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Full Length Research Paper

Ecophysiological factor effect on growth and ochratoxin A production by *Aspergillus ochraceus*

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Ochratoxin A represents an important problem in fungal food poisoning in some countries. It has toxic effect on human and animals. So far the potentiality of thirty four fungal species belonging to 12 genera isolated from Egyptian legume seeds to release ochratoxin A in their culture filtrates and some ecophysiological factors affecting its production were tested. It was found that nearly 56% of the tested fungi showed positive results as the production of ochratoxin A. Aspergilli particularly *Aspergillus ochraceus* was distinguished by its capacity to produce ochratoxin A when grown on a selected medium. The maximum ochratoxin A yield was achieved were showed after 9 days incubation at 25°C. An initial pH value of 6.0 was found to be optimum for ochratoxin production and growth of *A. ochraceus*. The influence of water activity (a_w) upon biosynthesis of ochratoxin A and biomass yields were also discussed briefly. The present study gives an account of observations made on the production of ochratoxin A and biomass in relation to the carbon substrate and mitogen compounds supplied in the bioprocess. Sucrose followed by glucose and maltose were more initiative for the growth and ochratoxin A production than cellulosic substrate. Peanut seeds represent the most favorable natural substrate than other substrates investigated. Yeast extract followed by ammonium nitrate were the best nitrogen compounds for biomass and ochratoxin A production by test fungus. In addition the impact of the level of selected carbon and nitrogen ratio on both experimental parameters were also elucidated.

Key words Ochratoxin A, Aspergillus ochraceus, Ecophysiological factors, carbon substrate, mitogen compounds.

INTRODUCTION

Ochratoxin A (OTA) is a nephrotoxic and mycotoxin with carcinogenic, immuno suppressive and teratogenic properties (Murphy et al., 2006; IARC, 2009). Ochratoxin A is a fungal secondary metabolite produced by some species of fungal genera *Aspergillus* and *Penscillium* (Khalesi and Khatib, 2011). Today it is known that, ochratoxin A is frequently produced by several *Aspergillus* species including *A. ochraceus*, *A. carbonarius*, *A. niger*, *A. awamori*, *A. albertensis*, *A. wentii*, *A. auricomus*, *A. sclerotiorum* and *Penicillium* species especially *P. verrucosum* (JECFA, 2001, Frisvad et al., 2004, Abbas et al., 2009, Lasram et al., 2010, Schmidt-Heydet et al., 2011 and Khalesi and Khatib, 2011). These fungal species that invade a broad range of food products such as cereal, legume seeds and derived products (Magnoli et al., 2006, Villa and Markaki, 2009 and Durate et al., 2010), in the field during development, harvest, storing of terminal shipment and processing, produce ochratoxin A if environmental conditions are favorable. The impact of ochratoxin A in food products is significant mainly on the effect on human and animal health and the problems in the trading of agricultural commodities (Astoreca et al., 2009).

Fungal growth and consequent ochratoxin A production is determined by a wide range of parameter, broadly classified into physical, chemical and by interactions involving factors (Nielsen, 2003). Ochratoxin A biosynthesis may be influenced by intrinsic ecophysio-

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logical factors, such as pH, moisture content and the composition of the medium substrate as well as extrinsic factors such as temperature (Medina et al., 2008, Bouras et al., 2009, Pose et al., 2010, Garcia et al., 2011 and Khalesi and Khatib, 2011). Predictions of the effects of environmental conditions and the interactions if these parameters on fungal growth and Ochratoxin A productin are important in the development of hurdle technology trends to stop spoilage and ochratoxin A contamination of the raw material destined to human and animal consumption. Thus, this work is intended to gain more information concerning the potentiality of some fungi isolated from different Egyptian legume seeds to produce ochratoxin A and some ecophysiological factors affecting the accumulation by selected fungus.

MATERIALS AND METHODS

Collection of seed samples

Ten samples (250 gm) of each peanut, soybean, cow pea broad bean and lupine seeds were collected from sharkia, Dakhalia and Kalubia Governorates, Egypt, during January 2009. These seed samples were transferred to the laboratory in clean plastic bags and were stored at 4.0°C till fungal analysis.

