PRODUCTION OF CHITOSAN BY SURFACE AND SUBMERGED FERMENTATION FROM *ASPERGILLUS NIGER* AND *RHIZOPUS NIGRICANS*

Zaghloul, R.A[1]; Abou-Aly, H.E[1];El-Housseiny,T. M[1];Ghonaimy,G.A[2]and Ashry, Noha,M[1]

1-Fac. Agric., Moshtohor, Benha University

2-Food Techn. Res. Inst. (FTRI), Agric.Res. Center (ARC)

ABSTRACT

This study was carried out to isolate chitosan producing fungi from different food sources. Forty five isolates of fungi were isolated from different natural contaminated sources, Twenty three isolates belonging to genus *Aspergillus* while, the other twenty two isolates were belonging to genus *Rhizopus*. All isolates were tested to choose the potential isolates according to the yield of fungal biomass. Results showed that, *Aspergillus* isolates gave an amount of biomass yield more than that obtained by *Rhizopus* isolates grown on all the tested media. The obtained results is clearly indicate that the highest records of fresh and dry weights of *Aspergillus* biomass were obtained on Karimi's medium while, the highest records of fresh and dry weights of *Rhizopus* biomass were observed on yeast peptone glucose medium. The selected fungal isolates which produced considerable biomass content were tested for their chitin and chitosan content using submerged and surface culture. It is important to mention that surface culture method gave higher records of biomass, chitin and chitosan production compared to submerged culture. So surface fermentation was used in subsequent experiment.

INTRODUCTION

Chitosan is a natural hydrophilic polysaccharide, it is a deacetylated derivative of chitin formed primarily by repeated units of β(1-4)-2-amino-2-deoxy-D-glucose (or D-glucose-amine) and comprising of (1,4)-linked aminodeoxy-β-D-glucan. It is non-toxic, biodegradable, biocompatible and highly polycationic biopolymer .Chitosan is found as a supporting material in many aquatic organisms; insects; terrestrial crustaceans; nematodes and fungi **(Nwe *et al*, 2010)**. An alternative source of chitosan is the cell wall of certain fungi especially *Aspergillus* and *Rhizopus* genera. **(Tan *et al*, 1996 and Suntornsuk *et al*, 2002).**Selection of a suitable media for the production of chitosan is a primary key factor and an extremely significant step. Substrates provide the required energy and substratum for the fungus to grow and produce the desired metabolite. Chitosan is known to be the best heavy metal adsorbent for all polymers so far characterized as chelating polymers **(Hu *et al*, 2004).** Fungal chitosan even exhibits greater collection ability than crustacean chitosan. This chelating ability is mainly due to its high content of amino groups and the abundant hydroxyl groups. The present work was carried out to determine the best fermentation method for chitosan production by *Aspergillus niger* and *Rhizopus nigricans*.

MATERIALS AND METHODS

Isolation of fungi

After collection of spoilage food samples from different locations. forty five fungal isolates were obtained using potato dextrose agar medium. The spoilage foods were washed with sterile water, then sub-culturing the fungi washed off water. The sub-culturing was carried out by using a sterile fresh medium of potato dextrose agar (PDA) and incubated at 28°C until fungal proliferation on medium surface. The isolation of pure fungal colony in culture medium was performed by using slants of a sterile fresh medium of PDA and incubated at 28°C for 5-7 days. The isolated fungi were maintained at 4°C according to the method described by **Al-Hindi *et al* (2011).**

Purification of fungal isolates

After isolation of fungi, the fungal isolates were purified by hyphal tip technique according to the method described by **Kishore (2007).**

Screening of fungal isolates

Primary screening

The primary screening was performed depending on the biomass production (fresh and dry weights) of fungal isolates. Forty five fungal isolates were tested to choose the best fungal isolates which gave the highest fresh and dry weight on different media namely Karimi's, PDA, YPG, Czapek's, Waksman's, MEP, malt extract and Richard's media.

