Microbial production of citric acid by *Aspergillus niger* on flower of *Madhuka longifolia*, flower of *Bauhinia variegate*, pithy pulp of fruit of *Adansonia digitata* and flower of *Bombax ceaba*

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ABSTRACT

The selected carbon sources which are flower of *Madhuka longifolia*, flower of *Bauhinia variegate*, pithy pulp of fruit of *Adansonia digitata* and flower of *Bombax ceaba* (100, 150, 200 and 250 g). Were cut in small pieces and crushed in mixture machine with 50 ml distilled water. After make final volume 1000 ml with distilled water, then after 100 ml this solution add in separate 250 ml Erlenmeyer flasks, After add KH_2PO_4 (100 mg), $NaNO_3$ (400 mg), $MgSO_4.7H_2O$ (20 mg), and adjust 4.5 initial pH with HCL and 0.1 NaOH. The flask was cotton plugged and autoclaved at 121^0 C for 15 minutes. After cooling at room temperature each medium inoculated with 1.0 ml (6.0×10^6) of *Aspergillus niger* (selected strains i.e. A, B, and C) conidial suspension and incubated at 28^0 C in static incubator for 8 days. After fermentation, the medium was diluted with distilled water (1:4 W/V). The medium was filtered and the filtrate was used for the subsequent analysis. In this study there were select four Carbons source which are flower of *Madhuka longifolia*, flower of *Bauhinia variegate*, pithy pulp of fruit of *Adansonia digitata* and flower of *Bombax ceaba.* And there was found that 200 g flower of *Madhuka longifolia longifolia* is best for citric acid production in all this three strains of *A. niger* which are A, B and C, the *A. niger* B is best for citric acid production.

Key Words: carbon sources, flower of *Madhuka longifolia*, flower of *Bauhinia variegate*, pithy pulp of fruit of *Adansonia digitata* and flower of *Bombax ceaba*, *Aspergillus niger*, citric acid

INTRODUCTION

Citric acid is a necessary constituent of various food preparations, pharmaceuticals, synthetic biodegradable detergents, cosmetics, alkyd resins and many other products. Currently, it is commercially produced by submerged (liquid state) fermentation of starch or sucrose based media (sucrose or glucose syrups) using *Aspergillus niger* (Lofty et al., 2007; Barrington and Kim, 2008). *A. niger* is being used commercially for citric acid production and it remains the organism of choice for commercial production because it produces more citric acid per time unit. Recently, a wide range of citric acid production has been reported in response to different levels of nutrient supplementation (Bari et al., 2009; Immandi et al., 2008). The main advantages of using *A. niger* are its ease of handling, its ability to ferment a variety of cheap raw materials and high yields.

A variety of fungi are reported to produce organic acids such as citric, oxalic, succinic and malic acid. Among them, citric acid production using the filamentous fungus *Aspergillus niger* is well known and widely used by industries producing food, beverages, chemicals and pharmaceutical products (Haq et al., 2001). Presently, citric acid production by *A. niger* is economically produced using submerged fermentation. However, the global demand for citric acid is growing faster than its production, implying that more economical processes are required to supplement or replace the present processes (Alvarez-Vasquez et al., 2000).

The important physico-chemical fermentation parameters influencing the growth of *A. niger* on a solid substrate and its production of citric acid are solid substrate composition, moisture content, particle size distribution, fermentation temperature, pH, fermentation time and inoculums density (Jianlong and Ping, 1998). Citric acid production is also known to be affected by inoculums density and fermentation time (Lee and Yun, 1999). Up to a specific limit, metabolite production generally increases with inoculums density (Kota and Sridhar, 1999).

MATERIAL AND METHODOLOGY

For the proposed research work the investigator has framed work into different parts.

