

**Fungal biomass production in mycological liquid medium by some ochratoxigenic fungi under static and shake culture conditions.***Jamal I. Elzwai¹, Mohammed A. Alshareef¹, Elfathi A. Elbarkoli¹ and Salima, A. Aldokali²¹ Food Science and Technology Department, Faculty of Engineering & Technology, Sebha University, Libya.² General Science Department, Faculty of Engineering & Technology, Sebha University, Libya.*Correspondence email: Jam.Elzwai@sebhau.edu.ly

Abstract In this study, fungal biomass production by some fungi which isolated from different cereal samples obtained from the south east of Libya was studied by using four mycological medium under shake and static condition. It has been found that fungal biomass was higher under shake culture than that of static condition. Its may be because of the adequate mass, heat transfer. *Aspergillus carbonarius* isolate produced higher fungal biomass on PDB under shake culture, whereas higher fungal biomass obtained on MEB under static condition. By *A. ochraceus* isolate it was higher on MEB under shake culture, while it was higher on CPDB under static condition. By *A. ochraceus* CBS 588.68, it was higher on CPDB under shake culture and on MEB under static condition. By *A. niger* 285522 it was higher on MEB under shake culture and on CPDB under static condition. The results indicates that ochratoxin A production was detected earlier under shake culture than static conditions on the mycological media using TLC technique.

Keywords: *Aspergillus*, *Penicillium*, Biomass, Static, Shake culture.**ايجاد الكتلة الخلوية بالايوساط الغذائية السائلة بواسطة بعض الفطريات المنتجة للاوكراتوكسين تحت****الظروف الساكنة والمتحركة*** جمال ابراهيم الزوي¹ و محمد عبدالله الشريف¹ و الفتحي ابوبكر البركولي¹ و سليمة ابوبكر الدوكالي²¹ قسم علوم و تقنية الاغذية - كلية العلوم الهندسية و التقنية - جامعة سبها، ليبيا² قسم العلوم العامة - كلية العلوم الهندسية و التقنية - جامعة سبها، ليبيا*للمراسلة: Jam.Elzwai@sebhau.edu.ly

المخلص تم في هذه الدراسة ايجاد الكتلة الخلوية لبعض الفطريات المعروفة و الفطريات المعزولة من عينات الحبوب المتحصل عليها من جنوب شرق ليبيا. حيث تم تنمية الفطريات على اربع انواع من الاوساط الغذائية الخاصة بتنمية الفطريات تحت ظروف تحضين ساكنة و متحركة. اوضحت النتائج ان انتاج الكتلة الخلوية كان اعلى في حالة التحضين المتحرك عنه في الحضين الساكن، حيث انتج فطر *A. carbonarius* اعلى كتلة خلوية على بيئة مرق PDB تحت ظروف التحضين المتحرك بينما فطر *A. ochraceus* isolate انتج كتلة خلوية على الوسط MEB تحت ظروف التحضين الساكنة، اما فطر *A. ochraceus* CBS588.68 فقد كانت اعلى كتلة خلوية على بيئة CPDB تحت ظروف التحضين المتحركة و على بيئة MEB تحت ظروف التحضين الساكنة. فطر *A. niger* 285522 انتج كتلة خلوية اعلى على الوسط الغذائي MEB تحت ظروف التحضين المتحركة و على ظروف التحضين الساكنة فقد كانت الكتلة الخلوية اعلى على الوسط الغذائي CPDB. كما اوضحت النتائج ان انتاج السم الفطري OTA قد انتج على الاوساط الغذائية تحت ظروف التحضين الثابتة قبل الاوساط الغذائية المحضنة تحت ظروف تحضين ساكنة باستعمال تقنية كروماتوغرافيا الطبقة الرقيقة.

الكلمات المفتاحية: اسبرجلس، بينيسيليوم، الكتلة الخلوية، مزرعة ثابتة، متحرك.

INTRODUCTION

Contamination of foodstuff with mycotoxins such as ochratoxins is a major matter of concern for human and animal health. Ochratoxin A (OTA) is a nephrotoxic and carcinogenic mycotoxin mainly produced by *Penicillium verrucosum* in temperate and cold climates and by *Aspergillus ochraceus* and related species belonging to section *Circumdati* in warmer and tropical climates. *Aspergillus carbonarius* and some species belonging to *Aspergillus niger* aggregate have been reported as OTA producers (2,4,5,9,17). The reported percentage of ochratoxigenic isolates belonging to the *A. niger* aggregate is much lower than *A. carbonarius* species (1). Ochratoxin A has

been detected worldwide in a wide range of food products, including cereals (12), poultry feeds (15), feedstuffs (10), green coffee beans (14), cocoa beans (7), wine grapes, dried vine fruits (9), peanuts (11) and beer (13).