This experiment was performed by adding 40 ml from each medium in 100 ml Erlenmeyer flasks. The media were inoculated by fungal isolate disks (5cm in diameter). The flasks were incubated at 28°C for 7 days. At the end of incubation period, fungal mycelia were harvested using filtration and weighed to calculate the fresh weights. Biomass of each isolate was washed with distilled water and dried at 60° C to a constant weight. The fungal isolates which gave higher fresh and dry weights were selected for secondary screening.

Secondary screening:

The selected fungal isolates which gave considerable biomass values were tested for their chitosan production using surface and submerged methods.

One hundred ml of the best media which were detected in the primary screening were dispended in 500 ml Erlenmeyer flasks. The flasks were autoclaved at 121°C for 15 min. The media were inoculated with a suitable spore suspension (104 spore / ml) of each selected isolates.

The flasks which were used for submerged technique were incubated at 28°C for 7 days using shaker at 150 rpm, while the flasks were used for the surface

Effect of optimal conditions

This experiment was carried out to compare the efficiency of one of two strains of either *Aspergillus* or *Rhizopus* when using the optimum conditions for chitosan production. A volume of 100 ml of either Karimi's medium and yeast peptone glucose medium was dispended in 500 ml Erlenmeyer flasks for growing *A.niger* or *R.nigricans* strains, respectively. .

Each flask was supplemented with sucrose as a carbon source and peptone as a nitrogen source for chitosan production from *R. nigricans*. Peptone was replaced

with urea as a nitrogen source for chitosan production from *A. niger*. Different media were initially adjusted at pH ranged from 4 to 4.5 for *A. niger* and pH 5 for *R. nigricans*. The flasks were autoclaved at 121°C for 15 min. Each flask containing Karimi's and YPG media was inoculated with one of the strains of *A. niger* (No.4 & 11) and *R. nigricans* (No.6 & 22), respectively using suitable spore suspension (10^4spore/ml).The flasks were incubated at 28°C for 7 day using surface method. At the end of different incubation periods, the produced chitosan was extracted and calculated as mg/l. culture productions which incubated at the same temperature and period without shaking.

Chitosan extraction

After the incubation period, fungal mycelia were harvested and washed with distilled water, then dried at 60°C to a constant weight. Chitosan was extracted from dried fungal mycelia according to the method described by **Synowiecki and Al-Khateeb (1997)**.

Identification of the selected fungal isolates

The purified fungi were microscopically identified according to their cultural and morphological features. Identification was also confirmed by Department of Fungal Taxonomy, Plant pathology Research Institute, Agricultural Research Center (ARC), Giza, Egypt .Stock cultures were maintained on PDA slants at 4°C and sub-cultured on fresh medium every 6-8 weeks.

RESULTS AND DISCUSSION

Isolation of fungi

Forty five isolates of fungi were isolated from different natural contaminated sources. According to morphological characteristics and microscopic examination, the obtained fungal isolates were initially found to be belonging to genera *Aspergillus* and *Rhizopus*. Twenty three isolates belonging to genus *Aspergillus* while, the other twenty two isolates were belonging to genus *Rhizopus*.

Primary screening

Data presented in **Tables (1&2)** revealed that all *Aspergillus* and *Rhizopus* isolates were grown very well on all the tested media used in this investigation.

It should also be noticed that, *Aspergillus* isolates gave an amount of yield biomass more than that obtained by *Rhizopus* isolates grown on all the tested media. These results are in agreement with those obtained by **Ghonaimy, (2010)** who found that *A. niger* gave the greater biomass yield on potato dextrose, yeast peptone dextrose and malt extract media than that of *R. nigricans* on the same previous media.

The obtained results in **Table (1)** clearly indicate that the highest fresh and dry weights of *Aspergillus* biomass were obtained on Karimi's medium followed by Richard, malt extract peptone, Czapek's, potato dextrose, yeast peptone glucose, malt extract and Waksman's media. The fungal isolates number A11, A4, A20, A5 and A3 gave the highest values of fungal biomass dry weights giving 7.50, 6.70, 6.13, 5.88 and 5.75 g/100 ml, respectively. Therefore, these five *Aspergillus* isolates were used for secondary screening.

