Characteristics, of Pumpkin and Bottle Gourd in Egypt and Potentially their Contamination by Mycotoxins and Mycotoxigenic Fungi

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Abstract: The objectives of this study were to characterize of pumpkin (*Cucurbita maxima*) and bottle gourd (*Lagenaria specie*) seed, tissue and some physical, chemical, antifungal activity of their oil. The results showed that the value of moisture content of seed was ranged between 6.88-8.43%.The fatty acid compositions consist mainly unsaturated fatty acids (oleic acid and linoleic acid). The saturated fatty acids palmitic and stearic acid present at lower levels. The free fatty acid percentage of pumpkin oil was 1.6 % and 1.05% for bottle gourd oil which suggests their use as edible oil. They have higher saponification values (186.00 and 194.10 mg KOH/gm oil, respectively) indicate that both oil has high molecular weight fatty acid and therefore provides good feedstock for lubricants, candles and soap production. The iodine values of pumpkin oil and bottle gourd oil were 100.6 and 102.65 gm/100 gm fat, respectively suggesting a high degree of unsaturation which makes both oil good for cooking and suitable for margarine production. All samples were free from aflatoxins and zearalenone. *Aspergillus niger* was the predominating fungal. Both pumpkin and bottle gourd (seed or tissue) was not a suitable subsutrate for toxic *Fusarium graminerum* R2118 to growth and produce zearalenone.Oilseed of both pumpkin and bottle gourd hadn't antifungal effect.

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Keywords: Pumpkin, bottle gourd, fatty acid, oil, mycotoxin, fungi, antifungal

1. Introduction

In Egypt, pumpkin and bottle gourd are planted primarily for the production of seeds that can be used for eating or the production of salad oil. Pumpkin (*Cucurbita maxima*) and bottle gourd (*Lagenaria specie*) belong to the family Cucurbitaceae. They are leafy green vegetable. Fruits are variable in size, color, shape and weight. They have a moderately hard rind, with a thick, edible flesh below, and central seed cavity. There are numerous seeds in the fruit. Most seeds are plump and tan or soft white. They are all covered with a testa that serves as a protectant around the seeds. They are consumed directly for human consumption as a snack food in many cultures throughout the world. They are excellent sources in both oil and protein **(Christian, 2006 andMurkovic a&Pfannhauser, 2000**).

Seeds could be utilized successfully as a good sources of edible protein (320 g/kg) and oil (450 g/kg for human consumption, as well as animal food; at the same time, it minimizes waste pollution (**Lazos, 1986**).Also, they are rich in Na, K, Fe, P, Mn and pectin (1.01%) **Egbekun *et al.*, (1998).** He obtained a new variety of pumpkin he called (Vitamin pumpkin) which contained 14 mg % of carotene of the deride weight of flesh.

Pumpkin and bottle gourd oils are considered the most nutritional oils available. They are considered an excellent source of essential fatty acids, antioxidants, vitamins and sterols. They are contains omega 3 which is known to promote energy levels, brain function and overall human vitality. Also contains high level of vitamin E as well as vitamin A and vitamin C. The phytosterols present in pumpkin seed oil are lowering the cholesterol levels (**Taylor *et al*, 2006, Bavec *et al*, 2002 and Murkovic, &Pfannhauser 2000**).

In addition pumpkin seeds have been used for healing purposes. They were applied for varied purposes such as healing wounds and burns, as a diuretic (to dispel insects, worms and parasites), hormone production, over active bladder and for prostate problems. Also, they are recommended in the literature that it can be used as a protection against colworm, tapeworm, seasickness and interruption of pregenancy (**Markovic andBastic, 1976**).

