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RESEARCH ARTICLE

Extraction ,Optimization of Uricase From *Aspergillus niger.*

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Abstract

The present study deals with production, extraction and optimization of the extracellular fungal uricase. The fungal flora will be isolated from soil .Optimization of some nutritional and physical factors in the basal medium in order to intensify the production of extracellular uricases will be carried out. The optimization factores recored the highest value of specific activity for producing uricase:(uric acid +2% yeast extract) media was recored (14.83 U/mg) , lactose was recored as a carbon source (8.23 U/mg), uric acid was recored (14.05U/mg) as a nitrogen source, the highest phosphourse source NaH2PO4 was recored (7.1 U/mg) ,and then the highest elements source was mangnous sulphate recored (1 2.64 U/mg) ,and the ascorbic acid was recored the highest specific activity (13.01U/ mg) as avitamin source , glycin was the highest amion acid source for producing enzyme (13.93 U/mg), the optimum PH and temperture were (6.0)and 30 ^{o} C recored (13.65 U/mg) , (15.24 U/mg) respectively.the incubation period was recored (15 .71 U /mg).

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Introduction:-

uricase or Urate oxidase (urate: oxygen oxidoreductase, EC 1.7.3.3) is an enzyme that catalyse the oxidation of uric acid to allantoin and plays an important role in purine metabolism [1]. This enzyme is widely present in most vertebrates but is absent in humans [2] .It was first found in bovine kidney. Various natural sources such as bacteria [3] , fungi [4] and eukaryotic cells [5] have also been found to be uricase producers. The first important application discovered for uricase was in clinical biochemistry as a diagnostic reagent for measurement of uric acid in blood and other biological fluids [6] . Higher primates (humans and apes) lack functional uricase and excrete uric acid as the end product of purine degradation [7 ; 8]. In some individuals, uric acid precipitates, leading to gout symptoms. Gout treatment generally includes allopurinol, which is a potent competitive inhibitor of xanthine dehydrogenase, an enzyme which catalyses the conversion of hypoxanthine to xanthine and xanthine to uric acid. However, in the case of gout associated with renal complications, direct injection of urate oxidase allows much more rapid resorption of urate nephrolithiases. Such injections are done to prevent or treat hyperuricemia disorders that may occur during chemotherapy.Gout is a painful disorder, characterized by uricemia, recurrent attacks of acute arthritis, deposition of sodium urate in and around joints, and in many cases, formationof uric acid calculi [9] .

Uricase catalyzes the oxidative opening of the purine ring of urate to yield allantoin, carbon dioxide and hydrogen peroxide. Determining the urine and urate concentration in blood is effective for the diagnosis of gout as urate accumulation is a causative factor of gout in humans. The enzyme is useful for enzymatic determination of urate in clinical analysis by coupling with 4-aminoantipyrine-peroxidase system [10] . Uricase can be also used as a protein drug to reduce the toxic urate accumulation [2] . Decomposition of uric acid by microorganisms regarding their identities, occurrence and uricolytic activities of different microorganisms isolated from soil was studied by [11] . Fortunately, microbial uricase have been obtained in the purified form by many reasearcheres [12 ;13]. The method for the determination of uric acid in blood serum by its reaction with uricase was described by [14] . Uricase production ability by microorganisms and some physicochemical parameters were tested to optimize uricase productivity was studied by [15] .

Material and method:-

The microorganism recorded in this study were isolated from soil sample.

Media:-

Three different media were used for uricase production. **Yeast extract sucrose medium**: Sucrose 150.0 g; yeast extract 20.0 g. [16] .

Czapek- Dox- medium:-

$NaNO_3$ 2.0 g; K_2HPO_4 1.0 g; KCl 0.5 g; $MgSO_4$ 0.5 g; $FeSO_4.7H_2O$ 0.01 g and sucrose 20.0 g. [17] .

