Keratinophilic Fungi Isolated from Soil of School Playgrounds in Abakpa Nike Town, Enugu State, Nigeria

Olisaka F. N.* Ohakwe R. O. and Oyeka C. A.
Department of Biological Sciences, Microbiology Unit, Godfrey Okoye University, Thinkers Corner, Nigeria.
* Corresponding author: frances@gouni.edu.ng

Abstract: In nature, keratinous substrates are broken down by keratinophilic fungus and a major habitat for this fungus is the soil. Their ability to breakdown keratin contributes to the pathogenicity potential of many of these fungi. Their actions may lead to worsen of respiratory diseases and development of a condition known as dermatomycosis, in both humans and animals. This study was aimed at isolating Keratinophilic fungi from the soil of the school playgrounds which are located at Abakpa Nike, Enugu state, Nigeria. Forty (40) soil samples were obtained from ten different school playgrounds using the hair bait techniques (HTB). Twenty-four (60%) yielded fungal growth. The growth on the baits were sub cultured into plates of Sabouraud dextrose agar (SDA) and identification of the isolates was based on macroscopic and microscopic characteristics. A total of 66 fungal isolates of 13 species from 5 genera were identified. *Aspergillus flavus* 13(32.5%) was the most dominant keratinophilic fungus, followed by *Microsporum gypseum* 10(25%), *Aspergillus fumigatus* 8(20%), *Trichophyton mentagrophytes* and *Aspergillus niger* 6(15%), *Trichopyton equinum* 5(12.5%), *Microsporum ferrugineum* and *Fusarium* 4(10%), *Microsporum audounii* 3(7.5%), *Microsporum distortum* and *Microsporum cookie* 2(5%*) Alterneria* 2(5%) and *Microsporum terrestre* 1(2.5%). The presence of keratinophilic fungi in school playground soils may present a serious risk to human health, especially for children and teenagers. The need for schools to practice and enforce hygiene protocols is important so as to curb the spread of these fungi and lower the risk of fungal infections.
Key word: *Aspergillus niger*, dermatomycosis, keratinophilic, playground, soil

INTRODUCTION

Fungi are one-celled or sophisticated multicellular creatures on earth. Most species are cosmopolitan creatures that can be found in almost any habitat that exists in every ecosystem, including the air (as spores) (Kambura *et al.,* 2016), although they spend most of their life primarily on soil and on plant materials, with some even making an appearance in the sea (Salano *et al*., 2018). In the concept of biocenosis, a class of fungi known as decomposers lives in the soil and decomposes dead organic matter, facilitating the recycling of chemical elements like carbon, nitrogen, and phosphorus in the cycle of matter (Ochei *et al*., 2000). A significant amount of the degradation of dead organic matter occurs in the soil, where reducers are in charge of the organic breakdown of plants and animal remains. Participating fungus can initiate the decay process, especially in the case of plants, by producing enzymes that break down the cellulose found in the cell wall of plants (Lange *et al.,* 2012).

Morphologically, fungi are categorized as microscopic multicellular filaments made up of sepate or non-septate hyphae; very few are unicellular in the form of yeast(s). Some are macroscopic, like mushrooms (Okafor, 2008). The formation of spores, a specific type of structure, helps fungi spread and survive. The very light, comparable to seeds, spores that allow fungi to reproduce can be suspended in the air and carried great distances by wind, rain, and insects. Evidently by these processes, spores can enter a building, the soil, exposed human tissues, and can be breathed (Kambura *et al.,* 2016).

Some saprotrophic fungal species are parasitic on other types of life; some infect plants, causing illnesses like cankers, scabs, and mildews, while others infect both humans and animals, causing diseases that affect both. Human diseases can be brought on by fungi by the creation of enzymes, poisons, and allergies. One of the key characteristics that contributes to fungi's ability to infect humans with disease is their capacity to flourish in a wide variety of

temperatures. According to various studies, fungi have been found in fruits, vegetables, raw food, cooked food, leather materials, moist cloths, house roofs, and raw and cooked food (Joanne *et al.,* 2013).

