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Optimization of Alpha Amylase Enzyme Produced by Some Fungal Species Isolated from Saudi Arabian Soil

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Abstract: **The production, extraction and optimization of extracellular α-amylase enzyme from some fungal species isolated from five different soil sites in Saudi Arabia. Six fungal species (*Aspergillus flavus, A. niger, A. terrus, Cladosporium herbarum, Cunninghamella echinulate* and *Penicillium italicum*) constituting 26 colonies were isolated from the five tested soil samples. The highest significant value of extracellular α-amylase (64.78 unit/ml) was showed in the culture filtrate of the *A. niger*. Optimization of some nutritional and physical factors in the basal medium in order to intensify the production of *A. niger* extracellular α-amylase was carried out. The highest productivity of *A. niger* α-amylase (74.56 and 76.63 unit/ml) occurred on fructose and potassium nitrate as carbon and inorganic nitrogen sources, respectively. The optimum pH and temperature for the maximum productivity of extracellular α-amylase activities were 7.0 and 20 °C, respectively.**

Keywords: **Fungi, Alpha amylase, *Aspergillus*, Isolation, Optimization.**

I. INTRODUCTION

Enzymes in biotechnology have stimulated the investigation of their production with the purpose of selecting promising enzyme producers and increasing their yield. In addition, the understanding of physiological mechanisms regulating enzyme synthesis could be useful for improving the technological process of edible and medicinal production. The physiology of enzymes has been extensively studied by [1]. Sources of commercial enzymes cover a wide range (from microorganisms to plants to animal sources). In commercial enzyme production, fungi and yeast contribute about 50 % of the total organisms. Microbes are preferred than plants and animals as they are cheap sources, their enzyme contents are predictable and growth substrates are obtained as standard raw materials [2].

Microorganisms in particular have been regarded as a treasure source of useful enzymes, because they multiply at extremely high rate and synthesize biologically active products which can be controlled by humans. In recent years, there has been a phenomenal increase in the use of enzymes as industrial catalysts. Microbial enzymes have advantage over the use of conventional chemical catalysts for numerous reasons: they exhibit high catalytic activity, a high degree of substrate specificity, can be produced in large amounts, highly biodegradable, no threat to the environment and are economically viable. Filamentous fungi have been widely used to produce hydrolytic enzymes for industrial applications [3]. Starch is a carbohydrate consisting of a large number of glucose units joined by glycosidic bonds.

Starch contributes greatly to the textural properties of many foods and is widely used in food and industrial applications as a thickener, colloidal stabilizer, gelling agent, bulking agent and water retention agent [4]. Amylases are ubiquitous enzymes, being widespread in animals, plants and fungi. Amylases account for approximately 30 % of world enzyme production and are one of the most important industrial enzymes which are in high demand in various sectors such as food, pharmaceuticals, textiles and detergents. Fungal sources of amylases are mostly terrestrial isolates (*Aspergillus* sp.,

Alternaria sp., *Fusarium* sp., *Penicillium* sp., and *Trichoderma* sp.). The applications of fungal producing amylases include conversion of starch to sugar syrup and the production of cyclodextrins in the pharmaceutical industry. The most important amylases for industrial and biotechnological applications is α-amylases. Each of these applications requires unique enzyme properties with respect to pH, temperature, specificity and stability [5, 6]. Alpha-amylase (E. C. 3.2.1.1) are extracellular endoenzymes that hydrolyze internal α-1,4 glycosidic linkage in starch and related substrate producing oligosaccharides and limit dextrin. Alpha-amylases find potential applications in pharmaceuticals, baking, brewing, textile (in desizing fabric), paper, syrup industries and detergent manufacturing processes [7].

The aim of the present study is the production of α-amylase enzyme from some fungal species isolated from Saudi Arabian soil. The research will also include extraction and investigation the nutritional and physical factors to reach the optimum conditions for extracellular alpha amylase enzyme.

II. MATERIAL AND METHOD

Collection of soil samples:

Five desert soil samples were collected from two different sites in Saudi Arabia. Two samples were isolated from AL-Madinah AL-Munawarah city (Al- Khalydiah, 4 Km east of prince Mohammed bin Abdulaziz road and AL-Qiblatain, 550 m west of Khalid bin Al-Waleed road) and three samples from Jeddah city (Wadi AL-Bayadah, 35 Km east of high way, Wadi AL-Muharraq, 6 Km east of high way and Wadi Qariat almuzareen, 20 Km east of high way). The soil samples were taken at a depth of 20 cm from the soil surface according to the method described by Oliveira, Cavalcanti [8]

The culture media:

Modified Czapek Dox agar medium:

Starch 30.0, Sodium nitrate ($NaNO_3$ 2.0, Dipotassium hydrogen phosphate (K_2HPO_4) 1.0, Magnesium sulfate ($MgSO_4 . 7H_2O$) 0.5, Potassium chloride (KCl) 0.5, Ferrous sulfate ($FeSO_4 .7H_2O$) 0.01, Agar 15.0. After solubilization, streptomycin 30 µg/ml and rose bengal 1:30000 were added to prevent bacterial growth.