Uric acid medium:-

uric acid 1.0 g; K_2HPO_4 1.0 g; $MgSO_4$ 0.5 g; NaCl 0.5 g; $FeSO_4$ 0.01 g and sucrose 20.0 g , Ingredient of each medium was dissolved in 1 liter distilled water and pH was adjusted to 6.5 - 7.0 [18] .

Fermentation conditions

Fifty mL aliquots of fermentation medium were dispensed in 250 mL Erlenmeyer conical flasks, inoculated with two discs (10 mm in diameter) from 7-day old cultures. The inoculated flasks were incubated on a rotatory incubator shaker at 150 rpm for 8 days at 30 °C after which the mycelium of each isolate was collected by centrifugation at 5000 rpm for 15 min at 4 °C. The cell free supernatant was used as a crude enzyme for further determinations [19] .

Enzyme assay:-

Uricase activity was assayed using the method described by(Klose *et al*., 1978). Standard reaction mixture contained 2.2 ml of 2 mmol/L uric acid dissolved in 0.1 mol/L sodium borate buffer (pH 8.5), 0.15 ml of 60mmol/L 4-aminoantipyrine, 0.15 ml of 20 U/ml peroxidase from horseradish,0.15 ml of 1.5% phenol, 0.15 ml of 0.1 mol/L sodium borate buffer (pH 8.5) and 0.2 ml of properly diluted enzyme solution to a final volume of 3.0 ml. The mixture was incubated at 25°C for 20 min and the increase in absorbance at 505 nm was spectrophotometrically measured. One unit of enzyme is defined as the amount of enzyme that produces 1.0 _mol of H_2O_2 per minute under standard assay conditions [19] .

Total protein concentration determination:-

Protein concentration was determined according to Bradford [20] with BSA as standard.

Factors affecting uricase production:-

Carbon sources:-

Sucrose was omitted from uric acid fermentation media and replaced by 1% of each glucose, fructose, lactose, starch, cellulose and glycerol [19].

Nitrogen sources:-

The liquid medium containing 0.5% uric acid was used as control. The following nitrogen sources wereused: (ammonium chloride., potassium carbonate , ammonium sulphate, pepton and sodium nitrate added to the medium as a sole sources of nitrogen [21].

Phosphate sources:-

The following phosphate sources: (sodium phosphate,magnesium phosphate and ammonium phosphate) were added fermentation medium at a concentration equimolar to the concentration of the basal phosphate source (K_2HPO_4) as control [21] .

Amino acid:-

Addition of the individual amino acid at 1.0 g/L of cysteine, cystine, arginine, glycine and tryptophane to the fungal culture. The pH was adjusted to 8.0 [19].

Vitamins:-

Some vitamins such as riboflavin, nicotinic acid, folic acid and ascorbic acid were added to the fungal culture at a concentration 1.0 g/L of each vitamin and sterilized by filtration [19] . **Elements**

Different elements such as Ca_2+ ($CaCl_2$), CO_2+ ($COCl_2$), Mn_2+ ($MnSO_4$), Mg2+ ($MgSO_4$), Fe3+($FeCl_3$), PO_4^{3}- (Na_2HPO_4), CN-1 (Na CN), I_3 (Iodine) and Hg+2 ($HgCl_2$) have been added to the culture medium at a concentration equimolar to the concentration of the basal element of the medium [19].

Effect of PH and Temperature:-

The medium containing the optimum source and concentration of the chosen nutritional factors was used as control. The following buffer solutions were used: - citrate buffer solution for pHs 4, 6; boric acid-borax buffer solution for pHs 7, 8 ; and carbonate bicarbonate buffer solution for pH 9 , each at 0.05M concentration. The liquid medium containing the optimum source and concentration of the chosen nutritional factors and optimum pH were used. Incubation was carried out at the following temperatures; 25, 30, 35, 40 , 45 ^{o}C, respectively for 10 days [21].

