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STUDY of the INFLUENCE of NUTRIENTS on CITRIC ACID PRODUCTION by *Aspergillus niger* UNDER SOLID STATE FERMENTATION USING RICE CHAFF and SESAMUM OIL CAKE as SUBSTRATE

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Abstract: The main aim of this study is to investigate about citric acid production by ETGP12 and ETGP18 strains of *Aspergillus niger* with the inclusion of certain nutrients along with the substrates. The substrates (rice chaff and sesamum oil cake) were fermented in the presence of different nutrients like ammonium carbonate and potassium dihydrogen phosphate at concentrations ranging from 0.1 to 0.5 mg/100g of substrates for a period of 72 and 96 hours and the same were tested even in mixed combinations of nutrients. In both the cases it was observed that these nutrients enhanced the rate of citric acid production initially but at higher concentrations the yield of citric acid is reduced.

Keywords: Sesamum oil cake, Rice chaff, Fermentation, *Aspergillus niger* , Citric acid.

I.INTRODUCTION:

Citric acid is a 6 carbon containing tricarboxylic acid which was first isolated from lemon juice. Today citric acid is a valuable product having applications mainly in food chemical cosmetic beverages and many other industries. In the recent years citric acid production is done using fungal fermentation using several strains of *Aspergillus niger* as it is much cheaper than the conventional method of producing it from citrus fruits. For this purpose different agro industrial residues (apple, pomace ,orange processing waste, coffee husk, oil cakes). To obtain maximum production of citric acid , standardization of media and fermentation conditions is crucial. And thus in our study we are assessing the effect of changes in the citric acid production by the addition of nutrients along with the substrate under solid state fermentation using two strains of *Aspergillus niger* (ETGP12 & ETGP18) along with *Aspergillus niger* ATCC9142 as a control culture. In this study by using sesamum oil cake and rice chaff as substrates mainly due to its low price and easy availability, it was designed to know the difference of inorganic nutrients and also the composition of sesamum oil cake (moisture -9.3 % , carbohydrate content -39.8 % , reducing sugars -14.1 % , protein -14.29 % , fats – 13.4 % along with traces of iron manganese and copper) and rice chaff (moisture -2.3 % , carbohydrate content -43.2 % , reducing sugars -18.6 % , protein -23.4 % , fats – 8.3 % along with traces of iron manganese and copper) which was most suited for our study.

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II.MATERIALS AND METHODS:

Sesamum oil cake and rice chaff was ground and dried at 52°C for 6 hours (Tran and Mitchell, 1995). The powders thus obtained were analysed for fermentable sugars and adjusted to pH 3.0 (Hang and Woodams, 1985). The samples thus prepared were taken out separately into 250ml Erlenmeyer flask and rehydrated to required moisture content by adding sterile water as per Hang and Woodams (1987). The cotton plugged flasks were autoclaved at 121°C for 15 minutes followed to cool to about 35°C. The contents of flasks were inoculated with 1ml of inoculum ($1x10^7$ spores/ml). Then the flasks were mixed thoroughly by gently beating on the palm of the hands and incubated in a slanting position at 35°C in an incubator (Plate 5) with 65-70% relative humidity for 7 days (Ramesh and Lonsane, 1990). The substrates prepared as earlier were fermented in presence of different nutrients like ammonium nitrate, urea, ammonium carbonate and potassium dihydrogen phosphate at concentrations ranging from 0.1 to 0.5 mg/100g substrate and the same were tested even in mixed combinations of nutrients as described by Shankaranand (1995) respectively.

III.RESULTS AND DISCUSSION

Results of the studies pertaining to the yield of the citric acid with strains of *A. niger* ATCC9142, ETGP12 and ETGP18 using sesamum oil cake and rice chaff wastes as the substrates are represented in figures 1-8. The optimized duration of the citric acid production was found to be between 72 and 96 h. Hence, the study on the effect of the various nutrients on the production of the citric acid using the sesamum oil cake and rice chaff was established for the period of 72 and 96 h. The data revealed that all the four nutrients used under the present study had enhanced the yield of citric acid with sesamum oil cake as the substrates. Supplementation of ammonium nitrate, urea, ammonium carbonate and potassium di hydrogen phosphate separately upto 0.25, 0.30, 0.35 and 0.20 mg/100g respectively tended to enhance citric acid yield, while higher concentration of the same nutrients decreased the citric acid yield at 72 hrs. Maximum yields of citric acid were 102.8, 103.8, 106.7, 101.3 g/kg concurrent with the 72.5, 73.2, 74.4 and 72.9% sugar conversion efficiency of *A. niger* ATCC9142 at the above mentioned concentrations of the nutrients respectively. Rice chaff was used as the substrate to produce the citric acid and it was supplemented with the ammonium nitrate, urea, ammonium carbonate and potassium di hydrogen phosphate separately up to 0.25, 0.30, 0.35 and 0.20 mg/100 g respectively and has enhanced the citric acid yield. The higher concentrations of the nutrients decreased the yield of the citric acid. The yields of citric acid at the above said concentration of the nutrients were 105.7, 107.8, 109.55 and 104.6 g/mg concurrent with the conversion efficiency of 72.2, 73.5, 75.6 and 71.4 % respectively.