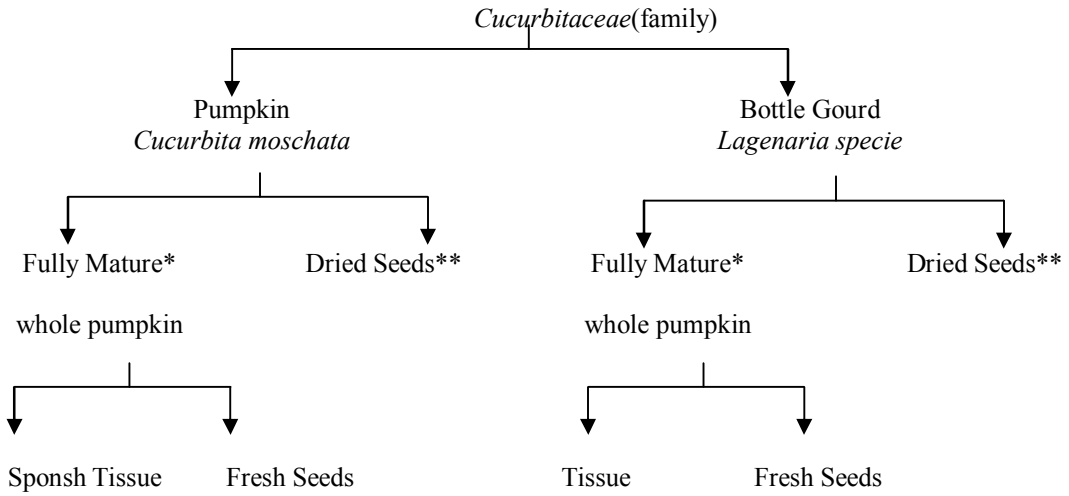
The seeds can be cooked and dried and served as snacks (e.g., Egypt) and might also be cooked, ground (West Africa) and fermented for use as a flavor enhancer in gravies and soups (**Nwokolo and Sim, 1987). Lugauskas *et al*. (2005)** found that, vegetables and fruit can become a good substrate for mycotoxin producing micromycetes. Mycotoxins are

toxic secondary metabolites producerd by many filamentous of fungi and contaminated various agricultural commodities in pre-harvest, harvest, post-harvest and in storage condition (**Kumar *et al.*, 2008**). Alfatoxins are one of the most dangerous food-borne mycotoxins in Africa and epidemiological studies of human populations exposed to diets naturally contaminated with aflatoxins revealed an association between the high incidence of liver cancer in Africa and dietary intake of aflatoxins (**MERCK, 2006**). Aflatoxins produced by toxigenic strains of *Aspergillus flavus* and *A. parasiticus,* chemically, aflatoxins belong to the group of bifuranocoumarins, aflatoxins B_1, B_2, G_1 and G_2 being the most toxic and the liver representing the main target organ. Zearalenone is a *Fusrarium* produced mycotoxin that has a chemical structure similar to estrogen and can produce an estrogenic response in animals. Pumpkin production in Egypt is 369 tons through years (2009-2010) which cultivated in Kana and Elkalubia Governorate.

This work aims to characterize Pumpkin and bottle gourd (seed, tissue and oil) cultivated in Egypt by evaluating their fatty acid compositions and study their antifungal activity, detection of some mycotoxin, and isolation of toxic fungi.

2. Materials and Methods

2.1. Samples



* Twenty samples obtained from the Agricultural Research Center Ministry of Agriculture, Egypt.
**Twenty samples perched from local market.

2.2. Determination of moisture content of pumpkin and bottle gourd

Twenty grams of sample were milled and dried in an oven at 105°C for 24 hr, then cooled in desiccator and re-weight to a constant weight. The moisture content was calculated as percentage of the dry weight.

2.3. Preparation of Samples for oil extraction

Pumpkin and bottle gourd seeds used in this work were obtained from local market in Cairo City, Egypt. The seeds were screened to remove bad seeds and then shelled manually. After that they were dried in an air oven at 60°C for 24 hrs and were ground using a mechanical grinder. They were then stored in a refrigerator at about 4°C for further use.