Isolation of fungal species from the five desert soil sites:

Soil samples were gathered according to the method described by Abou-Zeid and Abd EL-Fattah [9]. The soil is taken directly into a clean plastic bag. At least five samples were taken at random from each place and brought together into one composite sample which is mixed thoroughly and divided several times. Soil samples are kept in cool place during transportation and storage. The fungal count was determined by the dilution plate method. Soil to be diluted was sieved and the air dry soil was placed in graduated cylinder. Water was added to the soil until the total volume of 250 ml was reached. The suspension was stirred and poured into 1000 ml Erlenmeyer flask and shake for 30 minutes. Ten ml of this suspension were immediately withdrawn (while in motion) into a sterile 10 ml pipette and transferred into a 90 ml sterile water blank. Ten ml samples were transferred immediately through successive 90 ml sterile water blank until desired final dilution (1:1000) was reached. One ml of the desired dilution was transferred aseptically into each Petri dishe, then 12-15 ml of an appropriate modified Czapek Dox agar medium cooled to just above the solidifying temperature, were added to each dish. The dishes were rotated by hand in a broad swirling motion so that the soil suspension was dispersed in the agar. After incubation at 30 °C from 5 to 7 days, the resulting colonies were counted. The average number of colonies was multiplied by the dilution factor to obtain the number per gram of the original soil.

Purification of the isolated fungal species:

The isolated fungal species were purified by transferring a very small part of each representative colony to the Petri dishes of the culture media by streak plate method. After incubation for 7 days at 30 °C, a single colony was aseptically sub-cultured on a slant of modified Czapek Dox agar medium.

Identification of the isolated fungal species:

The developing fungal colonies were identified up to the species level by microscopic examination. This was made through the help of the following references: [10-14]

Cultivation of the isolated fungal species:

The original fungal stock culture was subcultured on plates containing modified Czapek Dox agar medium and incubated at 30 ˚C for 2-4 days. Discs of 1cm diameter were cut from the 2-4 day old cultures used for inoculating the conical flasks which contained 100 ml of sterile liquid medium each. The flasks were incubated for 10 days at 30 ˚C static incubator. Than the culture was filtered with filter paper to separate the mycelium. The filtrate was treated for preparing amylase enzymes.

Linear growth:

Known volumes of modified Czapek Dox agar medium were sterilized by autoclaving. Aliquots of about 15 ml of this medium were dispersed into three sterile Petri dishes, (8 cm diameter). Each dish was inoculated at its center with a fungal disc (1cm diameter) cut from the colony margin of 5 day old cultures. The plates were incubated at 30 °C for 3, 5 and 7 days. The mean of three diameters of each colony (cm) was measured [15].

Total dry matter (TDM):

Sterile modified Czapek Dox liquid medium was dispensed into 250 ml Erlenmeyer flasks (50 ml per flask). Each flask was inoculated with a fungal disc (1cm diameter) taken from the colony margin of 5 day old cultures. Triplicate flasks were incubated at 30 °C for 10 days, then filtered. The mycelium washed several times with distilled water and dried between filter paper. The mycelium was dried in oven at 80 °C till constant weight to get the dry weight expressed as g /50 ml growth medium. The filtrate was prepared for prepation of extracellular α-amylase enzyme [16].

Assay of α-amylase enzyme activity:

Activity of α-amylase enzyme was assayed according to [17-19]. The standard glucose (STD) was prepared by mixing 10 µl of the provided standard with 406 µl assay buffer (Potassium Phosphate, Sodium Chloride and Bovine Serum Albumin). Transfer 10 µl assay buffer, 10 µl glucose and 10 µl of each sample into separate wells of a clear flat-bottom 96-well plate. Prepare enough working reagent for each well by mixing 40 µl assay buffer, 0.5 µl substrate (starch), 1 µl enzyme A (α-glucosidase), 1 µl enzyme B (glucose oxidase). Transfer 40 µl working reagent to each well. Incubate for 15 minutes at room temperature (25 °C). Add 150 µl detection reagent (Ammonium iron, Sulfuric acid, Xylenol orange and Sorbitol) to each well. Mix and incubate for 20 minutes at room temperature (25 °C). Read optical density at 585 nm (540 – 610 nm) on a plate reader. One unit of α-amylase enzyme catalyzes the production of 1µmole of glucose per minute under the assay conditions. The α-amylase activity is calculated according to the following equation:

$$\text{α-amylase activity} = \frac{\text{O.D. S} - \text{O.D. B}}{\text{O.D. STD} - \text{O.D. B}} \times \frac{400}{\text{t (minutes)}} \times \frac{n}{1000} \quad \text{(unit/ml)}$$

O.D. S, O.D. STD and O.D. B are optical density values of the sample, glucose standard and assay buffer. The incubation time (t =15 minutes in the standard protocol. The dilution factor (n = 50).

