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RESEARCH ARTICLE

CITRIC ACID PRODUCTION FROM SOME LOCAL ISOLATES OF THE FUNGUS *ASPERGILLUS NIGER* BY RICE HUSKS FILTRATE MEDIUM

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ABSTRACT

The current study included isolation and identification some local isolates of the fungus *Aspergillus niger* from the soil of one of the gardens of Al-Qadisiya University and exclusion the isolates that have the ability to produce aflatoxins and screened to select the more efficient isolate in producing citric acid and biomass after growing the isolates on synthetic medium containing sucrose supplemented with NH_4NO_3, KH_2PO_4 and MgSO4.7H_2O and determine some of the optimal conditions for the production of citric acid from the selected isolation after growing it on the rice husks filtrate medium supplemented with some nutrients referred to in the synthetic medium.

The results showed isolation and identification 12 local fungal isolates of the fungus *A. niger* was then screening these isolates by detects its ability to produce aflatoxins and exclusion the toxic isolates, *A. niger* 4 showed high ability on the production of aflatoxins while *A. niger* 6 and *A. niger* 12 showed moderate ability on the production of aflatoxins at the CEA medium and weak ability at the production medium which contain agar, while *A. niger* 1 and *A. niger* 10 showed little ability on the production of aflatoxins, while the other isolates did not show the ability to produce aflatoxins.

Tasted ability of 7 local fungal isolates of the fungus *A. niger* on the production of citric acid by using synthetic medium of production, The results showed that for all fungal isolates studied the ability to produce citric acid but in varying degrees, As for the dry biomass for fungal isolates have values ranged between (12.64-19.73) g/l, According to the results of the statistical analysis that showed there were significant differences at the level of 5 % probability between the concentration of citric acid product of fungal isolation *A. niger* which amounted to 14.683 g/l and the other isolates and the absence of significant differences between the dry biomass of this isolation value which amounted to 18.96 g/l and isolation that gave the highest biomass has therefore selected fungal isolation *A. niger* 5 to be used in all subsequent production studies.

The optimum cultural and environmental conditions for the production of citric acid from the selected isolation were studied it was obtained the highest production of citric acid which amounted to 19.447 g/l by using the rice husks filtrate medium containing 15 % reducing sugars, 0.25 % ammonium sulfate, at temperature 30 ◌̊C and primary pH 4.

INTRODUCTION

The organic acids in addition to antibiotics and amino acid are considered important fermentation products, its include mainly Citric acid, Gluconic acid, Kojic acid and Itaconic acid (Brown *et al.*, 1987). Citric acid (2-hydroxy propane 1,2,3-tricarboxylic acid) is the most important of these organic acids, derived its name from the Latin word which means Citrus, the Swedish chemist Scheele Carl Wilhelm is the first isolation of citric acid from lemon juice in 1784, and also the citric acid produced naturally by metabolic pathways that take place in a living cell by tri carboxylic acid cycle (Swain *et al.*, 2011). Citric acid has many uses in the areas of food, chemical and pharmaceutical industries as it uses 70% of the citric acid in various food industries and 12% in the chemical, pharmaceutical, medical, and 18% in other industries (Soccol *et al.*, 2003). Also found inhibitory effect for citric acid on the growth of some fungi such as *Trichophyton mentagrophytes*, *Aspergillus fumigatus*, *Candida albicans* and *Malassezia furfur* (Shokri, 2011). In spite of the possibility of producing citric acid from plant, animal sources and chemical methods, but that commercial production has been mainly by microbial fermentation that characterized by this fermentation of the possibility of increasing production by improving the environmental conditions and genetic control for microorganism (Demain & Dana, 2007).

There are many microorganisms have the ability to produce citric acid such as bacteria including *Bacillus subtilis*, *B.*

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licheniformis and *Corynebacterium* spp. (Kapoor *et al*., 1983). and fungi such as *Aspergillus niger, A. wentii, A. awamori, A. flavus, A. nidulans, Mucor piriformis, Trichoderma viride, Penicillium janthinellum* and *P. restrictum* and yeasts such as *Candida tropicalis, C. lipolytica* and *C. intermedia* (Papagianni, 2007).

