Trakia Journal of Sciences, Vol. 9, No2, pp 33-39, 2011
Copyright © 2011 Trakia University
Available online at:
http://www.uni-sz.bg

ISSN 1313-7050 (print)
ISSN 1313-3551 (online)

Original Contribution

ANTIBIOTIC-PRODUCING FUNGI PRESENT IN THE SOIL ENVIRONMENT OF KEFFI METROPOLIS, NASARAWA STATE, NIGERIA

M. D. Makut*, O. A. Owolewa

Microbiology Unit, Department of Biological Sciences,Nasarawa State University, Keffi, Nigeria

ABSTRACT

An investigation was carried out to determine the presence of antibiotic-producing fungi in the soil environment of Keffi Metropolis. Soil samples were collected from ten different locations of the Keffi Metropolis for the isolation of fungi. Pour plate method involving serial dilution was used for the isolation of fungi. The media used for the isolation were Malt Extract Agar (MEA), Potato Dextrose Agar (PDA), and Plate Count Agar (PCA). Sensitivity test using *Candida albicans, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* as test organisms was employed to determine the ability of the fungal isolates to produce antimicrobials. Ten species of fungi were isolated from the soil samples and these included *Absidia corymbifera, Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, Cladosporium herbarum, Curvularia lunata, Penicillium* sp., *Rhizopus stolonifer* and *Trichoderma viride.* All the fungal isolates were found to inhibit the growth of at least one of the test pathogens. *T. viride* produced inhibition zone of 18mm against *E. coli, A. niger* produced 12mm inhibition zone against *S. aureus. Absidia. corymbifera* inhibited *P. aeruginosa* (10mm), while *Aspergillus. flavus* and *Penicillium* sp produced inhibition zone of 12mm, respectively, against *C. Albicans.* The results of this investigation demonstrate that strains of antibiotic-producing fungi are present in the soil of Keffi Metropolis, Nasarawa State, Nigeria, and these could be harnessed by the pharmaceutical industries for the production of antibiotics from local souces.

Key words: Antibiotic-producing fungi, soil, Keffi

INTRODUCTION

The term ‘antibiotic’ literally means ‘against life’. An antibiotic was originally defined as a substance, produced by one microorganism (1), or of biological origin (2) which at low concentrations can inhibit the growth of other microorganisms or infectious organisms (3). Antibiotics include a chemically heterogeneous group of small organic molecules of microbial origin that, at low concentrations, are deleterious to the growth or metabolic activities of other microorganisms (4). According to Talaro and Talaro (5), antibiotics substances produced by natural metabolic processes of some microorganisms that can inhibit or destroy other microorganism. Dutta (6) defined antibiotics as the miracle drugs of modern times that act as magic bullets shooting down the infective organisms that have invaded the human body and caused infections.

***Correspondence to:** *M.D.Makut, Microbiology Unit, Department of Biological Sciences,Nasarawa State University, P.M.B. 1028, Keffi, Nigeria, E-mail: makmakwin@yahoo.com*

Originally, the term antibiotics referred only to organic compounds, produced by bacteria and fungi, which are toxic to other microorganisms (3). Antibiotics represent the single contribution of drug therapy for the health care of increasing population of the world, and provide effective control of many microbial pathogens that have been the cause of death of human and animals (7). The advent of synthetic methods has, however, resulted in a modification of this definition and an antibiotic now refers to a substance produced by a microorganism, or to a similar substance (produced wholly or partly by chemical synthesis), which in low concentrations inhibits the growth of other microorganisms (1).

Antibiotic, generally, refers to antibacterial. However, because the term is loosely defined, it is preferable to specify compounds as being antibacterials, antifungals and antivirals (3, 8, 9).

It was not until 1940 with the discovery of penicillin, the first, best-known and most widely used antibiotic (2, 3, 10, 11, 12) in 1928 by an English Bacteriologist, late Sir Alexander Fleming that the first clinical trials of penicillin were tried on humans. This antibiotic was obtained from a blue green mould of the soil called *Penicillium notatum* (6). Penicillin was discovered accidentally in 1928 by Fleming, who showed its efficacy in laboratory cultures against many disease producing bacteria. This discovery marked the beginning of the development of antibacterial compounds produced by living organisms (11).

