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SCREENING FOR CITRIC ACID PRODUCING FUNGI FROM THE SOIL ENVIRONMENT OF JOS NORTH, PLATEAU STATE, NIGERIA

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ABSTRACT

The investigation was aimed at screening for citric acid producing fungi from the soil environment of Jos North Local Government Area using standard mycological methods. Serial dilution method was employed using Potato Dextrose Agar as culture medium for fungi isolation. Total Fungal Counts at the different locations were determined and expressed as CFU/ml. Farin Gada had the highest fungi count of 2.9×10^3 while Angwan Rogo had the least fungi count of 7.3×10^2. The frequency of occurrence for each species of fungus was also determined using standard mycological method. Aspergillus niger and Fusarium oxysporum had the highest frequency of occurrence of 80% while Saccharomyces cerevisiae had the lowest frequency of occurrence of 10%. Fungal isolates were identified using standard methods based on their cultural and morphological features. A total of ten (10) fungi species were isolated and identified (*Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Penicillium glaucum, Penicillium rugulosum, Penicillium chrysogenum, Fusarium oxysporum, Rhizopus stolonifer, Rhodotorula rubra* and *Saccharomyces cerevisiae*). Six species were found to produce citric acid at varying concentrations (*Aspergillus niger*, 3.2g/L; *Aspergillus flavus*, 2.3g/L; *Penicillium glaucum,* 2.0g/L; *Penicillium chrysogenum*, 1.9g/L; *Penicillium rugulosum,* 1.7g/L; and *Saccharomyces cerevisiae*, 0.7g/L). This investigation demonstrated that there are citric acid producing fungal strains in the soil environment of Jos North Local Government Area of Plateau State, Nigeria, which can be harnessed locally for industrial production.

Keywords: *Citric acid, Fungi, Soil, Jos North, Nigeria*

INTRODUCTION

Citric acid is one of the most common products which have a never ending demand in the global market. It plays a pivotal role in food and beverage industries and pharmaceutical, chemical cosmetic, and other industries for applications such as acidulation, antioxidant, flavor, enhancement, preservation, and plasticization and as a synergistic agent.

Citric acid [2-hydroxy 1,2,3 propane tricarboxylic acid ($CH_2COOH.COH.COOH.CH_2COOH$)] is ubiquitous in nature and exists as an intermediate in the citric acid cycle when carbohydrates are oxidized to carbon dioxide, and its accumulation is strongly influenced by the balance of nutrients. The type and concentration of the carbon source, especially glucose and sucrose, has a significant effect on citric acid production. In general, the final concentration of citric acid increases as the initial concentration of the carbon source is increased (Papassiopi *et al.*, 1999). In its pure form, citric acid is solid at room temperature, melts at 153ºC and decomposes at higher temperatures into other products (Rajoka *et al*., 1998). Citric acid is a weak organic acid most commonly found in citrus fruits. It is responsible for the tart taste of various fruits in which it occurs like lemons, limes, figs, oranges, pineapples, pears and goose-berries. Citric acid can be recovered from its calcium salt by adding sulphuric acid (Anon, 1975).

Citric acid is non-toxic and easily oxidized in the human body. Due to its high solubility, palatability and low toxicity, it can be used in food, biochemical and pharmaceutical industries. These uses have placed greater demand on increased citric acid production and search for more efficient fermentation process. The worldwide demand of citric acid is about 6.0 x 105 tons per year and it is bound to increase day by day (Ali *et al.*, 2002). Citric acid is a good, natural preservative and is also used to add an acidic (sour) taste to foods and soft drinks and other food products. Utilization of citric acid includes flavor enhancement, bacterial inhibition, pH adjustment and as an anti oxidant (Majunder *et al.*, 2010). Citric acid has many uses especially in the areas of food, chemical and pharmaceutical industries as 70% of the citric acid produced is used in various food industries, 12% in the chemical, pharmaceutical and medical sectors, while 18% in other industries (Soccol *et al*., 2003). Citric acid has also been found to have inhibitory effect on the growth of some fungi such as *Trichophyton mentagrophytes, Aspergillus fumigatus, Candida albicans* and *Malassezia furfur* (Shokri, 2011).