Factories producing citric acid used many strains of *A. niger* for the production of citric acid in large quantities with some improvements on the various production stages from time to time in order to increase production of citric acid (Hossain *et al*., 1984). The most important advantages of using *A. niger* in the production of citric acid is the ease of isolation and high susceptibility to fermentation of a large quantity of cheap raw material price and thus get high productivity as characterized fungus ability to convert existing sugars in the fermentation media to the citric acid with high efficiency ranging from (70-90) % (Meers & Milsom, 1987).

Recently there has been great interest in the process of microbiology development on cheap raw materials in the production of various organic acids processes in order to achieve higher production of organic acid required and the lowest cost, especially since the various food manufacturing processes, accompanied by many of the wastes, which are often rich in sugars which makes it possible to use them in the production of organic acids (Ali & Zulkali, 2011). Because of the increased interest in microorganisms and applications in nutrition, health Pharmaceutical as materials therapeutic and industry as materials cosmetics, detergents and various uses of citric acid this study aims to search for some isolates of the fungus *A. niger* from the local environment, which have the ability to produce citric acid and the possibility of using rice husks filtrate medium supplemented with some nutrients for the production of citric acid and determine some optimal conditions that increase the production.

MATERIALS AND METHODS

Preparation of Culture Media

Potato Dextrose Agar (PDA)

The preparation of this medium by dissolving 39 grams of it in the amount of distilled water in a glass beaker, adjust the pH at 5.6 and then complete the volume to 1 liter with distilled water then was sterilized by Autoclave for 15 minutes, the medium used for the purpose of isolation, identification and storage the isolates from the soil.

Coconut Extract Agar (CEA)

This medium was prepared according to the Dianese & Lin, (1976), as follows:

Taking the amount of 100 g of coconut commercially available in the market and added to 300 ml of distilled water, heated the mixture for 20 minutes, then was nominated mix by a piece of gauze and then added to the filtrate 1.5% Agar and complete volume to 300 ml with distilled water were then sterilization medium in the autoclave for 15 minutes, the medium used for the purpose of detection ability of isolated on the production of aflatoxins.

The production medium

Use the medium described by Ali *et al*., (2001) for the screening of fungal isolates used in this study for the production of citric acid, this medium is formed 15 % sucrose, 0.25% NH_4NO_3, 0.1% KH_2PO_4 and 0.025% $MgSO_4.7H_2O$, adjust the pH at 3.5, then the medium put in the flask 250 ml capacity by 25 ml/flask and then sterilization medium in the autoclave for 15 minutes.

Isolation and Identification

It was isolated fungi used in this study by taking a sample of agricultural soil (Gardens University of AL-Qadisiya) in March of 2014 by 1 kg was placed inside a nylon bags clean and was taken to the laboratory for the purpose of isolating fungi as it was followed dilution method in isolate the fungus, then it was taken 1 ml of fourth (1/10000) and fifth (1/100000) dilution each separately and placed in 15 petri dish each dilution was added to 25 ml of the PDA then incubated at 25 ° C for 5 days then purification fungus required after identification process which depending on the (Barnett & Hunter, 1972; Domsch *et al*., 1980; Moustafa, 1982).

Detection Ability of Isolated Fungi on the Production of Aflatoxins

Ability of isolated fungi on the production of aflatoxins was detected laboratory according to the way of Saito & Machida, (1999) with alteration of this way by using CEA medium and fermentation medium with agar in concentration 20 g/l and adjust the pH at 3.5, as it was prepared Petri dishes that contains cultural media, then inoculated with one disc diameter of 7 mm from fungal colonies under study by using cork borer and placed in the middle of the each dishes, then incubated dishes for 7 days at 25 ͦC and then dishes taken out of the incubator and turned upside down and then was added to the center of each cover of the dishes 0.2 ml from ammonia solution 25 % and then incubated for 2-7 days at 30 ͦC by two petri dish for each fungus and each medium, and check dishes during this period, to note the color rules change compared to the treatment of control if the colony base color change to red pink or yellow orange color with different degree, it shows that the fungus has the ability to produce aflatoxins, then was exclusion the toxic fungi which have the ability to produce aflatoxins from this study.