Due to their significance as pathogens that take advantage of humans and animals, keratinophilic fungi have recently attracted research on a global scale (Ganaie *et al*., 2010). According to their native environments, the keratinophilic fungus group's species have been separated into three groups. When humans serve as the natural hosts, anthropophilic: Zoophilic refers to the use of animals as natural hosts; geophilic refers to the use of dirt as a natural environment. The majority of keratinophilic fungi live in the soil and are not dermatophytes.

The primary habitat for the existence and activity of fungi is soil. Dermatophytes are generally saprobial and keratinophilic fungi of the mould origin that have developed to parasitize people and other animals. This creates superficial mycoses and lesions with a circular disposition (Henri *et al.,* 2006). Depending on the regions of the body affected, fungi found in the soil can also cause systemic, subcutaneous, and superficial infections. A superficial mycotic infection only affects the top most layer of the skin, nails, hair, and mucous membrane.

Mycoses are fungal infections that affect humans and can be found everywhere in the world (David *et al*., 2015). Some of the factors that that contribute to the prevalence of mycoses or human fungal diseases include; the ability for fungi to grow on normal human body temperature (37°C) the production of toxins and enzymes, host immunity and the use of immunosuppressive medications in organ transplants (David *et al.,* 2015). Natural factors in the environment can cause the prevalence of Keratinophilic fungi. These factors include the pH of the soil, temperature, climate, source of keratin, humidity and the amount of light in the environment (David *et al*., 2015). Different studies have been carried out on the prevalence of fungi in the soil (Sharma *et al*., 2014; Eze *et al*., 2019).

Taking into consideration the above background information, the aim of this study is to determine the prevalent keratinophilic fungi present in playground soils of schools located at Abakpa Nike town, Enugu. The findings of this study can be useful since there is paucity of data on the presence of keratinophilic fungi in the soils of school playgrounds as no study has been carried out on these soils in Enugu State.

MATERIALS AND METHODS

Study area: The study area for this research work were some primary and secondary school playgrounds in Abakpa Nike, Enugu. Abakpa Nike is a town in Enugu East local government area (LGA) of Enugu state.

Collection of soil samples: Forty (40) soil samples were taken from different sites of 6 primary schools and 4 secondary school playgrounds for a period of 4 months. The soil samples were collected from 4 distinct areas in each School playgrounds. Samples of soil were obtained from different locations within each school using a sterile disposable spoon and dispensed in sterile ziplock bags. The bags were properly sealed, labelled and delivered to the laboratory for further analysis (Eze *et al*., 2019).

Keratinophilic fungi isolation from substrate: A modified hair bait technique by Vanbreuseghem was used in the isolation of Keratinophilic fungi from soil (Vanbreuseghem, 1952). Short strands of human hair of ca.0.4 to 1.6cm in length was defatted in methanol solution for 24 hours and was further washed with distilled water and then dried in a hot air oven at 45 ^{o}C for 1 to 2 hrs. The keratin substrate was then cut into small pieces (2-3cm) and sterilized in an autoclave (Tarun *et al*., 2020). Then each soil sample was homogenized,10g portions was carefully weighed into a sterile petri dish. The soil was made moist with distilled water. The sterilized pieces of human hair were placed on the soil samples in sterile Petri dishes. Each Petri dish was

individually tagged with the date, location of and duration of incubation. The petri dishes were incubated for a month at room temperature (22–28°C). The baited hair samples were checked regularly for signs of fungal development, if any, while sterile water was intermittently introduced to provide the moisture essential for fungal growth. Subsequently, samples that had visible fungal growth were subcultured onto new petri plates (Mukesh and Meenakshi, 2010).