In spite of the possibility of producing citric acid from plant and animal sources, and by chemical methods, commercial production has been mainly by microbial fermentation which has the advantage of the possibility of increasing production by improving the environmental conditions and genetic control for the producing microorganism (Demain and Dana, 2007). A large number of microorganisms including fungi and bacteria such as Arthrobacter paraffinens, Bacillus licheniformis and *Corynebacterium* spp., *Aspergillus niger, A. aculeatus, A. carbonarius, A. awamori, A. foetidus, A. fonsecaeus, A. phoenicis and Penicillium janthinellum;* and yeasts such as *Candida tropicalis, C. oleophila, C. guilliermondii, C. citroformans, Hansenula anamola* and*Yarrowia lipolytica* have been employed for citric acid production (Grewal and Kalra, 1995; Pandey *et al.*, 2001; Soccol *et al.*, 2006). Most of them, however, are not able to produce commercially acceptable yields due to the fact that citric acid is a metabolite of energy metabolism and its accumulation increases in appreciable amounts only under conditions of drastic imbalances. Arzumanov *et al.*, (2000) reported that various fungi have been found to accumulate citric acid in their culture media, and these include strains of *Aspergillus niger, A. awamori, Penicillium restrictum, Trichoderma viride*, *Mucor piriformis* and *Yarrowia lipolytica*. At present, A. niger remained the organism of choice for citric acid production due to ease of handling, its ability to ferment a variety of cheap raw materials and high yields (Patil and Patil, 2014; Alsudani and Al-Shibli, 2015.)

Although a list of screening work for citric acid producing microorganisms have been reported in many developed countries, there is a dart of in many developing countries including Nigeria. However, in order to meet the rising demand for citric acid in its many applications, there is need for continual search for more efficient fungal strains from our environments especially the soil environment being the largest reservoir of microorganisms. This study thus aimed at isolating fungi from the soil environment of Jos North Local Government Area of Plateau State, Nigeria, and screening them for their ability to produce citric acid which could eventually be harnessed for local production of this industrially important organic acid.

MATERIALS AND METHODS

This research work was carried out in Jos North Local Government Area of Plateau State, Nigeria. Its headquarter is in the city center of Jos. Jos is in the middle belt of Nigeria, and is about 275km from Kaduna, 124 km from Bauchi and 164 km from Lafia(the Nassarawa State Capital). The town is situated on latitude $9^0$55" North and Longitude 8054" East. The town is about 1250m (about 4100 ft) above the sea level on the Delimi River with average

monthly temperatures ranging between 21°C and 25°C (Wikipedia, 2013).

Soil samples were collected from ten (10) different locations in Jos North Local Government Area of Plateau State. These locations were Angwan Rukuba, Nassarawa Gwom, Eto Baba, DogonDutse, Federal College of Forestry, Angwan Rogo, University of Jos main campus, Farin Gada, Ruso village and Naraguta village.

Using a sterilized spatula, 250g of soil sample was collected from each location into 400g-capacity Plastic containers which was previously washed and rinsed with 70% alcohol. The samples from the ten different locations were conveyed to Laboratory for analyses.

The soil samples from each sites was sieved and evaluated by the unified analysis technique so as to determine the soil type (Whitbread *et al.*, 1996, Pettijohn, 2006).

The pH of the soil samples were determined using digital pH meter according to standard methods of Association of Official Analytical Chemists (AOAC, 1990). 10g of each soil sample was measured into a 50ml distilled water in a 100ml conical flask and gently shaken to achieve a thorough mixture. The digital pH meter was later inserted into the solution and allowed for some minutes when it stops reading, the displayed value on the pH meter was recorded.

The soil temperature was determined by the use of a soil thermometer. At each site, the thermometer was inserted into the soil up to the depth of 5cm and then allowed to stay for 10 minutes, after which the temperature reading was obtained. The temperature values of three consecutive readings were recorded and the mean was determined for each site (Dix and Webster, 1995).