Inoculation of Production Medium

For the purpose of selecting fungal isolation that have good growth and more efficient in the production of citric acid was transferred one disc diameter of 7 mm of all fungal isolates from PDA medium aged one week to the flask contain fermentation medium with 3 flask of each isolation, then incubated on Shaker incubator with quickly shake 200r/min at 30 ͦC for 7 days.

Assay Method

The dry biomass was determined by filtering the culture medium through weighed filter paper Whatman No.1, mycelia were thoroughly washed with tap water and dried at 110 C for 24 hours and mycelia dry weight was calculated by using the sensitive balance.

The concentration of citric acid was estimated by the method of Marier & Boulet, (1958) in all steps in the study and depending on the standard curve for citric acid.

It was prepared standard citric acid solution concentration (10 mg/ml) by dissolving 1 g of citric acid in the amount of sterilize distilled water and then complete the volume to 100 ml with sterile distilled water to a final concentration of the citric acid (10 mg/ml) was then made the standard curve for the citric acid as follows:

1. Added sizes shown in the table below in test tubes and with three replicates for each size for a gradual concentration of the citric acid (1-10) mg/ml.

Tubes	Volume of standard citric acid (ml)	Volume of distilled water (ml)	Total volume (ml)	Citric acid concentration (mg/ml)
1	0	5	5	0
2	0.5	4.5	5	1
3	1	4	5	2
4	2	3	5	4
5	3	2	5	6
6	4	1	5	8
7	5	0	5	10

2. Add 1.3 ml of reagent Pyridine to 1 ml of each concentration of the citric acid concentrations prepared in the above table and.
3. Added 5.7 ml of acetic anhydride to each tube and shaken the tubes well and then placed directly in a water bath at 32 ◌°C for 30 minutes.
4. Absorption was read for each concentration at wavelength 420 nm by spectrometer device after device reset by tube (1) as a solution zero (Blank).
5. Painted the relationship between the absorption and concentration of citric acid for obtaining a standard curve as shown in Figure (1) it was estimated citric acid concentration to the production medium by reference to the standard curve.

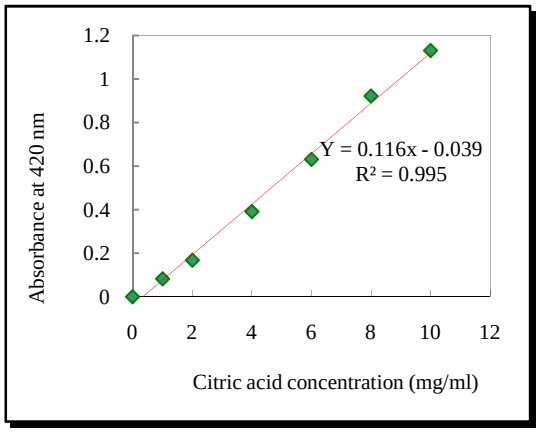

Figure 1 Citric acid standard curve.

Then it was determining fungal isolate that have good growth and heaviest productive for citric acid and used in other steps in this study.

Determine some of the Optimal Conditions for the Production of Citric acid

The effect of some factors in the production of citric acid from selected fungal isolate, these factors included the following: carbon source concentration (rice husks), type of nitrogen source and concentration (ammonium nitrate, yeast extract, ammonium carbonate, ammonium sulfate and potassium nitrate), incubation temperature and primary pH.

Carbon Source

Studied the possibility of using rice husks as a source of carbon for the production of citric acid from fungal isolate under study process has included several steps as follows:

Preparation of Glucose Standard Curve

Prepared the standard curve for glucose to the estimate the reducing sugars in the rice husks filtrate medium when prepared as well as estimating the remaining reducing sugars in the medium of production after fermentation process finishes have followed the method of Miller (1959) in the estimation of reducing sugars as follows:

Standard glucose solution concentration (10 mg/ml):

Prepared by dissolving 1 g of glucose in the amount of sterile distilled water and then complete the volume to 100 ml with sterile distilled water to a final concentration of glucose (10 mg/ml).

Sodium hydroxide solution:

This solution was prepared by dissolving 8 g of sodium hydroxide in the amount of sterile distilled water and then complete the volume to 100 ml with sterile distilled water.