Subculture of isolated fungi: With the help of a sterilized forcep, the baits that clearly demonstrated fungal growth were selected for subculturing into plate of Sabouraud dexterose agar (SDA) enriched with 0.05mg/ml chloramphenicol and 0.5 mg/ml cyloheximide. Chloramphenicol and cycloheximide-containing plates were intended to prevent the growth of bacteria and saprophytic fungi, respectively (Mukesh and Meenakshi, 2010).

Colony morphology of fungal isolates: Macroscopic characterization, such as colony color, texture, and reverse pigmentation, was used to characterize the different species for identification. In comparison to the color Atlas of Pathogenic Fungi, fungal hyphae formations, macroconidia, and microconidia were employed to identify them microscopically. The identication of keratinophilic fungi and their morphology were studied by Lactophenol Cotton Blue (LPCB) wet mount method and Slide culture techniques for better identification. On a clean glass slide, the mycelium was subjected to a few drops of LPCB. It was covered with a cover slip, and examined under low and high power microscope magnification.

Slide culture techniques for better identification: The culture plates that showed fungal growth at the end of the incubation were further subjected to slide culture techniques for identification using Riddell (1950) slide culture techniques. The Petri-dish containing a filter paper, U-shaped rod, microscopic slide and cover slip were sterilized by autoclaving at 121^0C at 15psi for 15 minutes. The sterilized slide was then placed on the U-shaped rod using sterilized forceps. A sterile scapel blade was used to cut out a 1cm square SDA block and it was placed on top of the slide. A wire loop was used to pick up a part of the fungus under investigation and then it was inoculated at the four sides of the agar block. A sterilized cover slip was gently placed on the inoculated agar block. About 4ml of sterile distilled water was poured aseptically into the plate to provide a humid environment favorable for fungal growth. The plates were then incubated at room temperature for at 5 to 7 days to allow growth and sporulation to occur.

RESULTS AND DISCUSSION

A total of forty (40) soil samples were collected from six (6) Primary schools and four (4) colleges located in Abakpa Nike Enugu for this study. A total of 24 (60%) of the soil samples yielded keratinophilic fungi (Table 1). Table 2, shows the distribution of keratinophilic fungi in School playgrounds and the percentage of recovered isolates in each school. A total of 11% of the isolates were recovered from Amen nursery and Primary school, Housing Estate primary School, Bishop Okoye Nursery and Primary School and Cocci International College, while a total of 9% of the isolates were recovered each from St Peter and Paul Nursery School, Msgr Raphael Eze Nursery and Primary School, Abakpa Nike Primary School, Daughters of Divine Love Juniorate, Modern International College and Nike Grammar School (Table 3). Generally, a total number of 66 fungal isolates of thirteen (13) different species belonging to 5 genera were isolated (Table 4). In this study, the keratinophilic fungi that were most often isolated were *Aspergillus flavus* 13 (32.5%), *Microsporum gypseum*, 10 (25%), *Aspergillus fumigatus* 8 (20%), *Trichophyton mentagroghytes* and *Aspergillus niger* 6 (15%) (Table 5). The microscopic and macroscopic characteristics of the isolates are shown in Table 6. The macroscopic and microscopic features of

Microsporum ferrugineum are shown in Figure 4b and 4a. Figure 5a and 5b shows the Morphological features of *Microsporum gypseum*. Morphological features of *Trichopyton mentagropytes* are shown in Fig 7a and 7b. The isolates of *A. flavus* formed yellow to dark green colonies, growth was rapid and widely spread. Microscopic examination on lactophenol cotton blue mounts of growth demonstrates characteristic coarsely rough colorless conidiophore, conidia heads are typically radiate. *Microsporum gypseum* was the second prevalent species in this study. The isolates of *M gypseum* were initially white and floccose, later powdery and to cinnamon-brown in colour colonies. Microscopic identification showed abundant, thin-walled macroconidia with 4-6 septate. Other dermatophytes identified were; *Trichophyton equinum* 5 (12.5%), *Microsporum ferrugineum* 4 (10%), *Microsporum audounii* 3 (7.5%), *Microsporum distortum* 2 (5%), *Microsporum cookie* 2 (5%) and *Trichopyton terrestre* 1(2.5%). Other non-dermatophytes isolated were; *Fusarium* 4 (10%) and *Alterneria* 2 (5%).

