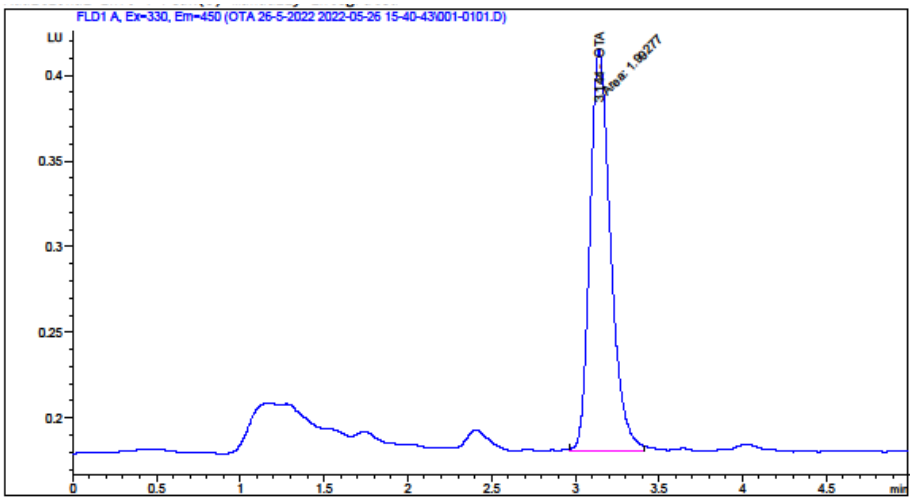

Fig. (11): Standard (STD) spiked in the HPLC chromatogram of Ochratoxin A (OTA)

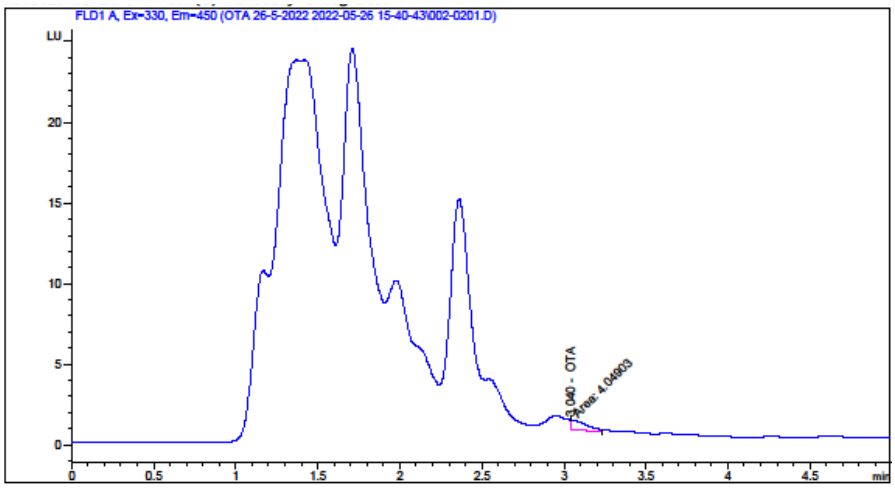

Fig. (12): HPLC chromatogram of Ochratoxin A (OTA) produced by *Aspergillus niger* isolate No. 25 from Location No. 1.