Di nitro salicylic acid

This solution was prepared by dissolving 1 g of 3,5-DNSA in 50 ml of sterile distilled water and then added for it 20 ml of sodium hydroxide solution and 30 g of potassium sodium tartrate then complete the volume to 100 ml with sterile distilled water.

It was then follow the steps in the preparation of standard curve for glucose process:

1. Added sizes shown in the table below in test tubes and with three replicates for each size for a gradual concentration of the glucose (1-10) mg/ml.
2. Add 1 ml of reagent (3,5-DNSA) to 1 ml of each concentration of glucose solution prepared in the above table and placed in boiling water bath for 5 minutes.
3. Cool the tubes in a snowy boiling water bath after the end of the period directly.

4. Add 5 ml of sterile distilled water for each test tube was shaken well.
5. Absorption was read for each concentration at wavelength 540 nm by spectrometer device after device reset by tube (1) as a solution zero (Blank).
6. Painted the relationship between the absorption and concentration of glucose for obtaining a standard curve as shown in Figure (2).

Tubes	Volume of standard glucose (ml)	Volume of distilled water (ml)	Total volume (ml)	Glucose concentration (mg/ml)
1	0	5	5	0
2	0.5	4.5	5	1
3	1	4	5	2
4	2	3	5	4
5	3	2	5	6
6	4	1	5	8
7	5	0	5	10

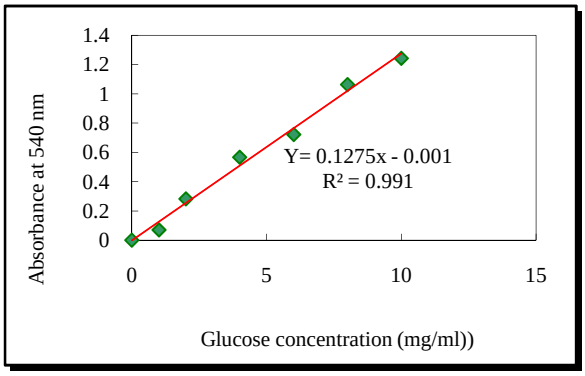

Figure 2 Glucose standard curve.

Prepare Rice Husks Filtrate Medium

Followed the way of Ang *et al*., (2009) in the preparation of the rice husks filtrate medium, it was washing rice husks well with distilled water to remove impurities and dust and then dried using oven at 55 ◌°C for 24 hours, then was taken 100 g of rice husks and put in flask capacity of 500 ml and added to it amount of distilled water and then added to it 10 ml of nitric acid and complete size with distilled water to 500 ml and then was boiling solution at 100 ◌°C for 8 hours, and then leave the solution to cool then filtrated by using the filtering filter paper Whatman No. 1. It was estimated reducing sugars in rice husks filtrate by reference to the glucose standard curve, then prepared a different concentration of the rice husks filtrate and these concentrations are (11, 13, 15, 17 and 19) % reducing sugar.

Used the filtrate as a medium to dissolve the components of the citric acid production medium with the exception of sucrose, adjust pH of the medium at 3.5, then put the medium in the flasks 250 ml capacity by 25 ml/flask and then sterilization medium in the autoclave for 15 minutes and then inoculated with one disc diameter of 7 mm by 3 replications and incubated in Shaker incubator quickly shake 200 r/min at 30 ◌°C for 7 days. After the end of the incubation period filtrate the fermentation media through filter paper Whatman No.1 and leave the filtrate to estimate the amount of citric acid and sugar remaining and dried the filter paper in the oven was to determine the biomass As stated in the above.

Nitrogen source

Chosen the concentration of 15 % of the rice husks filtrate as a source of carbon in the all other steps, prepared this concentration and added to the components of production medium except the source of nitrogen as it was five nitrogen sources tested to determine the source of the best ones in the production of citric acid from the selected isolate and this sources are (ammonium nitrate, yeast extract, ammonium carbonate, ammonium sulfate and potassium nitrate) has been used all these sources concentration of 0.25% with the treatment of the control, which includes not add any nitrogen source. Then was determined the optimal concentration for the optimal source of nitrogen by made gradual concentration and these concentrations were (0.1, 0.25, 0.5, 0.75 and 1.0)%.