Another antibiotic, streptomycin was isolated in 1944 by Waksman, a Microbiologist, from a species of soil bacteria, called *Streptomyces griseus*, particularly tubercle bacilli, and has proved to be very valuable against tuberculosis (6). A vigorous search for more antibiotics was on at this time and in 1947, another antibiotic, chloromycetin was discovered by Burkholder (6, 12, 13). It was isolated from *S. venezuelae*. It has a powerful action on a wide range of infectious bacteria both Gram positive and Gram negative (6).

The ability to produce antibiotics has been found mainly in fungi of the group Aspergillales, and in a few other bacteria (2). The streptomycetes are remarkable for the chemical diversity of antibiotics that they produce (5). Altogether about 2,000 antibiotics have been characterized so far; but only 50 are used therapeutically (2).

As more antibiotics were discovered, designed and studied, scientists found that they had different properties. Some of these properties include their source, range of activity and their kinds. These were used to classify them (12).

Perhaps one of the few most important discoveries regarding the beneficial use of fungi for humans was the identification in 1928 by Sir Alexander Fleming, that an isolate of *Penicillium notatum* produced a substance capable of killing Gram positive bacteria (6, 14, 15). This compound was subsequently identified as penicillin and was the first member of the β-lactam class of antibiotics to be discovered. These compounds function by inhibiting peptidoglycan synthesis in bacteria and their use has reduced the importance of the Gram positive as a cause of disease (10, 14, 16).

Subsequent to the identification of penicillin production by *P. notatum*, screening experiments revealed that *P. chrysogenum* was a superior producer of penicillin. A typical fermentation yields three types of Penicillin, namely, Penicillin F, Penicillin G and Penicillin V (1, 2, 5).

Antibiotics produced by fungi, are widely used in current chemotherapy especially the penicillin, cephalosporin and fusidic acid, which have antibacterial and antifungal activity (17). A number of antibiotic drugs have been discovered from soil-inhabiting microorganisms which include fungi (20% of isolated antibiotics), actinomycetes (70%) and eubacteria (10%) (18, 19).

The last three decades are characterized by the novel discoveries of microorganisms capable of producing compounds, as potential source of new antibiotics (20).
This investigation aimed at determinining the diversity of mesophilic fungi in the soil environment of Keffi metropolis and making an assessment of their antibiotic-producing potentials.

MATERIALS AND METHODS

Study Area

This research was carried out in Keffi town, which is in the Northern part of Nigeria. Keffi is about 58km from Abuja, the Federal Capital Territory (FCT) and 128km from Lafia, the Nasarawa State capital. The town is situated on latitude 8˚5 North of the equator and longitude 7˚50 East. The town is about 850 meters above the sea level (21).

Sample Collection

Soil samples of 250g each were collected aseptically from ten different locations in Keffi town. Spatula was used to collect the soil samples into plastic containers of 300g capacity which were previously washed with 70% alcohol. The samples were immediately taken to the Microbiology Laboratory, where they were analyzed. The ten locations where the samples were collected included Angwan Lambu, Angwan Rama, Angwan Tofar, Angwan Woje, Dadin Kowa, High Court, Kofar kokona, Nasarawa State University Main Campus, Pyanku Campus, and Yelwa.

Determination of Soil Temperature

The temperature of the soil at the ten different sites was determined by the use of thermometer. The thermometer was inserted into the soil up to depth of 5cm and allowed to stay for 10minutes, after which the temperature reading was obtained. The average of three consecutive readings was recorded for each site (22).

Determination of Soil pH

The soil pH values were determined by digital pH meter using standard methods of Watson and Brown (23). Using this method, 3g of soil sample was weighed into a beaker containing 3ml of distilled water, which was stirred for five seconds and allowed to stand for 10minutes. The electrode of the pH meter was then inserted into the slurry and swirled carefully. The reading was taken thereof and the average of the consecutive readings was recorded for each site.

Soil Types

The soil types were determined by the Unified Soil Classification System in the Geology and Mining laboratory (Nasarawa State University, Keffi) according the Methods of Whitbread *et al*. (24) and Pettijohn (25).

ISOLATION AND IDENTIFICATION OF FUNGAL ISOLATES

The soil fungi were isolated by both the Direct Soil Inoculation and the Soil Dilution Techniques using the pour plate method. The Media used for the isolation were Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) Plates were incubated for 5 days at 28˚C (26).

Pure cultures of fungal isolates were identified using both macroscopic (cultural) and microscopic (morphological) features with reference to Barnett and Hunter (27) and Domsch *et al*. (28).