Results pertaining to the yield of citric acid by *A. niger* ETGP12 using sesamum oil cake and rice chaff added with different levels of nutrients for 96 h fermentation. The addition of the ammonium nitrate, urea, ammonium carbonate and potassium di hydrogen phosphate separately up to 0.25, 0.30, 0.35 and 0.20 mg/100 g respectively to the sesamum oil cake has enhanced the citric acid yield, while higher concentrations of the nutrients decreased the yield. Maximum citric acid yields were 105.7, 108.6, 111.2 and 105.2 g/kg concurrent with 75.2. 76.35, 79.6 and 74.5% sugar conversion efficiency of *A. niger* ETGP12. Similarly for rice chaff, the citric acid yield was found to be high with the decrease in the concentration of the nutrients, such as 108.3, 111.0, 114.1 and 109.9 g/kg s the highest citric acid produced with the sugar conversion efficiency as 71.1, 72.4, 74.5 and 70.3% respectively..

The addition of the ammonium nitrate, urea, ammonium carbonate and potassium di hydrogen phosphate separately up to 0.25, 0.30, 0.35 and 0.20 mg/100g respectively to the rice chaff has enhanced the citric acid yield, while higher concentrations of the nutrients decreased the yield. Maximum citric acid yields were 98.8, 101.4, 108.3 and 95.5 g/kg concurrent with 68.9. 70.2, 75.4 and 66.3% sugar conversion efficiency of *A. niger* ETGP18 for the sesamum oil cake for 72 h.Nitrogen constituent has a profound effect on yield of citric acid because the type of nitrogen source and its concentrate effect the development of fungus

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considerably. In the present study ammonium carbonate was found to be best nitrogen source required for growth of fungus .Among different concentration tested, 0.20% ammonium nitrate was optimized for maximal citric acid accumulation. It might be due to the fact that at the concentration of ammonium nitrate, the mycelia growth was resulting in greater citric acid production. At low ammonium nitrate concentration, less acid production might be due to lower supply of free nitrogen for mycelia growth. On the other hand due to large quantity of ammonium nitrate (other than 20%) the biomass grew slower and production decreased sharply. The concentration of phosphate in the fermentation medium is also very important for the production of citric acid.

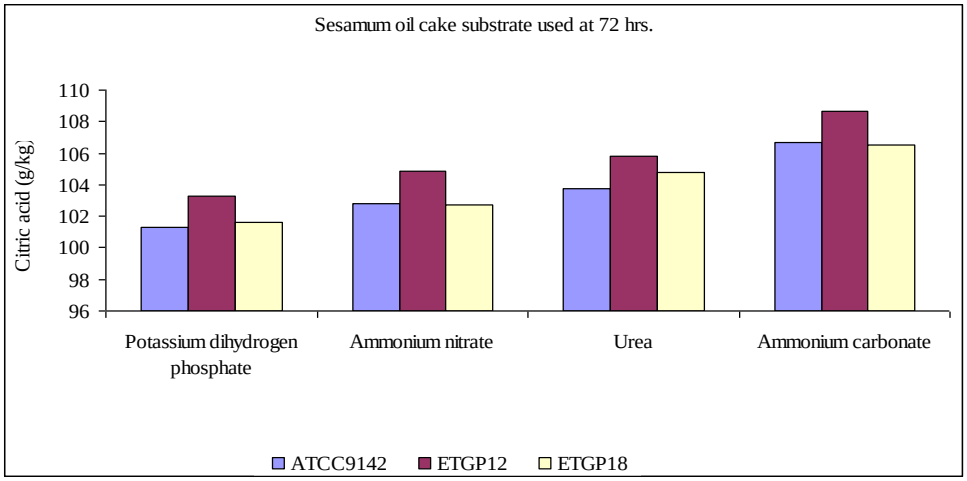

Fig.1 Production of citric acid using sesamum oil cake as the substrate using different nutrients.