1) **Isolation of organism (*Aspergillus niger*):**
   a) **Isolation of Rhizosphere Soil From 54 places and 23 species of trees of Nandurbar District:**

The natural source of *Aspergillus niger* is soil. For the isolation of high citric acid yielding strains of *Aspergillus niger,* for this propose soil samples from 54

b) Serial dilution method:

In this way, 54 different samples were collected. Each sample was diluted in serial dilution so as to get 1/100, 1/1000 and 1/10000 dilution of each sample. These dilutions (0.1 ml) of each sample were spread separately on sterile Potato-Dextrose agar (PDA) medium containing streptomycin and Penicillin to avoid growth of bacteria.

c) Classification, Identification and Maintenance of the fungal cultures:

The young *Aspergillus niger* colonies were picked up and transferred to potato dextrose agar (PDA) slants. The cultural and morphological characteristics of *A. niger* isolate were observed.

The PDA slants were then inoculated by transferring a small amount of *A. niger* conidia from the petri plates and incubating at 30^0 C (4-6 days) for maximum sporulation. Culture was kept in refrigerator at 4^0C for further study.

d) Screening isolated cultures for citric acid production:

To study the *Aspergillus niger* strain producing organic acid from carbon substrates can be detected by incorporation of pH indicators bromocresol dye in Potato Dextrose agar medium and growing fungal strain on it. A color change of medium from blue to yellow in the vicinity of colony indicates organic acid production.

e) Preparation of conidial inoculation:

Conidial inoculation was used in the present study. Conidia from 4-6 days old slant culture were used for the inoculation. Ten milliliters of sterilized distilled water was added to slant having profuse conidial growth on its surface. An inoculum needle was used to break the conidial clamps. The tubes were shaken vigorously to obtain a homogenous mixture of the conidial suspension. The homogenous mixture of the conidial suspension was added in distilled water and makes a final 100 ml. The soil suspension was further diluted to 10^4 - 10^6 times. One milliliter of this diluted suspension was transferred to submerged fermentation medium for estimation of citric acid.

f) Designing of media for fermentation of citric acid production:

Growth and production of microorganism are strongly affected by the medium composition such as concentrations of carbon, nitrogen, phosphorous, and potassium. Thus,

citric acid productivity by *A. niger* can be improved by optimizing the medium composition. In this respect there was select for Carbon source which are flower of *Madhuka longifolia*, Flower of *Bauhinia variegate*, pithy pulp of fruit of *Adansonia digitata* and flower of *Bombax ceaba.*

g) Fermentation technique:

The selected carbon sources which are flower of *Madhuka longifolia*, flower of *Bauhinia variegate*, pithy pulp of fruit of *Adansonia digitata* and flower of *Bombax ceaba.* Were cut in small spices and crushed in mixture machine with 20 ml distilled water and the mixture containing KH_2PO_4, $NaNO_3$, $MgSO_4.7H_2O$, and adjustment of pH of medium was added into individual 250 ml cotton plugged conical flasks. The flasks were autoclaved at 15.0 lbs/in^2 pressure for 15 minutes. After cooling at room temperature the flasks were inoculated with 1.0 ml of conidial suspension and incubated at 30^0 C in incubator. The ingredients of the flasks were then filtered and filtrate was used for the estimation of citric acid.

h) Estimation of citric acid production:

After fermentation medium was diluted with distilled water (1:4 W/V). The medium was then filtered and filtrate was used for the subsequent analysis.

Citric acid was determined titrimetrically by using 0.1 NaOH and phenolphthalein as indicator and calculated as % according to the formula:

