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Phenol Utilization by *Aspergillus flavus* Isolated from River and Wastewater

**Adetitun, D. O. and Akanbi, A. T.*
Department of Microbiology, Faculty of Life Sciences, University of Ilorin, Nigeria

Abstract
Six fungal species were isolated from Oyun River and wastewater from Lagos Hostel within the University of Ilorin. Streak plate method was used for the isolation of the fungi on Potato Dextrose Agar incubated at 25 ^{o}C for 72hours. The fungi were identified as Chrysonilia sitophilia, Penicillium chrysogenum, Candida albicans, Aspergillus niger, Rhodotorula sp., and Aspergillus flavus. Biodegradation of phenol was done using Aspergillus flavus at the following concentrations, 0.2mM, 0.4mM, 0.6mM, 0.8mM, and 1.0mM over fifteen days' incubation period with sampling every three days under aerobic conditions. The depletion of phenol was measured by spectrophotometer. Aspergillus flavus showed the highest disappearance value of 0.048 at concentration of 1mM, followed by 0.023 at concentration of 0.178 mol^{-1}, followed by 0.022 at concentration of 0.6mM, followed by 0.02 at concentration of 0.8mM, and lastly 0.018 at concentration of 0.2mM. The highest degradative value occurred at 1mM (0.048) and the lowest at 0.2mM (0.018). This shows that Aspergillus flavus is a potential phenol degrader and can be used in biotechnology for control of ecological oil spillage/pollution.

Keywords: Phenol, Wastewater, Utilization, *Aspergillus flavus*

Introduction
A greater quantity of water is found in the earth's interior (1). Rivers have been used as a sink for wastes from agriculture and industry due to its flow and ecological nature; rivers are able to regenerate themselves to admit staggering amount of tributaries. However, all rivers have limited absorptive capacity for sewage and fertilizer from cropland or farmland (2). It is estimated that 80% of ill-health in less developed countries stems from lack of safe water and adequate sanitation. Official government statistics suggest that only 55% of Africans had reasonable access to safe water supply and this is likely to be an overestimation. Even fewer Africans had access to adequate sanitation facilities, with rural coverage rates estimated at 20-35% (3, 4). Not only is freshwater essential to life but it is also a relatively scarce resource, and is likely to become more so with the impacts of global warming and population growth (5).
A characteristic of freshwater is that it has low concentrations of dissolved salts and other total dissolved solids. Sea water and brackish water are not freshwaters (6). Freshwater creates a hypotonic environment for aquatic organisms. This is problematic for some organisms with previous skins or with gill membranes, whose cell membranes may burst if excess water is not excreted. Freshwater is host to numerous microorganisms that affect human health directly. Polluted drinking water is a major source of illness and death throughout the world (7, 8). Industrial effluents are a source of drinking water contamination (9). Domestic, industrial and agricultural users produce large quantities of waste products and waterways provide a cheap and effective way of disposing of many of these. During dry weather, the flow of some rivers consists almost entirely of treated effluents. It is essential that the effluent discharged into a watercourse is of high quality and the degree of pollution is such that the self-purifying capacity of the river is not overloaded (5). Water is considered to be polluted when it is altered in composition or condition so that it becomes less suitable for any or all of the functions and purposes for which it would be suitable in its natural state (10).
Wastewater is any water that has been adversely affected in quality by anthropogenic influence (11). Sewage fungi are an aquatic periphyton organism associated with polluted water. These fungi form a slimy growth that are found in sewage and sewage-polluted waters. Some sewage fungi are *Fusarium aquaeductuum, Geotrichum candidum, Leptomitus lacteus Carchesium polypinum, Flexibacter spp, Achyla spp. Flavobacterium spp. Didymosphenia spp. Leptothrix ochracea.* (12).
Phenol is a monosubstituted aromatic compound and is recognized as major environmental pollutant. It is produced through natural and anthropogenic processes. It is found in nature as a constituent of coal tar. The major part of phenol present in the environment is due to industrial use (13). The heavy production and use of phenol in industrial activities make it a major eco contaminant in most wastewater facilities, such as oil refineries, pharmaceuticals and plastic industries (14, 15).
It has been reported in literature that a few microorganisms can utilize phenol as the sole source of carbon and energy (16, 17). Numerous bacterial species have been isolated and characterized as phenol-degrading microbes

**Corresponding Author's E-mail: adetitun.do@unilorin.edu.ng*

(18). Therefore, identifying new and or indigenous phenol-degrading fungi is key in the bioremediation of the phenol-polluted localities. The aim of this study was to determine the type and fungal load in a river and wastewater and to determine the phenol utilization of the predominant fungus, *Aspergillus flavus* in the presence of nickel nitrate.