Isolation of seed borne fungi

Different fungal species were isolated from seeds according to Roy and Kumarin (1991) with some modifications (Shindia et al., 2001). The surface sterilized seed samples were plated on Czapek's Dox agar medium to which rosebengal (65 ppm) and dihydrostreptomycin (40 ug/ml) were added as bacteriostatic agents (Martin, 1950). The plates were incubated at 30°C for 7 days. The developing moulds were isolated and identified according to universal keys of identification of fungi including, Gilman (1957), Barron (1968), Ellis (1971), Raper and Fenell (1977), Pitt (1979), Carmichael et al. (1980), Domsch et al. (1980) and Nelson et al. (1983) and others. The fungal cultures were maintained on potato dextrose agar slants and incubated at 28°C for 7 days (Smith and Onions, 1983). The spore suspension of each fungal species was prepared by suspending the spores from the slant in 10 ml of sterilized distilled H_2O (Shindia et al., 2001).

Medium and cultivation conditions

The fermentation medium for ochratoxin production was composed of (g/l), 40.0 sucrose, 20 yeast extract and 1L distilled H_2O **(**Scott et al., 1970). The pH was adjusted to 5.0. The production medium was dispensed in the fermentation flasks, each contain 50 ml sterile medium. The fermentation flask was inoculated with 1 ml spore suspension of each fungal species. The submerged flasks were shaken at 3.7 Hz and 28°C for 7 days, using a shaker incubator (New Brunswick Scientific Edison, N.J.USA.). At the end of incubation period the culture flasks were fittered through whatman No. 1 filter paper. The culture filtrates were assayed for ochratoxin A production. Three replicates were employed for each treatment.

Ochratoxin A extraction

At the end of fermentation process, three replicates/treatments were destructively sampled and stored at -20°C until OTA analysis was carried out according to **(AOAC, 2007)**. A 50 ml of culture fittrate of tested organism or 25 g portion of a finely ground legume seeds sample was added to a 500 ml Erlenmyer flask along with 12.5 ml H_2O and then add 125 ml $CHCl_3$. The mixture was shaken for 30 min and filtered through a microfiber filter to remove particulate matter.

Separation and clean up of ochratoxins (AOAC, 2007).

A ball of glass wall plug was placed in bottom of chromatographic tube. Weight 6 g $NaHCO_3$ diatomaceous earth mixture transfer to tube and add 50 ml $CHCl_3$ extract to the tube and wash with 70 ml of n-hex-ane followed by 70 ml chloroform discarding washing-ochratoxin solution elute with 100 mL CH_3 CooH – benzene (2: 98) from the column. The elution was concentrated by evaporation on steam under nitrogen and transfere quantitavely with $CHCl_3$ to 4 dram (15 mL) vial. The vial was capped and saved for qualitative and quantitative detection.

Qualitative detection of ochratoxin A

Thin layer chromatography (TLC) using glass plates (20 x 20 cm) coated with 0.3 mm slice gel DG (Merk) and activated at 110°C for 30 min were used for qualitative analysis. Benzen: methanol: acetic acid (18: 1: 1) solvent system was used for developing the plates. Extracted ochratoxin samples were detected by visual comparison of the fluorescing zone of samples and standard ochratoxins spotted an the sample plate under short and long UV-wave (min, UVIS, Duouv source, Dosage, Hedel berge). Ochratoxins A should be found in R_f 0.4-0.8 typically at 0.65 (AOAC, 2007).

Quantitative detection of ochratoxin A by HPLC (AOAC, 2007)

Ochratoxin A detection in positive samples and standards

was performed by HPLC for each treatment following the protocol adopted by AOAC (2007) with some modifications. The HPLC apparatus used for ochratoxin A determination was waters (474) system. Equipped with quaternary pump fluorescence detector set system (excitation at 360 nm; emission at 460 nm). The chromatography column was phenomenes C_{18} (250 x 4.6 nm; 5 μm particle size). The mobile phase was pumped at 1.0 mL/min and consisted of an isocratic system 30% H_2O, 60% methanol and 10% CH_3 CN. Ochratoxin A was quantified on the basis of HPLC fluorometric response compared with Ochratoxin A standard (Sigma Aldrich Co. St Louis, Mo USA). Data were collected and integrated using (total chrom Navigator) chromatography manger soft ware.

Toxic effect of ochratoxin A on bacterial growth

Sterile petridishes were filled with 20 mL of nutrient agar medium mixed with 2 mL of *Lactobacillus subitilis* culture (Shindia et al., 2001 and Abdel- Khalik, 2008). After solidification of the medium, paper discs (6.0 cm diameters) were soaked with 20 μl ochratoxin A solution, air dried and put on the test plates. The plates were incubated overnight at 4°C and then 24h at 28°C. The inhibition zones were observed and measured.

