above mixture was autoclaved at 121°C for 15 minutes. The broth was taken and 2.4 grams of PD agar was added, mixed thoroughly. The were used for growing the inoculums. 100 ml of purified water was repeatedly washing the pre-treated biomass with distilled water and was hydrolysed with 1% NaOH solution. The pH was brought to neutral by were ground to a fine powder using a blender. The areca biomass was haemocytometer with a phase contrast microscope. spore concentration in 1% triton x-100 and determined by using a needed (Devanathan et al; 2007). Spore counting was done by reading the colony and microscopic examination of four isolated colonies were and bhat 1988]. The cultures were identified based on morphology of isolate and culture are the most commonly used microorganisms in SSF due to their importance have been produced in submerged fermentation [SMF] compared to other microorganisms. Aspergillus and Trichoderma species are very well known for cellulose production. Almost all fungi of the genus Aspergillus synthetize cellulase, therefore this genus has the potential to dominate enzyme industry. The enzymes of industrial production have been produced in submerged fermentation [SMF] because of the ease of handling and good control of environmental factors such as temperature, aeration, agitation and pH (Singh et. al. 2007). However, solid state fermentation (SSF) techniques are better adapted to enhance the yield (Ghildyal et al, 1985) along with reducing the cost of enzyme production. Filamentous fungi such as Aspergillus niger are the most commonly used microorganisms in SSF due to their ability to grow on low water substrates such as lignocellulosics. Fungi are advantageous as the enzyme production rate is higher compared to other microorganisms. Aspergillus and Trichoderma are the most commonly used microorganisms in SSF due to their ability to grow on low water substrates such as lignocellulosics. INTRODUCTION The enzymes produced by fungi are broad array like endoglucanases or CMcases and β-glucosidases. This complex of cellulases combines and acts on celluloses. For example complete cellulose hydrolysis into glucose is brought about by endoglucanases and β-glucosidases, cellobiohydrolyses degrade cellulose by removing cellulose from nonreducing ends of cyclodextrins. Finally, β-glucosidases hydrolize cellobiose in to glucose and remove glucose units from non-reducing ends of cyclodextrins. These enzymes find multiple uses in production of food, animal feed, textiles, fuel, chemical, pharmaceuticals and in waste management. MATERIALS AND METHODS Microorganism - Five soil samples were collected in an Areca farm of Tarkiree Taluk in chickmagalur district of Karnataka state, India. To isolate and culture Aspergillus niger, the samples and were serially dilutd and spread on plates as sterile triplicates of CMC agar [wood and bhat 1988]. The cultures were identified based on morphology of the colony and microscopic examination of four isolated colonies were further maintained on PDA agar slants, and later stored at 4°C until needed (Devananathan et al; 2007). Spore counting was done by reading spore concentration in 1% triton x -100 and determined by using a haemocytometer with a phase contrast microscope. The waters of PD agar were added, mixed thoroughly. The above mixture was autoclaved at 121°C for 15 minutes. The broth was further supplemented with a g/litre of Sucrose, K,HPO₄, Sodium Nitrate, MgSO₄, KCl, FeSO₄(Trace) for minerals. Substrate - Powdered Arecaut spade prepared as above was used in the study as the lignocellulosic substrate. Areca spade was procured from Tarikere Taluk in chickmagalur district of Karnataka state, India. The substrate was sterilized at 121°C. Submerged fermentation (SmF) of Areca Spade Biomass - Submerged fermentation was carried out in 250 ml flasks. PD broth was combined with areca spade powder, at 1% W/V. The flasks with the PD broth and Areca biomass were sterilized in an autoclave and inoculated with 1x10⁹ spores of Aspergillus at density from spore suspension prepared by flooding the 7-day old slants with 2 ml of distilled water. The inoculated flasks were incubated at 30°C for 7 days on a rotary shaker. The composition of the medium contained g/l distilled water Dextrose 20; Potato Starch 4.0; Agar 15.0; and added supplements sucrose, K,PO₄, Sodium Nitrate, MgSO₄, KC1, FeSO₄(Trace). culture broth of the flasks was filtered threw pre weighed whatman No. 1 filter paper to separate mycelial mat and culture media filtrate (Narasimha et al; 2006). Cellulase enzymes in the filtrate were measured. Dry mycelial mat weight on the filter paper was determined. Solid state fermentation of Areca Spade Biomass - Solid state fermentation was carried out in 250 ml flasks that contained 10 grams of the Areca Spade powder which was moistened with mineral salt solution, the contents were sterilized, pH was maintained at 6. The 7day old slant was flooded with 2 ml of distilled water and inoculated, the contents were mixed well and incubated at 30°C in a humidified incubator for 7 days with a gentle shake. Post fermentation the contents of the flask were mixed with 100 ml of 0.2 M [pH 4.8] Sodium acetate buffer and the contents were shaken at 180 rpm on a shaker and the filtrates of the above were centrifuged at 6000 rpm for 10 minutes and further subjected to enzyme assessment. Enzyme Essay – Ghosh method (1987) was used to assay the activity of Carboxymethyl cellulase's (CMcase) endoglucanase activity. Diluted enzyme with citrate buffer was added to 1.8 ml of CMC solution and equilibrated, in a water bath at 50°C, with DES solution, boiled exactly for 50 min with DNS solution and cooled in an ice bath. Absorbance of contents was measured at 540 nm and determined the glucose concentration by a standard graph. For Fpase activity a strip of filter paper (What man no.1) was kept immersed in 1 ml of 0.05 M Sodium citrate buffer (of pH 5.0), reducing sugars were determined by DNS method. β – Glucosidase was estimated by NPG method 1 ml of ρNPG and 1.8 ml of acetate buffer are incubated at 50°C. Enzyme dilution series were added to the substance mix, enzyme blanks are