Materials and Methods

Collection of water sample

Wastewater sample was collected from Oyun River (Plate 1) within the University campus and the Hostel area of the University of Ilorin into a labelled sterile sample bottle. The sample was collected between 7.00 am and 11.00 am. Grab samples were taken on a different day. The samples were collected according to standard methods (19). Water samples from the river were obtained from 1 cm below the surface. The samples were immediately transported to the laboratory for further analysis.

Plate 1: Aspects of Lower Oyun River where Sample was taken from

Mycological Analysis of Wastewater Sample

Serial dilution was done up to 10-4 using sterile pipettes. Exactly 10-2 and 10-4 dilutions of each sample were poured on prepared Potato Dextrose agar (PDA) and the inoculum was spread on the surface of the agar in each plate using a sterile spreader. The PDA plates were incubated at 25oC for 72hours. Pure cultures were obtained by subculturing different and distinct colonies onto sterile potato dextrose agar plates containing 1% streptomycin. The pure cultures obtained were transferred onto agar slants in McCartney bottles and incubated at 250C for 3 days. The bottles were then stored in the refrigerator until needed. The isolates were characterized macroscopically and microscopically. The macroscopic aspect of characterization of the isolates was done with the use of the naked eye noting the color of mycelia, spores, sizes and shape both at the front and reverse sides of the plates. The colonies were also observed microscopically in which their spores and vegetative structures were specifically noted (20, 21).

Cultivation with phenol as sole carbon and energy sources

Mineral salts medium lacking carbon and energy source was prepared to test phenol degradation capacity. The medium contained KH2PO4 (0.125g/L), Na2HPO4 (0.350g/L), MgSO4.7H2O (0.050g/L), KNO3 (0.075g/L), (NH4)2SO4 (0.250g/L) (26). The weighed salts were mixed with distilled water in a beaker and stirred using magnetic stirrer for proper mixing. Exactly 30ml from the well homogenized medium was dispensed into 50ml culture tubes. These were then sterilized in an autoclave for 15minutes at 121oc. The phenol concentrations applied to the medium after sterilization are 0.2mM, 0.4mM, 0.6mM, 0.8mM and 1.0mM. Phenol acted as sole carbon and energy source. Exactly 0.5ml of *Aspergillus flavus* inoculum were taken from suspension and introduced into each reaction tube. *Aspergillus flavus* was used in this experiment because it was the most occurring organism. Each reaction set up was done in duplicates. Control reactions which consisted of mineral salts medium and phenol but lacking Aspergillus flavus was also set up at different concentrations. The absorbance was read at 3 days' interval starting from day 0 at wavelength 400nm using the spectrophotometer. Distilled water was used as blank (22).

Results and Discussion

Six fungal species were isolated and identified from the river and wastewater, they are *Chrysonilia sitophilia, Penicillium chrysogenum, Candida albicans, Aspergillus niger, Rhodotorula sp.* and *Aspergillus flavus*. In table

1 is shown the daily distribution of fungi isolated from Lagos Hostel (one of the female hostels) effluent. The most occurring fungus was *Candida albicans* which was present from day 1 to day 5 and the least is *Chrysonilia sitophilia*. The table also portrayed the daily distribution of fungi isolated from the Lagos Hostel Compound C. The most frequent fungus was *Penicillium chrysogenum* and the least frequent was *Aspergillus flavus*. The table further depicts the daily distribution of fungi isolated from Oyun river upper area. The most occurrence fungus was *Penicillium chrysogenum* and the least occurring fungus was *Aspergillus flavus*. Table 1 also shows the daily distribution of fungi isolated from Oyun river lower area. *Aspergillus flavus* is the most occurring while the least occurring was *Chrysonilia sitophilia*. Table 2 portrays the total count of the fungal isolates. None of the identified fungus fall under the category described as sewage fungi.