Biomass estimation

The content of each fermentation flask was filtered and mycelial residues were washed with ethanel and distilled H_2O, then dried at 80°C to constants mass (Shindia et al., 2001).

Statistical analysis

The obtained results were conducted to one-way ANOVA. The data were statistically analyzed as complete randomized block design. Means were compared using the least significant difference (LSD) at $P<0.05$ (Snedecor and Cochran, 1982).

RESULTS AND DISCUSSION

Potentiality of production of ochratoxin A among test seed borne fungi: The aim of this experiment was to investigate the capacity of thirty four fungal species to produce ochratoxin A in their culture filtrates. Thirty four fungal species belonging to 12 general isolated from different seeds were examined (Table 1). The results show that all the tested fungi survived well in the experimental media. No correlation existed between biomass production and the released ochratoxin A in their filtrates. Table (1) reveals that only, the 16 species of Aspergilli and 3 of penicillia could produce ochratoxin A in their cultures. The remaining tested fungal species failed to produce ochratoxin A in their culture filtrates under experimental connotations. *Aspergillus ochraceus* was obviously the best producer of ochratoxin A followed by *A. carbonarius, A. niger, A. flavus, A. tamari, A. oryzae, A. parasiticus* and *A. fumigatus*. The fungal species with ochratoxin A production below 50% of that of *A. ochraceus* were considered low producers of ochratoxin A and will not be considered for further discussion (Table 1). In connection with our screening study, several fungal species particularly Aspergilli were listed by other investigators as ochratoxin A producers in culture media and foodstuffs, such as *A. ochraceus, A. niger, A. carbonarius, A. awamori, A. alliaceus, A. melleus, A. sclerotium and A. glaucus* (Harris and Mantle, 2001; Cabanes, 2002, Magnoli et al., 2003, Frisvad et al., 2004, Romero et al., 2005, Marin et al., 2006, Astoreca et al., 2009, Romero et al., 2010; Palumbo et al., 2011). Their results and the studies of others (Albrouch et al., 2011, Brera et al., 2011; Sonjaka et al., 2011) were supportive to our observation about the ochratoxin A production by the reported fungi this a wide variations from low to high as well as non production of ochratoxin A which appeared in the present study may be attributed to the inherited differences in the biological activities of different fungal species surviving in single environmental niche. These differences proved the complementary action of these fungi towards one another in their living environment such as storage legume seeds.

The screening study in this work was extended to elucidate the effect of extracted ochratoxin A produced by different producers of the most active species of *Aspergillus* on bacterial strain. High purified ochratoxin A produce by *Aspergillus* spp were inoculated with bacterial strain (*L. subtilis*). After 24h of incubation it was recorded that ochratoxin A could inhibite bacterial growth and this inhibition varied according to the *Aspergillus* species producing it. Maximum inhibition of *L. subitilis* growth was observed by ochratoxin A produced by *A. ochraceus* followed by *A. carbonarius* and *A. niger* (Figure 1). This variation in ochratoxin A effect can be discussed as, effect of ochratoxin A depends on its concentration and *A. ochraceus* consideration the highest producer for ochratoxin A and so it had the highest effect on *L. subitilis* growth. The results of other investigators (Heller et al., 1975 and Ali- Vehams et al., 1998) were in agreement with our observations about the inhibitory effect of ochratoxins on bacterial growth. They also reported that ochratoxins reduce protein and RNA synthesis of bacterial cells.

Aspergillus ochraceus gave the highest amounts of ochratoxin in culture filtrates under bioprocess conditions. These findings justified the selection of *A. ochraceus*