Incubation Temperature

Prepared the rice husks filtrate medium and added to it ammonium carbonate with optimum concentration that has been reached previously, then put the medium in the flasks 250 ml capacity by 25 ml/flask and then inoculated with selected isolate was study the effect of different incubation temperature(20, 25, 30, 35 and 40) ◌°C in the production of citric acid .

Primary pH

The effect of primary pH in the production of citric acid from the Selected isolate, put the medium in the flasks 250 ml capacity by 25 ml/flask and adjusted the pH to (2.5, 3, 3.5, 4, 4.5 and 5) by using both hydroxide sodium and hydrochloric acid and then incubated the flasks in the optimal production conditions that have been previously reached.

RESULTS AND DISCUSSION

Fungal Isolates

It was Isolate 12 local fungal isolate of the fungus *A. niger* from the soil (Table 1).

Table 1 Fungal isolates

Number of Isolates	Fungui
1	*Aspergillus niger*
2	*A. niger*
3	*A. niger*
4	*A. niger*
5	*A. niger*
6	*A. niger*
7	*A. niger*
8	*A. niger*
9	*A. niger*
10	*A. niger*
11	*A. niger*
12	*A. niger*

Production of Aflatoxins

The screening of fungal isolates under study and that detects its ability to produce aflatoxins and exclusion the toxic isolates, which have the ability to produce aflatoxins by using ammonia solution of 25 % as indicator for this ability as soon as contact

with the surface of the fungal colony vapor ammonia, the color base change to red pink or yellow orange color with different degree depending on the amount of aflatoxin product (Saito & Machida, 1999).

The results showed the ability of some isolates to produce aflatoxins at the CEA medium and production medium with agar (Table 2), and the percentage of positive isolates for this test 41.66 % as isolation *A. niger* 4 showed high ability on the production of aflatoxins while *A. niger* 6 and *A. niger* 12 showed moderate ability on the production of aflatoxins at the CEA medium and weak ability at the production medium which contain agar, while *A. niger* 1 and *A. niger* 10 showed little ability on the production of aflatoxins, while the other isolates did not show the ability to produce aflatoxins.

These results are compatible with (Amadi & Adeniyi, 2009) which found some isolates of the fungus *A. niger* that isolated from some stored seeds ability to produce aflatoxins.

Table (2) Detection ability of fungal isolates to produce aflatoxins

Isolates	CEA	Production medium with agar
A. niger 1	*	*
A. niger 2	-	-
A. niger 3	-	-
A. niger 4	+++	***
A. niger 5	-	-
A. niger 6	**	+
A. niger 7	-	-
A. niger 8	-	-
A. niger 9	-	-
A. niger 10	+	*
A. niger 11	-	-
A. niger 12	++	*

+ Light red pink, * Light yellow orange (The ability to produce a few aflatoxin)
++ Moderate red pink, ** Moderate yellow orange (The ability to produce moderate aflatoxin)
+++ Dark red pink, *** Dark yellow orange (The ability to produce high aflatoxin)
- Inability to produce aflatoxin

Screening Fungal Isolates by the Production of Citric acid and Biomass

Tasted ability of 7 local fungal isolates of the fungus *A. niger* on the production of citric acid by using synthetic medium of production, The results showed that for all fungal isolates studied the ability to produce citric acid but in varying degrees, As for the dry biomass for fungal isolates have values ranged between (12.64-19.73) g/l, according to the results of the statistical analysis that showed there were significant differences at the level of 5 % probability between the concentration of citric acid product of fungal isolation *A. niger* which amounted to 14.683 g/l and the other isolates and the absence of significant differences between the dry biomass of this isolation value which amounted to 18.96 g/l and isolation that gave the highest biomass has therefore selected fungal isolation *A. niger* 5 to be used in all subsequent production studies (Table 3).

The screening fungal isolates and choose the more efficient for the production of citric acid in a number of studies have made (Makut & Ade-lbijola, 2012) screening fungi that have been isolated from soil and noted the high ability for the isolates of the fungus *A. niger* in the production of citric acid in comparison to other isolates. (Dashen *et al*., 2013) screening 14 fungal isolates of the fungus *A. niger*, isolated from various sources on the basis of their ability to produce citric acid and found the isolate *A. niger* CP3 excelled the other isolates in the total amount of citric acid produced which reached 12.81 g/l.