Penicillium chrysogenum is common in temperate and subtropical regions and can be found on salted food products (23). It has rarely been reported as a cause of human disease. (24). The airborne asexual spores of *Penicillium chrysogenum* have been reported as important human allergens (25). *P. chrysogenum* has been used industrially to produce penicillin and xanthocillin X, to treat pulp mill waste and to produce the enzymes polyamine oxidase, phosphogluconate dehydrogenase, and glucose oxidase (26).

Candida albicans is a type of yeast that is commonly used as a model organism for biology. It is generally referred to as a dimorphic fungus since it grows both as yeast and filamentous cells. It has several different morphological phenotypes. It is a common member of human gut flora and does not seem to proliferate outside mammalian hosts (27). Its presence in water can be due to passage of human excreta. It is found in the gastrointestinal tract and mouth of healthy adults (28, 29). It is usually a commensal organism, but can become pathogenic in immunocompromised individuals under a variety of conditions (29, 30). *Aspergillus niger* causes a disease called black mould on certain fruits and vegetables. It is ubiquitous in soil and is commonly reported from indoor environments (31). Rhodotorula is a common environmental inhabitant. It can be cultured from soil, water, milk, fruit juice, and air samples.

Aspergillus flavus is saprotrophic, pathogenic and it is best known for its colonization of cereal grains, legumes, and tree nuts (32). Postharvest rot typically develops during harvest, storage, and/or transit. Aspergillus flavus infections can occur while hosts are still in the field (pre-harvest), but often show no symptoms (dormancy) until postharvest storage and/or transport. In addition to causing pre-harvest and postharvest infections, many strains produce significant quantities of toxic compounds known as mycotoxins, which, when consumed, are toxic to mammals (33). *A. flavus* is also an opportunistic human and animal pathogen, causing aspergillosis in immunocompromised individuals (34). A. *flavus* is found globally as a saprophyte in soils and causes disease on many important agriculture crops. Common hosts of the pathogen are cereal grains, legumes, and tree nuts. Specifically, *Aspergillus flavus* infection causes ear rot in corn and yellow mold in peanuts either before or after harvest infection can be present in the field, pre-harvest, postharvest, during storage, and during transit. It is common for the pathogen to originate while host crops are still in the field; however, symptoms and signs of the pathogen are often unseen. *A. flavus* has the potential to infect seedlings by sporulation on injured seeds. In grains, the pathogen can invade seed embryos and cause infection, which decreases germination and can lead to infected seeds planted in the field. The pathogen can also discolor embryos, damage seedlings, and kill seedlings, which reduce grade and price of the grains. The incidence of *Aspergillus flavus* infection increases in the presence of insects and any type of stress on the host in the field as a result of damage. Stresses include stalk rot, drought, severe leaf damage, and/or less than ideal storage conditions (33). In general, excessive water conditions and high temperatures of storage of cereals and cowpea makes the occurrence of *Aspergillus flavus* aflatoxin production to rise (34). Adetitun and Abioye (35) reported that *Aspergillus niger* isolated from air had the capability to utilize phenol. This is in agreement with the findings of this work.

Table 1: Daily Distribution of Fungi Isolated from the sampling Sites

Isolates		Time (Day)					Percentage Occurrence (%)
		1	2	3	4	5	
Chrysonilia sitophilia	Lagos Hostel	+	-	-	-	+	40
	Compound C	+	-	-	-	-	20
	Oyun Upper	+	-	-	-	-	20
	Oyun Lower	+	-	-	-	-	20
Penicillium chrysogenum	Lagos Hostel	+	-	-	-	+	40
	Compound C	+	+	+	+	+	100
	Oyun Upper	+	+	+	+	+	100
	Oyun Lower	-	-	+	-	+	
Candida albicans	Lagos Hostel	+	+	+	+	+	100
	Compound C	-	-	+	+	+	60
	Oyun Upper	-	-	+	+	+	60
	Oyun Lower	-	-	-	+	+	40
Aspergillus niger	Lagos Hostel	+	-	-	+	+	60
	Compound C	-	-	-	-	-	0
	Oyun Upper	-	-	-	-	-	0
	Oyun Lower	-	-	+	-	+	40
Rhodotorula sp.	Lagos Hostel	+	+	+	-	+	80
	Compound C	-	-	-	-	-	0
	Oyun Upper	-	-	-	-	-	0
	Oyun Lower	-	-	-	-	-	0
Aspergillus flavus	Lagos Hostel	-	+	-	-	+	40
	Compound C	-	+	-	-	-	20
	Oyun Upper	-	+	-	-	-	20
	Oyun Lower	-	-	+	+	-	40