Potato Dextrose Agar was prepared according to the manufacture's specification. That is, 39 g of powder were dispensed in Erlenmeyer flask containing 1L (1000ml) of distilled water. The flask was covered with cotton plug and mixed thoroughly. The media was sterilized using autoclave at 121°C for 15 minutes. After cooling to a temperature of about 40^0C,30mg each of Penicillin and Streptomycin powder were measured and poured into the medium to inhibit bacterial growth. The medium was gently shaken to achieve a thorough mixture before pouring into the sterilized petri dishes. About 10ml of the sterilized media was distributed to each sterilized petri dishes (glass/disposable). The plates were left undisturbed until the agar solidified.

Preparation of samples were done by weighing ten (10) grams of the soil samples from different sources and suspended in 100ml of sterilized distilled water. Suitable serial dilutions of soil samples from each sites were carried out. One (1) millimeter of each diluents at 10^{-2} was inoculated on the solidified agar plates and incubated in 3 replicate at 25°C for two 2 days to determine the colony forming unit (CFU/g), after which the incubation continued for another 5 days and daily observations were made to determine the presence of filamentous fungi (Nester *et al*., 1998; Wainright *et al.,* 1993).

Pure cultures of isolates were obtained by repeated sub-culture on Potato Dextrose Agar (PDA). The fungal isolates were identified based on the basis of their cultural and morphological features. The cultural characteristics were determined by their appearance on culture plates while the morphological features were determined microscopically with reference to Compendium of Soil Fungi (Domsch *et al*., 1980).

The percentage frequency of occurrence for each species of fungus was determined by the method of Sampo *et al*., (1997) using the formula; A/B x 100; where; A = Number of plates in which species appear and B = Total number of plates incubated for each sites.

The concentration of citric acid in culture was estimated titrimetrically (AOAC, 1995) as reported by Imandi *et al.*, (2007) and Khosravi *et al.,* (2008). The fungal isolates were inoculated into Nutrient Broth and incubated at 30^0C for 7 days (Sikander *et al*., 2001; Magnuson and Linda, 2007).

A 10ml culture broth was added to 50ml of distilled in an Erlenmeyer flask to which 3 drops of phenolphthalein was added using a dropper and thereafter, 0.1M solution of NaOH was slowly titrated into the broth/water solution with continual swirling to keep it thoroughly mixed. When the end-point of the titration was reached the indicator changes from colourless to light pink. The amount (volume) of NaOH used (titre) was read off from the burette and the figure recorded. The burette was filled for subsequent test. The quantity of citric acid produced by each funal species was computed and expressed as gram per litre (g/L) according to the methods of Association of Analytical Chemists (AOAC, 1995).

RESULTS AND DISCUSSION

The results of the physico-chemical properties of soil samples of the different locations in Jos North Local Government Area are presented in Table 1. The soils of Angwan Rukuba, Federal College of Forestry, University of Jos main campus, Farin Gada and Naraguta village are loamy, while those of Naraguta village are loamy, while those of Nassarawa Gwom and Dogon Dutse are sandy, and those of Eto Baba, AngwanRogo and Ruso village are clay. Soil samples from Eto Baba, Ruso village, Farin Gada, Angwan

Rukuba, Dogon Dutse, Nassarawa Gwom, and Angwan Rogo had alkaline pH with values that ranged from 7.1 and 8.2, while soil samples from Naraguta village, University of Jos main campus and Federal College of Forestry had low pH values which ranged from 6.4 (acidic) and 7.0 (neutral).The temperature of the soil at all sites during the time of this investigation (rainy season/September, 2014) ranged between 22^0C and 29^0C, with Nasarawa Gwom having the lowest, while Farin Gada had had the highest.

Table 2 shows the results of the Total Fungal Counts of the soil of the different locations. The fungal counts were expressed as colony forming units per gram (CFU/g). Angwan Rukuba, Federal College of Forestry and Farin Gada had high fungal counts of 2.5 x 10^3, 2.9 x 10^3 and 2.7 x 10^3 respectively, followed by those of the Eto Baba, University of Jos main campus, and Ruso village which had counts of 1.9 x 10^3, 1.8 x 10^3 and 1.7 x 10^3 respectively. Also Nassarawa Gwom, Dogon Dutse and Naraguta village had counts of 1.4 x 10^3, 1.0 x 10^3, and 1.5 x 10^3, respectively. Angwan Rogo had the lowest count of 7.3 x 10^2.