Selection of the most efficient fungal species producing α-amylase enzyme:

All isolated fungal species were assayed for their α-amylase activities on starch substrate. From the statical analysis, the fungal species that showed the highest significant extracellular α-amylase activity was selected for further experiments.

Optimization of the growth medium for the maximum production of α-amylase by the selected fungal species:

To study the effect of nutritional and physical factors, the relevant components of the modified Czapek Dox medium were substituted by different corresponding sources. Triplicate flasks were used for each treatment. Extracellular α-amylase activity was measured in the culture filtrate of the most efficient selected fungal species.

Nutritional factors:

Effect of different carbon sources on α-amylase activity

The following carbon sources: cellulose, fructose, glucose, maltose, starch (control) and sucrose were used in the preparation of the modified Czapek Dox medium. Triplicate flasks containing modified Czapek Dox liquid medium with different carbon sources were inoculated with the most efficient selected fungal disc and incubated at 30 °C for 10 days. Alpha-amylase activity was measured in the culture filtrate of the most efficient selected fungal species [20].

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Effect of different nitrogen sources on α-amylase activity:

The liquid medium containing the chosen carbon source from the previous experiment was used. The following nitrogen sources were added to the medium:- inorganic nitrogen sources {ammonium sulfate, potassium nitrate and sodium nitrate (control)} and organic nitrogen sources (casein, peptone and yeast extract). The flasks inoculated with the selected fungal disc were incubated at 30 °C for 10 days. Alpha- amylase activity was measured in the culture filtrate of the most efficient selected fungal species [21].

Physical factors:

Effect of different pH values on α-amylase activity:

The medium containing the optimum sources of the chosen nutritional factors were used. The following buffer solutions were used:- potassium biphthalate / sulfamic acid for pH 3, sodium phosphate dibasic/ potassium biphthalate for pH 5, sodium phosphate dibasic/ phosphate monobasic buffer for pH 7 (control), sodium bicarbonate / sodium carbonate buffer for pH 9 and sodium phosphate tribasic/ sodium phosphate dibasic for pH 11. The flasks were inoculated with the selected fungal discs and incubated at 30 °C for 10 days. Extracellular α-amylase activites were assayed [22].

Effect of different temperatures values on α-amylase activity:

The triplicate flasks of liquid medium containing the optimum source of the chosen nutritional factors and the optimum pH were used. Incubation was carried out at the following temperatures:- {10, 20, 30 (control), 40, 50 and 60 °C} for 10 days. The culture filtrates were used as extracellular α-amylase enzyme sources for assaying α-amylase activities of the selected fungal species [23].

III. RESULTS

The experiment was carried out to isolate some amylolytic fungal species from five desert soil sites. Two samples were isolated from AL-Madinah AL-Munawarah city (Al- Khalydiah, 4 Km east of prince Mohammed bin Abdulaziz road and AL-Qiblatain, 550 m west of Khalid bin Al-Waleed road) and three samples from Jeddah city (Wadi AL-Bayadah, 35 Km east of high way, Wadi AL-Muharraq, 6 Km east of high way and Wadi Qariat almuzareen, 20 Km east of high way). Total of 26 fungal colonies were captured all over the experiment from all five tested soil samples constituting 6 fungal species. Six fungal species (*Aspergillus flavus, A. niger, A. terrus, Cladosporium herbarum, Cunninghamella echinulate* and *Penicillium italicum*) related to four genera were isolated from the five tested soil samples. *A. flavus* was the most dominant fungal species (6 colonies) and it was isolated with moderate occurrence and population density 23.07 %. Low occurrence was showed by *A. niger* (R. D. 15.38 %), *A. terrus* (R. D. 19.23 %), *Cladosporium herbarum* and *Cunninghamella echinulate* (R. D. 11.53 %) and *Penicillium italicum* (R. D. 19.23 %) (Table I).

TABLE I. Total counts, Relative density and frequency of occurrence of isolated fungal species all over the tested five soil samples located in five different sites in Saudi Arabia.