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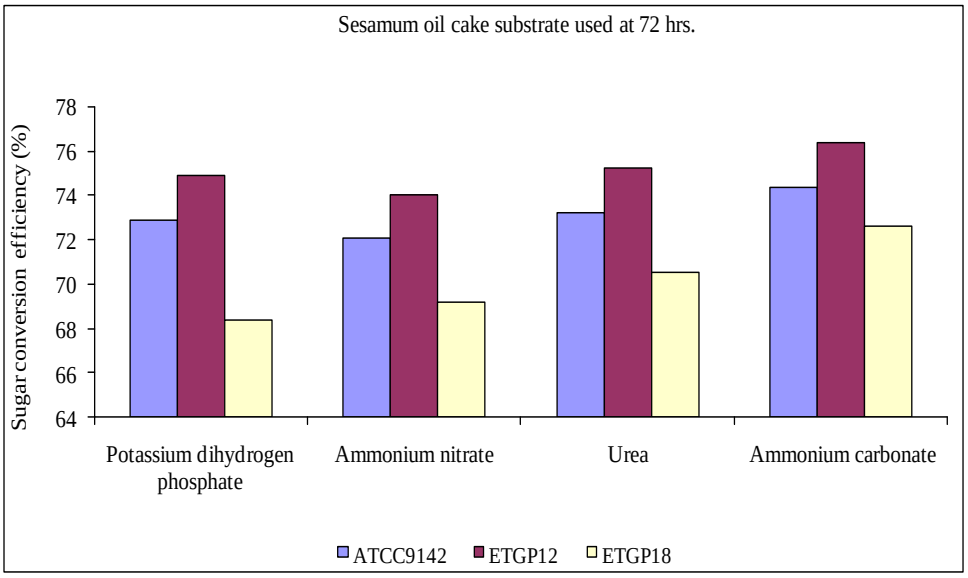

Fig.2 Sugar conversion efficient using sesamum oil cake as the substrate using different nutrients.

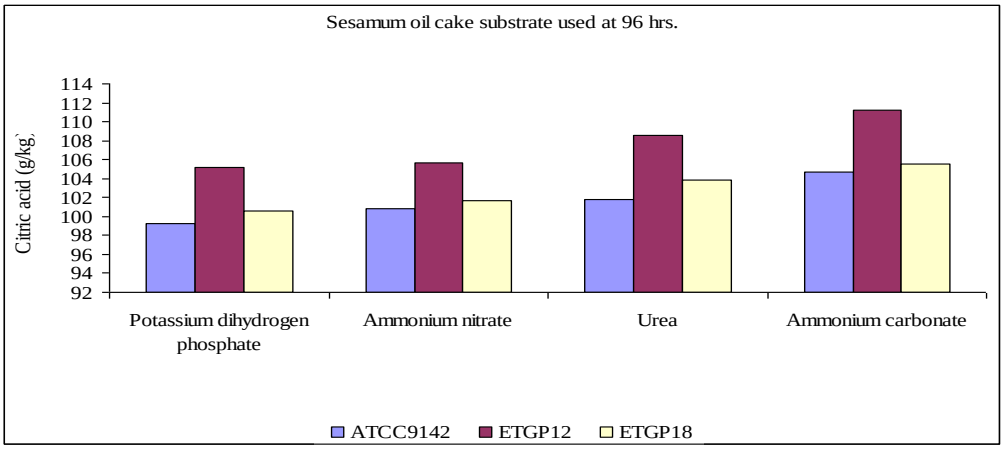

Fig. 3 Production of citric acid using sesamum oil cake as the substrate using different nutrients.