Table 3 Screening fungal isolates by the Production of Citric acid and Biomass

Isolates	Citric acid concentration (gm/l)	Biomass (gm/l)	Final pH
A. niger 2	1.824±0.321 f	17.17±0.561 b	2.60
A. niger 3	5.174±0.41 c	12.64±0.32 d	2.41
A. niger 5	14.683±0.526 a	18.96±0.443 a	1.85
A. niger 7	10.135±0.423 b	17.26±0.218 b	1.73
A. niger 8	2.513±0.326 e	19.73±0.377 a	2.30
A. niger 9	10.973±0.052 b	14.62±0.51 c	1.67
A. niger 11	3.796±0.071 d	16.86±0.623 b	2.14

- Represents the results shown in the table the rate of three replicates ± standard error.
- The rates which have the same characters do not differ significantly among themselves for the vertical comparisons by Duncan test at the level of 5 % probability

Optimal Conditions for the Production of Citric acid

Carbon Source

Compared the production of citric acid and biomass for *A. niger* 5 by using two culture media, the first synthetic medium contain sucrose 15 % as a source of carbon, NH_4NO_3, KH_2PO_4 and $MgSO4.7H_2O$ The second is rice husks filtrate supplemented with ammonium nitrate and salts and the same concentrations as in the first medium.

Tested different concentrations from rice husks filtrate ranged between (11-19) % reducing sugars to determine the optimal concentration of them for the production of citric acid and biomass of the fungus under study.

The results showed the highest values of production of citric acid were using 15 % reducing sugars as the citric acid concentration reached 14.709 g/l with significant differences from the other concentrations used at a level of 5 % probability, also gave the same concentration highest biomass of the fungus reached 17.97 g/l with significant differences from the other of concentrations used at a level of 5 % probability (Figure 3).

Depending on these results were chosen the concentration 15 % reducing sugars as the best concentration of the carbon source was used in all stages of this study.

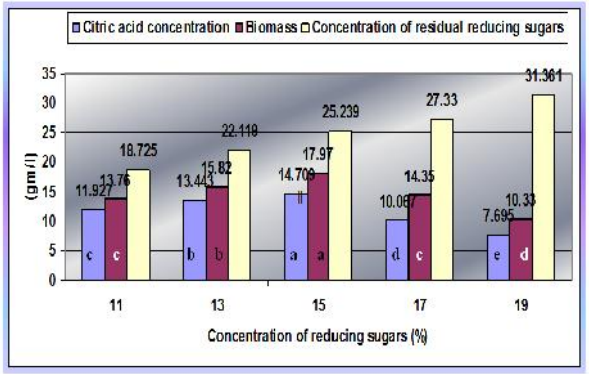

Figure 3 The effect of the concentration of reducing sugars in rice husks filtrate medium in the production of citric acid from *A. niger* 5.

- **The rates which have the same characters do not differ significantly among themselves for the vertical comparisons by Duncan test at the level of 5 % probability.**

Rice husks filtrate medium contains some components that support the growth of fungi and the production of citric acid, as contain 40-50% cellulose and 25-30% of lignin and 15-20% inorganic salts and 8-15% water (Giddel & Jivan, 2007). When treated initially chemically using one of the acids or bases, such as nitric acid, phosphoric acid, sulfuric acid, sodium hydroxide and calcium hydroxide before it is used in the preparation of fungal culture media these complex sugars convert into simplest sugars can fungus used in growth and to do different metabolic activities (Ang *et al*., 2009).

Some studies also reported the ability of some fungi especially fungal isolates of the fungus *A. niger* to produce extracellular enzyme analyzes the complex cellulosic materials and convert them into simplest sugars can fungus used it and do different metabolic activities (Reddy & Pushpa, 2012; Jantasila *et al*., 2012).

As can be seen from the results the low concentration of citric acid product and the biomass of the fungus when using concentrations higher than 15 % have been the reason for this is due to the osmosis effect caused by high concentrations of sugars for the fungi cells (Peksel & Kubicek, 2003).