Fungal species	Total colony count No./g dry soil	Relative density (%)	Occurrence*
Aspergillus flavus	6	23.07	M
A. niger	4	15.38	L
A. terrus	5	19.23	L
Total Aspergilli	15	57.69	-
Cladosporium herbarum	3	11.53	L
Cunninghamella echinulate	3	11.53	L
Penicillium italicum	5	19.23	L
Total count	26	100	-
Number of fungal species	6	-	-

*Occurrence: High (H): 4-5 sites, Moderate (M): 2-3 sites and Low (L): 0-1 sites.

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Linear growth:

The maximum significant linear growth (7.82 cm) was showed by *A. niger* while the lowest growth (3.04 cm) was showed by *C. echinulate* after 7 days incubation period. The growth of the remaining fungal species was decreased gradually as follow *A. flavus* > *C. herbarum* > *A. terrus* >*P. italicum* (Fig. 1).

Total dry matter (TDM):

A significance difference was showed between the maximum growth of *Aspergillus flavus* (0.44 g/50 ml) and the lowest dry weight (0.32 g/50ml) of *Cunninghamella echinulate*. Non significance difference was investigated between the highest growth of *A. flavus* and *A. niger*, *A. terrus* and *Cladosporium herbarum* (Fig. 2).

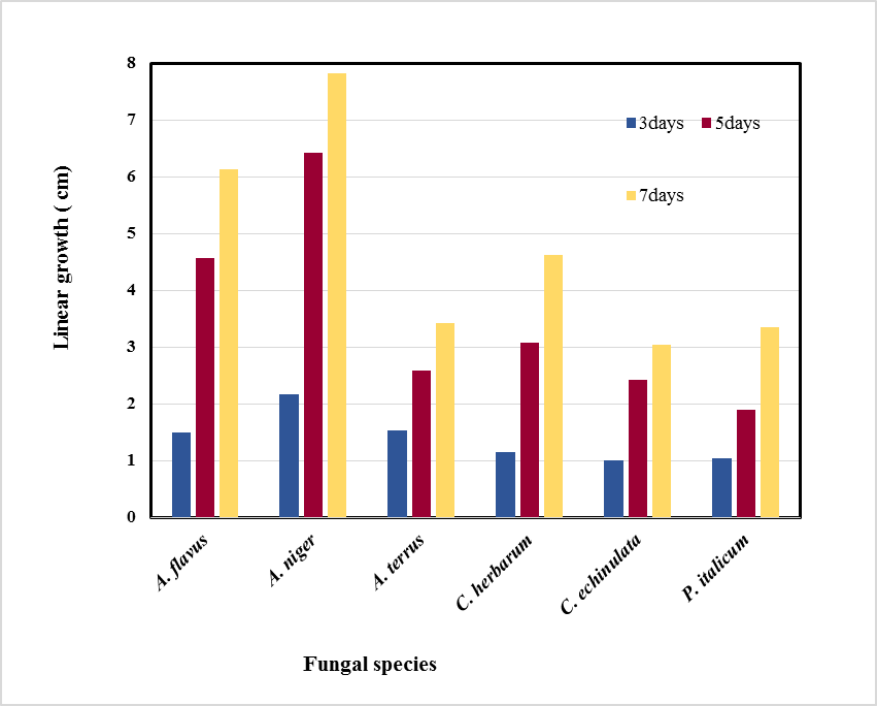

Fig. 1. Linear growth (cm) of the six isolated fungal species after different incubation periods (3, 5 and 7 days) at 30 °C on modified Czapek Dox agar medium.