Given their propensity for infection, keratinophilic fungi are closely related to dermatophytes. Due to their degradation of hard keratin tissues, they have keratinophilic and keratinolytic characteristics as a result. Dermatophytes and keratinophilic fungi are found in abundance in soils that have been supplemented with keratin substrate, such as hair, feathers, horn and hoof and skin (Marchisio, 2000). The study reavealed that the non-dermatophytic fungus *Aspergillus flavus*, was the most widespread with the exception of *Aspergillus fumigatus* (20%) and *Aspergillus niger* (15%), which came in third and fifth, respectively. This is in agreement with Agu *et al*. (2013). These organisms are frequent saprophytes in soil and plant waste, therefore it is not unexpected that the results are identical. Some of them are frequently found in lab waste. *Aspergillus sp* has been linked to mycotic keratitis and has been found to be airborne. When breathed, particularly by people with compromised immune systems, they are known to induce opportunistic aspergillosis (Arastehfar *et al*., 2021). Aspergillus also produces aflatoxin, a dangerous mycotoxin, produced as a secondary metabolite when it infests foods like grains and nuts, further causing food spoilage. Aflatoxin induces significant reactions from its victims at relatively low concentrations. Furthermore, in the order of the second and fourth most prevalent keratinophilic fungi, *Microsporum gypseum* (25%) and *Trichophyton mentagrophytes* (15%) continue to be the two most common dermatophytes. Similar study has been carried out by Oyeka and Okoli (2003), Agu *et al*. (2013), and Eze *et al*. (2019), who isolated various *Microsporum* and *Trichophyton* species from Nigerian soils.

Among other fungi, *Microsporum* and *Trichophyton* species were also isolated by Singh *et al*. (2009) and Bisen and Tiwari (2015) in the corresponding research on keratinophilic fungi in soil. The three genera of dermatophytes—*Epidermophyton*, *Microsporum*, and *Trichophyto*n - are grouped together, and they can spread either directly through direct contact with an infected host or indirectly through indirect contact with contaminated objects or the environment. In low- and middle-income nations like Nigeria, where potential risk factors for infection like poor hygiene, crowding, age (young and old immunosuppressed individuals), and low socioeconomic factors are still present, fungi infections are a significant public health concern among school-age children (Olaide *et al*., 2014). The reason why children, particularly schoolchildren, are frequently affected is due to *M. gypseum* and *T. mentagrophytes'* (geophilic and zoophilic) presence in the school playground soils. These organisms are the aetiological agents of *Tinea pedis* and *Tinea unginium,* respectively (Kamal *et al*., 2012). Contact with the earth increases their susceptibility to diseases. Minor and major injuries can also occur in the playing fields and

children's playgrounds, and in most cases children pay no attention to the injury and the wounds are not disinfected creating a source of traumatic implantation of dermatophytic agents of subcutaneous mycoses Katarzyna *et al*. (2014). Keratinophilic fungi played a significant role in the natural degradation of keratinized residues as reported by Sharma and Rajak (2003). The other isolated dermatophytes *T. equinum, M. ferrugineum, M. audounii, M. distortum, M. cookie* and T. terrestre and non-dermatophyte Fusarium and alterneria were recorded in decreasing order. *T. terrestre* has been recorded from hospital dust of Kanpur Uttar Pradesh, India, by Singh *et al*. (2009). While Adefemi *et al*. (2011) reported *M. audounii* as a causative agent of *Tinea capitis* and *Tinea corporis*. Five (12.5%).