TEST ORGANISMS

The test organisms used for this study were *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The organisms were obtained from Federal Medical Centre, Keffi (Nasarawa State, Nigeria), where they were isolated from patients. Isolates of these organisms were brought to the laboratory of the Microbiology Unit, Nasarawa State University. The organisms were sub-cultured and their identities were verified using standard methods of Cowan and Steel (29).

DETERMINATION OF PERCENTAGE OCCURRENCE FREQUENCY OF FUNGAL ISOLATES

The percentage frequency of occurrence for each species of fungus isolated was determined by the methods of Sampo *et al*. (30). This was computed by the formula:

A/B x 100; where A = Number of plates in which the species appeared, and B = Total number of plates incubated for each sites.

DETERMINATION OF ANTIBIOTIC PRODUCTION BY THE FUNGAL ISOLATES

Methods used by Williams and Cross (31) was employed to determine the antibiotic production potential by fungal isolates *in vitro* against common human pathogens. The human pathogens used for this study were *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Each fungal isolate was streaked on Nutrient Agar as a straight line and incubated at 30˚C. After two days of incubation, the human pathogenic test organisms (*Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) were streaked perpendicular to the streaked line of the growing fungus. This was then incubated at 37˚C for 24 hours, after which the Zone of Inhibition of each test organism from the streaked line of the growing fungus was measured.

RESULTS

Table 1 shows the results of the Physico-Chemical Properties of soil of the ten different locations in Keffi Metropolis, while **Table 2** shows the results of the Total Aerobic Plate Count and the Fungal Count of Soil samples of these locations. **Table 3** shows the result of Percentage Frequency of Occurrence of Fungal Isolates from the soil samples. **Table 4** shows the Zone of Inhibition of Test Organisms by Perpendicular Streak method of the fungal Isolates.

Table 1.*Physico-Chemical Properties of soil sample of the different locations of Keffi Metropolis*

Locations	**Soil Types**	**pH**	**Temperature**
Angwan Lambu	Loamy	8.3 ± 1.7	26 ± 0.5
Angwan Rama	Sandy	5.9 ± 0.7	25 ± 0.5
Angwan Woje	Sandy	6.3 ± 0.3	26 ± 0.5
Dadin Kowa	Clay	7.0 ± 0.4	26 ± 0.5
G.R.A	Loamy	6.7 ± 0.1	25 ± 0.5
High Court	Loamy	7.4 ± 0.8	25 ± 0.5
Main Campus	Clay	6.5 ± 0.3	26 ± 0.5
Kofar Kokona	Sandy	6.0 ± 0.6	25 ± 0.5
Pyanku Campus	Sandy	6.5 ± 0.1	26 ± 0.5
Yelwa	Sandy	5.7 ± 0.9	26 ± 0.5

Table 2. *Total Aerobic Plate Count and Total Fungal Count (TFU/ml) of isolates from the different locations of Keffi Metropolis*

Sites	Aerobic Plate Count (CFU/ml)	Total Fungal Count (TFU/ml)
A	$2.7 \times 10^6 \pm 0.0$	$4.7 \times 10^3 \pm 1.9$
B	$4.5 \times 10^6 \pm 1.8$	$2.6 \times 10^3 \pm 0.2$
C	$2.3 \times 10^6 \pm 0.4$	$1.6 \times 10^3 \pm 1.2$
D	$1.1 \times 10^6 \pm 1.6$	$1.4 \times 10^3 \pm 1.4$
E	$2.7 \times 10^6 \pm 0.0$	$2.6 \times 10^3 \pm 0.2$
F	$2.2 \times 10^6 \pm 0.5$	$2.3 \times 10^3 \pm 0.5$
G	$4.0 \times 10^6 \pm 1.3$	$3.0 \times 10^3 \pm 0.2$
H	$1.1 \times 10^6 \pm 1.6$	$2.1 \times 10^3 \pm 0.7$
I	$2.7 \times 10^6 \pm 0.0$	$4.6 \times 10^3 \pm 1.8$
J	$3.7 \times 10^6 \pm 1.0$	$3.0 \times 10^3 \pm 0.2$

KEY
A = Angwan Lambu; B = Angwan Rama; C = Angwan Woje; D = Dadin Kowa; E = G.R.A; F = High Court;
G = Kofar Kokona; H = Main Campus; I = Pyanku Campus, J = Yelwa