Results and discussion:-

Fermentation medium:-

Two disks of *A.niger* were inoculated in 250 conical flasks containing 50 mL of each of the following medium: uric acid, Czapek dox and yeast extract sucrose. The inoculated flasks were incubated at 30 °C on an incubator shaker 150 rpm for 8 days at pH 8.0. The results in (Table 1) showed that the modified uric acid(2% yeast) medium was the most favorable medium for uricase production (14.83U/mg), while Czapek dox medium (7.48 U/mg) was the lowest one. This may be due to that several fungi can utilize uric acid as a sole source of nitrogen or to satisfy their requirements of nitrogen and carbon. These results were in confirmity with what obtained by [22].

Table 1: Effect of different fermentation media on uricase production by *Aspergillus niger.*

Media	Specific activity (U/mg)
Uric acid	10.56
Czepak -dox	7.48
Yeast extract sucrose	12.17
Uric acid (2%yeast)	14.83

Carbon sources:-

This experiment was designed to select the favourable carbon source for uricase production. The results in (Figure 1) show that the highest amount of uricase enzyme (8.23U/mg) was produced in the medium containing lactose. These results were in disagreement with those of [18] who showed that *A. flavus* produced the highest amount of uricase in the medium containing sucrose.

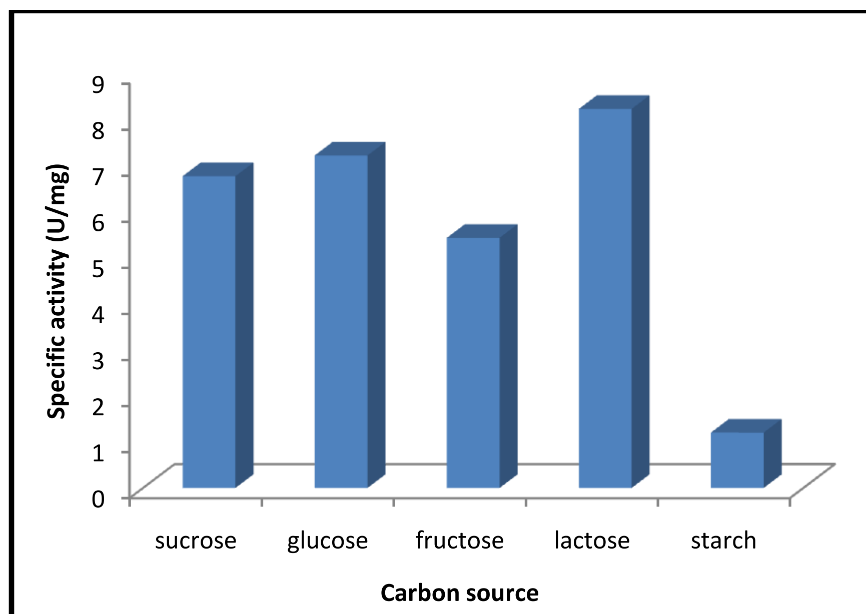

Figure 1. Effect of different carbon sources on uricase enzyme production

Nitrogen sources:-

The present experiment was conducted to test the suitability of different nitrogen sources for uricase production. The results presented in (Figure 2) show that *A. niger* recorded highest amount of uricase (14.05 U/mg) when the fermentation medium contained uric acid. Similar results were obtained by other workers i.e. [23 ; 18] , who produced uricase in medium containing uric acid as a sole nitrogen source.