Table 1: Prevalence of keratinophilic fungi and dermatophytes isolated from soil samples of various schools

Schools	AM	SS	MR	HE	BO	AN	CC	DD	MC	NG	Total
No of samples studied	4	4	4	4	4	4	4	4	4	4	40
No of samplespositive	3	3	2	3	3	2	2	3	1	2	24
Positive samples %	75	75	50	75	75	50	50	75	25	50	60

Keys: AM = Amen Nursery and Primary School; SS = St Peter and Paul Nursery and Primary School; MR = Msgr Rapheal Eze Nursery and Primary School; HE = Housing Estate Primary School; BO = Bishop Okoye Primary School; AN = Abakpa Nike Primary School: AC= Cocci International College; DD= Daughters of Divine Love Juniorate; MC = Modern International College; NG = Nike Grammar School.

Table 2: Distribution of keratinophilic fungi in different school playgrounds

Name of fungus	Distribution of Keratinophilic fungi in different school playgrounds									
	AM	SS	MR	HE	BO	AN	CC	DD	MC	NG
M. gypseum	02	--	--	02	--	03	--	01	01	01
T. mentagrophytes	--	02	--	--	02	--	02	--	--	--
T. equinum	01	--	02	--	--	--	--	--	--	02
M. ferrugineum	--	01	--	01	--	--	01	--	--	01
M. audounii	--	--	--	--	02	--	--	--	01	--
M. distortum	--	--	--	--	--	01	--	01	--	--
M. cookie	01	--	01	--	--	--	--	--	--	--
T. terrestre	--	--	--	--	--	--	01	--	--	--
A.flavus	02	--	01	--	02	02	02	--	02	02
A. fumigatus	--	02	--	02	--	02	--	02	--	--
A. niger	--	--	01	02	--	--	--	01	02	--
Fusarium	01	01	01	--	--	--	01	--	--	--
Alterneria	--	--	--	--	01	--	--	01	--	--
Total colonies	7(11%)	6(9%)	6(9%)	7(11%)	7(11%)	6(9%)	7(11%)	6(9%)	6(9%)	6(9%)

Keys: AM = Amen Nursery and Primary School; SS = St Peter and Paul Nursery and Primary School; MR = Msgr Rapheal Eze Nursery and Primary School; HE = Housing Estate Primary School; BO = Bishop Okoye Primary School; AN = Abakpa Nike Primary School: AC= Cocci International College; DD= Daughters of Divine Love Juniorate; MC = Modern International College; NG = Nike Grammar School.

Table 3: Frequency of occurrence of keratinophilic fungi isolated

Isolates	Total no. of fungal isolates	Percentage occurrence (%)
Dermatophytes		
Microsporum gypseum	10	25
Trichophyton mentagrophytes	6	15
Trichophyton equinum	5	12.5
Microsporum ferrugineum	4	10
Microsporum audounii	3	7.5
Microsporum distortum	2	5
Microsporum cookie	2	5
Trichophyton terrestre	1	2.5
Non-dermatophytes		
Aspergillus flavus	13	32.5
Aspergillus fumigatus	8	20
Aspergillus niger	6	15
Fusarium	4	10
Alterneria	2	5
Total	66	