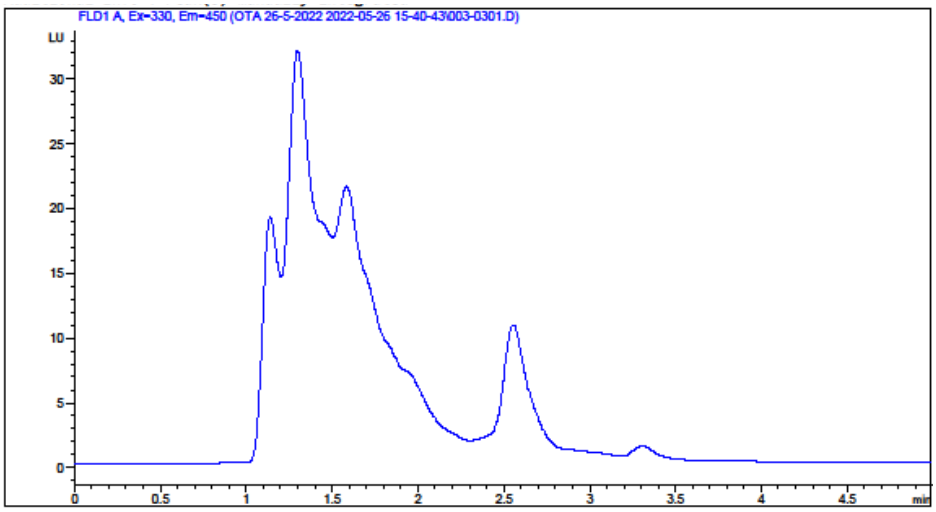

Fig. (13): HPLC chromatogram appeared negative reaction of Ochratoxin A (OTA) produced by *Aspergillus niger* isolate No. 12 from Location No. 2.

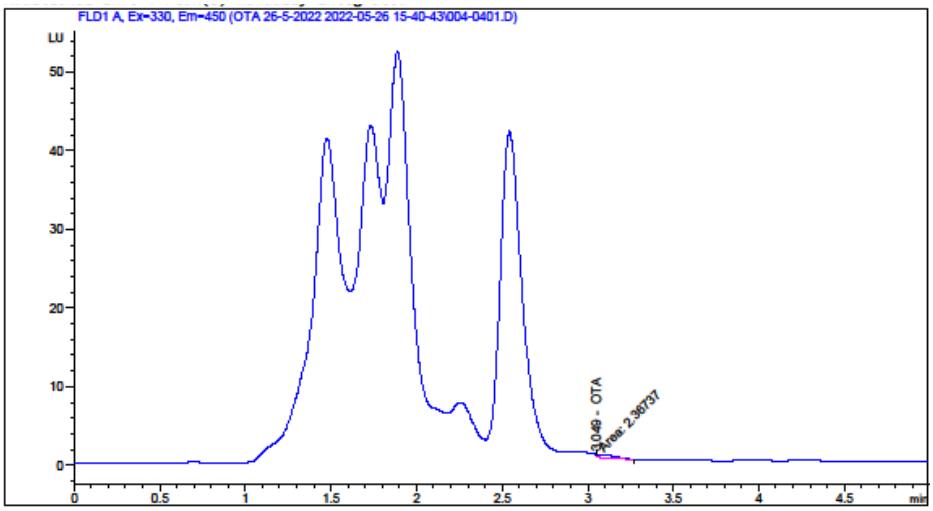

Fig. (14): HPLC chromatogram of Ochratoxin A (OTA) produced by *Aspergillus niger* isolate No. 31 from Location No. 3.

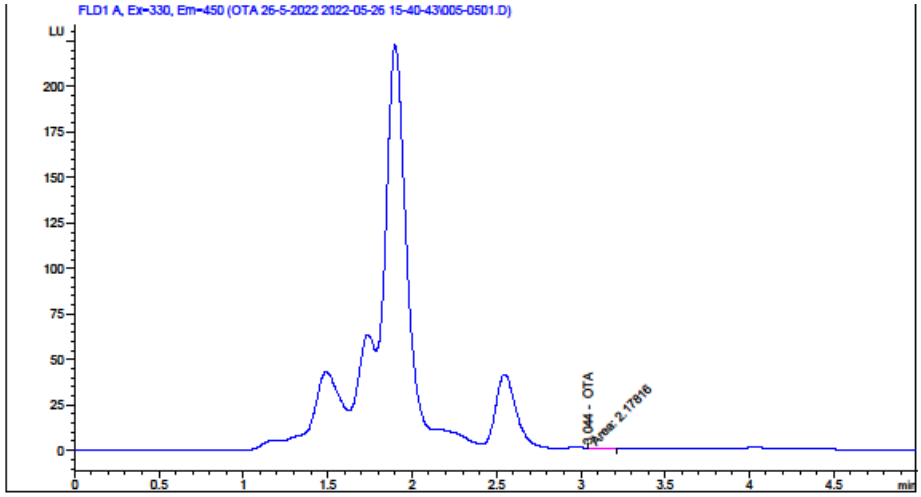

Fig. (15): HPLC chromatogram of Ochratoxin A (OTA) produced by *Aspergillus niger* isolate No. 35 from Location No. 4.