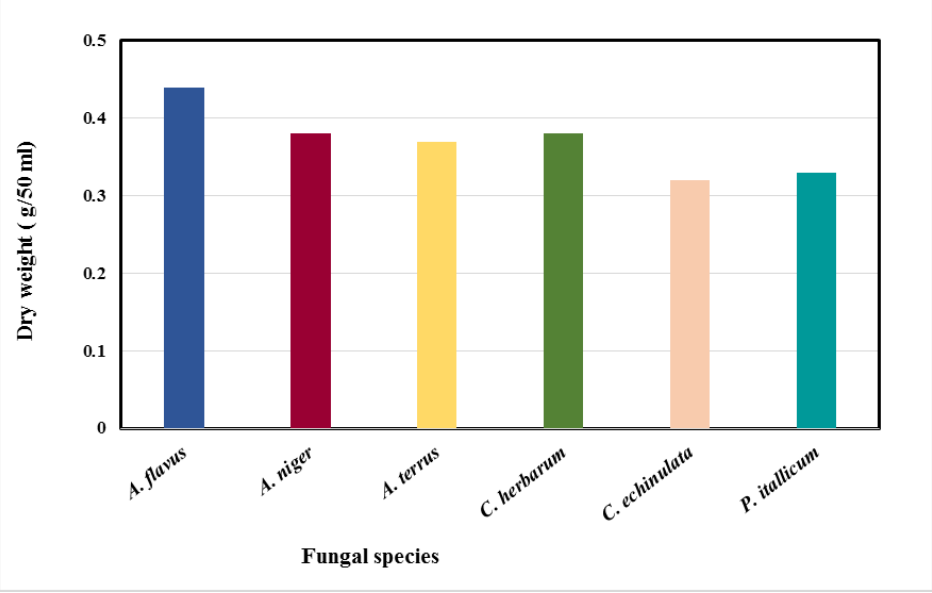

Fig. 2. The dry weight (g/ 50 ml) of the six isolated fungal species after 10 days incubation period at 30 °C on modified Czapek Dox medium

Assay of α-amylase enzyme of all isolated fungal species:

The maximum significant value of extracellular α-amylase enzyme was shown in the culture filtrate of *Aspergillus niger* (64.78 unit/ml), whereas the minimum value was shown in *A. terrus* (4.63 unit/ ml). The extracellular α-amylase enzyme activities for the remaining fungal species were in descending order: *Cladosporium herbarum* (33.92 unit/ml) > *Cunninghamella echinulate* (31.79 unit/ml) > *Aspergillus flavus* (22.48 unit/ml) > *Penicillium italicum* (7.99 unit/ml). From the statistical analysis, *A. niger* showed the highest significant extracellular α-amylase productivity which recovered from AL-Khalidiyah in AL-Madinah AL-Munawarah, it was selected for further studies (Table II).

TABLE II. Assay of extracellular α-amylase enzyme activities for the six isolated fungal species after 10 days incubation period at 30 °C on modified Czapek Dox medium.

Fungal species	α-amylase activity (unit/ml)
Aspergillus flavus	22.48
A. niger	64.78 *
A. terrus	4.63
Cladosporium herbarum	33.92
Cunninghamella echinulate	31.79
Penicillium italicum	7.99
L.S.D at 0.05	27.16

*High significance

Optimization of the growth medium for the maximum production of α-amylase by *A. niger* Nutritional factors:

Effect of different carbon sources:

The result reveal that fructose was the best carbon source for the maximum significant α-amylase productivity (74.56 unit/ml) by *A. niger*. On the other hand, the lowest α-amylase activity (17.42 unit/ml) was shown with sucrose as carbon source. The activities of α-amylase enzyme were 46.07, 44.88, 38.76 and 30.02 unit/ml with starch, cellulose, glucose and maltose as carbon sources, respectively. Therefor, fructose was proved to be the best carbon source for maximum α-amylase activity by *A. niger*. Thereby, it was used as suitable carbon source in further experiments (Fig. 3 A).

Effect of different nitrogen sources:

Inorganic nitrogen source (potassium nitrate) was the best nitrogen source used by *A. niger* with highest significant activity (76.63 unit/ml). A highly decrease in extracellular α-amylase (22.26 unit/ml) was showed when using ammonium sulfate as a nitrogen source. Thereby, potassium nitrate was the best nitrogen source for enzyme activity of *A. niger*. Accordingly, it was used for the next experiments. The production of α-amylase by *A. niger* with sodium nitrate, yeast extract, peptone cand casein were recorded in descending order 53.72, 52.29, 41.13 and 29.33 unit /ml, respectively (Fig. 3 B).

Physical factors:

Effect of pH values:

The gradual increase and decrease in pH values of the culture medium above and below pH 7 were accompanied with decrease in extracellular α-amylase activity. The highest significant α-amylase activity (83.50 unit/ml) was showed at pH 7. On the other hand, The maximum inhibitory effect (3.20 unit/ml) of α-amylase activity was showed at alkaline pH 11. The pH of the culture medium was accordingly adjusted at pH 7 and it was used in the next experiment (Fig. 3 C).

Effect of different temperature values:

Non significance differences was showed in the higher production values of α-amylase enzyme (84.39, 81.82 and 79.00 unit/ml) at 20, 30 and 40 °C, respectively. On the other hand the lowest activity of α-amylase (16.19 unit/ml) was showed at 60 °C (Fig. 3 D).