This results agree with (Hossain *et al*., 1984) which found the best production of citric acid can be obtained by using sugars concentrations ranging from 14-22 % and that these high concentrations of sugars is working to increase the effectiveness of glycolysis process in addition to inhibition the work of the enzyme α-Ketoglutarate dehydrogenase and thus increase the production of citric acid. Also agree with (Ali *et al*., 2001) which found the concentration 15 % of the sucrose used as a source of carbon it was the optimal concentration in the production of citric acid from the isolation of the fungus *A. niger*.

Nitrogen Source

Studied the effect of nitrogen source in the production of citric acid from local fungal isolate *A. niger* 5 has used five different nitrogen sources, including ammonium nitrate, yeast extract, ammonium carbonate, ammonium sulfate and potassium nitrate was used as a concentration of these sources 0.25 %.

The results showed the best source of nitrogen for the production of citric acid and biomass of the fungus *A. niger* 5 is ammonium nitrate, reaching production of citric acid 14.225 g/l and the biomass of the fungus 17.22 g/l. The results also showed that the use of ammonium sulfate as a source of nitrogen for the production of citric acid had given similar results for the use of ammonium nitrate as the amount of citric acid produced it reached to 13.417g/l and the biomass of the fungus reached to 16.62 g/l (Figure 4). As the results of the statistical analysis showed no significant differences in the amount of citric acid produced by *A. niger* 5 when using ammonium nitrate and ammonium sulfate as a source of nitrogen compared to other sources used at a level of 5 % probability. Because of that ammonium nitrate locally banned substance use at the present time and it can not be easily obtained and made available in large quantities so it was chosen ammonium sulfate as the best source of nitrogen was used in all subsequent stages of the study.

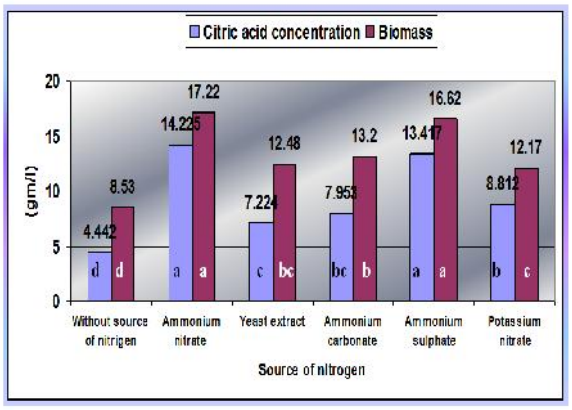

Figure 4 The effect of the type of nitrogen source in the production of citric acid from *A. niger* 5.

- **The rates which have the same characters do not differ significantly among themselves for the vertical comparisons by Duncan test at the level of 5 % probability.**

1. This results agree with (Bhattacharjee & Baruah, 2015) which studied the effect of different nitrogen sources in the production of citric acid from fungal isolate *A. niger* S-6 and found that ammonium sulfate gave the highest amount of citric acid produced, which reached to 38 g/l compared with the other sources.
2. After it was determined ammonium sulfate as the best source of nitrogen for the production of citric acid was determined the optimum concentration of the source of nitrogen to produce it, and the results showed that the highest production of citric acid was by using concentration 0.25 % of ammonium sulfate as the citric acid product concentration reached to 14.257 g/l (Figure 5). As t is noted that an increase of the concentration of ammonium sulfate from 0.25 % biomass of the fungus increases with the decrease in the amount of citric acid

produced may be due reason to used the fungus to the source of nitrogen in the growth and building of proteins instead on the citric acid production (Kareem *et al*., 2010).

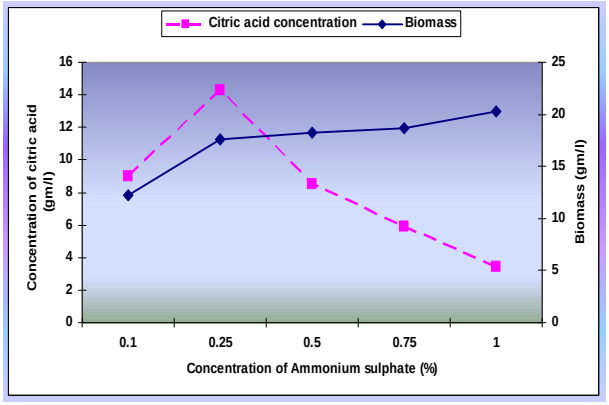

Figure 5 The effect of the concentration of ammonium sulfate in the production of citric acid from A. niger 5**.**

The optimum concentration of ammonium sulfate obtained in this study and the existence of the optimal concentration of carbon source obtained in this study also may help in the preparation of the balanced medium in terms of the ratio between carbon and nitrogen (C/N Ratio), which achieves an increase in the production of citric acid, has been found (Pandey, 2003) the production of citric acid decreases when the carbon to nitrogen ratio less than 100, although the amount of nitrogen necessary for the production of citric acid should be limited with the availability of large amounts of carbon source during production phase to be converted to the citric acid by enzymes responsible for it.