In addition, data in **Table (2)** showed that the highest records of fresh and dry weights of fungal biomass were observed on yeast peptone glucose medium,

followed by Karimi's, malt extract peptone, Czapek's, Waksman's, potato dextrose, malt extract and Richard media.

The fungal isolates number R6, R15, R18, R20 and R22 gave higher fungal dry biomass (0.75 g/100ml) comparing to the other *Rhizopus* isolates when they were grown on yeast peptone glucose medium.

Therefore, *Aspergillus* and *Rhizopus* isolates that produced much more higher biomass content were selected for further studies on their potent medium.

Table 1: Fungal biomass production (g/100ml) of *Aspergillus* isolates in different media.

Isolate No.	Media															
	M_1		M_2		M_3		M_4		M_5		M_6		M_7		M_8	
	fresh	dry	fresh	dry	fresh	Dry	fresh	Dry	fresh	Dry	fresh	dry	fresh	Dry	fresh	dry
A1	16.58	5.08	7.00	0.75	7.75	1.83	5.58	0.70	4.95	0.33	7.08	2.20	3.45	1.08	10.33	3.95
A 2	11.70	2.05	7.58	0.83	6.75	1.00	4.83	0.58	5.95	0.58	8.70	1.58	3.83	0.33	10.08	3.20
A 3	14.83	5.75	6.83	1.33	6.05	1.05	5.20	0.58	6.83	3.75	6.95	0.83	4.33	0.33	8.45	2.20
A 4	18.75	6.70	5.95	1.83	6.83	1.70	4.20	1.70	5.20	0.58	8.45	2.33	3.70	0.33	9.45	3.33
A 5	18.38	5.88	7.45	0.95	6.20	1.45	6.58	1.45	5.45	1.58	6.70	0.33	3.95	0.20	5.20	1.45
A 6	18.30	5.28	6.20	1.45	7.20	1.20	5.20	0.45	5.08	0.20	7.15	0.95	3.83	0.20	9.58	3.20
A 7	13.25	1.88	7.20	2.08	5.20	0.83	3.58	0.20	4.08	0.33	6.70	0.83	3.45	0.70	5.83	1.08
A 8	15.05	4.43	4.20	0.83	6.20	0.58	3.83	0.45	3.70	0.20	6.95	1.08	3.20	0.45	7.83	1.45
A 9	14.75	2.43	6.83	1.20	6.25	0.55	4.45	0.58	2.20	0.08	7.33	2.33	3.33	0.33	7.70	2.20
A 10	11.55	3.05	6.08	1.45	6.83	1.58	3.33	0.25	0.45	0.08	6.08	0.83	3.33	0.45	6.58	1.33
A 11	15.00	7.50	7.25	2.00	5.95	0.45	3.83	0.20	3.83	0.33	5.70	0.83	3.08	0.83	10.08	2.95
A 12	12.13	4.38	6.33	0.58	5.33	0.58	9.08	1.83	5.45	0.33	6.95	1.20	5.20	0.20	11.50	4.00
A 13	7.38	1.88	6.70	0.58	4.70	0.58	6.08	1.20	4.20	0.33	7.83	1.83	5.83	0.45	8.13	1.05
A 14	11.88	3.75	5.83	0.33	4.58	0.33	5.45	1.70	4.08	0.95	7.70	1.83	4.83	0.33	8.75	1.55
A 15	10.25	3.63	7.45	0.70	4.45	0.70	6.08	1.95	4.58	1.08	8.58	1.58	3.33	0.20	11.75	2.25
A 16	10.63	3.88	5.33	0.45	4.45	0.45	5.83	1.95	4.70	0.20	7.08	1.33	3.70	0.45	9.75	2.00
A 17	14.25	5.25	7.20	0.95	5.70	0.95	7.20	1.83	5.95	0.20	8.20	2.58	4.33	0.95	9.38	3.25
A 18	10.38	1.88	5.83	0.33	4.83	0.33	4.95	1.08	4.33	0.20	7.83	2.83	5.45	0.70	9.25	2.50
A 19	14.13	2.25	6.83	0.45	3.95	0.45	5.45	0.95	4.83	0.33	8.45	1.83	3.58	0.45	10.50	3.00
A 20	14.50	6.13	7.08	0.20	5.70	0.20	6.08	2.45	4.70	0.95	7.08	1.83	4.33	0.70	8.50	1.63
A 21	10.50	3.75	6.45	0.83	4.45	0.83	6.45	2.45	4.95	0.70	8.70	3.08	3.33	0.58	10.00	1.63
A 22	16.00	5.38	6.70	0.58	2.83	0.58	5.95	1.20	3.95	0.33	8.95	3.33	3.33	0.45	6.75	1.75
A 23	12.88	4.25	7.08	0.58	7.20	0.58	5.58	1.70	3.95	0.45	11.08	2.58	6.20	0.70	9.00	0.75