2.4. Oil extraction process

The oil contents of both seeds were determined by complete extraction using soxhlet extractor. 100 g of the shelled ground seed was packed into a weighed thimble, which was introduced into the soxhlet system. The extraction process was carried out six hours and the temperature was corresponded to the boiling point of the solvent used. Solution obtained of the extracted oil was stripped of the solvent using vacuum distillation at a reduced temperature. The oil obtained was weighed and the percentage oil content was calculated (**Attah and Ibemesi, 1990**).

Solvent used for oil extraction was petroleum ether (60-80°C) which was obtained from El Nasr Company for Chemicals, Cairo, Egypt.

2.5. Testing of the physical and chemical properties of oil

Fatty acid composition of Pumpkin and bottle gourd seeds were determined using gas liquid chromatographic analysis of the oil methyl esters. Modification of the oil to its methyl esters was

made using 2% H_2SO_4 as a catalyst in the presence of dry methyl alcohol in excess. The chromatographic analysis was made using Perkin Elmer Chromatograph (Waltham, MA, USA). Samples were separated on a DB-5 column coated with 5% phenyl and 95% methylpoly siloxan with 60 m length, 0.32 mm inside diameter, and 0.25 mm thickness. Detector temperature was 250˚C, injection temperature was 230˚C, and the column temperature was programmed from 150˚C to 240 ˚C at 3˚C /min. Oils were tested for some other characteristics including % fatty acid, saponification number, iodine value, and peroxide value using Standard Tentative methods of analysis(**AOCS,1991**).

2.6. Mycotoxins analysis

Mycotoxins analysis by extraction, purification, thin layer Chromatography (TLC) for qualitative detection of Aflatoxins B_1, B_2, G_1 and G_2 and earalenone (ZEN) according to **AOAC (2007).** Finally for quantitative determination of mycotoxins. High performance liquid Chromatography (HPLC) Technique was employed according to **Martins *et al.*, (2008)**. The quantitative determination of mycotoxins was carried out compared of standard mycotoxins (Sigma).

2.7 Fungi isolation and Identification from pumpkin and bottle gourd

2.7.1. Isolation of fungi from seed

the dilution plate method as described by **Johnson and Curl (1972)** was used for isolation pumpkin and bottle gourd seeds fungi by immersion in 1% sodium hypochlorite solution for 1 min then washed with sterilized water, PDA (Potato Dextrose Agar medium) was used as cultivation and isolation medium, chloramphenicol (0.5 mg ml^{-1}) as bacteriostatic agent. Five plates were used for each sample incluated at 28^{o}C for 7 days and the developing fungi were identified, counted and calculated as percentage for 100 seed of each tested sample. Taxonomic identification of fungi (based on purely morphologically macro-and microscopic characteristics) was carried out according to **Booth (1977), Pitt (1991), Samson *et al.*, (2002) and Summerell *et al.*, (2003).**

2.7.2. Isolation fungi of Tissue

Fungi count were determined from 10 gm cut samples (pumpkin) or ground samples (bottle gourd) with 90 ml of sterile 30 m MK_2 HPO_4 by using Aerobic Plate Count (APC) (**Barnet and Hunter, 1987**) fungi counts were expressed on logarithmic Scale (base 10), (**Torkar and Vengust, 2008).**

2.8.Culture conditions for production of aflatoxins by *Aspergillus flavus*NRRL3251 and Zearalemone by *Fusarium graminearum* R 2118

100 g of pumpkin seed and tissues as well as bottle gourd put in flasks autoclaved, after cooling, 30 ml of sterilized water was added and spores of each fungi were inoculated for 2 weeks at 25^{o}C. The cultures dried at 55^{o}C for 25 h, finely ground and analysis for mycotoxins detected, **Ichinoe *et al.*, (1983).**

2.9. The ability of extracted oil as antifungal

Effect of oil extracted from pumpkin and bottle gourd seeds on mycotoxigenic fungi. *Aspergillus flavus* NRRl 3251 and *fusarium graminearum* R2118 obtained from standard Association of Australia. North Sudnay. The teted fungi were grown on (PDA) medium. Seed oil as antifungal activity was determined using three methods:

1- Diffusion plate methods as reported by **Terras *et al.*, (1995**) by hole of 8 mm. diameter bore was mad on the plate. The hole filled with 50, 100, 150 ul of oil. Plates were performed in triplicate. The zone of inhibition produced by the extract was compared with control.