Table 3. *Percentage Frequency of Occurrence of Fungal Isolates*

Fungal Isolates	Sites										Occurrence Frequency (%)
	A	B	C	D	E	F	G	H	I	J	
Absidia corymbifera	-	+	-	-	-	-	+	-	-	+	30
Alternaria alternata	+	-	+	-	-	-	-	-	+	-	30
Aspergillus flavus	-	-	+	-	+	+	-	-	-	-	30
Aspergillus fumigatus	-	+	-	-	+	-	-	+	-	+	40
Aspergillus niger	+	-	+	+	-	+	+	-	+	+	60
Cladosporium herbarum	-	+	-	-	+	+	-	-	-	-	30
Curvularia lunata	-	+	-	+	-	-	+	-	+	-	40
Penicillium sp.	+	-	+	+	+	+	-	+	-	-	60
Rhizopus stolonifer	-	-	-	+	+	-	-	+	-	-	30
Trichoderma viride	+	-	-	+	-	-	+	+	-	-	40

KEY
- = Absent
+ = Present
A = Angwan Lambu; B = Angwan Rama; C = Angwan Woje; D = Dadin Kowa; E = G.R.A;
F = High Court; G = Kofar Kokona; H = Main Campus; I = Pyanku Campus; J = Yelwa

Table 4. *Zone of Inhibition (mm) of Test Organisms by Perpendicular Streak Method of Fungal Isolates*

Fungal Isolates	Zone of Inhibition (mm) of Test Organisms			
	C. albicans	*E. coli*	*P. aeruginosa*	*Staph. aureus*
Absidia corymbifera	10 ± 0.6	15 ± 3.9	10 ± 2.7	11 ± 2.6
Alternaria alternata	6 ± 3.4	10 ± 1.1	6 ± 1.3	0 ± 0.0
Aspergillus flavus	12 ± 2.6	8 ± 3.1	0 ± 0.0	12 ± 3.6
Aspergillus fumigatus	11 ± 1.6	0 ± 0.0	13 ± 5.7	14 ±5.6
Aspergillus niger	10 ± 0.6	15 ± 3.9	7 ± 0.3	16 ± 7.6
Cladosporium herbarum	8 ± 1.4	14 ± 2.9	0 ± 0.0	11 ± 2.6
Curvularia lunata	9 ± 0.4	18 ± 6.9	0 ± 0.0	6 ± 2.4
Penicillium sp.	12 ± 2.6	13 ± 1.9	6 ± 1.3	8 ± 0.4
Rhizopus stolonifer	9 ± 0.4	0 ± 0.0	7 ± 0.3	6 ± 2.4
Trichoderma viride	7 ± 2.4	18 ± 6.9	9 ± 1.7	0 ± 0.0

DISCUSSION

The results of the Physico-chemical properties of soil samples show that soil environments of Angwan Rama, Angwan Woje, Kofar Kokona, Pyanku Campus and Yelwa are sandy, while the soil sample from Dadin Kowa and University Main Campus are clay, and the remaining three sites (i.e. Angwan Lambu, High Court and G.R.A) are loamy soils. The pH values of the soil samples show that Yelwa was the most acidic with pH of 5.7 and Angwan Lambu was the most alkaline with pH of 8.3. Dadin Kowa and High Court were slightly alkaline (7.0 and 7.4 respectively), while other locations had pH values that ranged from 5.9 to 6.5. The temperature of the soil environments of Keffi at the time of this investigation (rainy season) revealed that the soil environment of Keffi had temperature range between 25^0C and 26^0C.

Table 2 shows the results of the Total Aerobic Plate Count and Total Fungal Count. The highest Total Plate Aerobic Counts were observed in Angwan Rama, Kofar Kokona and Yelwa with counts of 4.5 X 10^6, 4.0 X 10^3 and 3.7 X 10^6 cfu/ml, respectively. Dadin Kowa and University Main Campus had the counts of 1.1 X 10^6 cfu/ml which was the least count, while Angwan Lambu, G.R.A and Pyanku had the same counts of 2.7 X 10^6 cfu/ml, respectively. Angwan Woje and High Court had respective counts of 2.3 X 10^6 and 2.2 X 10^6 cfu/ml. Also, Table 2 shows that Angwan Lambu and Pyanku had the highest Fungal Counts of 4.7 X 10^3 and 4.6 X 10^3, respectively). Kofar Kokona and Yelwa both had the same count of 3.0 X 10^3. In the same vein, Angwan Rama and G.R.A had the similar counts of 2.6 X 10^3, while high Court, University Main Campus, Angwan Woje and Dadin Kowa had the least counts of 2.3 X 10^3, 2.1 X 10^3, 1.6 X 10^3 and 1.4 X 10^3, respectively.