A: *Aspergillus* M_3: Potato dextrose medium M_6: Malt extract peptone medium

M_1: Karimi medium M_4: Czapek medium M_7:Malt extract medium

M_2: Yeast peptone glucose medium M_5: Waksman's medium M_8: Richard medium

Table 2: Fungal biomass production (g/100 ml) of *Rhizopus* isolates on different media

Isolate No.	Media															
	M_1		M_2		M_3		M_4		M_5		M_6		M_7		M_8	
	fresh	dry	fresh	dry	fresh	dry	Fresh	dry	fresh	Dry	fresh	dry	fresh	dry	fresh	dry
R 1	3.25	0.45	6.25	0.58	5.50	0.23	5.01	0.40	6.50	0.28	6.00	0.40	3.38	0.10	2.30	0.17
R 2	3.00	0.50	4.13	0.50	5.50	0.25	4.65	0.41	4.75	0.28	5.25	0.40	2.75	0.08	3.18	0.10
R 3	3.50	0.53	5.75	0.50	4.25	0.28	6.00	0.50	4.00	0.28	5.63	0.40	2.75	0.08	2.86	0.15
R 4	2.25	0.45	5.13	0.48	3.50	0.30	5.53	0.40	3.75	0.23	5.50	0.43	2.13	0.10	2.99	0.18
R 5	3.50	0.50	5.38	0.55	3.63	0.25	5.00	0.54	5.13	0.28	4.38	0.43	2.38	0.08	3.15	0.20
R 6	3.50	0.55	6.38	0.75	4.75	0.23	4.22	0.51	5.10	0.33	9.13	0.58	2.50	0.10	2.43	0.11
R 7	2.75	0.50	4.75	0.75	5.00	0.28	4.35	0.43	5.13	0.28	4.25	0.53	2.88	0.15	2.55	0.08
R 8	3.50	0.53	5.63	0.55	3.75	0.28	8.15	0.59	5.00	0.28	5.75	0.43	2.63	0.13	2.35	0.10
R 9	3.50	0.45	5.75	0.50	3.25	0.28	5.65	0.43	5.38	0.28	4.88	0.48	2.00	0.08	2.34	0.08
R 10	3.50	0.50	5.88	0.50	4.13	0.25	4.64	0.50	4.38	0.25	5.00	0.45	3.25	0.10	3.15	0.10
R 11	3.50	0.48	5.38	0.60	3.25	0.25	5.00	0.45	5.50	0.28	4.63	0.50	2.38	0.08	1.30	0.16
R 12	3.00	0.50	5.63	0.55	3.75	0.15	5.00	0.52	4.50	0.33	5.25	0.43	2.50	0.20	2.11	0.10
R 13	2.50	0.50	5.50	0.50	3.25	0.35	5.55	0.61	3.50	0.28	6.00	0.53	2.25	0.18	1.99	0.08
R 14	2.63	0.50	5.25	0.58	3.00	0.23	5.20	0.43	4.50	0.33	5.75	0.45	2.75	0.20	2.40	0.19
R 15	2.88	0.55	5.88	0.75	3.00	0.20	9.10	0.58	5.50	0.33	5.00	0.58	4.25	0.15	2.65	0.20
R 16	3.00	0.50	5.75	0.55	3.00	0.30	5.70	0.45	4.75	0.30	5.00	0.55	2.25	0.18	2.40	0.21
R 17	3.25	0.50	5.50	0.63	3.50	0.28	7.00	0.56	4.00	0.30	4.00	0.50	2.50	0.23	3.25	0.15
R 18	3.50	0.53	5.63	0.75	3.00	0.28	4.38	0.48	5.00	0.30	8.25	0.60	3.75	0.18	2.23	0.13
R 19	2.25	0.50	5.50	0.58	3.50	0.08	5.65	0.49	3.50	0.25	5.75	0.50	1.50	0.20	2.52	0.08
R 20	3.00	0.55	5.13	0.75	4.00	0.33	5.50	0.42	4.75	0.35	7.00	0.58	3.00	0.20	3.68	0.14
R 21	3.25	0.50	5.13	0.60	3.50	0.33	5.00	0.40	4.75	0.35	5.00	0.43	3.25	0.20	2.37	0.08
R 22	2.88	0.60	6.88	0.75	3.50	0.35	4.00	0.50	4.25	0.28	5.75	0.60	2.50	0.20	2.26	0.15