2-A 5-m diameter disk was placed on the surface of the agar and inoculated with a suspension of the test fungi. Two perpendicular diameters of the growth Zone were measured for which the average growth area was calculated. Each concentration was mixed with sterilized semi-solidified PDA medium and then poured into sterilized Petri dishes (10 ml in each plate)

3-Liquid culture, mycelial growth of *A. flavus* NRRL 3251 and *F.* ferminearum R 2118 and mycotoxin produced were determined in potato-dextrose both liquid media (PDA) at different concentration of oil 6.25, 12.5 and 25, 50 and 100 ml according to (**Mehdi *et al.*, 2008 and Neslihan *et al.*, 2008).**

3. Results and Discussion

3.1. Moisture content

The moisture content of pumpkin and bottle gourd (seed, freash seed and tissue) was determined .Table 1 showed that the highest moisture content was found in sponsh tissue from pumpkin which recorded 74.73% while the smallest value for

bottle gourd seed 8.43% which there are no big different between the moisture content between seed and fresh seed, **Chinyere *et al*. (2009)** found that moisture content of bottle gourd was 7.92%.

Table 1: Moisture content on pumpkin and bottle gourd

Sample	Moisture Content (%)		
	Seed	Fresh Seed	Tissue
Pumpkin	6.88	53.73	74.73
Bottle Gourd	8.43	8.51	9.00

3.2. Oil content

The oil content of pumpkin and bottle gourd seeds are 35.7 and 28%, respectively.

3.3. Fatty acid composition of pumpkin and bottle gourd oil

Fatty acid composition of pumpkin and bottle gourd oils estimated by gas-liquid chromatography are listed in Table 2. Their compositions differ slightly from the reported in the literature (**Stevenson et al, 2007, Al-Khalifa, 1996, Lazos et al, 1995, Markovic and Bastic, 1976**) due to the difference in climatic and soil conditions. Pumpkin and bottle gourd oils contain the same saturated, monounsaturated and poly-unsaturated fatty acids.

It is clear that pumpkin consists palmitic acid (15.52 %) and stearic acid (9.27 %) and polyunsaturated linoleic acid (42.05 %) lower than those present in bottle gourd oil which contains 18.75 %, 10.39 % and 57.66 %, respectively. The monounsaturated oleic acid (33.16 %) presents in pumpkin is higher than that in bottle gourd oil (13.20 %). The high percentage of unsaturated fatty acids (75.21 %, 70.86 %) in pumpkin and bottle gourd oils, respectively indicating that both oil is good for cooking and suitable for margarine production.

Table (2): Chemical composition of pumpkin and bottle gourd oil

Fatty Acid %	Pumpkin	Bottle Gourd
Palmitic Acid, $C_{16:0}$	15.52	18.75
Stearic Acid, C18:0	9.27	10.39
Oleic Acid, $C_{18:1}$	33.16	13.20
Linoleic Acid, $C_{18:2}$	42.05	57.66

3.4. Physical and chemical properties.

Some physical and chemical properties of pumpkin and bottle gourd oils are given in Table 3. It is clear that the two oils are nearest in studied value except iodine; pumpkin oil appearance is green, so it doesn't need any refining step before use but bottle gourd is red. The obtained values of refractive index and specific gravity are similar for both pumpkin and bottle gourd oils as mentioned in the literature. The low percentage of free fatty acid and peroxide values of pumpkin and bottle gourd oils also indicate that the ability of its using edible purposes. The saponification numbers of both oil were 186.00 and 194.10 mg KOH/gm oil which is relatively higher compared to other vegetable oils. This indicates high molecular weight due to the presence of higher number of carbon atoms and the ability of use in both oils in candle, soap production (liquid soaps and shampoos) and as chemical feedstock's for lubricants.