Legend: + = Present; - = Absent

In figure 1 is shown the reduction of 0.2mM phenol by Aspergillus flavus. The amount of phenol present at day 0 was 0.03. By day 15 it had reduced to 0.012. This is 60% disappearance of phenol. The control remained unchanged. Figure 2 indicates the reduction of 0.4mM phenol by Aspergillus flavus. The concentration of phenol at day 0 was 0.06 and was reduced to 0.037 at the end of the experiment. This signified 38% disappearance of phenol. The reading of the control experiment was unchanged.

Table 2: Total count of fungal isolates at the different sampling sites

Sampling Day	Fungal colony count ($cfuml^{-1}$)/Sampling Site							
	Lagos Hostel		Compound C		Oyun Upper		Oyun Lower	
	10^2	10^4	10^2	10^4	10^2	10^4	10^{-2}	10^4
1	71	19	69	2	15	1	1	0
2	166	84	78	18	9	1	22	0
3	14	5	40	30	10	1	14	5
4	44	24	80	52	30	10	8	1
5	70	43	154	64	46	17	88	56

The utilization of 0.6mM phenol by *Aspergillus flavus* is indicate in figure 3. Phenol concentration at day 0 was 0.06 and was reduced to 0.038 at the end of the experiment. This signified 38% disappearance of phenol. Figure 4 depicts the reduction of 0.8mM phenol by Aspergillus flavus. About 25% utilization of phenol was observed here. The utilization of phenol at day 0 was 0.081. This was reduced to 0.061 at day 15. The control remains unchanged too. Figure 5 shows the reduction of 1mM phenol by *Aspergillus flavus*. The amount of phenol present at day 0 was 0.4 and it was reduced to 0.352 at the end of the experiment. About 12% utilization of phenol was observed here.

From these experimental results it was observed that the higher the phenol concentration used the lower the amount utilized. Higher phenol concentrations had lower reduction rates while lower phenol concentrations had higher reduction rates. The higher phenol concentrations may be toxic to the fungus used to challenge it.

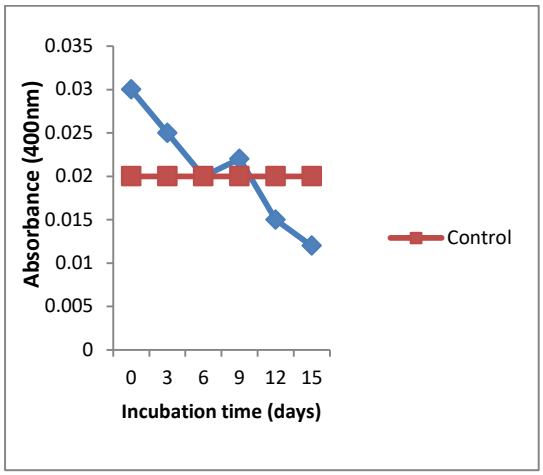

Figure 1: Reduction in concentration of 0.2mM phenol after 15 days.

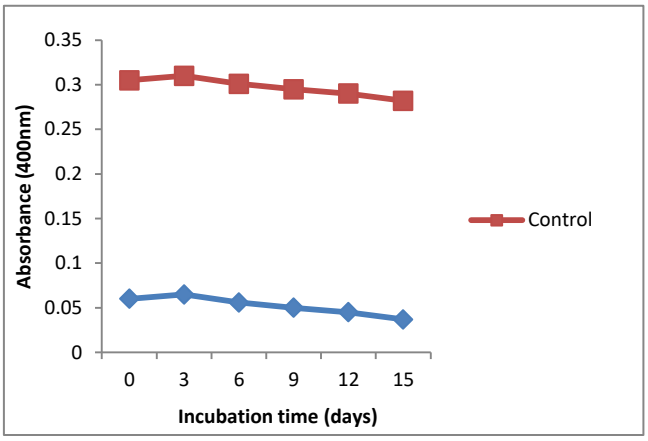

Figure 2: Reduction in concentration of 0.4mM phenol after 15 days.