Biochemistry
Madhuri B Dept of Biochemistry, S. V. University, Tirupati. 517502. A. P. India.
Narasimha G Dept of Virology, S. V. University, Tirupati. 517502. A. P. India.
Balaji M* Dept of Biochemistry, S. V. University, Tirupati. 517502. A. P. India. *Corresponding Author

ABSTRACT
Areca palm (Chrysalidocarpus lutescens) a widely used plant having feathery arching brands with 10 leaflets. All these plants produce much of waste in additions to greeny and nuts. This waste of spade is used for the production of various molecules that are used in industry and pharma sector. Fermentation techniques are used to generate economically important enzymes for industrial and pharmaceutical purposes. Cellulase enzyme degrades the cellulose in between β-1, 4 glucosidic link found in lignocellulosic complex which under physical treatment is slower to degrade. The present study of Aspergillus niger for cellulose production was carried in solid state (SS) and submerged (SM) fermentations for production of cellulase enzyme. Cellulase production in SSF after 72 h of fermentation was 9.02 and in SMF activity was 2.98 per ml of cultured broth at pH 6 and temperature at 30°C. Both SMF and SSF were supplemented with lactose and lactobionic acid, which acted as cellulase production inducers. The aim of the present work was to study the effect of Areca palm spade as substrate for Aspergillus niger and its cellulase production under SMF and SSF.

KEYWORDS
Aspergillus niger, Areca nut, Areca spade, Fermentation, lignocellulosuses

Substrate - Powdered Arecaut spade prepared as above was used in the study as the lignocellulosic substrate. Areca spade was procured from Tarikere Taluk in chickmagalur district of Karnataka state, India. The substrate was sterilized at 121°C.

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prepared with acetate buffer, substrate blanks were prepared with 0.1%NPNG solution and tubes were incubated at 50°C for 30 min. 5 ml of glycine buffer was added to stop the reaction, and absorbance of liberated p-Nitrophenol products was measured at 450 nm based on the substrate blank. Enzyme solution net absorbance was read by substracting readings of enzyme blanks. P-Nitrophenol release was determined on the basis of the known concentration p-Nitrophenol diluted by glycine at 480 nm. The enzyme activity was calculated on the basis of linear range between the absorbance and enzyme concentration (Zhang, Hong and Ye, 2001-Aug).

One unit of enzyme activity (CM case and Fpase) was defined as the amount of an enzyme which releases 1 µ mole of reducing sugars per min with glucose as standard. The values of enzyme activity were expressed as U/ml for SmF and u/g of dry mycelial bran or mat [DDM] for SSF.