On the other hand, the unsaponifiable matter is about 0.6 % which is considerably higher and indicated that their using in the therapeutic purposes.

In addition, the estimated iodine values (100.6 gm/100 gm fat and 102.65 gm/100 gm fat) for pumpkin and bottle gourd oils, respectively) indicates high unsaturation fatty acid content. So, they are classified in the category of semidrying oils group because iodine values are relatively low compared to soybean and cotton seed oils which have values of 132 and 107 gm/100 gm fat, respectively. This property suggests their uses in surface coating industry, production of alkyd resin, shoe polish, and varnishes when mixed with iron oxide.

Table (3) Characterization of pumpkin and bottle gourd oil

Property	Pumpkin	Bottle gourd
Appearance	green	red
Specific Gravity	0.912	0.919
Refractive Index	1.465	1.469
Percentage Fatty Acid, %	1.60	1.05
Peroxide Value, meq / kg	2.50	2.06
Saponification Value, mgKOH/gm oil	186.00	194.10
Iodine Value, gm/100gm fat	100.60	102.65

3.5. Proximate analysis of pumpkin and bottle gourd

Proximate analysis of pumpkin and bottle gourd whole seed are presented in Table 4. It is clear that pumpkin kernels contained a high percentage of crude protein 35% than bottle gourd. This is similar to high protein seed such soybeans and cowpea (22.7%); however, it is higher than others such as lima beans (19.8%) and chickpeas (19%) (**Oshodi, 1993, Alfawaz, 2004 and Lazos, 1986**). The high oil and protein contents make the seed has a potential source of commercial vegetable oil and protein. The carbohydrate content of pumpkin is 10.56% of the kernel was lower than that reported by (**Lazos, 1986, El-Adawy *et al*., 2001 and Kamel *et al*. 1982**)

but it is 15.22% for bottle gourd. The crude fiber content of pumpkin kernel was similar to that reported by (**Kamel *et al.*, 1982**).

Table 4. Proximate analysis of pumpkin and bottle gourd seed.

Analysis	Pumpkin	Bottle Gourd
Crude Protein, %	35.00	22.48
Total Ash, %	3.80	2.68
Crude fiber, %	14.94	5.65
Carbohydrate, %	10.56	15.22

The most characterization and proximate analysis of bottle gourd were nearest of that reported by **Chinyere et al. (2009)**.

3.6. Natural occurrence of mycotoxins:

Sixty samples of all pumpkin as well as bottle gourd (seed, fresh seed and tissue) were screened qualitatively and quantatively for both Aflatoxin (B_1, B_2, G_1 and G_2) and Zearalenone, the results found that all samples were completely free from any mycotoxins.These results are not agreement with **Lugauskas et al., (2005)** who, detected 0.0112mg/kg of zearalenon in shelled pumpkin seed, while, **Najmus *et al.*, (2009)** who detected aflatoxin G_1 in four samples of pumpkin.

3.7. Fungi isolation and identification

Pumpkin and bottle gourd were subjected to isolation and identification of fungi Tables 5 and 6. The fungal species isolated from seed were *Aspergillus flavus, A. niger, Fusarium spp., Rizopuus oryzae* and *Altenaria alternate. Aspergillus niger* was the predominating fungal.

Lugauskas *et. al.* , (2005) noticed that *Aspergillus, Penicillium, Mucor, Rhizomucor, Rhizopus* genera can spread quicker than other fungi, and are able to contaminate and damage a wide range of products of plant origin, also remain viable under extreme environmental conditions like humidity shortage, low air temperature, the influence of ultraviolet radiation, and other physical fators. *Rizopus oryzae* and *Altenaria alternate* were isolated from only bottle gourd, *A. niger* had been found in sterilized and non-sterilized pumpkin seed at 74.19% and 66.67%, respectively. Also Table (5) shows the kinds and count of fungi expressed.