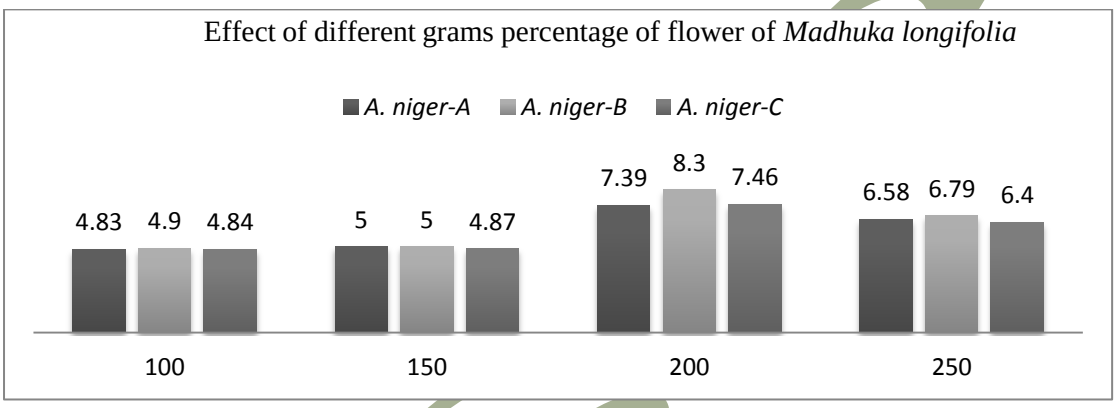
$$\% \text{ citric acid} = \frac{\text{Normality X volume of 0.1 M NaOH X Equivalent weight of citric acid X dilution factor}}{\text{Weight of sample (g) X 10}}$$

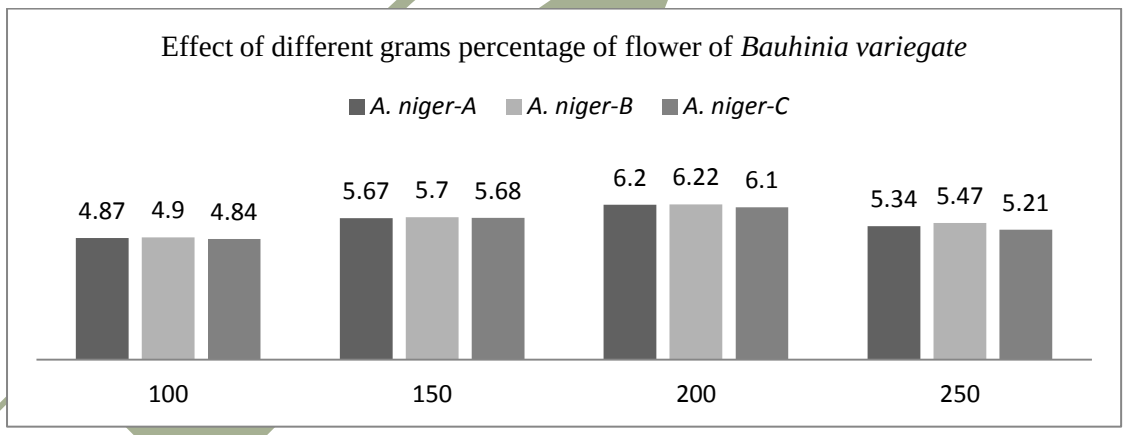
(Kareem, S. O.et al. 2010)