Table 4: Characteristics of the different fungal isolates

Isolates	Macroscopic	Microscopic
Aspergillus flavus	Colony was rapidly growing, widely spread and floccose in texture. Colour varies from yellow to dark green	Conidiophores were colourless, rough, thick wall. Conidia were pyriform to globose, rough- walled, colorless
Aspergillus fumigatus	Colony was rapidly growing and velvety in texture, Colour is initially white and later green to dark	Conidiophores were short, smooth, densely crowded. Flask-shaped vesicles. spherical to globose, and form a columnar mass that was green in colour
Aspergillus niger	Compact, clusters of dark colonies	Hyaline conidiophore phylides borne on vesicles, abundant of dark walled conidia with septate hyphae
Trichopyton mentagrophytes	Usually white, fluffy on top with yellow bottom	Clusters of microconidia cigarshaped macroconidia with terminal rat-tail filament
Trichopyton terrestre	Moderately rapid growth, initially white in colour and fluffy in texture with pale yellow granular center	Macroconidia were abundant, clavate round ends, smooth and thin-walled. Microconidia were pyriform, on short stalks along the hyphae
Microsporum distortum	Colony was velvety to fluffy raised centre, white to tan in colour.	Septate hyphae, macroconidia were irregular and distorted and abundant microconidia with club shape
Microsporum ferrugineum	Colony was downy, white to intense yellow colour	No distinguishing spores, septate, giving term bamboo hyphae
Trichopyton equinum	Colony was flat, white to buff in colour	Microconidia were abundant, pyriform stalked around the hypae
Microsporum audounii	Fluffy white and granular colony. reverse is tan yellow	Macroconidia were spindle-shaped with terminal knob, they are thick walled. Microconidia are clavate in form.
Microsporum gypseum	Colony was initially white and floccose, later powdery to granular and to cinnamonbrown in colour.	Abundant, thin-walled macroconidia with 4-6 septate
Microsporum cookie	Colony was flat, powdery, Huge, long, thick, rough-walled white to yellowish in colour spores with more than 8 septa	
Fusarium	Fast growing colony, white and Largest spores were sickle-shaped cottony. later rose to red color contains several small spore at both sides	
Alterneria	Rapid growing colonies, Large hand grenade-shape spores, grayish to black to brown borne in chains, septate	

CONCLUSION

Distinct isolates of keratinophilic fungus were isolated from Different school playgrounds. This may have been caused by variations in the organic content of the soil. The distribution of keratinophilic fungi in the soil is significantly influenced by the organic matter concentration of the soil. The current study demonstrates the abundance of keratinophilic fungus in the playground soils of primary and secondary schools. The school children are thus exposed to the risk of contracting superficial mycoses (dermatophytosis) through direct contact with an infected host or through indirect contact with contaminated formicites in the environment.

REFERENCES

Adefemi, S. A Odeigah L. O. and Alabi, K. M. (2011) Prevalence of Dermatophytosis among primary school children in Oke-lyo community of Kwara State. *Nigerian Journal of Clinical Practice,* 14(1), 23-28.

Agu, G. C., Shoyemi, W. R., Thomas, B. T. and Gbadamosi, K.P. (2013). Presence of keratinophilic fungi in schools playing grounds in Sagamu, Ogun State, *Nigeria. New York Science Journal,* 6(12):127-13.

Arastehfar, A., Carvalho, A., Houbraken, J., Lombardi, L., Garcia-Rubio.R., Jenks, J. D., Rivero- Menendez, O., Aljohani, R., Jacobsen, L. D., Berman, J., Osherov, N., Hedayati, M. T., Ilkit, M., Armstrong-James, D., Gabaldon, T., Meletiadis J., Kostzewa, M., Pan, W., Lass-Florl, C., Perlin, D. S. and Hoenigl, M. (2021). *Aspergillus fumigatus* and *aspergillosis. Studies in Mycology, volume* 100(1): 100115- 100115(1)

Bisen, P. and Tiwari, S. (2015). A Review on Keratinophilic fungi of Madhya Pradesh. *IOSR Journal of Pharmacy and Biological Sciences;* 10, 18-22.

David, D.W. and Harish, G.C. (2015). Burden of serious fungal infections in Trinidad and Tabago. Blackwell Verlag, *Journal of Mycoses* 58. 80-84.

Eze, E. M., Ezebialu, C. U., Unegbu, V. N. and Nneji, I.R. (2019) Prevalence of keratinophiic fungi and other dermatophytes from soils of Nnewi in Anambra State, Nigeria. *Novel Research in Microbiology Journal,* 3(3): 379-386.

Ganaie, M. A., Sood, S., Rizvi,G. and Kahn, T. A. (2010). Isolation and identification of keratinophilic fungi from different soil samples in Jhansi City India: *Plant pathol. J.* 9(4): 194-197.