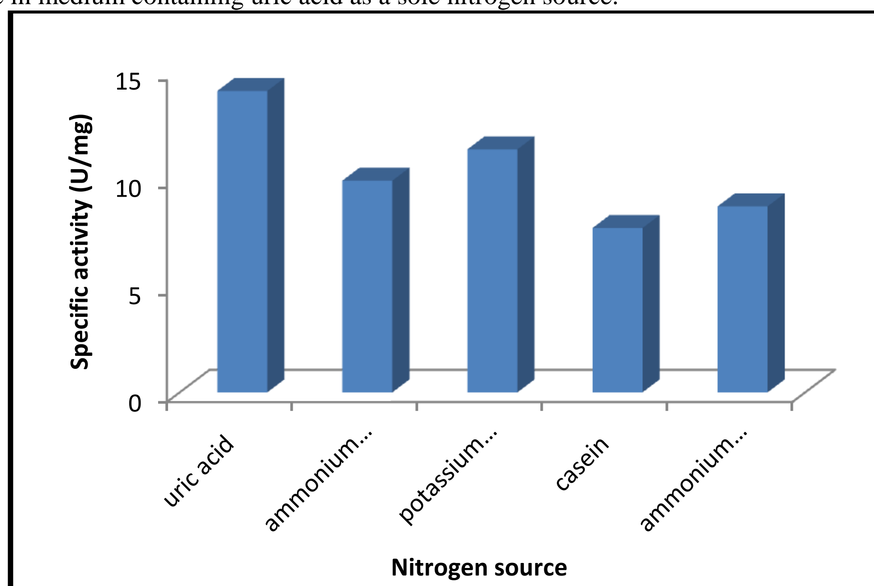

Figure 2. Effect of different nitrogen sources on uricase enzyme production.

Phosphours sources:-

The results presented in (Figure 3) show that dihydrogen sodium phosphate gave the highest amount of uricase (7.1 U/mg). It was obvious that potassium phosphate is more suitable than sodium phosphate. These results were agree with those of [10] who found that KH_2PO4 gave the highest amount of uricase as compared with K_2HPO4. Hydrogen ion plays an important role for the acidic range balance.

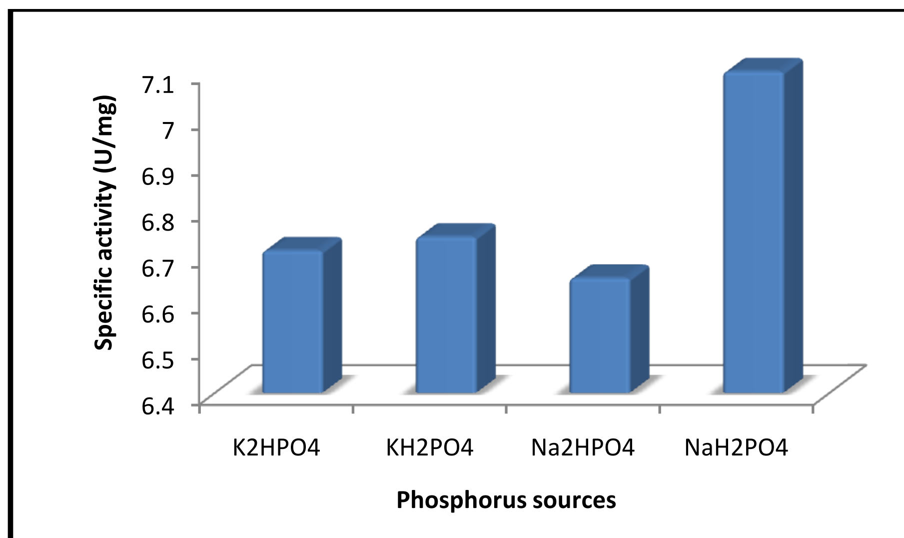

Figure 3. Effect of different phosphours sources on uricase enzyme production.

Elements:-

It is clear from (Figure 4) that mangnous sulphate gave the highest amount of uricase (12.64 U/mg). On the other hand, calcium chloride gave the less amount of uricase (6.14 U/mg).