The objective of this study was to investigate the production of fungal biomass in four mycological liquid medium under static and shaken culture conditions.

MATERIALS AND METHODS**Organisms**

Organisms used in this study were *A. ochraceus* CBS 588.68, *A. niger* 285522, and two fungal isolates which isolated from Wheat barley samples

obtained from the South East of Libya and were identified according to their morphological appearance as described by (16) as *A. carbonarius* and *A. ochraceus*. All moulds were sub cultured on malt extract agar slopes and stored at 4°C.

Mycological liquid media

Malt extract agar (MEA), Potato dextrose agar (PDA), Czapek Dox agar (CPDA) and Yeast extract sucrose broth (YESB) (Oxoid, UK). Broths from (MEA, PDA and CPDA) were prepared from solid medium by filtering a suspension of each medium through Whatman filter paper No. 1 to remove the agar from the media. Nine milliliters of each liquid media were distributed in universal bottles and sterilized at 121°C for 15 minutes.

Preparation of spore suspension

The mould spores were harvested from agar slopes with 9ml of sterile distilled water. Spores counted by using an Improved Neuberg counter to give a spore concentration of 2.1×10^6 spores/ml

Inoculation of the media

Static culture conditions

One hundred microlitres of fungal spore suspension (1.7×10^6 spores/ml) of *A. carbonarius* isolate, (2.3×10^6 spores/ml) of *A. ochraceus* isolate, (2.6×10^6 spores/ml) of *A. ochraceus* strain CBS 288.68, and (2.1×10^6 spores/ml) of *A. niger* strain 285522 were inoculated in each universal bottle containing 9ml medium (10 bottles of each medium for each mould), and incubated at 25°C for 10 days. A bottle was taken each day for biomass determination.

Shaken culture conditions

Spore suspension (0.5ml) of *A. carbonarius* isolate (1.7×10^6 spores/ml), *A. ochraceus* isolate (2.3×10^6 spores/ml), *A. ochraceus* strain CBS 288.68 (2.6×10^6 spores/ml) and *A. niger* strain 285522 (2.1×10^6 spores/ml) was inoculated in each 25ml medium in 50ml conical flasks. The flasks were then incubated on an orbital incubator (shaken speed =100rpm) at 25°C for 10 days. A flask was taken each day for analysis.

Determination of mycelial dry weight

After incubation, content of the flasks and the bottles were filtered through previously weighed Whatman filter paper No 1, it was then dried in an oven at 70°C over night.

Ochratoxin A analysis

The filtrate (5ml) of each sample was analysed for OTA production by extracting with 5ml chloroform. The bottles were vigorously shaken to ensure a good extraction. The solvent was evaporated to dryness by using a hot water bath. The residue was re-dissolved in 100µl chloroform and 10µl was spotted on TLC plates. The plates were developed in a solvent tank containing toluene-ethyl acetate-(88%), formic acid, chloroform (70:50:20:50) for one hour after which the plates were allowed to dry and examined under ultraviolet light ($\lambda=365\text{nm}$).

RESULTS AND DISCUSSION

Under shaken culture conditions, the biomass produced by *Aspergillus carbonarius* isolate was 7.5mg/day on PDB, 4.75mg/day on MEB, 2.65mg/day on CPDB and 0.95mg/day on YESB (Figure 1). Ochratoxin A production by *A.*

carbonarius J001 on MEB was detected at day 5 of incubation, day 6 on PDB and CPDB and at day 8 on YESB (Table 1).

The *Aspergillus ochraceus* isolate growth was visually noticed at day 2 of incubation on MEB, PDB and CPDB under shaken culture, but it was noticed at day 3 on YESB. Fungal biomass production based on dry weight increased with incubation time to reach the maximum weight at the end of incubation time, while on MEB, the biomass was 6.65mg/day, 3.55mg/day on PDB, 4.15mg/day on CPDB and 0.95mg/day on YESB (Figure 2). Ochratoxin A production was detected at day 7 on MEB, PDB and CPDB and day 9 on YESB (Table 1).