Table 3 shows that *Aspergillus niger* and *Penicillium* sp had the highest percentage frequency of occurrence of 60% each, while *Aspergillus fumigatus*, *Curvularia lunata* and *Trichoderma viride* respectively had 40%. Other fungal isolates, namely *Absidia corymbifera*, *Alternaria alternata*, *Aspergillus flavus*, *Cladosporium herbarum* and *Rhizopus stolonifer*, all had 30% as their percentage occurrence frequency.

The results of the Zone of Inhibition in **Table 4** shows that all the fungal isolates have antimicrobial activity against at least three of the test pathogens, which indicates that these fungi produce some form antimicrobial substance(s) was responsible for inhibiting the test organisms. *Absidia corymbifera*, *Aspergillus niger* and *Penicillium* sp. Were found to inhibit all the four test organisms, while the remaining isolates inhibited at least three of the pathogens tested. *Absidia corymbifera* was found to inhibit *C. albicans*, *E. coli*, *P. aeruginosa* and *S. aureus* with inhibition zones of 10mm, 15mm, 10mm and 11mm, respectively. *Alternaria alternata* inhibited all test organisms except *S. aureus* with inhibition zones of 10mm against *E. coli* and 6mm against *C. albicans* and *P. aeruginosa*. The three species of *Aspergillus*

isolated showed variable extents of inhibition. *A. flavus* was found to inhibit C. *albicans*, *E. coli* and *S. aureus* by producing inhibition zones of 12mm, 8mm and 12mm, respectively, but had no effect on *P. aeruginosa*. *A. fumigatus* inhibited *C. albicans*, *P. aeruginosa* and *S. aureus* by producing inhibition zones of 11mm, 13mm and 14mm, respectively. Similarly, inhibition zones of 10mm, 15mm, 7mm and 16mm were respectively produced against C. *albicans*, *E. coli*, *P. aeruginosa* and *S. aureus* by *A. niger*. *Cladosporium herbarum* was also found to inhibit C. *albicans* (8mm), *E. coli* (14mm) and *S. aureus* (11mm). C. *albicans*, *E. coli*, *P. aeruginosa* and *S. aureus* were inhibited by *Curvularia lunata* by producing inhibition zones of 9mm, 18mm and 6mm, respectively. Inhibitory activity of *Penicillium* sp. was observed against C. *albicans* (12mm), *E. coli* (13mm), *P. aeruginosa* (6mm) and *S. aureus* (8mm). *Rhizopus stolonifer* inhibited C. *albicans* (9mm), *P. aeruginosa* (7mm) and *S. aureus* (6mm) while *Trichoderma viride* showed inhibition zones of 7mm, 18mm and 9mm respectively against C. *albicans*, *E. coli*, *P. aeruginosa*.

CONCLUSION

All the fungal isolates inhibited *Candida albicans* while all, except *Aspergillus fumigatus* and *Rhizopus stolonifer* inhibited *Escherichia coli*. Of all the isolates, only three, *Aspergillus flavus, Cladosporium herbarum and Curvularia lunata*, did not inhibit *Pseudomonas aeruginosa*. *Staphylococcus aureus* was also not inhibited by *Alternaria alternata* and *Trichoderma viride*. This investigation reveals that all fungal species isolated from the soil environment do produce some form of antimicrobials.

Although, this investigation is a primary study, further investigations needs to be embarked upon to determine the type of antimicrobial substance(s) produced or the type of effect they cause on the pathogens, whether static or cidal.

ACKNOLEDGEMENTS

The authors are grateful to the Microbiology Unit, Department of Biological Sciences, Nasarawa State University, Keffi, Nigeria, for providing the laboratory items used in this investigation.