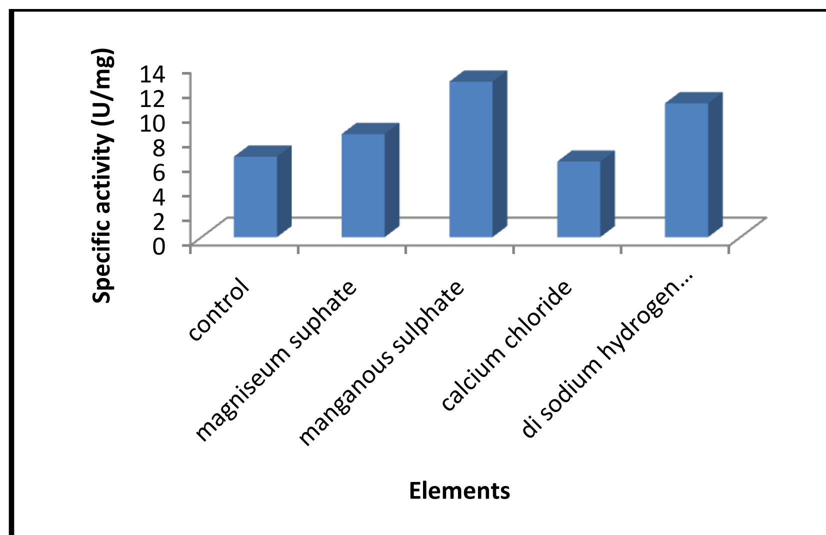

Figure 4: Effect of some elements on uricase enzyme Production.

Vitamins:-

Data presented in (Figure 5) show that nicotinic acid and ascorbic acid had a high stimulatory effect on uricase production by the experimental organism (11.78 and 13.01 U/mg), respectively. Also, folic acid and thamin appeared to stimulate uricase production but at a lesser extent (7.63 and 8.32 U/mg, respectively). Such findings were obtained by [18]. who found that the production of uricase was hardly affected by the incorporation of most studied vitamins irrespective of the fungus. However, the addition of vitamin C, molasses and vitamin B12 slightly stimulated this process in *A. terreus, A. flavus* and *Trichoderma sp*., respectively. They also demonstrated that addition of nicotinic acid, folic acid and riboflavin exhibited various inhibitory effects against uricase production.

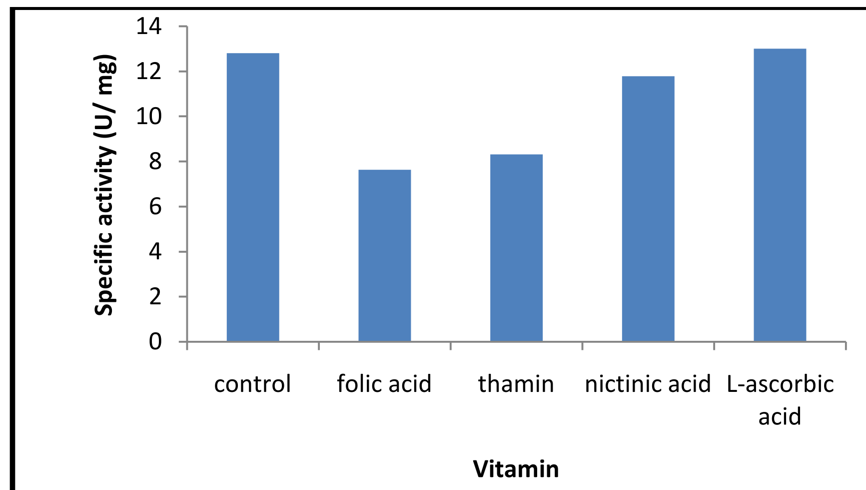

Figure 5. Effect of different vitamins on uricase enzyme Production.

Amino acids:-

It is clear from the results presented in (Figuren 6) that the presence of glycin in the medium gave the highest amount of uricase (13.93 U/mg) followed by control(5.63 U/mL). On the other hand cyctine gave the lowest amount of uricase (2.88 U/mg).