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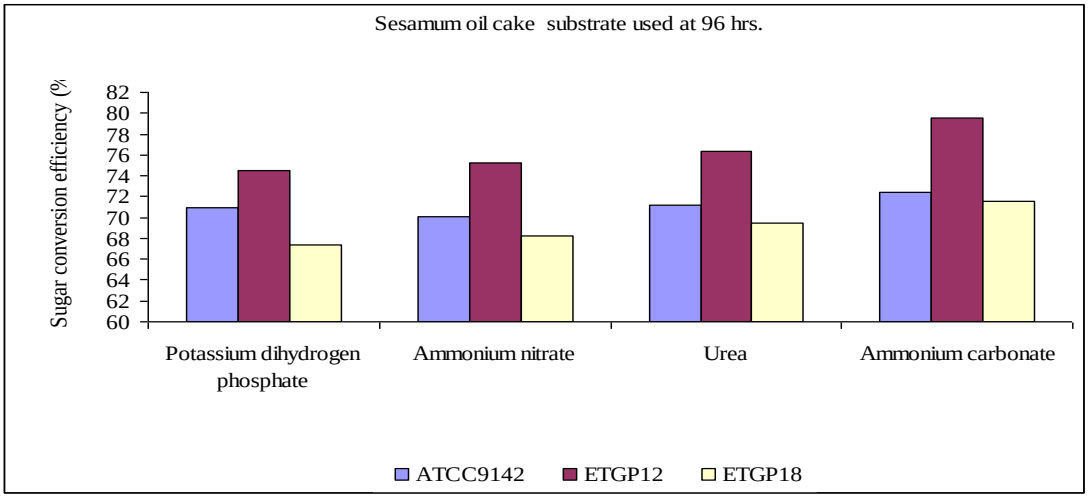

Fig.4 Sugar conversion efficient using sesamum oil cake as the substrate using different nutrients.

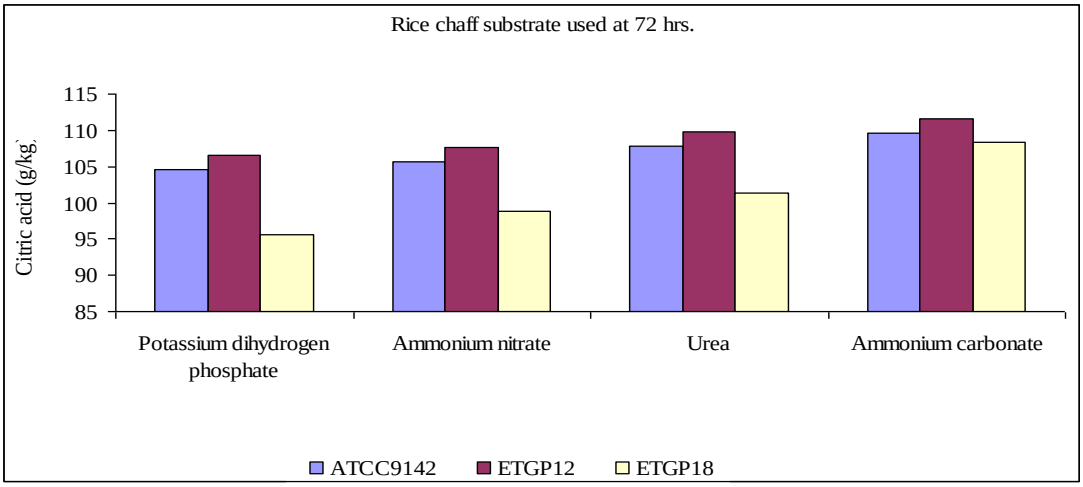

Fig. 5 Production of citric acid using rice chaff as the substrate using different nutrients.

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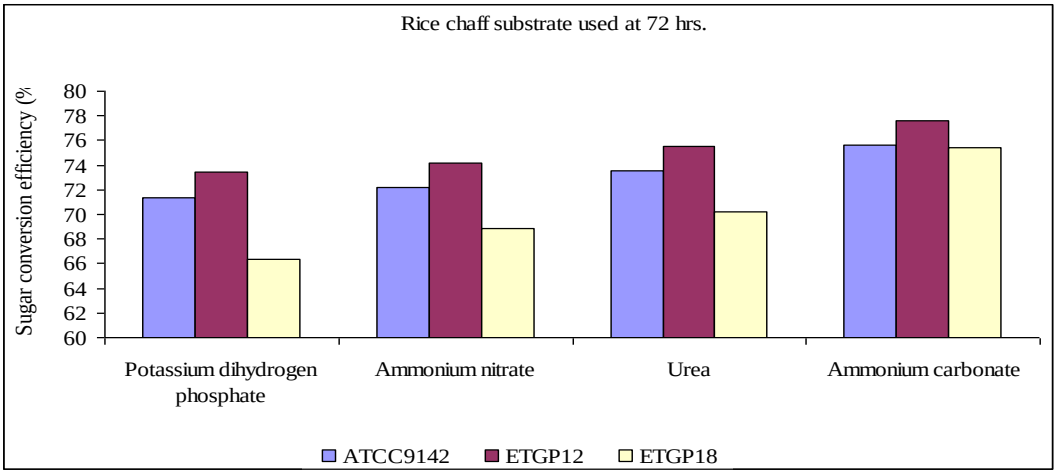

Fig. 6 Sugar conversion efficient using rice chaff as the substrate using different nutrients.