Table 3 shows the results of percentage frequencies of occurrence of fungal isolates from different locations of Jos North Local Government Area. From the results obtained, Aspergillus niger and Fusarium oxysporum had the highest frequency of occurrence of 80%, followed by Aspergillus flavus and Penicillim rugulosum which had 60% and 50% respectively. Aspergillus fumigatus ,Penicillium glaucum and Rhizopus stolonifer had the same 30%, while Penicillium chrysogenum and Rhodotorula rubra had 40% and 20% respectively. Saccharomyces cerevisiae had the lowest frequency of occurrence of 10%.

Table 4 shows the results of fungal isolates citric acid producers from the different locations in Jos North Local Government Area. From the result obtained, *Aspergillus fumigatus, Rhodotorula rubra, Rhizopus stolonifer* and *Fusarium oxysporum* did not produce citric acid, while *Aspergillus niger*,*Penicillium glaucum*, *Penicillium chrysogenum*, *Penicillum rugulosum, Aspergillus flavus* and *Saccharomyces cerevisiae* produced citric acid at varying concentrations. *Aspergillus niger* was (3.2gL^{-1}), *Aspergilus flavus* (2.3gl^{-1}), *Penicillium glaucum* (2.0gL^{-1}),*Penicillium rugulosum*(1.7gL^{-1}), *Penicillium chrysogenum* (1.9gL^{-1}) and *Saccharomyces cerevisiae* (0.7gL^{-1}).

The result of this study demonstrated that the soil types found in Jos North Local Government Area varies at different locations ranging from sandy, clay and loamy, respectively. The soil also has varying temperature and pH at different locations. The temperature of the soil at all the sites during the time of this investigation (rainy season) ranged between 22^0C and 29^0C. The locations with loamy soil type habours more diverse fungi species as compare to location with sandy and clay soil type respectively. It was also observed that fungi can survive a wide range of temperature. The soil samples from different locations in Jos North Local Government Area range from slightly acidic to highly alkaline as shown by the pH values in the result obtained.

This study also demonstrated that the counts of fungi in the local soil of Jos North Local Government Area, is relatively high which other workers had earlier reported similar findings (Hussain *et al.,* 2007; Khan *et al.*, 2007; Makut and Ade-Ibijola, 2012,).

The soil of Jos North Local Government Area has a relatively large diversity of fungi, as reflected in this study. The most abundant species is Aspergillus niger which could be as a result of its ability to rapidly colonize and degrade available organic matter. Also the asexual spores produced are said to be resistant to many environmental stress which enable the organism to survive during inactive period. Fusarium oxysporum are also relatively high in diversity and this could be attributed to vegetation as this organism is commonly associated with plant debris and can survive in the soil or plant debris as active hyphae especially on cultivated soil(Burgess and Summerell, 1992). Other fungal isolates with high abundance were *Aspergillus flavus and Penicillium rugulosum while Aspergillus fumigatus, Penicillium glaucum, Penicillium chrysogenum, Rhizopus stolonifer* and *Rhodotorula rubra* were relatively low with *Saccharomyces cerevisiae* being the least in distribution. This may be attributed to the effect of environmental conditions on species diversification of fungi in the soil (Zachow *et al.,* 2009).

Aspergillus fumigatus, Fusarium oxysporum, Rhizopus stotonifer and *Rhodotorula rubra* did not produce citric acid, while *Saccharomyces cerevisiae,Penicillium rugulosum,Penicillium glaucum,Penicillium chrysogenum, Aspergillus flavus* and *Aspergillus niger* produced citric acid at varying concentrations of (0.7gL^{-1}, 1.7gL^{-1}, 2.0gL^{-1}, 1.9gL^{-1}, 2.1gL^{-1} and 3.2gL^{-1}) respectively. Aspergillus niger found to produced the highest quantity of citric acid (3.2gL^{-1}), followed by Aspergillus flavus (2.1gL^{-1}), *Penicillium, glaucum* (2.0gL^{-1}) *Penicillium chrysogenum* (1.9gL^{-1}), *Penicilliumrugulosum* (1.7gL^{-1}) a*nd Saccharomyces cerevisiae* (0.7gL^{-1}).