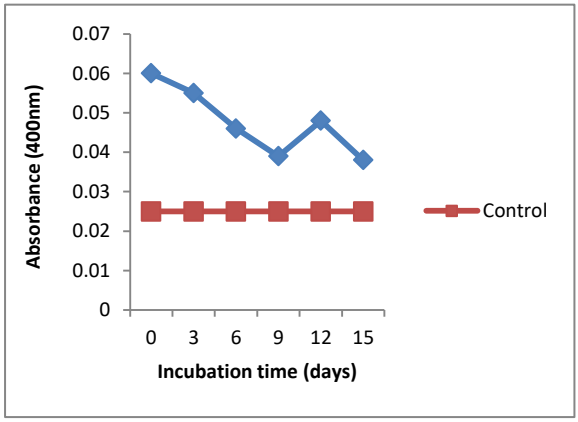

Figure 3: Reduction in concentration of 0.6mM phenol after 15 days.

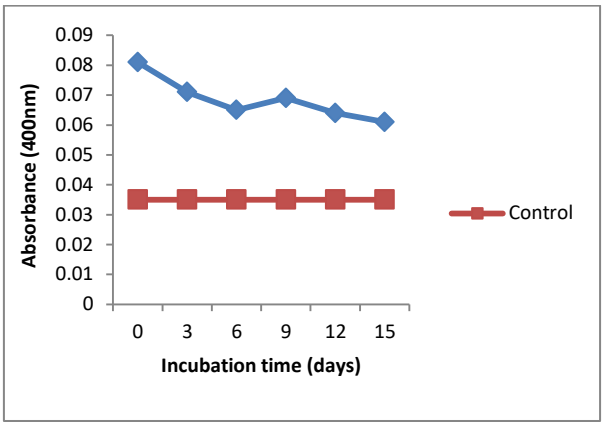

Figure 4: Reduction in concentration of 0.8mM phenol after 15 days.

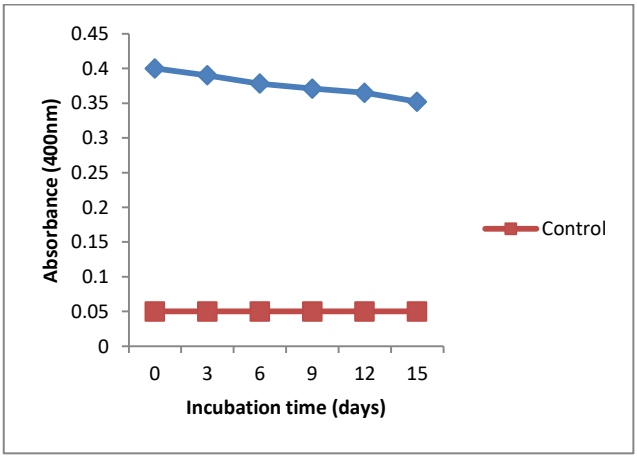

Figure 5: Reduction in concentration of 1mM phenol after 15 days.

Alternaria sp. and *Penicillium sp.* isolated from indoor environment by Jacob and Alsohaili (36) showed ability to degrade phenol. They determined ability to grow with phenol by increase in biomass (dry weight) and phenol depletion by gas chromatography. In our work phenol depletion was measured using spectrophotometer. In the

report of Bernats and Juhna (37), the batch flasks degradation of phenol by monocultures of *Trametes versicolor, Phanerochaete chrysosporium, Gloeophyllum trabeum* and *Irpex lacteus* was examined. Their results showed that white-rot fungus, T. Versicolor was the best of the species.