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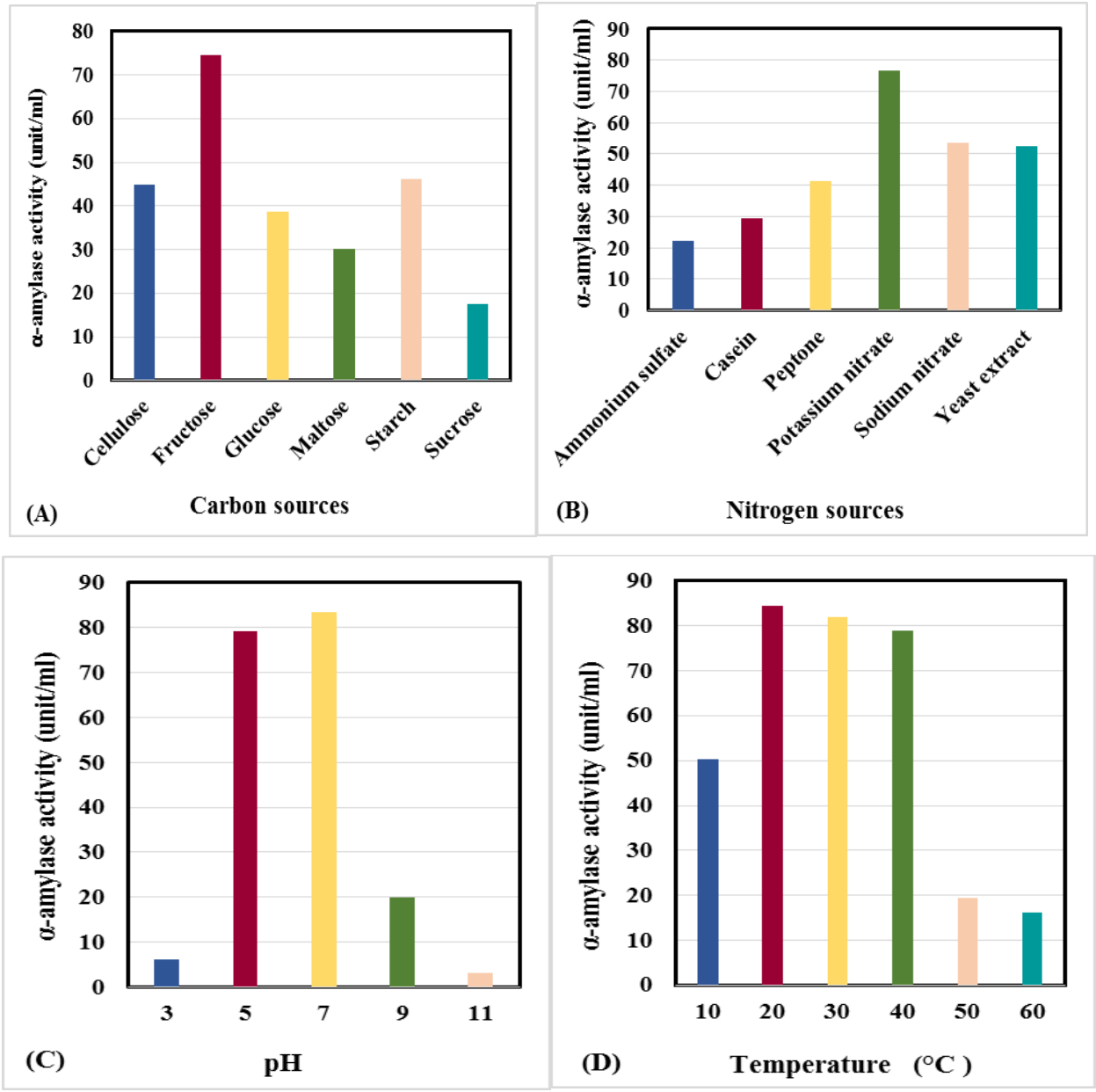

Fig. 3. Effect of different (carbon and nitrogen sources) and (pH and temperatures values) on α-amylase activity (unit/ml) produced by *A. niger* after 10 days incubation period at 30 °C.

IV. DISCUSSION

In the present work, the isolation of fungal species was carried out from five soil samples. Six fungal species (*Aspergillus flavus*, *A. niger*, *A. terrus*, *Cladosporium herbarum*, *Cunninghamella echinulate* and *Penicillium italicum*) related to four genera were isolated from the five tested soil samples. The dominance of *A. flavus* in the tested desert soil may refer to the fact that many species of *Aspergillus* demonstrate oligotrophy where they are capable of growing in nutrient depleted environments or environments in which there is a complete lack of key nutrients. The hyphal mode of fungal growth and their good tolerance to low water activity conditions make fungi efficient and competitive in natural microflora for bioconversion of solid substrates in the soil [24]. Drought stresses accompanied by elevated soil temperatures are conducive to *A. flavus* invasion in non-irrigated fields [25]. *A. flavus* is a filamentous fungus distributed worldwide that has the ability to colonize a broad range of organic substrates [26].