These findings have further confirmed the superior of *Aspergillus niger* as the industrial species for the production of citric acid as reported by several workers (Rohr *et al*., 1993; Kirimura *et al*., 2000; Makut and Ade-Ibijola, 2012). The results also agree with that of Torres (1994) where it was reported that some species of fungi other than *Aspergillus niger* also do produce citric acid.

Table 1: Physico-chemical properties of soil samples of the different locations in Jos North Local Government Area

Sites	Soil Type	pH	Temperature (0C)
A	Loamy	7.7±0.39	23±0.54
B	Sandy	8.0±0.14	22±0.59
C	Clay	7.1±0.57	25±0.45
D	Sandy	7.8±0.22	24±0.48
E	Loamy	7.0±0.83	25±0.53
F	Clay	8.2±0.51	26±0.66
G	Loamy	6.9±0.92	24±0.52
H	Loamy	7.7±0.28	29±0.50
I	Clay	7.6±1.14	25±0.47
J	Loamy	6.4±0.40	25±0.51

KEY: A = AngwanRukuba, B = Nassarawa Gwom, C = Eto Baba, D = DogonDutse, E = Federal College of Forestry, F = AngwanRogo, G = University of Jos Main Campus, H = FarinGada, I = Ruso village, J - Naraguta village.

Table 2: Fungal count (CFU/g) of the soil in different locations of Jos North Local Government Area

Sites	Fungal Count (CFU/g)
A	2.5 X 10^3±0.23
B	1.4 X 10^3±0.47
C	1.9 X 10^3±0.36
D	1.0 X 10^3±0.52
E	2.9 X 10^3±0.44
F	7.3 X 10^2±0.39
G	1.8 X 10^3±0.51
H	2.7 X10^3±0.29
I	1.7 X 10^3±0.48
J	1.5 X 10^3±0.55

KEY: A = AngwanRukuba, B = Nassarawa Gwom, C = Eto Baba, D = DogonDutse, E = Federal College of Forestry, F = AngwanRogo, G = University of Jos Main Campus, H = FarinGada, I = Ruso village, J - Naraguta village.

Table 3: Percentage frequencies of occurrence of fungi isolated from the different soil locations of Jos North Local Government Area

Fungal Isolates	A	B	C	D	E	F	G	H	I		Occurence (%)
Aspergillus niger	+	-	+	+	-	+	+	+	+	+	80
Aspergillus flavus	-	+	-	-	+	+	+	-	+	+	60
Aspergillus fumigatus	-	+	-	-	+	-	-	-	-	+	30
Fusarium oxysporum	+	+	-	+	+	+	+	+	+	-	80
Penicillium glaucum	-	-	-	-	-	+	-	-	+	+	30
Penicillium rugulosum	+	-	+	-	-	+	-	+	-	+	50
Penicillium chrysogenum	-	+	+	-	-	+	+	-	-	-	40
Rhizopus stolonifer	-	+	-	-	-	-	-	+	+	-	30
Rhodotorula rubra	-	+	-	-	-	-	+	-	-	-	20
Saccharomyces cerevisiae	-	-	-	-	-	-	+	-	-	-	10

KEY: + = Present, - = Absent, A = AngwanRukuba, B = Nassarawa Gwom, C = Eto Baba, D = DogonDutse, E = Federal College of Forestry, F = AngwanRogo, G = University of Jos Main Campus, H = FarinGada, I = Ruso village, J = Naraguta village.

Table 4: Citric acid production by fungi isolated from different soil locations of Jos North Local Government Area

Fungal Isolates	Citric Acid Production	Amount of Citric Acid produced (g/L)
Aspergillus niger	+	3.2± 0.02
Aspergillus flavus	+	2.3±0.01
Aspergillus fumigates	-	0.0±0.00
Fusarium oxysporum	-	0.0±0.00
Penicillium glaucum	+	2.0±0.01
Penicillium rugulosum	+	1.7±0.01
Penicillium chrysogenum	+	1.9±0.02
Rhizopus stolonifer	-	0.0±0.00
Rhodotorula rubra	-	0.0±0.00
Saccharomyces cerevisiae	+	0.7±0.02