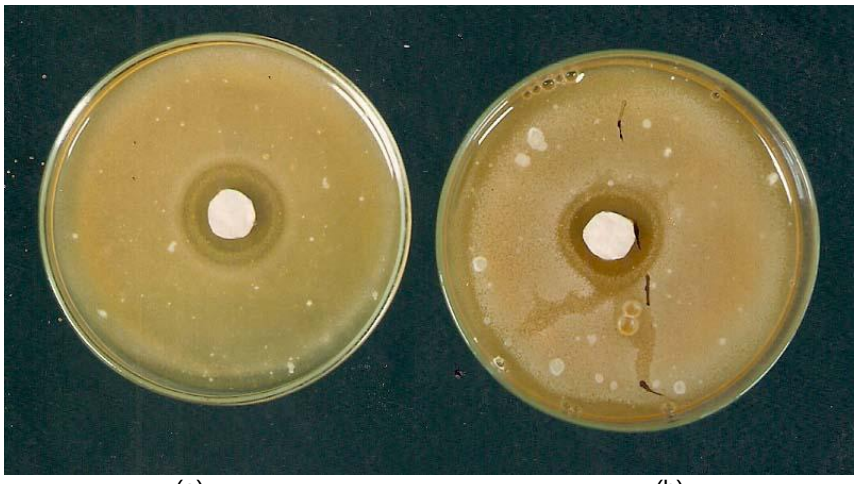
Table 1. Screening for ochratoxin A and biomass production by the tested fungal species after 7 days at 30°C.

Fungal species	Dry biomass g/L	Ochratoxin A ug/ml
Aspergillus avenaceus	7.11±0.2	7.11±0.8
A. candidus Link	6.51±0.2	6.16±1.2
A. carbonarius (Bain.) Thom	8.90±0.25	17.11±1.0
A. carreus (Tiegh) Blochwitz	5.13±0.22	7.11±0.6
A. flavus Link	8.95±0.34	12.13±1.2
A. flavipes (Bain & Sart) Thom & Church	5.90±0.35	9.08±0.8
A. fumigatus Fresenius	6.60±0.22	17.00±1.5
A. nidulans Apinis	6.11±0.20	21.01±1.0
A. niger van Tieghem	9.52±0.30	6.08±0.5
A. ochraceus Wilhelm	8.11±0.1	11.30±0.5
A. ornatus Raper, Fennell & Tresner	7.02±0.15	11.50±0.5
A. oryzae (Ahlb.) Cohn	8.00±0.15	11.30±0.85
A. tamari Kita	8.10±0.20	8.11±0.8
A. parasiticus Speare	6.8±0.30	6.70±1.0
A. terreus Thom	7.80±0.33	5.0±0.75
A. versicolor (Vuill) Tirabosohi	5.11±0.23	6.0±0.5
Penicillium chrysogenum Thom	6.50±0.26	3.0±0.5
P. citrinum Thom	5.11±0.18	0.0
P. oxalicum Currie and Thom	4.80±0.25	0.0
P. purpurogenum Stoll	5.70±0.12	0.0
Alternaria alternate (Fris) Keissler	4.11±0.22	0.0
Cladosporium oxysporum Berkand Cust	4.59±0.28	0.0
Fusarium moniliferme sheldon	5.13±0.16	0.0
F. solani (Matt) App. and Wollen	5.60±0.24	0.0
Humicola grisea	4.01±0.18	0.0
Malbranchea cinnomenae	3.60±0.32	0.0
Paecilomyces variotii Brain	5.18±0.12	0.0
Paecilomyces violaceae	5.00±0.24	0.0
Stachybotrys atra Cord	4.12±0.21	0.0
Trichoderma koningii Oudamans	6.11±0.16	0.0
T. viride Pers	5.90±0.11	0.0
Mucor circinoloids (Bain) van Tieghem	240±0.15	0.0
M. racemosus Fres	290±0.35	0.0
Rhizopus oryzae Went and Prinsen geerlings	3.11±0.22	00

for further experimentation.

Time course of ochratoxin A production and growth of A. ochraceus

The growth of *A. ochraceus* and its production of ochratoxin A in culture both were determined during the bioprocess which prolonged for 14 days. (Figure 2) reveals a correlation between level of ochratoxin A and extends of fermentation period. Negligible production of ochrotoin A was observed in the initial 24hr of bioprocess, though adequate growth was maintained under these conditions. Optimum production of the ochratoxin A was obtained after 9 days of fermentation. Beyond this period the ochratoxin was found to drop gradually with a further extension of the incubation to 14 days. The data (Figure 2) also indicate that, no correlation was observed between the released amounts of ochratoxin A in culture filtrate and the growth of *A. ochraceus*. Optimum growth was obtained after 5 days of fermentation periods. These results coincide with those of many workers who concluded that, in the first, the fungal strain must grow exponentially and form enough biomass production, then many secondary metabolites such as ochratoxin A are optimally prevaliled during deceleration and maximum stationary phases (Demain, 1972, Smith, 1996, Calvo et al., 2002 and Astoreca et al., 2009). Previous investigators found that an incubation period 10 days was optimal for ochratoxin A production by several *Aspergillus* species (Marin et al., 2006, Medina et al., 2008 and Spadro et al., 2010). On the other hand an incubation period from 5 to 7 days was most favorable for the biosynthesis and release ochratoxin A by different Aspergilli (Varga et al., 2002, Belli et al., 2004 and 2005).