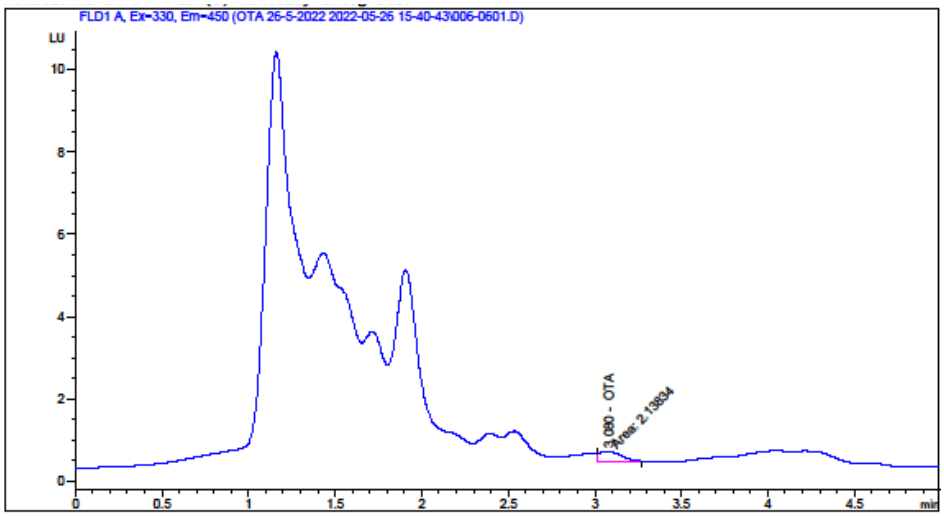

Fig. (16): HPLC chromatogram of Ochratoxin A (OTA) produced by *Aspergillus niger* isolate No. 39 from Location No. 5.

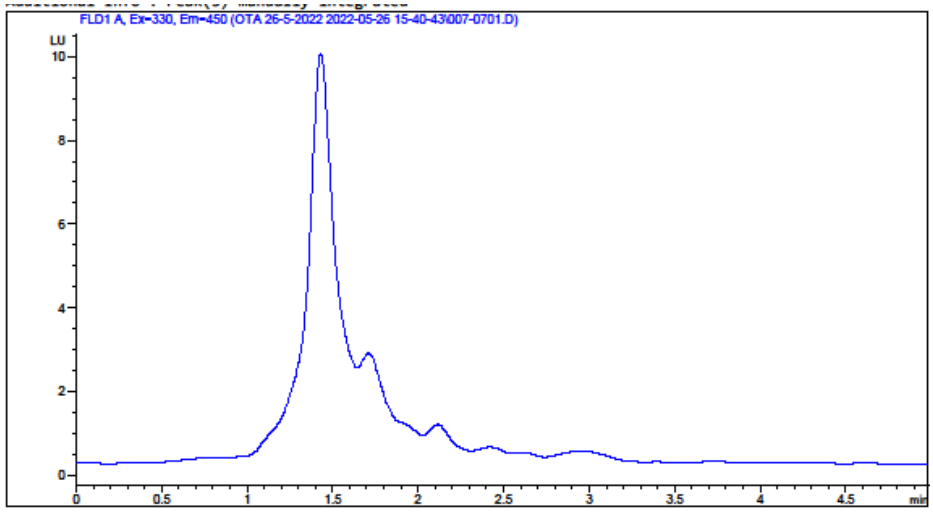

Fig. (17): HPLC chromatogram appeared negative reaction of Ochratoxin A (OTA) produced by *Penicillium* sp., isolate No. 41 from Location No. 7.

4. Conclusion

To the best of our knowledge, in Egypt, there is currently a lack of information on the toxigenic fungi contamination of sugarcane juice. Here, we reported the potential contamination by toxigenic fungi of sugarcane juice. The obtained results indicates that, a wide variety of fungal species were present in collected sugarcane juice samples. A number of harmful fungi were identified which produce number of mycotoxins and caused potential health hazard to human. There is need to improve the quality of sugarcane juice in order to save the human health. By testing the *in vitro* toxin production of fungal species isolated, this study showed their capability of producing Aflatoxin (AFs) and Ochratoxin A (OTA), and therefore, this might be the cause of possible contamination of sugarcane plants. These data demonstrate the need for further investigations aimed at assessing the possible risk for human health, due to the consumption of sugarcane juice contaminant by toxigenic fungi.

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