Key: + = Positive , - = Negative

CONCLUSION

The assertion that certain species of fungi other than *Aspergillus niger* do produce citric acid is to a large extent verified by this investigation. The present study has also established that the soil environment Jos North Local Government Area, Plateau State, Nigeria has citric acid producing fungi, *Aspergillus niger, Aspergillus flavus, Penicillium rugulsum, Penicillium chrysogenum, Saccharomyces cerevisiae* and *Penicillium glaucum*, which produce this organic acid at varying quantities. The results thus demonstrated that the soil environment of Jos North Local Government Area, Plateau State, Nigeria has species of fungi that are capable of producing citric acid in appreciable amounts which can be harnessed as industrially useful fungi that could be used in

industrial production of this important organic acid.

However, there is need for further studies especially in area of strains development in order to develop high yielding strains that can produce much higher quantities of the desired product so as to increase the economic viability of such production.

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REFERENCES

Ali, S., Haq, I. U., Qadcer, M. A. and Iqbal, J. (2002). Production of citric acid by *Aspergillus niger* using cane molasses in a stirred fermenter. *Electronic Journal of Biotechnology*,5(3):125-130.

Alsudani, A.A. and Al-Shibli, M.K. (2015). Citric acid production from some local isolates of fungus *Aspergillus niger* by rice husks filtrate medium. *International Journal of Recent Scientific Research,* 6(8): 5625-5633

Anon, P. (1975). Food additives solvent extraction process for citric acid. United States Drug Administered and Fed Registered Drug Report, 40 (4): 9080-9082.

Arzumanov, T.E.; Shishkanova, N.V. and Finogenova, T.V. (2000). Biosynthesis of citric acid by Yarrowia lipolytica repeat-batch culture on ethanol. *Applied Microbiology and Biotechnology,* 53: 525-529.

AOAC (1995).Official methods of analysts. Association of Official Analytical Chemists, 16th Edition. Washington, D. C. Pp. 135-136.

Burgess, L.W. and Summerell, B.A. (1992). Mycogeography of Fusarium: Survey of Fusarium species from subtropical and semiarid grassland soils from Queensland Australia. *Mycological Research*, 96(9):780–784.

Demain, A. and Dana, C.A. (2007). The business of biotechnology. *Industrial Biotechnology Journal,* 3 (3): 269-283.

Dix N.J. and Webster J.W.(1995). Fungal ecology.Chapman and Hall.London, U.K. Pp. 45-48.

Dosmch, K.H.I., Gams, W. and Anderson, T. (1980).Compendium of soil fungi, Volume I and II. Academic Press, London.

Grewal, H. S. and Kalra, K.L (1995).Fungal production of citric acid. Biotechnology Advances,13: 209-234.

Hussain S., Arshad M., Saleem M. and Zahir Z.A. (2007). Screening of soil fungi for in vitro degradation of endosulfan, *World Journal of Microbiology and Biotechnology*, 23, 939-945

Imandi.S, B., Bandaru V. V. R., Somalanka, S. R. and Garapati. H. R. (2007).Optimization of medium constituents for the production of citric acid from byproduct glycerol using doehlert experimental design.*Enzyme and Microbial Technology,*40: 1367-1372.

Khan, K.H., Shaukat, S.S. (1990). Citric acid production with mixed strains of *Aspergillus niger* in submerged culture. *Acta Microbiol. Hung*. 37: 9–13.

Khosravi. D, K. and Zoghi, A. (2008). Comparison of pretreatment strategies of sugarcane bagasse: experimental design for citric Acid production. *Rinresnun-e Technology*.99:6986-6993.

Kirimura, K., Yoda, M., Shimizu, H., Sugano, S., Mizuno, M., Kino, K., Usami, (2000). Contribution of cyanide-insensitive respiratory pathway catalyzed by alternative oxidase, to citric acid production in *Aspergillus niger. Bioscience*, Biotechnology, and Biochemistry,64: 2034-2039

Magnuson J.R. and Linda, L.L. (2007).Organic acid production by filamentous fungi. In: Tkacz J. and Lange, L. (eds) Advances in Fungi Biotechnology for Industry, Agriculture and Medicine. Kluwer Academic Publishers. Rich Land. Pp. 307-340.