Lowry Test for Protein Estimation -To know the concentration of the cellulases produced by Aspergillus niger Lowry test was done for extracellular proteins secreted by Aspergillus niger in to the fermented substrate. In this estimation three reagents were used. Reagent A consists of 2 gm sodium potassium tartrate x 4 H2O, 100 gm sodium carbonate, and 500 ml 1N NaOH, H2O to one litre keeps 2 to 3 months. Reagent B is composed of 2 gm sodium potassium tartrate x 4 H2O, 1 gm of copper sulphate (CuSO4 x 5H2O), 90 ml H2O, 10 ml 1N NaOH. The reagent can be kept for 2 to 3 months. Reagent C consists of 1 volume Folin-Ciocalteu reagent diluted with 15 volume water. A series of dilutions were prepared of 0.3 mg/ml BSA in the same buffer containing the unknowns, to give concentrations of 30 to 150 micrograms/ml (0.03 to 0.15 mg/ml). Added 1.0 ml each dilution of standard, to 0.90 ml reagent A in separate test tubes and mixed. Tubes were incubated 10 min in a 50 degrees C bath, then cooled to room temperature. Added 0.1 ml of reagent B to the series of tubes, mixed, incubated for 10 min at room temperature. Rapidly added 3 ml of reagent C to each tube, mixed and incubated for 10 min at 50°degree in water bath, and cooled to room temperature. Final assay volume was 5 ml and absorbance was measured at 650 nm in 1 cm cuvettes. A standard curve of absorbance versus micrograms protein was plotted, and determined the amount of protein of extracellular enzymes using from this curve.

Effect of fermentation parameters on SmF and SSF - The Aspergillus niger and the areca spade powder were used for the production of fermentation product under various conditions.

Effect of Time on Enzyme Production - Enzyme production was determined at different time periods from 4th to 8th day of incubation by maintaining P0 at 5.5 and temperature at 30°C.

Effect of Temperature - The effect of temperature was optimized on Areca Spade Biomass (ASB) at different ranges from 25°C to 45°C, maintaining P0 at 5.5 for 8 days of incubation.

Effect of P0 - The effect of P0 was checked by fermenting ASB with Aspergillus niger at different P0 values from 4.5, 5.5, 6.5, 7.5 and 8. ASB was then incubated at 30°C for 7 days.

Effect of Carbon Source - Different Carbon Sources like Glucose, Maltose, Lactose, Sucrose and Starch were supplemented to the medium to determine the infusing capacity of molecule by incubating at 30°C and 7 days of time period.

Effect of Nitrogen Source - Various Nitrogen supplement sources like NaNO3, (NH4)2SO4, NH4Cl, NH4NO3, and KNO3 were studied at 30°C and P0 5.5 for 8 days of incubator.

RESULTS AND DISCUSSION

Tarricere is abundant in areca nut farms and also Aspergillus niger in these farms. The local strain of Aspergillus niger has natural capacity to degrade the lignocellulosic components by secreting lignocellulltyic enzymes. These enzymes were studied and analysed by using enzyme assay and to confirm the presence of cellulases like Fpase, β-glucosidase and Endoglucanase. These enzymes convert the substrate into monomers which are used as sources of industry and pharmacy. Aspergillus niger was isolated by serial dilution of soil sample cultured from the areca farm. The pure culture of fungal colonies of white colony morphology were collected from PD agar plates and then turned black during sporulation, the sporulated organism was microscopically examined stained with lacto phenol cotton blue dye using for morphological confirmation. The identification feature like hyaline septate hyphae, conidial head, conidiophores, globose shaped vesicles, sterigmata confirmed Aspergillus niger.

Cellulases Secreted by Aspergillus niger - Cellulolytic enzymes were secreted in to the broth and substrate of areca biomass by Aspergillus niger. After 7days of incubation the filtrate of SmF and SSF contained Fpase, CMCase and β-glucosidase. CMCase as the most prominent enzyme forming the larger moiety than Fpase and β-glucosidase.