Production of fungal biomass by *A. ochraceus* CBS 588.68 under shaken culture was high on CPDB with a total weight of 5.2mg at the end of incubation time. The weights were 4.9, 2.7 and 0.35mg/day on MEB, PDB and YESB respectively (Figure 3). Ochratoxin A production was detected at day 8 on MEB and PDB and at day 9 on CPDB and YESB (Table 1).

The maximum biomass produced by *A. niger* 285522 was 4.65mg/day on MEB, 2.45mg/day on PDB, 0.45mg/day on CPDB and 0.35mg/day on YESB (Figure 4). Production of fungal biomass by *A. carbonarius* isolate, *A. ochraceus* isolate, *A. ochraceus* CBS 588.68 and *A. niger* 285522 could have increased beyond 10 days of incubation in all liquid media used under shaken culture. Ochratoxin A production by *A. niger* 285522 was detected at day 7 on MEB and PDB, day 8 on CPDB and at day 9 on YESB (Table 1).

Production of fungal biomass on liquid medium under static condition by *A. carbonarius* isolate increased with incubation time. The fungal biomass produced was 3.05mg/day on MEB, 1.75mg/day on CPDB and 1.35mg/day on PDB and YESB (Figure 1). Production of OTA by *A. carbonarius* isolate on liquid medium under static conditions was detected at day 7 on MEB and PDB, day 8 on CPDB and YESB (Table 2).

Aspergillus ochraceus isolate grew on liquid medium under static conditions and produced fungal biomass. The biomass was 4.35mg/day on MEB, 1.75mg/day on CPDB, 2.25mg/day on PDB and 0.55mg/day on YESB (Figure 2). Ochratoxin A production by *A. ochraceus* isolate on liquid medium was detected at day 8 on MEB and PDB and it was detected at day 9 of incubation on CPDB and YESB (Table 2).

Aspergillus ochraceus CBS 588.68 produced fungal biomass under static condition, the biomass on MEB was 2.65mg/day, on PDB 1.85mg/day, CPDB 1.45mg/day and YESB 1.25mg/day, (Figure 3). Formation of OTA by *A. ochraceus* CBS 588.68 was detected at day 8 on MEB and on day 9 on PDB, CPDB and YESB (Table 2).

The highest fungal biomass produced by *A. niger* 285522 was on CPDB, the weight was 9.55mg/day, 2.35mg/day on MEB, 2.65mg/day on PDB, and 1.85mg/day on YESB (Figure 4). Ochratoxin A production was detected at day 6 on MEB and PDB and at day 8 and 9 on CPDB and YESB under static condition (Table 2).

Therefore, it was found that the production of fungal biomass on liquid medium by all moulds tested was higher under liquid shaken culture than that of static condition. It may be that in the shaken culture there was adequate mass and heat transfer which enhanced fungal biomass and OTA production.

(8) studied 13 toxigenic fungal strains for zearalenone production, among these strains they found that one strain, *Fusarium culmorum*, produced zearalenone in liquid yeast extract sucrose medium at a temperature of 24°C for 2 days. They found that ochratoxin A produced by *Aspergillus ochraceus* occurred after 2 to 4 days of incubation on liquid medium at 24°C. (6) examined a liquid medium containing skimmed milk, potato extract and 2% sucrose and concluded that this medium supported higher penitrem mycotoxin production by *Penicillium*

crustosum than other liquid medium. The study also noticed that there is an increase on toxin production by increasing the amount of sucrose to 4%, but when the amount of sucrose was increased to 16%, the amount of toxin was decreased by more than 50%. Study by (3) indicated that isolates of *Mycobacterium verrucaria* vary widely in terms of toxins production when cultured on various media and some fungal isolates produced large numbers and quantities of toxin on solid media such as rice, whereas others produced more in liquid media. Another study by (18) suggested potato dextrose broth (PDB) supplemented with manganese chloride better supported the production of patulin by *Penicillium expansum* when compared with malt extract broth and 5% glucose yeast extract peptone broth.