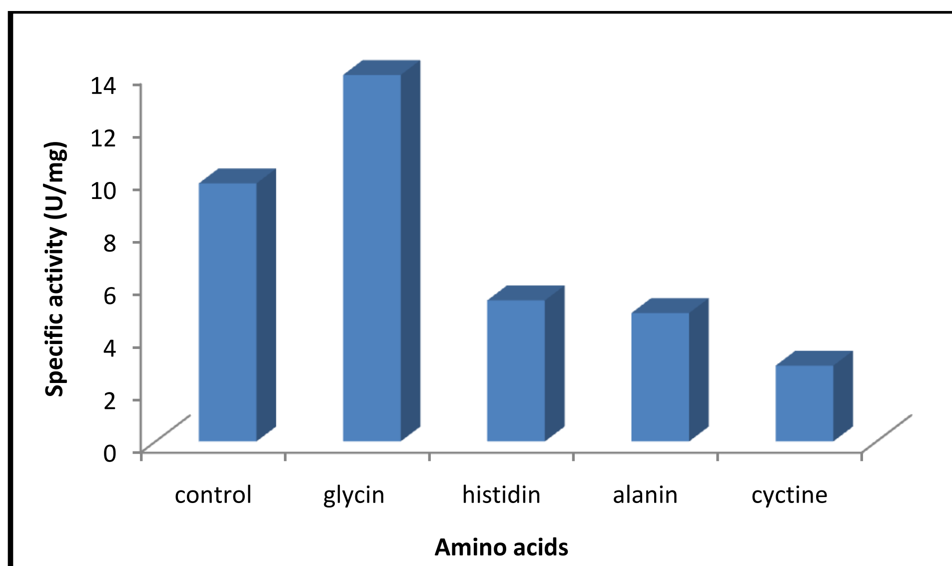

Figure 6: Effect of different amino acids on uricase enzyme production.

pH:-

The purpose of this experiment was to determine the optimum pH-value of fermentation medium suitable for uricase production. *Aspergilus niger* was inoculated in fermentation medium adjusted at different pH values (5.0, 6.0, 7.0, 8.0, and 9.0). All complementary methods were adopted as mentioned before. Results presented in (Figure 7) show that the optimal pH for uricase production (13.65 U/mg) by *A. niger* was pH 6.0. More or less than this point decreased uricase production. These results agreement with those of [24 ; 23] who found that pH 6.0 was optimum for uricase production from *A. flavus* and Mucor hiemalis, respectively. [25] showed that the highest production of uricase by *A. wentii* was at pH 10. In this connection, the optimum pH for uricase production by *A. flavus* S.79 was reported at pH 9.2 [26].

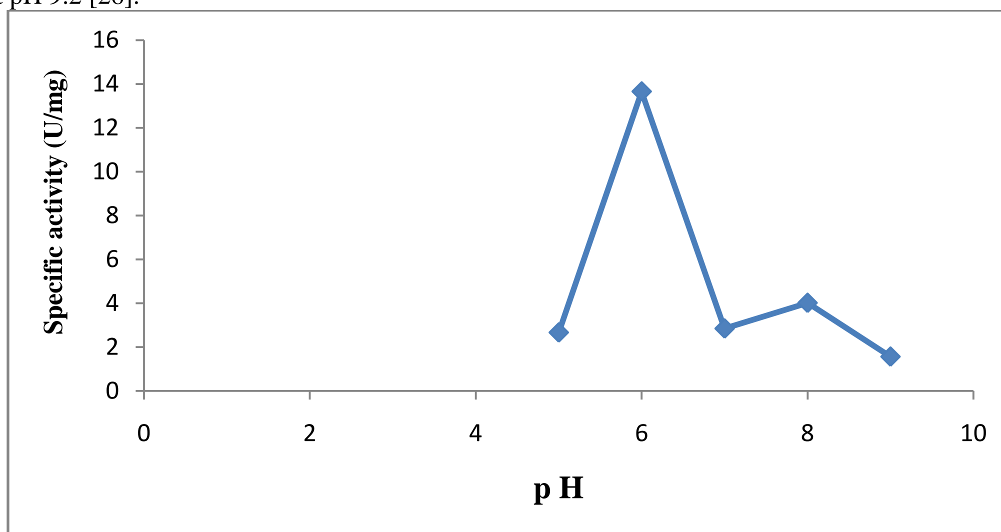

Figure 6: Effect of different PH on uricase enzyme production.