(a) (b)

(c)

Figure 1. Effect of ochratoxin A on bacterial strain, *Lactobacillus subtilis*:
(a) Bacterial growth inhibited by ochratoxin A produced by *A. ochraceus*.
(b) Bacterial growth inhibited by ochratoxin A produced by *A. carbonarius*.
(c) Bacterial growth inhibited by ochratoxin A produced by *A. niger*.

Effect of the initial pH value

Ochratoxin A and dry mycelial mass profile with respect of initial pH of the fermentation medium is shown in (Figure 3). The results revealed that an initial pH of 6 was found to be optimal for both the growth of fungal strain and ochratoxin A production. Above and below this pH value the yield of biomass and ochratoxin A was substantially lower it is also from the data on dry biomass that the tested organism was able on survive over a much wider range in pH. These data agree with the findings of Klich et al. (2009) who found that the optimum levels of ochratoxin A of three isolates of *A. alliacens* were obtained at pH 6.0. This is contrast to the situation in *Penicillum nordicum* shows highest production of ochratoxin A at neurtral to slightly alkaline. Conditions, ranged between pH 6-8 (Geisen, 2004**)**. In addition, Spadro et al. (2010), recently, found that the maximum fungal growth and ochratoxin A production by *A. ochraceus* was detected at pH 4.0.

Effect of incubation temperature

the incubation temperature has a significant on the production of ochratoxin A and growth of *A. ochraceus* (Figure 4). The optimal temperature for ochratoxin A and biomass production by tested organism was found to be 25°C. Above and below this temperature the biomass and ochratoxin A release dropped. It was also found that the fungus failed completely to develop mycelia and hence no ochratoxin A production at 50°C. from these observations, it can be concluded that the incubation temperature consider a critical factor controlling fungal growth and ochratoxin A production (Frisvad and Samson, 1991). In accordance with our findings are those obtained by Pardo et al. (2006), Medina et al. (2008) and Alborch et al. (2011) who reported that, 25°C

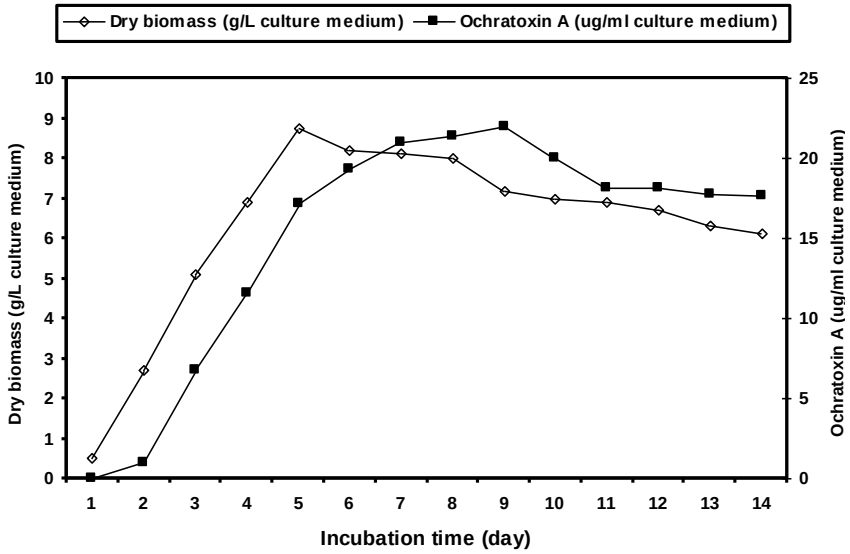


Figure 2. Time course of growth and ochratoxin A production *Aspergillus ochraceus* during 14 days.