Krastanov et al. (38) reported that microorganisms possess enzymes capable of decomposition various aliphatic and aromatic toxic compounds. The observed ability of A flavus to reduce phenol in this experiment may be due to the release of degrading enzymes. Fungi has unique ability for degradation even under conditions unfavorable for other microorganisms (38). This is a big plus for fungi. Aspergillus sp. among others was able to tolerate phenol and was used to degrade phenol to varying capacities. Higher starting cell densities of the organisms lead to quicker degradation time for full phenol degradation and it also increased the rate of degradation as reported by Santos and Linardi (39). Pizzolitto et al. (40) reported the inhibitory effect of natural phenolic compounds on the growth of Aspergillus parasiticus. Also, in a related study by Senani-Oularb et al. (41) on olive mill wastewater containing phenol the inhibition of the growth of Aspergillus flavus and aflatoxin B1 production was observed. These are not in concord with this present report. This may be due to the fact that the phenol used by Pizzolitto et al. (40) was natural compounds. The degradation of phenol in this study by A flavus and the data generated are indicative of the possibility of using A. flavus in protecting the environment from phenol pollution.

References

1.Crockett C: How did Earth get its water? Science News 187(10):18. 2015.
2.Suarez RAB: Freshwater Ecosystems. http:html.rincondelvago.com/ecosistema. The classification system presented here is based on the (2007) phylogenetic study by Hibbett et al. 2008.
3.Kirkwood A, Safe Water for Africa. Africa Health 20(6): 9-10. 1998.
4.Hart, C.A. (2000). Escherichia coli and diarrhoea disease. Postgraduate Doctor Africa. 24(2):32-36.
5.Mason C: Biology of Freshwater Pollution. Pearson Education Limited (4th edition), Edinburgh Gate pp.375. 2002.
6.Robert B Jackson SR: Water in a changing world. Ecological Applications 11(4):1027 1045. 2001.
7.Ghazanfar SA, Hussain HSN: Aquatic and marsh plants of Kano State. Nigerian Field 51 (3-4): 115-128. 1986.
8. Lean G, Hinrichsen D, Markham A: Atlas of Environment. Hutchinson, London. pp 58. 1990.
9. National Summary of Water Quality Conditions, Inventory Report to Congress, 1994.
10. Olayemi AB: Bacteriological Water Assessment of an Urban River in Nigeria. International Journal of Environmental Health Research 4:156-164. 1994.
11. Engin GO, Demir I: Cost analysis of alternative methods for wastewater handling in small communities. Journal of Environmental Management 79: 357-363. 2005.
12. Eikelbloom, D. H. (2000). Activated Sludge Information Systems
13. EPA: Toxicological Review of Phenol. US Environmental Protection Agency, Washington D.C. 2002.
14. Whiteley AS, Bailey MJ, Bacterial Community Structure and Physiological State Within an Industrial Phenol Bioremediation System. Applied Environmental Microbiology. 66: 2400–2407. 2000.
15. Arutchelvan V, Kanakasabai V, Nagarajan S, Muralikrishnan V: Isolation and Identification of Novel High Strength Phenol Degrading Bacterial Strains from Phenol-Formaldehyde Resin Manufacturing Industrial Wastewater. Journal of Hazardous Materials 127: 238-243. 2005.
16. Paller G, Hommel RK, Kleber HP: Phenol Degradation by Acinetobacter calcoaceticus NCIB 8250. Journal of Basic Microbiology 35: 325-335. 1995.
17. Barbosa TCP, Luz AP, Bedin ML, Gabilan NH: Effect of Ceramic Plant Effluent on Phenol Degradation by Acinetobacter calcoaceticus. International Biodeterioration and Biodegradation 37(1):122. 1996.
18. Geng A, Soh A, Lim C, Loke L: Isolation and Characterization of a Phenol-Degrading Bacterium from an Industrial Activated Sludge. Applied Microbiology and Biotechnology 71:728-735. 2006.
19. APHA: Standard Methods for the Examination of water and wastewater.13th edn. Washington, DC: American Public Health Association 1976.
20. Aneja M, Gianfagnaa TJ, Hebbarb PK: Trichoderma harzianum produces nonanoic acid, an inhibitor of spore germination and mycelial growth of two cacao pathogens. Physiol. And Mol. Plant Path 67: 304–307. 2005.
21. Onions AHS, Alisopp D, Eggins HOW: Smith's Introduction to Industrial Mycology. Seventh Edition 1981.
22. Vecchioli GI, Del Panno MT, Painceira MT: Use of selected autochthonous soil bacteria to enhance degradation of hydrocarbons in soil. Environ. Pollut 67:249-258. 1990.
23. Garcia IG, Pena PRJ, Venceslada JLB, Martin AM, Santos MAM, Gomez ER: Removal of phenol compounds from olive mill wastewater using *Phanaerochaete chrysoporium, Aspergillus niger, Aspergillus terreus, and Geotrichum candida*. Process Biochem 35: 751-758. 2000.