The obtained result was in accordance with Nasser [27] who stated that *Aspergillus* and *Penicillium* contributed the greatest number of species isolated from 40 soil samples collected from different places in soil of Saudi Arabia. Also,

Al-Hazmi [28] found that the highest frequency of the fungal isolates from different sites in Jeddah, Saudi Arabia belonged to the genus *Aspergillus* with a frequency percentage of 65.3 % followed by the genus *Penicillium* which constitute 16.32 % of the total isolates. *Aspergillus* species were *A. flavus, A. niger , A. terreus* and *A. carneus*, while *Penicillium species* were *P. citrinum, P. rubrum* and *P. canesens*. Saleem and Ebrahim [20] found that the most common species were collected from AL-Madinah AL-Munawwarah, Saudi Arabia were *Aspergillus flavus, A. fumigatus, A. niger, A. terreus, Penicillium chrysogenum* and *Rhizopus stolonifer* while, *Cunninghamella echinulate* was showed with low occurrence. Binsadiq [29] isolated some species (*Alternaria aletrnata, Aspergillus flavus, A. niger, A. thomi, A. zonatus, Chaethomium piluliferum, Cladosporium herbarum, Fusarium solani, Pencillium chrysogenum, P. notatum* and *Ulocladium chlamydosporium)* from the eastern region of Saudi Arabia including coastal area.

In the present work, *A. flavus* has the highest dry weight gain. A direct relationship was existed between the highest induction of the tested extracellular α-amylase enzyme activity of *A. niger* with the highest significant linear growth. According to statistical analysis, *A. niger* was selected as the most efficient producer for extracellular α-amylase enzyme. The obtaining data was agree with Khan and Yadav [30] who isolated some fungal species from soil and screened for alpha amylase production. *Aspergillus niger* was showed best activity among all the isolates. Also, Varalakshmi, Kumudini [31] reported that *Aspergillus* species was the best amylase producer among some fungal isolates. Alpha-amylase from fungal sources, especially *Aspergillus* spp. has gained more attention because of the easy availability and high productivity of the fungi, which are also suitable for genetic manipulations. Different species of the genus *Aspergillus* such as *A. niger, A. oryzae, A. flavaus, A. tamarie* and *A. fumigatus* have been frequently used for the production of α-amylase [32]. Abu, Ado [33] stated that *Aspergillus niger* that is capable of producing high amounts of amylase is used for commercial production of α-amylase. Studies on fungal amylases especially in developing countries have concentrated mainly on *Aspergillus niger*, probably because of their ubiquitous nature and non-fastidious nutritional requirements of these organisms. Fungal sources of amylases are confined to terrestrial isolates, mostly to *Aspergillus* and *Penicillium* species [34].

Optimization of the tested culture conditions for the selected fungal species (*Aspergillus niger*) was carried out to improve α-amylase productivity. The highest significant value of *Aspergillus niger* extracellular α-amylase was recorded with fructose as carbon source and this may be due to the constitutive nature of α-amylase enzyme. The obtained results agree with Lalitha, Shravya [35] who produced maximum extracellular α-amylase activity in the culture filtrate of *Trichoderma viride* supplemented with fructose as a carbon source. The choice of fructose over any other sugar suggests that the use of dissacharides being energy expensive. However the organism did not prefer glucose which has ultimately get converted to fructose 6 phosphate which would be energy saving as entry of fructose into glycolysis that would minimize the energy. The differences in enzyme productivity when using different substrates in solid state fermentation refer to many factors consist of the nature of internal structure of substrate, porosity degree and that effect on the microbial cell penetration and its growth. So, it preferred to select highly porosity substrate or brittle materials as nutritious media because of easy of growth inside it. As well as enzyme productivity is highly affected by the size of media granules therefore the substrate which has large granules size increases the enzyme production by increasing the surface area of exposed material for microorganism [36]. Aiyer [37] stated that the non metabolizable sugars like arabinose, raffinose, mesoinositol, sucrose and galactose did not support α-amylase production by *Bacillus licheniformis.* Also, Hassan and Karim [38] found that the complex sugars like sucrose and maltose are relatively more difficult to be utilized by *B. subtilis* and required longer time to be decomposed into simpler sugar. Among the various substrates tested for amylase activity, wheat bran was found to be the best substrate for amylase production by *Penicillium Purpurogenum* [39].