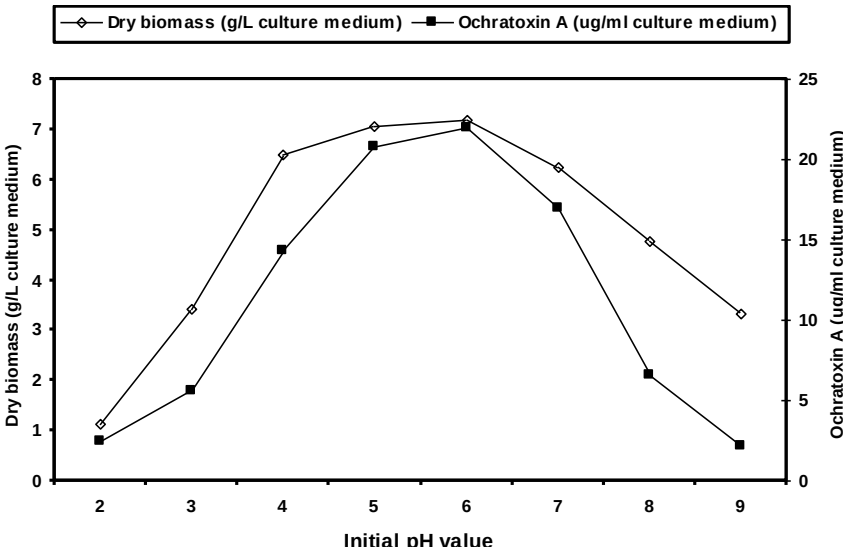


Figure 3. Effect of an initial pH value on the growth yield and ochratoxin A production by *Aspergillus ochraceus*.

was optimal incubation temperature for the growth and ochratoxin A production by *A. ochraceus, A. carbonarius* and *A. lacticoffeatue.*

Effect of water activity (a_w)

Water activity is perhaps the most critical factor influencing germination, growth and establishment of fungi as well as their mycotoxin production on nutrient rich substrates **(**Bouras et al., 2009). The effect of a_w (0.83-0.997) on growth and ochratoxin A production by *A. ochraceus*, cultured in yeast extract sucrose medium as studied (Figure 5). It was demonstrated that the growth rate and ochratoxin A production by *A. ochraceus* increased with the increment of a_w. Thus, the maximum biomass and ochratoxin A production by tested fungus were observed at 0.997 a_w at 25°C. No growth and consequently biosynthesis of ochratoxin A by *A. ochraceus* in culture fittrate were recorded at 0.83 (a_w)

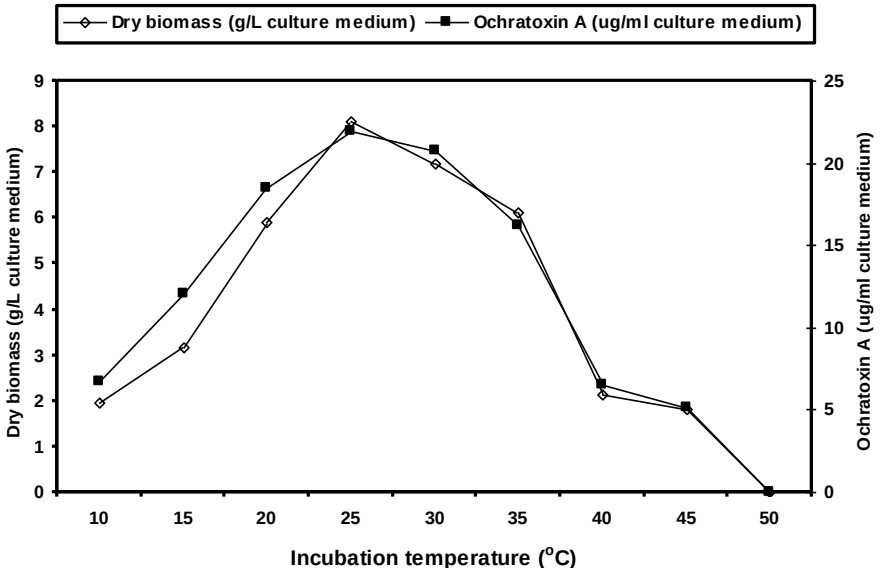


Figure 4. Effect of incubation temperatures on the growth and ochratoxin A production *Aspergillus ochraceus*.