24. Samson RA, Houbraken J, Thrane U, Frisvad JC, Andersen B: Food and Indoor Fungi. CBS-KNAW- Fungal Biodiversity Centre, Utrecht, Netherlands pp. 398. 2010.
25. Hoog GS, Guarro J, Gené J, Figueras MJ: Atlas of Clinical Fungi. 2nd Edition. Centraalbureau voor Schimmelcultures, Utrecht, Netherlands 2000.
26. Shen HD, Chou H, Tam MF, Chang CY, Lai HY, Wang SR: "Molecular and immunological characterization of Pen ch 18, the vacuolar serine protease major allergen of Penicillium chrysogenum". Allergy 58 (10): 993–1002. 2003.
27. Odds FC: Candida and Candidosis: A Review and Bibliography (2nd ed.). London; 31Philadelphia: Bailliere Tindall 1988.
28. Kerawala C, Newlands C, eds: Oral and maxillofacial surgery. Oxford: Oxford University Press pp. 446, 447. 2010.
29. Erdogan A, Rao SS: "Small intestinal fungal overgrowth". Curr Gastroenterol Rep 17 (4): 16. 2015.
30. Martins N, Ferreira IC, Barros L, Silva S, Henriques M: "Candidiasis: predisposing factors, prevention, diagnosis and alternative treatment". Mycopathologia 177 (5–6): 223–240. 2014.
31. Samson RA, Houbraken J, Summerbell RC, Flannigan B, Miller JD: "Common and important species of fungi and actinomycetes in indoor environments". Microogranisms in Home and Indoor Work Environments CRC. pp. 287–292. 2001.
32. Masayuki M, Katsuyai G: Aspergillus: Molecular Biology and Genomics. Horizon Scientific Press p. 157. 2010.
33. Agrios GN: Plant Pathology. Fifth Edition. Elsevier Academic Press, Amsterdam p. 922, 2005.
34. Amaike S, Nancy P K: *Aspergillus flavus*. Annual Review of Phytopathology 49: 107–133. 2011.
35. Adetitun DO, Abioye AO: Mycological Pollutants in Air and Phenol Utilization by Aspergillus niger. Ilorin Journal of Science. 4(1); 61-76. 2017.
36. Jacob HJ, Alsohaili S: Isolation of Two Fungal Strains Capable of Phenol Biodegradation. Journal of Biological Sciences 10: 162-165. 2010.
37. Bernats M, Juhna T: Factors Governing Degradation of Phenol in Pharmaceutical Wastewater by White-rot Fungi: A Batch Study. The Open Biotechnology Journal 9(1-M10): 93-99. 2015.
38. Krastanov A, Alexieva Z, Yemendzhiev H: Microbial degradation of phenol and phenolic derivatives. Engineering in Life Sciences 13(1):76-87. 2012.
39. Santos VL, Linardi VR: Biodegradation of phenol by a filamentous fungi isolated from industrial effluents—identification and degradation potential. Process Biochemistry 39(8): 1001-1006. 2004.
40. Pizzolitto RP, Barberis CL, Dambolena JS, Herrera JM, Zunino MP, Magnoli CE, Rubinstein HR, Zygadlo JA, Dalcero AM: Inhibitory Effect of Natural Phenolic Compounds on *Aspergillus parasiticus* Growth. Journal of Chemistry. 547925:1-7. 2015
41. Senani-Oularbi N, Riba A, Moulti-Mati F: Inhibition of *Aspergillus flavus* growth and aflatoxin B1 production by olive mill wastewater. Bioscience Research 15(1):369-380. 2018.