Table: 01: Production of hydrolases from aspergillus niger in the presence of areca biomass.

| Enzyme  | SmF (U/ml) | SSF (U/g) |
|---------|------------|-----------|
| Fpase   | 2.20       | 3.36      |
| CMCase  | 2.69       | 8.02      |
| β-Glucosidase | 2.20 | 7.20      |

Maximum enzyme production was recorded around 30°C both under SmF and SSF. Around 80% of Cellulase production was observed around 35°C of temperature whereas 50% of cellulase around 20°C, 25°C and 40°C. Maximum enzyme concentration was recorded at 30-35°C. According to a study by (Devanathan et al. 2007), 37°C was said to be the optimum temperature for cellulose production by Aspergillus niger.

Fig-01: Determination of temperature optima for the production of hydrolases.

Fig-02: Determination of PH optima for the production of hydrolases.

P0 change also affected the product stability, growth of the fungi and caused morphological changes in microorganisms. The P0 effect can be described by Michaelis constant in the P0 range 3 to 8. The maximum cellulose was produced at P0 6.0. Short incubation period for enzyme production offers the potential for inexpensive production of enzyme. Incubation time necessary for optimal production varied between different enzymes produced from the substrate (Smith et al, 1996). In this study the enzyme production steadily increased along with incubation and reached the maximum at 96h (4days incubation) then started declining after, the lowest enzyme concentration being formed at P0 6.0. The maximum enzyme concentration was observed at P0 3.5.

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Table.02: estimation of enzyme in the presence of different carbon sources as supplements to and biomass

| Supplement | CHG-Ch | Fpe | Ch-Summary | CHG-Ch | Fpe | Ch-Summary |
|------------|--------|-----|------------|--------|-----|------------|
| Sugarcane  | 0.6%   | 0.3%| 0.3%       | 0.6%   | 0.3%| 0.3%       |
| Glucose    | 0.05   | 0.01| 0.01       | 0.10   | 0.02| 0.01       |
| Table Milk | 0.5%   | 0.1%| 0.1%       | 0.5%   | 0.1%| 0.1%       |
| Yeast      | 0.5%   | 0.1%| 0.1%       | 0.5%   | 0.1%| 0.1%       |
| Formic     | 0.5%   | 0.1%| 0.1%       | 0.5%   | 0.1%| 0.1%       |
| Cane        | 0.5%   | 0.1%| 0.1%       | 0.5%   | 0.1%| 0.1%       |

P value is <0.005

Bioassay and secretion of enzyme is also influenced by the moisture content in SSF. The ratio of 1:2 is found to be ideal for high enzyme production lower than optimal moisture content results in no solubility of nutrients from the substrate and therefore decreased enzyme production (Lonsane et al., 1985). Higher moisture levels will also reduce the yield by interfering the oxygen transfer and by increasing porosity i.e. inter particle spaces, thus hindering the growth there by affecting enzyme synthesis.

Table.03: Production of enzyme in sm process with the dilution of biomass and h2o in the presence of Aspergillus niger.

| Substrate | Distilled Water | Enzyme activity (g/L) | Ch-Summary | Ch-Summary | Ch-Summary |
|-----------|-----------------|-----------------------|------------|------------|------------|
| 1.50      | 2.40 ± 0.01     | 2.00 ± 0.01           | 2.00 ± 0.01|
| 1.40      | 2.00 ± 0.01     | 2.50 ± 0.01           | 2.50 ± 0.01|
| 1.60      | 2.00 ± 0.01     | 2.50 ± 0.01           | 2.50 ± 0.01|
| 1.80      | 2.00 ± 0.01     | 2.00 ± 0.01           | 2.00 ± 0.01|
| 2.00      | 2.00 ± 0.01     | 2.00 ± 0.01           | 2.00 ± 0.01|

μ Moles of enzymes produced in a minimum per mg protein the substrate present in Areca nut spade biomass under different dilution conditions. So 1:10 of w/v of moisture content can be allowed for synthesis of cellulases by Aspergillus niger. Inoculum size should also be optimum so as to facilitate proper usage of substrate by microorganisms 5 to 15% of inoculums size is optimum for SmF and 10% for SSF.

In this study Aspergillus niger was comparatively cultured under SSF and SMF. The SSF condition provided proper utilisation of the substrate for enzyme production; comparatively minimum inoculation was needed for SSF. SSF has less liquid wasteage, proper usage of substrate was achieved, cellulases produced in SSF were greater than enzymes produced by SmF.

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