Temperature:-

An experiment was designed to determine the optimum temperature for fungal growth to produce high quantity of uricase enzyme. Uric acid modified medium was inoculated with the fungal strain, maintained on an incubator shaker at different degrees of temperature (25, 30, 30, 35, 40 and 45 °C) for 10 days at pH 6.0. Results given in (Figure 7) show That uricase production increased as incubation temperature increased until reached maximum

(15.24 U/mg) at 30 °C and then decreased. These results were in agreement with those of [26 ; 24 ; 18 ; 23] who found that the optimum temperature for uricase production was 30 °C.

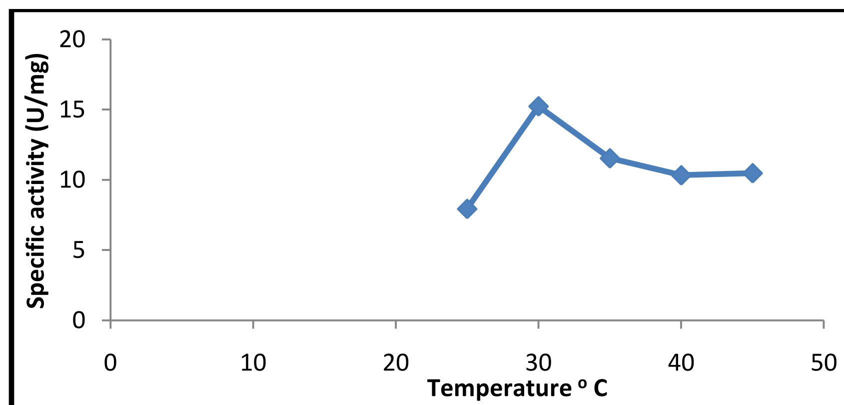

Figure 7: Effect of Different temperatures on uricase enzyme production.

Incubation period:-

The goal of this experiment was to select the optimum incubation period for uricase production by *A. niger* when grown on uric acid medium at 30 °C under shaken conditions for different periods of time. Results in (figure8) indicated that 7 days incubation was optimum for uricase production recored (15 .71 U /mg) by *A. niger*. These results disagree with those of [18] who produced uricase from *A. flavus, Aspergillus terreus* after 4 days incubation and from *Trichoderma sp.* after 6 days .[27] produced maximum uricase by *Hyphomyces* after 5 days incubation.

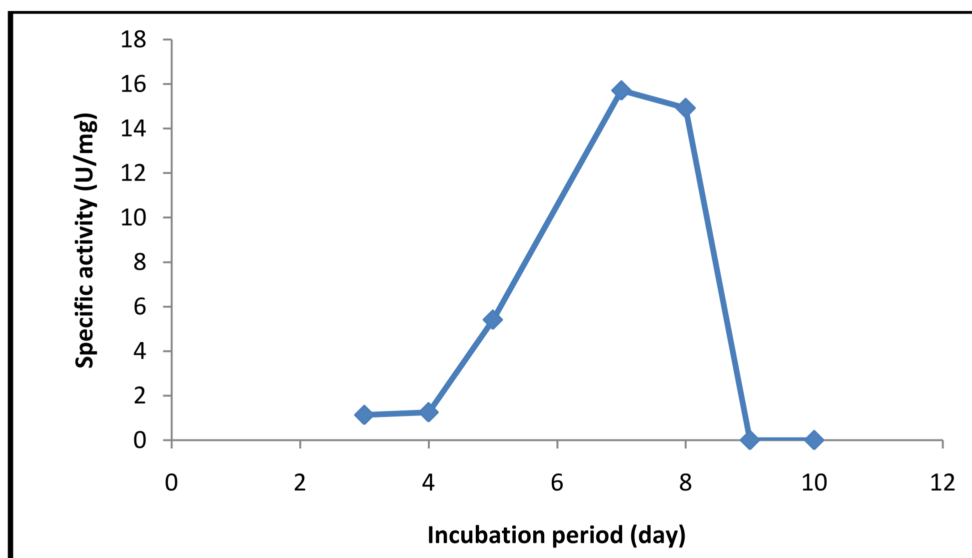

Figure 8: Effect of incubation period on uricase enzyme production by *A. niger*.

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