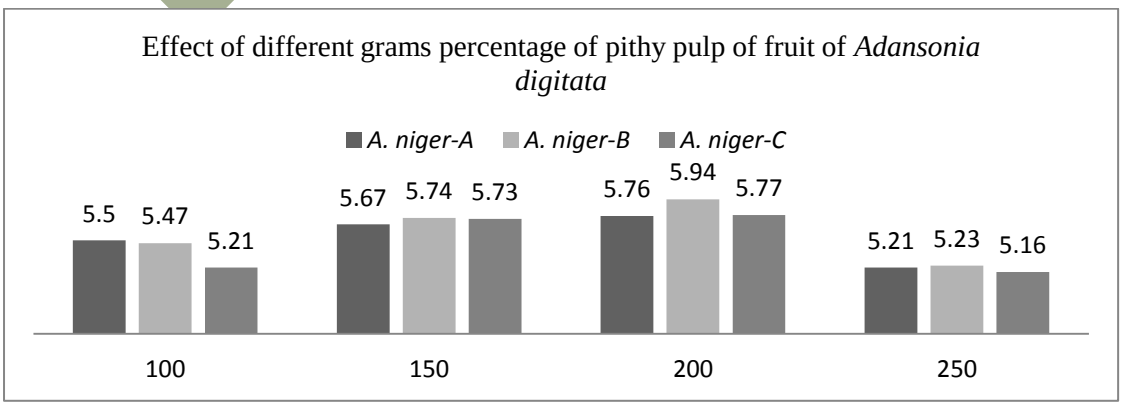
RESULT AND DISCUSSION

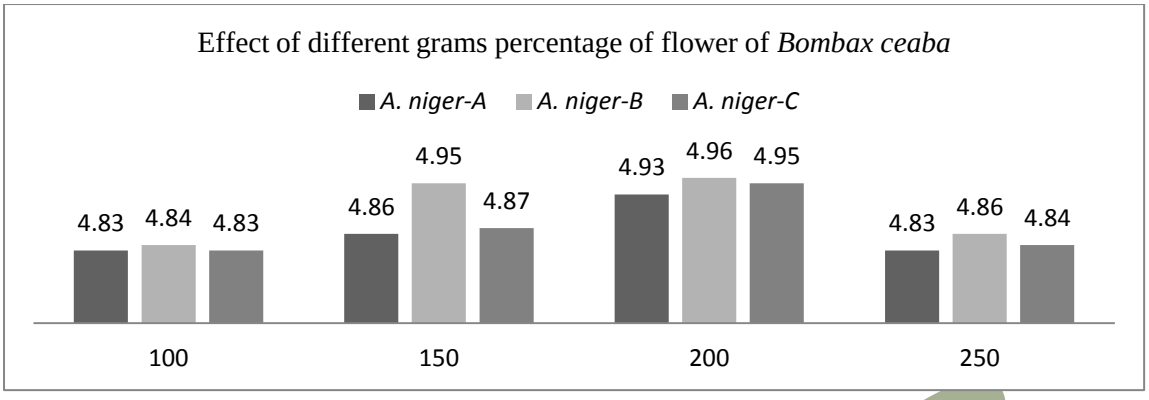
The selected carbon sources which are flower of *Madhuka longifolia*, flower of *Bauhinia variegate*, pithy pulp of fruit of *Adansonia digitata* and flower of *Bombax ceaba* (100, 150, 200 and 250 g). Were cut in small pieces and crushed in mixture machine with 50 ml distilled

water. After make final volume 1000 ml with distilled water, then after 100 ml this solution add in separate 250 ml Erlenmeyer flasks, After add KH_2PO_4 (100 mg), $NaNO_3$ (400 mg), $MgSO_4.7H_2O$ (20 mg), and adjust 4.5 initial pH with HCL and 0.1 NaOH. The flask was cotton plugged and autoclaved at 121^0 C for 15 minutes. After cooling at room temperature each medium inoculated with 1.0 ml (6.0 X 10^6) of *A. niger* (selected strains i.e. A, B, and C) conidial suspension and incubated at 28^0 C in static incubator for 8 days. After fermentation, the medium was diluted with distilled water (1:4 W/V). The medium was filtered and the filtrate was used for the subsequent analysis.









Kareem, S. O. et al. (2010) were investigated that a solid state fermentation was developed for citric acid production from pineapple waste by *Aspergillus niger* KS-7. The medium was supplemented with different concentration of glucose, sucrose, ammonium nitrate and ammonium phosphate. It was found that pineapple waste with 15% (w/v) sucrose and ammonium nitrate (0.25% w/v) gave the optimum citric acid secretion (60.61 g/kg) in the presence of methanol (2% v/v) when fermented for 5 days at 30 °C with the initial moisture content of 65%. The yield was more than 90% based on the amount of fermentable sugar consumed. These results present the use of pineapple peel as a cheap medium for the production of commercially valuable organic acid by *A. niger*.

In this study there were select four Carbons source which are flower of *Madhuka longifolia*, flower of *Bauhinia variegate*, pithy pulp of fruit of *Adansonia digitata* and flower of *Bombax ceaba.* And there was found that 200 g flower of *Madhuka longifolia longifolia* is best for citric acid production in all this three strains of *A. niger* which are A, B and C, the *A. niger* B is best for citric acid production.

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