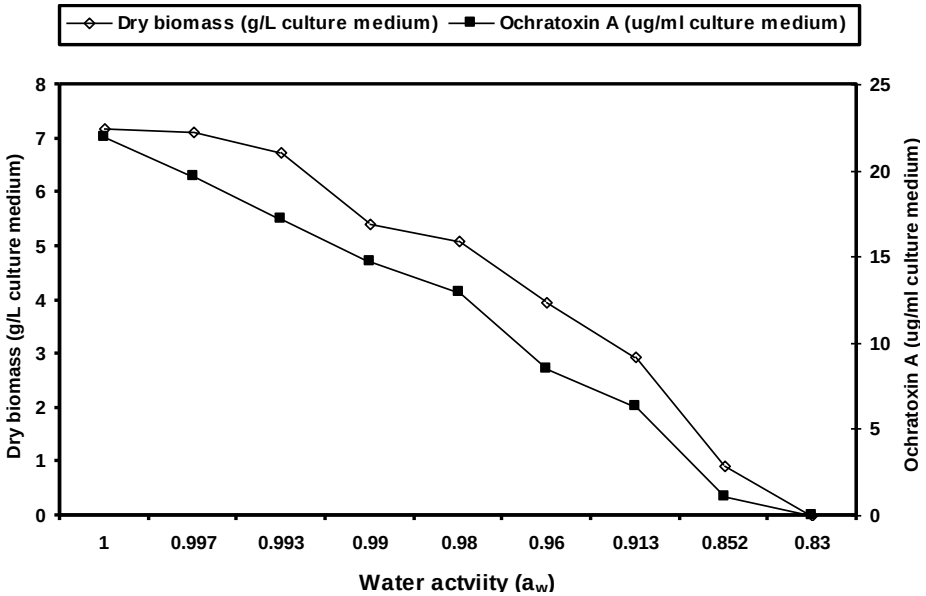


Figure 5. Effect of water activity (a_w) on the growth and ochratoxin A production *Aspergillus ochraceus* on yeast extract sucrose basal medium.

similar findings were reported by Pardo et al., (2004), Astoreca et al., (2007) and Selouane et al., (2009) for growth and ochratoxin A production by *A. ochraceus*, *A. niger* and *A. carbonarius*, respectively. Also, Lasram et al. (2010) reported that the optimal a_w for growth and achratoxin A production by *A. carbonarius* was 0.99 and their results an agreement with our data from our data and from what the authors discussed regarding the effects of a_w on OTA production, it seems that to control OTA formation, decreasing the a_w lower than 0.85 can be the first way to solve this problem.

Effect of substrate

Although different factors can influence the expression of the ochratoxigenic character of fungi, among them the fermentation substrate plays a significant role **(**Medina et al., 2004). The carbon substrates were selective in their effects on ochratoxin A production and growth of *A.*

Table 2. Effect of different substrates on the growth and ochratoxin A production by *Aspergillus ochraceus*.

Carbon substrate	Concentration %	Dry biomass (g/L culture medium) Mean±SE	Ochratoxin A (ug/ml culture medium) Mean±SE
Sucrose	4	7.17±0.15	21.95±0.50
Glucose	4	7.60±0.20	20.50±0.40
Fructose	4	7.05±0.20	18.95±0.45
Maltose	4	7.23±0.22	19.11±0.45
Lactose	4	5.56±0.20	15.55±0.52
Starch	1	6.10±0.26	16.35±0.48
Pectin	1	4.95±0.24	11.06±0.63
Suagrcane bagasse	1	6.90±0.20	17.27±0.58
Xylan	1	3.86±0.25	7.77±0.46
Cellulose	1	2.75±0.30	6.15±0.50

Table 3. Effect of some foodstuffs as carbon substrate on the growth and ochratoxin A production by *Aspergillus ochraceus*.

Foodstuffs substrate	Dry biomass (g/L culture medium) Mean±SE	Ochratoxin A (ug/ml culture medium) Mean±SE
Control*	7.17±0.30	21.95±0.55
Peanut	6.24±0.26	18.32±0.46
Soybean	6.14±0.20	16.95±0.63
Broad bean	5.58±0.20	11.65±0.68
Cowpea	5.23±0.25	9.77±0.70
Lupine	3.37±0.35	6.15±0.55

* Control: basal medium containing sucrose as carbon source.

ochraceus (Table 2). The present results reveal that, sucrose followed by glucose and maltose were the best substrates for biomass and ochratoxin A production by *A. ochraceus* during the incubation period of bioprocess cellulosic substance was the least carbon substrate for production of experimental parameters. Preferential utilization of some carbon substrate by the test fungus depends on the membrane carrier protein, which have at least a degree of substrate specificity. Thus, it transports sucrose in preference to other tested sugar because it usually has a constitutive carrier for this sugar is reported by other investigators (Lai et al., 1970, Deacon, 1997 and Muhlencoert et al., 2004). The preference fin usage of some carbon substrate by several ochratoxigenic fungi was also reported by Medina et al (2004), (2008) and Abbas et al (2009) and their results were supportive to our findings.