Majumder, L., Khalil, I., Munshi, M. K. and Alam, K. (2010). Citric acid production by *Aspergillus niger* using molasses and pumpkin as substrates. *European Journal of Biological Sciences*, 2 (1): 01-08.

Makut, M.D. and Ade-Ibijola, O.B. (2012). Citric acid producing fungi found in the soil environment of Keffi metropolis, Nasarawa State, Nigeria. *International Research Journal of Microbiology*, 3(7): 240-245.

Nester, E.W., Anderson, D.G., Roberts, E.C., Nancy, N.P. and Martha, T.N. (1998). *Microbiology*, A Human Perspective, 1st edition. WCB McGraw-Hill,New York. Pp. 307-312.

Pandey, A., Soccol, C.R., Rodriguez-Leon, J.A., Nigam, P.(2001). Production of Organic Acids by Solid-State Fermentation. In: Solid-State Fermentation in Biotechnology – Fundamentals and Applications. Asiatech Publishers Inc., New Delhi, India. Pp113–126.

Patil, G. and Patil, V.R. (2014). Production and partial characterization of citric acid by local isolates of *Aspergillus niger* using Sorghum. *International Research Journal of Pharmacy,* 5(3): 229-231.

Papassiopi, N., Tambouris, S., Kontopoulos, A. (1999). Removal of heavy metals fomcalcareous contaminated soils by EDTA leaching. Water, Air and Soil Pollution,109:1-15

Pettijohn, F.J. (2006). Sedimentary Rock (3rd edition). CBS Publishers, New Delli. Pp. 34-50.

Rajoka, M.I., Ahmad, M.N., Shahid, R., Latif, F. and Parvez, S. (1998). Citric acid production from sugar-cane molasses by cultures of *Aspergillus niger*. *Biologia*, 44 (1): 241-253.

Sampo, S., Bergero, R., Buffa,G.andLuppi-Mosca, A.M. (1997). Soil fungal communities in young and old Alnus viridiscoenosis. *Mycopathologia,* 89:837- 845.

Shokri, H.(2011). Evaluation of Inhibitory Effects of Citric and Tartaric Acids and Their Combination on the Growth of Trichophyton mentagrophytes, Aspergillus fumigatus, Candida albicans and Malassezia furfur. *World Journal of Zoology*, 6 (1): 12-15.

Soccol, C. R. and Vandenberghe, L.P.S. (2003).Overview of Applied Solid-State Fermentation in Brazil. *Biochemical Engineering Journal* 13:205-218.

Soccol, C. R., Vandenberghe, P. S.,Rodrigucs, C. and Pandey, A. (2006).New Perspectives for Citric Acid Production and Application.*Food Technology and Biotechnology*, 44(2): 141 -14).

Sikander, A., Ikram, H., Qadeer, M.A. and Javed, I. (2001). Production of citric by Aspergillus niger using cane molasses in a stirred fermentor. *Electronic Journal Biotechnology,* 5:113-118.

Torres, N. V., Lopez, J. C., Rivero, M. G. and Rojas, M. G. (1998).Kinetics of growth of *Aspergillus niger* during submerged, agar surface and solid-state fermentations. *Process Biochemistry,* 33,103-107.

Wainright, P.O., Hinkle, G., Sogin, M.L. and Stickel, S.K. (1993). Monophyletic origins of the Metazoa: an evolutionary link with fungi. *Science*, 260:340-342.

Wikipedia (2013). Location of Jos North Local Government Area in Plateau State, Nigeria. Wikipedia Free Encyclopedia. https//en.wikipedia.org/wiki/Jos North. Retrieved: 20th October, 2013.

Whitbread, A.M., Lefory, R.D.B. and Blaire, G.J. (1996). Changes in soil physical properties and soil organic fractions with a cropping on red brown earth soil. *Australian Society of Agronomy*, 15: 345-355.

Zachow, C., Pirker, H., Westendorf, C., Tilcher, R. & Berg, G. (2009). The Caenorhabditis elegans assay: a tool to evaluate the pathogenic potential of bacterial biocontrol agents. *European Journal of Plant Pathology*, 125: 367-376