*R: *Rhizopus* isolates
M1: Karimi medium
M2: Yeast peptone glucose medium
M3: Potato dextrose medium
M4: Czapek medium
M5: Waksman's medium
M6: Malt extract peptone medium
M7:Malt extract medium
M8: Richard medium

Secondary screening

Chitosan production by *Aspergillus* isolates

Data in **Fig .1** clearly indicate that all *Aspergillus* isolates produced considerable amounts of chitosan using both culture methods. *Aspergillus* isolate (A4) gave the highest amount of chitosan by using submerged culture. While, *Aspergillus* isolate (A5) gave the lowest records of chitosan. The lowest amount of produced chitosan by isolate A5 was 250 mg/l. It could be mentioned that the isolates A4 and A11 isolates gave the highest values of chitosan production when grown in submerged fermentation, since the amounts of produced chitosan were 370 and 340 mg/l, respectively.

These results are in harmony with those reported by **Hu *et al* (2004)** who found that chitosan was produced by incubating the *Aspergillus spp* and *Rhizopus spp* at 26°C in rotary shaker incubator with asset speed at 200 rpm. They added also the amount of produced chitosan was ranged from 203 to 646 mg/l for *Aspergillus spp* while, it was ranged from 69.5 to 89.8 for *Rhizopus spp*. Also, **Ghonaimy (2010)** studied chitosan production by *Aspergillus niger* and *Rhizopus nigricans* using different shaking rates i.e. 100, 125, 150, 175, 200 and 250 rpm at 28°C for 7 days. He found that fungal growth gradually increased by increasing the shaking rate up to 175 rpm. The amount of produced chitosan was 0.87 g/l and 0.67g/l for *Aspergillus niger* and *Rhizopus nigricans*, respectively.

Regarding chitosan production, data showed that isolate A4 was more efficient to produce chitosan (410 mg/l) followed by isolate A11 (370 mg/l).

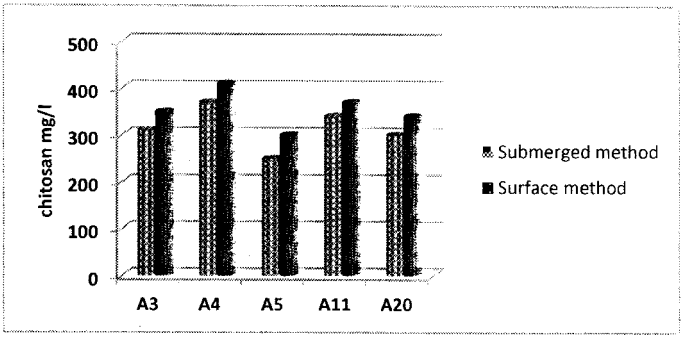

Fig.1: Chitosan production (mg/l) by selected *Aspergillus* isolates using submerged and surface methods.

These results are in agreement with those obtained by **Wu *et al* (2005)** who grew *Aspergillus niger* and *Mucor rouxii* on Yeast peptone glucose medium for chitosan production and incubated at 28°C without shaking.