Table 5: Isolation and identification of fungi from pumpkin.

Samples	Fungi (%)			
	A. favus	*A. niger*	*Fusarium spp.*	*Rizopus oryzae*
Seed(100)				
Sterilized	22.58	74.19	3.23	ND*
Non-Sterilized	31.58	66.67	1.75	ND
Fresh Seed(100)	30.00	60.00	ND	10.00
Sponsh				
Tissue(FungalCount) CFU/g	$1.84x10^2$	$36.83x10^2$	ND	ND

*ND: Not Detect

Table 6: Isolation and identification of fungi from bottle gourd.

Samples	Fungi (%)					
	Aspergillus			Other		
	flavus	*niger*	*ochraceus*	*Fusarium garminearum*	*Rizopus oryzae*	*Alternaria alternate*
Seed (%)						
Sterilized	44.45	37.04	ND	11.11	3.70	3.70
Non-sterilized	ND*	60.60	3.30	2.50	30.30	3.30
Fresh seed(100)	33.33	ND	ND	66.67	ND	ND
Sponsh						
Tissue(FungalCount) CFU/g	$45.03x10^2$	$9.83x10^2$	ND	ND	ND	ND

*ND: Not Detect

The present results revealed that, there were two Aspergelli species *A. flavus* and *A. niger* that had been found in tissue. The lowest actual counts of *A. flavus* on APC were observed 1.83 x 10^2 cfu/g isolated from pumpkin tissue while the highest counts were found in bottle gourd tissue which recorded 45.03 x 10^2 cfu/g.These results are accepted with **Lugauskas *et al.* (2005)** who reported that, sometime micromycetes develop directly on the surface of vegetable and fruit or in their inner tissues.

3.8. Production of mycotoxins

The results of the ability to produce aflatoxins (B_1, B_2, G_1 and G_2) by *A. flavus* NRRL 3251 and zearalenone toxin by *F.graminearum* R 2118 in pumpkin and bottle gourd (seed and tissue) are presented in Table 7. Table 7 reveals that, zearalenone toxin was not determined at any concentration in both pumpkin and bottle gourd, and aflatoxins were not determined in pumpkin sponsh tissue. On the other hand the ability of *A. flavus* (toxigenic fungi) to produce aflatoxins in bottle gourd tissue was very high than other substrate at concentration 11.08, 6.21, 9.92 and 4.75 ug/kg for aflatoxins (B_1, B_2, G_1 and G_2), respectively. **Lugauskas *et al.* (2005)** studied toxin producing micromycetes on imported products of plant origin, who found that, a strain growing on a particular type of vegetable or fruit could synthesize and excrete different toxin secondary metabolites.

The data demonstrate that bottle gourd fresh seed not a suitable substrate for toxic *A. flavus* NRRL 3251 to grow and produce aflatoxins, maybe because the low moisture content (8.51%) or the seed very hard and have compound which inhibit the fungal growth .

Table (7): Aflatoxins production by *A. flavus* NRRL 3251 and zearalenone by *F. graminearum* R2118 in pumpkin and bottle gourd.

Substrate	Aflatoxins ug/K				Zearalenone ug/k
	B_1	B_2	G_1	G_2	
Pumpkin					
Fresh Seed	5.71	4.82	4.47	3.01	ND*
Sponsh Tissue	ND	ND	ND	ND	ND
Bottle gourd					
Fresh Seed	2.91	2.00	ND	ND	ND
Tissue	11.08	6.21	9.92	4.75	ND

*ND: Not Detect

3.9. Effect of oil on mycotoxigenic fungi:

The effect of oil seed as antifungal was studied by three methods at different concentrations. The results appeared that the seed oil showed not antifungal activity but the toxigenic fungi grow well and produce mycotoxins at high concentration. It could be seen that the oil concentration increased the growth and toxin production increases as shown in figures 1 → 4.