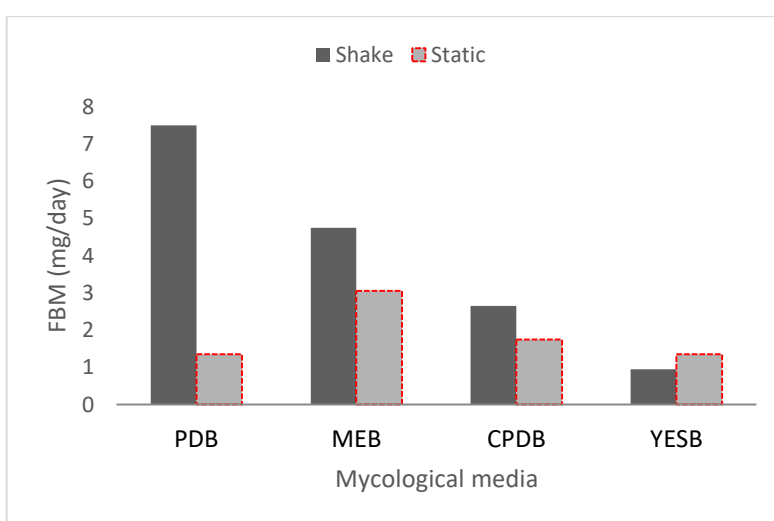


Figure 1. Production of fungal biomass by *A. carbonarius* isolate under shake and static condition on 4 mycological media for 10 days.

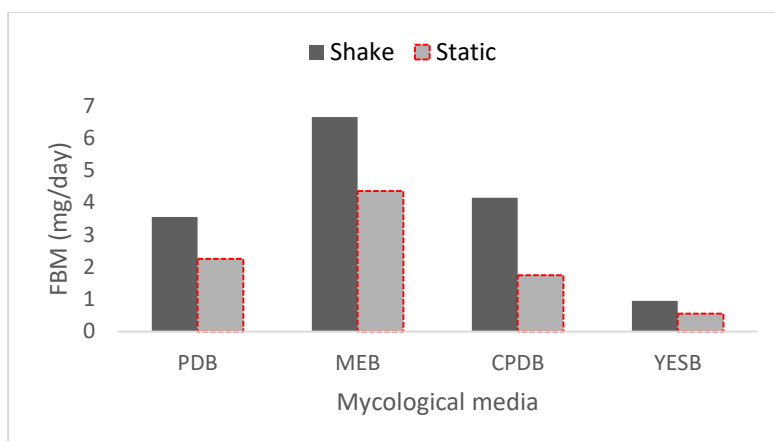


Figure 2. Production of fungal biomass by *A. ochraceus* isolate under shake and static condition on 4 mycological media for 10 days.

MEB: Malt Extract Broth

PDB: Potato Dextrose Broth

CPDB: Czapek Dox Broth.

YESB: Yeast Extract Sucrose Broth

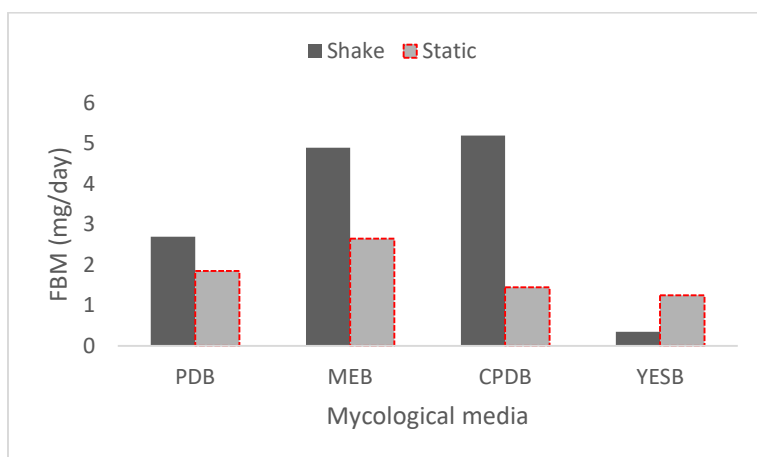


Figure 3. Production of fungal biomass by *A. ochraceus* CBS 588.68 under shake and static condition on 4 mycological media for 10 days.

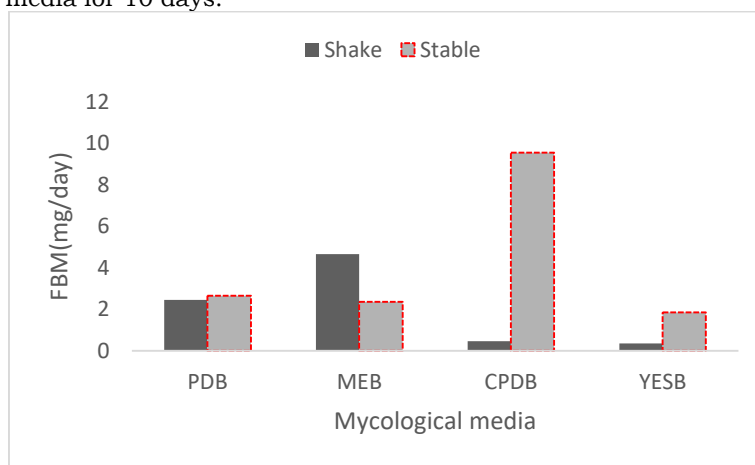


Figure 4. Production of fungal biomass by *A. niger* 285522 under shake and static condition on 4 mycological media for 10 days.