Maghsoodi *et al* (2008) grew *Aspergillus niger* in solid state fermentation for chitosan production. They found that the highest yield of chitosan was 17.053 g/kg dry substrate.

Chitosan production by *Rhizopus* isolates

Data in **Fig.2** emphasized that all *Rhizopus* isolates produced considerable amounts of chitosan when using submerged and surface culture methods. *Rhizopus* isolate R6 gave the highest records of chitosan when submerged was used. Whereas, *Rhizopus* isolate R15 gave the lowest records of chitosan. The lowest amount of chitosan was produced by isolate R15 giving 22 mg/l.

Regarding the chitosan production, data showed that the *Rhizopus* R6 and R22 isolates gave higher records of chitosan production, since the amounts of produced chitosan were 40 and 30 mg/l, respectively.

As regard to chitosan production, data revealed that isolate R22 was more efficient to produce chitosan (46 mg/l) followed by isolate R6 (43 mg/l).

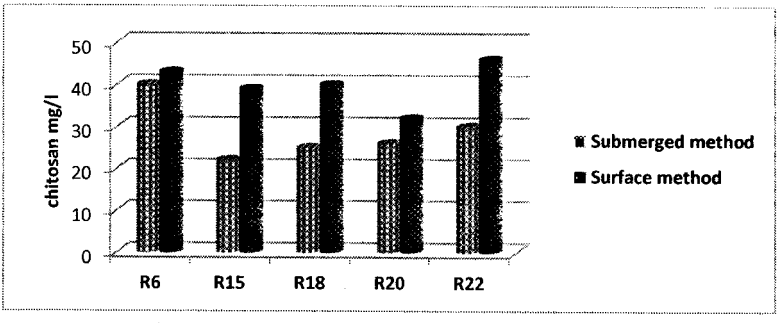

Fig. 2: Chitosan production (mg/l) by selected *Rhizopus* isolates using submerged and surface methods

Summing up, from data in **Figs (1&2)** it is important to mention that surface culture method gave higher records of chitosan production compared to submerged culture. This increase in biomass would subsequently result in a higher chitin and chitosan production. So surface fermentation was used in subsequent experiment.

These results are in agreement with those reported by **Crestini *et al* (1996) and Maghsoodi and Yaghmaei (2010)** who found that the chitosan yield was more than 20-50 times with solid substrate fermentation than those obtained with submerged fermentation. In additions, **Nwe *et al* (2011)** reported that the yield of chitosan obtained from mycelia of *G. butleri* grown on SSF was higher than that of *G. butleri* and *A. coerulea* grown on SMF, because the yield of mycelia obtained from SSF was higher than that from SMF.

Effect of the optimal conditions on chitosan production

Chitosan production by the two selected strains of either *Aspergillus* or *Rhizopus* was achieved at the optimal conditions which included both environmental and nutritional conditions those obtained in the previous experiments in the current study.

All previously obtained optimal conditions were applied. *Aspergillus niger* A4 and A11 were inoculated in modified Karimi medium containing sucrose 5 % and urea and incubated at 28°C for 7 days at pH 4.5. On the other hand, *R. nigricans* R6 and R22 were grown in modified yeast peptone glucose medium containing 2.5 sucrose and peptone as a nitrogen source and pH was adjusted at 5 while incubation temperature was 28°C for 7 days. Biomass, chitin and chitosan values were recorded in **Fig (3).**

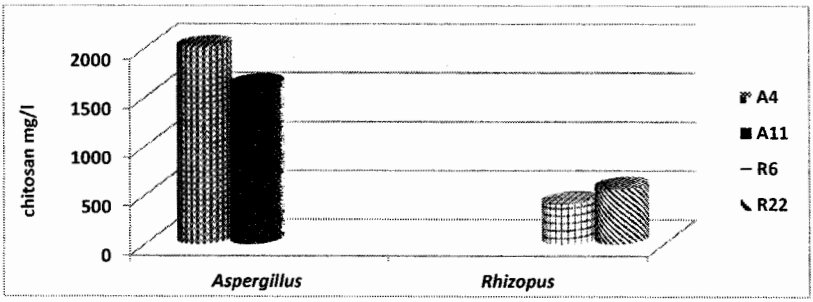

Fig 3: Effect of the optimal conditions on chitosan production by *Aspergillus niger* (A4 and A11) and *R. nigricans* (R6 and R22).