It is seen that toxin fungi was able to grow and produce toxin. Oil seed pumpkin and bottle gourd contains protein 35 and 22%, respectively. The oil is rich in vitamins at level 14 mg, it is probably responsible for growth toxic fungi and produced toxin. In these respect, **Mellon and Cotty, 1998** mentioned that the nuts and oilseeds that have been studied most thoroughly for their association with mycotoxin especially aflatoxins are also rich in protein.

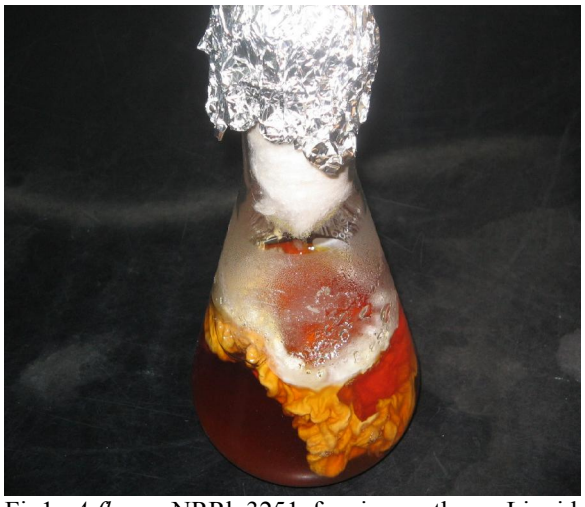


Fig1. *A.flavus* NRRl 3251 fungi growth on Liquid culture.

Fig 2. *F.graminearum* R2118 growth on Liquid culture.

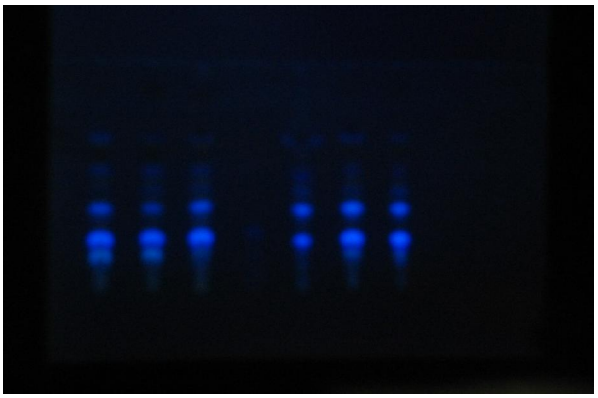


Fig 3. TLC showed aflatoxins(B_1, B_2, G_1,and G_2) extacted frrom Liquid culture.

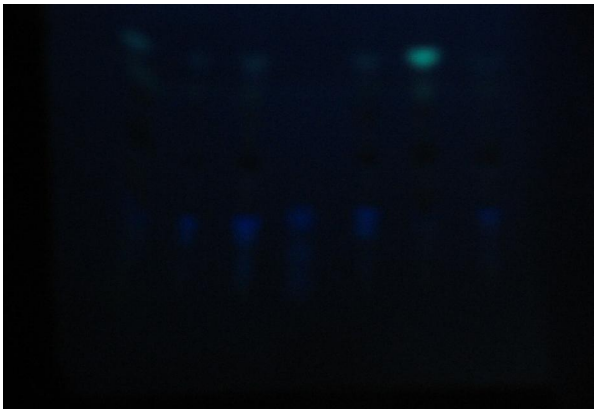


Fig 4. TLC showed Zeralenone toxin extacted from Liquid culture.

Conclusion

Pumpkin and bottle gourd in Egypt were free from aflatoxins and zearalenone, oil are a good for cooking and suitable for margarine production and therefore provides good feedstock for lubricants, candles and soap production. Pumpkin seed are a good source of protein must supplement in food and diets to increase the health implications.

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