Maximum significant value of α-amylase activity was manifested with potassium nitrate followed by sodium nitrate, that would be due to the nitrate source is less energy expensive than the rest organic nitrogen source. While, minimum α-amylase activity was showed with ammonium sulfate, which may be refer to decrease in pH in the culture medium. Akcan [40] found that sulfate salts decrease α-amylase production. The inhibitory effects of it may be related to the pH changes associated with their use in the medium. The obtained result coupled with, Abou-Elela, El-Sersy [41] who stated that the optimization of α-amylase enzyme production by *Nocardiopsis aegyptia* was showed with potassium nitrate as the best nitrogen source. Also, Ba Othman [42] recorded the maximum α-amylase activity of *Aspergillus oryzae* with potassium nitrate as a nitrogen source. The best nitrogen source for α-amylase production by *Aspergillus niger* was peptone followed by sodium nitrate. While, ammonium sulfate was poor nitrogen source for amylase production [43]. Rinku, Liji [21] investigated the effect of organic and inorganic nitrogen source on α-amylase production from *Aspergillus niger*. Media

supplemented with peptone showed maximum production of α-amylase enzyme. The maximum α-amylase activity produced by *Penicillium* sp. was shown on medium containing yeast extract as a nitrogen source [44].

Neutral pH value (7) was used in the production medium had a highest significant effect on the productivity of α-amylase enzyme by *A. niger*. It is known that the pH of the medium affects the availability of certain metallic ions, permeability membranes and enzymatic activities. The optimum temperature for extracellular *A. niger* α-amylase was at 20 °C. While, the minimum α-amylase activities showed at pH 11 and 60 °C, respectively. The obtained data was agree with Sundar, Liji [45] who recorded the maximum activity of *Aspergillus niger* α-amylase at pH 7.0 and room temperature 28 °C. Also, Hegde, Ramesha [46] recorded the maximum amylase activity of *Discosia* sp. at pH 7, while the minimum activity was showed at pH 11. Also, Tiwari, Jadhav [47] stated that the optimum pH for maximum α-amylase production by *Penicillium rugulosum* was pH 7. The optimum temperature for α-amylase enzyme activity of *Aspergillus niger* was found to be at room temperature (22 °C) in both solid and submerged fermentations [31]. Da Silva, Maller [48] studied the effect of environmental parameters on amylase production by *Aspergillus* species. *A. niger* produced high levels of amylolytic activity in liquid medium with initial pH 6.5. De Oliveira, Silvestre [49] stated that the amylase enzymes which produced from *Candida parapsilosis, Rhodotorula mucilaginosa* and *Candida glabrata* exhibited maximum activity at a pH of 7.0. Suganthi, Benazir [50] stated that when *Aspergillus niger* inocubated at 37 °C, the maximum yield of α-amylase enzyme was showed in the pH range 7 – 8. Abou-Elela, El-Sersy [41] recorded that the optimum temperature and pH for maximum production of α-amylase enzyme by *Nocardiopsis aegyptia* were 25 °C and 5.0, respectively. Juwon and Emmanuel [51] stated that the changes in pH do affect the affinity of enzymes for substrates, especially when the active site has been altered. A decreased saturation of the enzyme with the substrate as a result of the decrease in affinity may be due to the effect of pH on the stability of enzymes. This leads to a considerable denaturation and subsequent inactivation of the enzymes. Fadahunsi and Garuba [52] ststed that the optimum temperature for amylase production by *Aspergillus flavus* was at 30 ºC. Above the optimum temperature, amylase production was found to decrease at 60 ºC. The rate of enzyme catalysed reactions increase with temperature. This occurs only within the temperature range at which an enzyme is stable and retains full activity. Effect of temperature on the activity of an enzyme may be dependent on the stability of the enzyme substrate affinity. It might however have an effect on increase in reaction rate and increase in thermal denaturation [53]. Also, Hussain, Siddique [32] stated that at high temperatures enzymatic activity can be destroyed because enzymes are proteinaceous molecules. The optimum pH for α-amylase production by *Aspergillus awamori* was found to be significantly high at pH 6 when compared to other pH values. The α-amylase production by *Aspergillus awamori* increased significantly and reached the maximum value at 40 °C. Above 40 °C, there was a significant decline in α-amylase yield [54]. Ali, El-Nagdy [55] investigated the effect of pH and temperature on α-amylase productivity from *Aspergillus flavus*. The highest α-amylase enzyme production was recorded at pH 5.5. *A. flavus* has ability to produce α-amylase enzyme when incubated at temperature 20 °C and 25 °C. Alpha amylase productivity sharply increased and recorded the highest productivity at 28 °C.

V. CONCLUSSION

The use of the tested meso-neutrophilc extracellular α-amylase enzyme which was produced from *A. niger* that isolated from Saudi Arabian soil was preferable in industrial field to produce a large amount of the enzyme at the lowest cost, rather than the use of high expensive chemicals which have harmful effect on the environment and human health, where the most advantageous working conditions prevailing for application of α-amylase in biotechnology at moderate temperature and neutric conditions. Therefore, an ideal α-amylase should be able to function under these conditions.

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