Data clearly indicate that the *A. niger* A4 gave higher records of biomass, chitin and chitosan production at the optimal conditions than A11strains.The amounts of dry weight of chitosan production were 2000 and 1610 mg/l for *Aspergillus sp* A4 and *Aspergillus sp* A11 strains, respectively, while chitosan production did not exceed 1888 and 1398 mg/l in all the previous experiments.

Concerning the *Rhizopus* strains data showed that *R. nigricans* R22 gave higher records of biomass, chitin and chitosan production at optimal conditions than *R. nigricans* R6. It is not a surprising result that the highest amounts of chitosan

production were observed when using the optimal conditions. The amounts of dry weight of chitosan production were 410 and 560 mg/l for *Rhizopus sp* R6 and *Rhizopus sp* R22 strains, respectively, while they did not exceed 401 and 551 mg/l in all the previous experiments.

These results are in harmony with **Tan *et al* (1996)** who reported that, the extractable chitosan content varied widely among the fungi evaluated: even between species of the same genus. This variation is clearly seen in *Rhizopus spp*, the extractable chitosan ranged from 102.5 mg to 278.5 mg /l substrate.

Hang (1990) recorded the highest yield of extractable chitosan from *Rhizopus oryzae* was 700 mg/l. Maximum chitosan yield was 0.9121 g/l which produced from *Aspergillus niger* **(Maghsoodi *et al.*, 2009 b).** Similar result was obtained by **Logesh *et al* (2012)** who produced chitosan from endolichenic fungi such as *Aspergillus niger* and *Rhizopus oryzae*, they found that the maximum chitosan was 1.34 g/l and 270-700mg/l, respectively.

CONCLUSION AND RECOMENDATION

In view of the obtained results, it was clearly that the chitosan can be produced by several *Aspergillus* isolates on Karimi's medium and *Rhizopus* isolates on yeast peptone glucose medium. From the morphological characteristics and physiological properties, it was clear that the isolates could be identified as *Aspergillus niger* and *Rhizopus nigricans*. Surface culture method gave higher records of chitosan production compared to submerged culture. So we recommended using surface fermentation in experiments compared to submerged fermentation. *Aspergillus niger* and *Rhizopus nigricans* gave the highest amounts of chitosan production when using the optimal conditions.

REFERENCES

Al-Hindi, R. R.; Al-Najada, A. R. and Mohamed, S. A. (2011). Isolation and identification of some fruit spoilage fungi: screening of plant cell wall degrading enzymes. J. Microbiol. Res., 5(4): 443-448.

Crestini, C.; Kovac, B. and Sermann, G. G. (1996). Production and isolation of chitosan by submerged and solid-state fermentation from *Lentinus edodes*. Biotech. Bioeng., 50: 207-210.

Ghonaimy, G. A. (2010). Optimization of culture conditions for chitosan production by *Aspergillus niger* and *Rhizopus nigricans*. Annals Agric. Sci., Moshtohor, 48(1): 79-90.

Hang, Y. D. (1990). Chitosan production from *Rhizopus oryzae* mycelia. Biotech. Lett., 12(12):911-912.

Hu, K. J.; Hu, J. L.; Ho, K. P. and Yeung, K. W. (2004). Screening of fungi for chitosan producers and copper adsorption capacity of fungal chitosan and chito-anaceous materials. Carbohydrate Polymers, 58:45–52.

Kishore, C. (2007). Studies on diagnosis and management of fungal wilt diseases of carnation and gerbera under protected cultivation. MSc. Thesis, Fac. Agric., Agric. Sci., Univ., Dharwad, U.S, p:16.

Logesh, A. R.; Thillaimaharani, K. A.; Sharmila, K.; Kalaiselvam, M. and Raffi, S. M. (2012). Production of chitosan from endolichenic fungi

isolated from mangrove environment and its antagonistic activity. J. Tropical Biomedicine., 2:140-143.