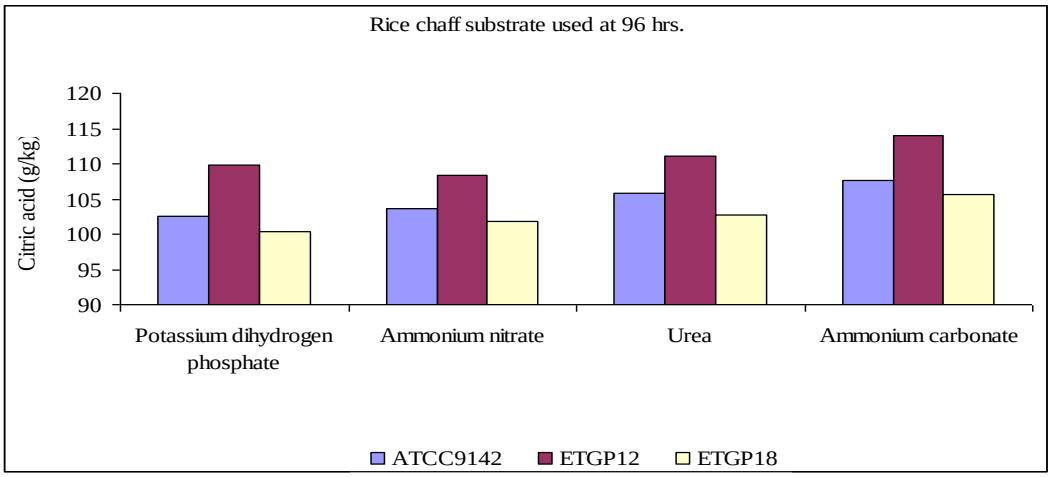

Fig. 7 Production of citric acid using rice chaff as the substrate using different nutrients.

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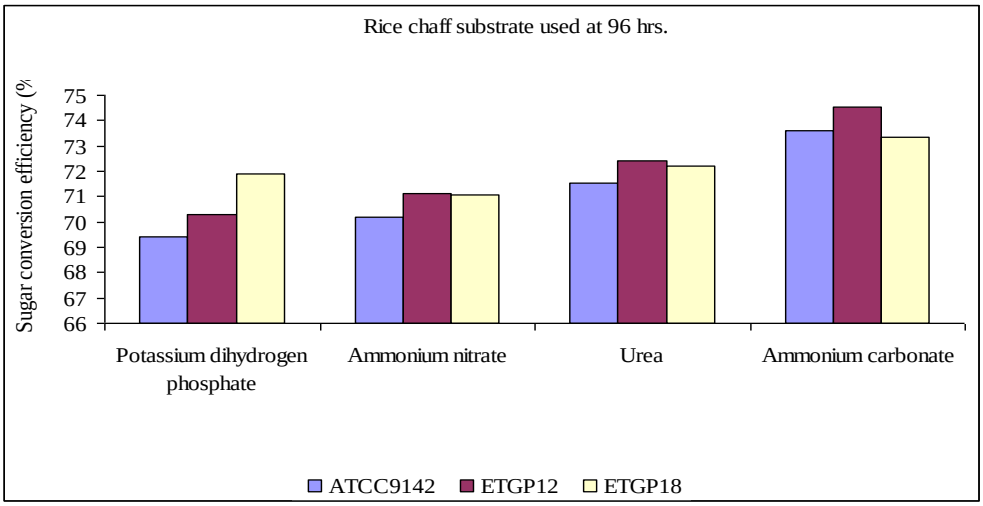

Fig. 8 Sugar conversion efficient using rice chaff as the substrate using different nutrients.

IV. CONCLUSION

The maximum production of citric acid (101.3g/kg &104.6g/kg) for sesamum oil cake & rice chaff respectively) was obtained when potassium dihydrogen phosphate was used as phosphate source. It might be due to the fact that phosphate was readily available to the mycelium using this phosphate source in the medium. Among different concentrations 0-10% potassium dihydrogen phosphate was found to be best concentration for optimum mycelial growth which resulted in greater production of citric acid (101.3 g/ and 104.6 in sesamum oil cake & rice chaff respectively). A higher concentration of phosphate in fermentation medium promotes more growth and less acid production(Khan et al , 1973).in general a phosphate concentration of about 0.1-0.15% in fermentation medium appears to be adequate.

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