The experiments were extended to determine the ochratoxin A as well as biomass production by tested fungus as grown on different legume seeds as sole carbon substrate in the fermented media. The results of this study (Table 3) showed that, peanut seeds was the best suitable source for the growth and ochratoxin A production followed by soybean and broad bean seeds. The superiority of peanut seeds as foodstuff energy substrate over the other test seeds may be attributed to the presence of high percentage of carbon content as well as some growth factors. Other investigators reported that carbon compounds present in food commodities play a vital role in fungal metabolism and mycotoxin production (Reddy et al., 1998). Kokkenon et al (2005) and Khalesi and Khatib (2011) also concluded that the growth substrate had selective effect on the mycotoxin ability of test fungi, *P. nordicum*, *P. crustosum* and *P. verrucosum* strains and their observations are in agreement with our results.

Effect of different nitrogen sources

The impact of a range of nitrogen sources on ochratoxin A and growth rate of *A. ochraceus* shown in (Table 4). All seven tested nitrogenous compounds were able to support growth of *A. ochraceus*. The final biomass and ochratoxin A production varied considerably, however, maximum production of both experimental parameters were evident in presence of yeast extract followed by ammonium nitrate and potassium nitrate. Minimum biosynthesis of ochratoxin A and biomass production by

Table 5. Effect of different carbon/nitrogen ratio on the growth and ochratoxin A production by *Aspergillus ochraceus*.

C*/N** ratio	Dry biomass (g/L culture medium) Mean±SE	Ochratoxin A (ug/ml culture medium) Mean±SE
10:1	7.92±0.30	15.50±0.72
20:1	8.11±0.35	21.95±0.85
30:1	8.05±0.15	20.95±0.85
40:1	7.65±0.15	19.37±0.80
50:1	7.04±0.26	17.12±1.00
60:1	6.11±0.30	16.52±1.00
70:1	4.50±0.34	14.09±0.88
80:1	3.19±0.32	7.16±1.20
90:1	2.60±0.32	3.75±0.70
100:1	1.96±0.20	2.30±0.70

*C = Sucrose **N = Yeast extract.

A. ochraceus were obtained only in presence of urea. This may be due to its toxic effects in high doses and/or the pH variation occurred after the addition in the bioprocess. Other tested inorganic and organic nitrogen compounds were favorable for the ochratoxin A production but comparatively less inducible than control. Other investigators, had conformal the selective induction of nitrogen sources on biomass and ochratoxin A production by *A. ochraceus* (Medina et al., 2004, Muhlencoert et al., 2004 and Abbas et al., 2009). In addition, ochratoxin A production by modifying the bioprocess with different nitrogen compounds were done by Ferreira and Pitout (1969) and Medina et al., (2008) who found that the best toxin production was obtained with organic nitrogen or ammonium nitrate. Their results confirmed our data. The prominent effect of complex organic nitrogen such as yeast extract may be attributed to the fact that such complex organic nitrogen give on hydrolysis a number of some intermediate compounds structurally available as precursors of mycotoxin and some growth factors as well as major minor elements that may be used for biosynthesis of enzyme responsible for mycotoxin anabolic pathway (Anonymous, 1999).

Effect of carbon/nitrogen ratio

varying C/N ratio (1:10 – 1:100) exerted a stimulatory effect on the growth and ochratoxin A production by *A. ochraceus* (Table 5). The best C/N ratio for maximum ochratoxin A production and growth rate of tested fungus was found to be 20:1 C/N ratio in bioprocess. Above this optimal C/N ratio. The biomass and ochratoxin A production almost reduced. Other investiagors (Medina et al., 2004, Muhlencoert et al., 2004, pardo et al., 2006, Medina et al., 2008) had confirmed the concentration of carbon and nitrogen dependence of biomass and ochratoxin A production by different ochratoxigenic fungi especially *A. ochraceus*.

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