Maghsoodi, V. and Yaghmaei, S. (2010). Comparison of solid substrate and submerged fermentation for chitosan production by *Aspergillus niger*. Transactions C: Chem. Chem. Eng., 17 (21):53-157.

Maghsoodi, V.; Razavi, J. and Yaghmaei, S. (2009 b). Production of chitosan by submerged fermentation from *Aspergillus niger*. Transactions C: Chem. Chem. Eng., 16(2):145-148.

Maghsoodi, V.; Yaghmaei, S.; Beigi, S. M. (2008). Influence of different nitrogen sources on amount of chitosan production by *Aspergillus niger* in solid state fermentation. J. Chem. Chem. Eng., 27(1) 47-52.

Nwe, N.; Furuike, T. and Tamura, H. (2010). Chitin and chitosan from terrestrial organisms. **In:** Chitin, chitosan, oligosaccharides and their derivatives: Biological activities and applications. Florida: Taylor and Francis Group., p:3.

Nwe, N.; Furuike, T.; and Tamura, H. (2011). Production, properties and applications of fungal cell wall polysaccharides: chitosan and glucan. Adv. Polym. Sci., 244:187-208.

Suntornsuk, W.; Pochanavanich, P. and Suntornsuk, L. (2002). Fungal chitosan production on food processing by-products. Process Biochem., 37: 727–729.

Synowiecki, J. and Al-Khateeb, Nadia, A. A. (1997). Mycelia of *Mucor rouxii* as a source of chitin and chitosan. Food Chem., 60(4):605-610.

Tan, S. C.; Tan, T. K.; Wong, S. M. and Khorb, E. (1996). The chitosan yield of zygomycetes at their optimum harvesting time. Carbohydrate Polymers., 30:239-242.

Wu, T.; Zivanovic, S.; Draughon, F. A.; Conway, W. S. and Sams, C. E. (2005). Physicochemical properties and bioactivity of fungal chitin and chitosan. J. Agric. Food Chem., 53: 3888-3894.

إنتاج الشيتوزان بواسطة فطريات أسبرجلس نيجر وريزوبس نيجريكانس باستخدام طرق التنمية السطحية والمغمورة

راشد عبدالفتاح زغلول[1] -حامد السيد أبوعلى [1]- طلعت محمد الحسينى[1] – غنيمى عبدالفتاح غنيمى[2] – نهى محمد عشرى[1]

1- كلية الزراعة بمشتهر – جامعة بنها

2- معهد بحوث وتكنولوجيا الأغذية- مركز البحوث الزراعية – الجيزة

تم إجراء هذه الدراسة لعزل الفطريات المنتجة للشيتوزان من مصادر مختلفة . تم عزل 45 عزلة فطرية من مصادر طبيعية ملوثة، وأوضحت نتائج العزل أن 23 عزلة تنتمي إلي جنس *Aspergillus* بينما22 عزلة تنتمي إلي جنس*Rhizopus* . تم اختبار كل العزلات لاختيار أفضلها في إنتاج الكتلة الحيوية . أوضحت النتائج أن عزلات *Aspergillus* أعطت كمية من الكتلة الحيوية أعلي من المنتجة بواسطة عزلات *Rhizopus*علي كل البيئات المختبرة . أشارت النتائج المتحصل عليها إلي أن بيئة Karimi's أعطت أعلي كمية من الكتلة الحيوية الرطبة والجافة لعزلات *Aspergillus* ، بينما بيئة Yeast peptone glucose أعطت أعلي كمية من الكتلة الحيوية الرطبة والجافة لعزلات *Rhizopus*. تم تقييم العزلات التى أعطت أعلى كتلة حيوية فى الانتخاب الأولى لاختيار أكثرها كفاءة في إنتاج الشيتوزان باستخدام طريقتي التنمية السطحية والمغمورة. ومن الجدير بالذكر أن النتائج المتحصل عليها أشارت إلي أن طريقة التنمية السطحية للفطريات أعطت أعلي إنتاج للكتلة الحيوية والشيتين والشيتوزان مقارنة بطريقة التنمية المغمورة.