MEB: Malt Extract Broth

PDB: Potato Dextrose Broth

CPDB: Czapek Dox Broth.

YESB: Yeast Extract Sucrose Broth

Table 1. Production of ochratoxin A by ochratoxigenic fungi on four liquid media under shake culture at 25°C for 10 days as determined by TLC technique.

Fungi	Medium	Ochratoxin A detection on TLC.									
		day 1	day2	day3	day4	day5	day 6	day 7	day 8	day 9	day 10
<i>A.carbonarius</i> isolate	MEB	ND	ND	ND	ND	+	+	+	+	+	+
	PDB	ND	ND	ND	ND	+	+	+	+	+	+
	CPDB	ND	ND	ND	ND	ND	+	+	+	+	+
	YESB	ND	ND	ND	ND	ND	ND	+	+	+	+
<i>A.ochraceus</i> isolate	MEB	ND	ND	ND	ND	ND	+	+	+	+	+
	PDB	ND	ND	ND	ND	ND	+	+	+	+	+
	CPDB	ND	ND	ND	ND	ND	ND	ND	ND	+	+
	YESB	ND	ND	ND	ND	ND	ND	ND	ND	+	+
<i>A.ochraceus</i> CBS 588.68	MEB	ND	ND	ND	ND	+	+	+	+	+	+
	PDB	ND	ND	ND	ND	+	+	+	+	+	+
	CPDB	ND	ND	ND	ND	ND	ND	ND	ND	+	+
	YESB	ND	ND	ND	ND	ND	ND	ND	ND	+	+
<i>A. niger</i> 285522	MEB	ND	ND	ND	ND	ND	+	+	+	+	+
	PDB	ND	ND	ND	ND	ND	+	+	+	+	+
	CPDB	ND	ND	ND	ND	ND	ND	ND	ND	+	+
	YESB	ND	ND	ND	ND	ND	ND	ND	ND	+	+

+ = OTA detected.

ND= Not detected

MEB= Malt extract broth.

PDB= Potato dextrose broth.

CPDB=Czapek Dox broth

YESB= Yeast extract sucrose broth.

Table 2. Production of ochratoxin A by ochratoxigenic fungi on four liquid media under static culture conditions at 25°C for 10 days as determined by TLC technique.

Fungi	Medium	Ochratoxin A detection on TLC.									
		day 1	day 2	Day3	day 4	day 5	day 6	day 7	day 8	day 9	day10
<i>A. carbonarius</i> isolate	MEB	ND	ND	ND	ND	ND	ND	+	+	+	+
	PDB	ND	ND	ND	ND	ND	ND	+	+	+	+
	CPDB	ND	ND	ND	ND	ND	ND	ND	+	+	+
	YESB	ND	ND	ND	ND	ND	ND	ND	+	+	+
<i>A. ochraceus</i> isolate	MEB	ND	ND	ND	ND	ND	ND	+	+	+	+
	PDB	ND	ND	ND	ND	ND	ND	+	+	+	+
	CPDB	ND	ND	ND	ND	ND	ND	+	+	+	+
	YESB	ND	ND	ND	ND	ND	ND	ND	ND	+	+
<i>A. ochraceus</i> CBS 588.68	MEB	ND	ND	ND	ND	ND	ND	ND	+	+	+
	PDB	ND	ND	ND	ND	ND	ND	ND	+	+	+
	CPDB	ND	ND	ND	ND	ND	ND	ND	ND	+	+
	YESB	ND	ND	ND	ND	ND	ND	ND	ND	+	+
<i>A. niger</i> 285522	MEB	ND	ND	ND	ND	ND	ND	+	+	+	+
	PDB	ND	ND	ND	ND	ND	ND	+	+	+	+
	CPDB	ND	ND	ND	ND	ND	ND	ND	+	+	+
	YESB	ND	ND	ND	ND	ND	ND	ND	ND	+	+

+ = OTA detection

ND= Not detected

MEB= Malt extract broth.

PDB= Potato dextrose broth.

CPDB= Czapek Dox Broth.

YESB= Yeast extract sucrose broth

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