Incubation Temperature

For the purpose of knowing the effect of different degrees of incubation temperature in the production of citric acid from *A. niger* 5, it was used five different temperatures. The results showed that the highest production of acid and biomass of by using temperature 30 ̊C as the amount of citric acid produced reached to 15.321 g/l and the biomass reached to 19.73 g/l (Figure 6), so the use of a temperature 30 ̊C in all subsequent stages of the study.

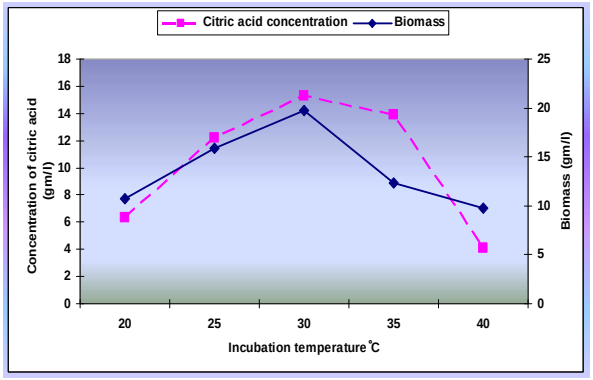

Figure 6 The effect of incubation temperature in the production of citric acid from *A. niger* 5.

It also noted the results of the low amount of citric acid produced at high temperature may be the reason for this is the produce other acids instead of citric acid such as oxalic acid (Vergano *et al*., 1996). (Anastassiadis *et al*., 2008) found that incubation temperatures ranging between 28-32 ̊c is the best for the production of citric acid, although the incubation temperature 30 ̊C is the optimum temperature for production.

As well as (Vasanthabharathi *et al*., 2013) that studied the effect of different incubation temperatures in the production of citric acid from *A. niger* and found that the temperature of 30 ̊C has given the highest production of citric acid.

Primary pH

Studied the effect of primary pH in the production of citric acid from fungal isolate under study, and as noted in Figure (7) the best primary pH of the citric acid production was 4, as the amount of citric acid produced reached to 19.447 g/l.

The results also showed that the increase of pH increased biomass of the *A. niger* 5 and decreased the amount of citric acid produced and can be explained by increasing the biomass of the fungus because the fungus under study from filamentous fungi which grow well under weak acidic conditions and that range values between (3 -6) (Fawole & Odunfa, 2003). As for the decrease in the amount of citric acid produced it can be attributed the reason for that to the decrease in the activities of the enzymes responsible for the production of citric acid and increase in the activities of the enzyme responsible for the formation oxalic acid and gluconic acid (Paul *et al*., 1999).

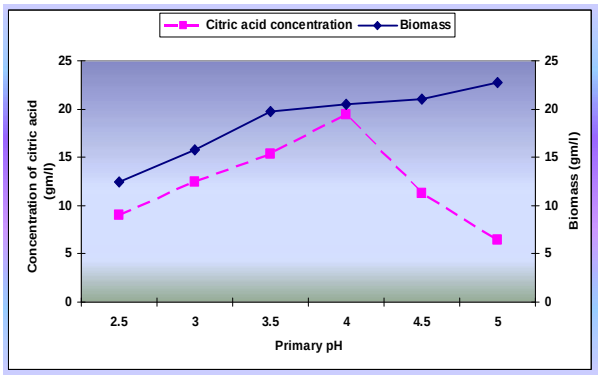

Figure 7 The effect of primary pH in the production of citric acid from *A. niger* 5.

This results agree with (Rao & Reddy, 2013) which found that the primary pH 4 is optimal for the production of citric acid from the fungus *A. niger* at the medium of oat bran.

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