REFERENCES

1. Denyer, S.P., Hodges, N.A. and German, S.P., Hugo and Russell's Pharmaceutical Microbiology, 7th edn. Blackwell Science, India, 2004.
2. Schlegel, H.G., General Microbiology, 7th ed. Cambridge University Press, Cambridge, 2003.
3. Hugo, W.B. and Russell, A.D., Pharmaceutical Microbiology, 5th edn. Blackwell Science, U K , 1998.
4. Thomashow, L.S. and Weller, D.M., Current Concepts in the Use of Introduced Bacteria for Biological Disease Control: Mechanisms and Antifungal Metabolites. In Stacey G and Keen N (eds), Plant-Microbe Interactions. Chapman and Hall, New York. pp187-235, 1995.
5. Talaro, K. and Talaro, A., Foundations in Microbiology, 4th edn. McGraw Hill, New York, 2002.
6. Dutta, A.C., Botany for Degree Students 18th edn. Oxford University Press, New York, 2005.
7. Robbert, J.E., Speedie, M.K. and Tyler, V.E., Antibiotics. In Balado D (ed), Pharmacognosy and Pharmacobiotechnogy. Williams and Wilkins, England, 1996.
8. Brooks, G.F., Butel, J.S., Morse, S.A., Jawetz, Melnick and Adelberg's Medical Microbiology, 23rd edn. McGraw Hill Companies, Singapore, 2004.
9. Cheesbrough, M., District Laboratory Practice in Tropical Countries, 5th edn. Cambridge University Press, Cambridge, 2006.
10. Berg, J.M., Tymoczko, J.L. and Stryer, L., *Biochemistry*, 5th edn. W.H. Freeman and Company, New York, 2002.
11. Taylor, D.J., Green, D.P.O. and Stout, G.W., Biological Science, 3rd edn. Cambridge University Press, Cambridge. 2003.
12. Sommer, C.V., Antibiotics. In Shapp MG, Gerald FC, Feder B, and Martin LA (eds), The New Book of Knowledge, pp 306-312, 2006
13. Cupp, M.J., Antibiotics. In Anderson MA, Dede C, Fontana L, Pandkkar KN, Taylor W and Waugh SL (eds), The World Book Encyclopedia Vol. 1, pp 550-552, 2004.
14. Walsh, G., Biopharmaceuticals, 2nd edn. John Wiley and Sons, England, 2003
15. Krishna, V.S., Biotechnology II, 1st edn. New Age International Publishers, New Delhi, 2006.

16. Nester, E.W., Anderson, D.G., Roberts, C.E., Pearsall, N.N. and Nester, M.T., Microbiology: A Human Perspective, 4th Edn. McGraw Hill, New York, 2004.
17. Dobashi, K., Matsuda, N., Hamada, M., Naganawa, H., Takita, T. and Takeuchi, T., Novel antifungal antibiotics octacosamicins A and B: Taxonomy, fermentation and isolation, physico-chemical properties and biological activities. *J. Antibiotics.* 41: 1525-1532, 1998
18. Bredy, J., Recent developments of antibiotic research and classification of antibiotics according to chemical structures. *Advanced Applied Microbiol.* 18: 309-406, 1974
19. Lechevalier, H., Production of some antibiotics by members of different genera of microorganisms. *Advanced Applied Microbiol.* 19: 25-45, 1975
20. Antibi, E. and Fishlock, D., Biotechnology, Strategies of Life. MIT Press, Cambridge, 1986.
21. Akwa, V.L.,Binbol, N.L., Samaila, K.L. and Marcus, N.D., Geographical Perspective of Nasarawa State. Onaivi Publishing Company, Keffi, Nigeria, 2007.
22. Dix, N.J. and Webster, J.W., Fungal Ecology. Chapman and Hall, London, 1995.
23. Watson, M.E. and Brown, J.R., pH and Lime requirements. *Soil Sci. Soc. Am. J.,* 221: 13-16, 1998.
24. Whitbread, A.M., Lefroy, R.D.B. and Blair, G.J., Changes in physical properties and soil organic fractions with a cropping on a red brown earth soil. *Australian J. Agro.* 12*:* 23-29, 1996.
25. Pettijohn, F.J., Sedimentary *Rock* (3rd Edition). CBS Publishers, New Delhi, 2000.
26. Aneja, K.R., Experiments in Microbiology, Plant Pathology and Tissue Culture. Wiley Eastern Limited, New Delhi, 1993.
27. Barnett, H.L. and Hunter, B.B., Illustrated Genera of Imperfect Fungi (4th edn). APS Press, Minnesota, 1998.
28. Domsch, K.H., Gams, W. and Anderson, T., Compendium of Soil Fungi. Academic Press, London, 1980.
29. Cowan, M, and Steel, L., Preparation of Test Organisms. *Nig. J. Microbiol*. 22: 56-60, 1965.
30. Sampo, S., Begero, R., Buffa, G. and Lumpimosa, A.M., Soil Fungi. Academic Press, London, 1997.
31. Williams, S.T. and Cross, T. Actinomycetes. In: J.R. Norris, D. W. Robbins, (Eds), *Methods in Microbiology*. Academic Press, New York, pp295-334, 1971.