Henri, D.L.V.B., Jose, D.L.F., Sandra, H.R.C., Rodrigo, H. F.T., Selene, D. A.C. (2006). Isolation of *Microsporum gypseum* from the haircoat of healthy wild fields kept in captivity in Brazil. *Brazilian journal of Microbiology* 37-148-152.

Joanne, W., Linda, S. and Chris, W. (2013). Prescott's Microbiology. *African edition.* MC Graw Hil LANGE 9 Edition.

Katarzyna, G., Piotr, K., Joanna, B. and Anna, W. (2014). Soil of Recreational Areas as a Reservoirof Keratinolytic Mold Fungi and Dermatophytes Potentially Pathogenic for Humans. *Pol. J. Environ. Stud.* 24(3): 993-1002.

Kambura, A., Mwirichia. R., Kasili, R., Karanja, E., Makonde, H. and Boga, H. (2016). Diversity of Fungi in Sediments and water samples from the hot springs of Lake Magadi and Little Magadi in Kenya. *African Journal of Microbiology Research,* 10(10), 330-338.

Kamal, R. A., Radhika, J. C., Sharma, P. S. and Ashish, A. (2012). Biodiversity of dermatophytes: an overview Rev. *Plant Pathol. Vol. 3, Indian Society*

of Mycology and Plant Pathology Scientific Publishers (India), Jodhpur pp. 299-314.

Lange, L., Bech, L., Busk, P.K., Grell, M.N., Huang, Y., Lange, M., Linde, T., Pilgaard, B., Roth, D. and Tong, X. (2012). The importance of fungi and of mycology for a global development of the bioeconomy. *IMA Fungus* 3(1): 87.

Marchisio V. F. (2000). Keratinophilic fungi: their role in nature and degradation of keratinic substrate. In: Kushwaha RKS, *Guarro J (eds.) Biology of Dermatophytes and other Keratinophilic Fungi. Revista Iberoamericana de Micologia, Spain, Bilbao,* pp. 86-92.

Moalaeih H, Zeynie F, Mhmoudi M, Pit M. (2006). Identification of Keratinophilic fungi in Dry-farming soil samples from South and Razavi Khorasan provinces in Iran. *J Sabzevar Univ Med Sci*,13(2):64–73.

Mukesh, S. and Meenakshi, S. (2010) Incidence of dermatophytes and other keratinophilic fungi in the schools and college playground soils of Jaipur, India. 4(24): 2647-2654.

Nosratabadi M, Kordbacheh P, Kachuei R, Safara M, Rezaie S, Afshari M. Isolation of keratinophilic fungi from the soil of Greater Tunb, Abu-Musa, and Sirri islands in the Persian Gulf, Iran. *Curr Med Mycol.* 3(2):13-19.

Ochei, J. and Kolhatkar, A. (2000). Medical Laboratory Sciences: Theory and practice. *Tata McGraw-Hill publishing company limited, New Delhi.*

Okafor, J. I. (2008). Fungal Diseases: A Serious Threat to Human Existence in Recent times. Forty-first inaugural lecture of the University of Nigeria. Nsukka.

Olaide, O. O., Olaniyi O., Olayinka, A. O., Akinlolu, G. O. and Olumayowa, A. O. (2014). The prevelance and pattern of superficial Fungal Infections among School Children in lle-Ife South-West Nigeria. Doi: 10.1155/2014/842917

Oyeka, C. A. and Okoli, I. (2003), Isolation of dermatophytes and non-dermatophytic fungi from soil in Nigeria. *Mycoses* 46(8): 336-338.

Riddell, R.W. (1950) Permanent Stained Mycological Preparation Obtained by slide culture. *Mycologia* 42, 265-270.

Salano, O. A. Makonde, H. M. Kasili, R. W. and Boga, H.L. (2018). Isolation and Characterization of Fungi from a Hot Spirng on the Shore of Lake Bogoria, Kenye. *Journal of yeast and Fungal Research* 9(1). 1-13.

Sarmiento, M. M., Mangiaterra, M., Bojanich, M. V., Basualdo, J. Á., & Giusiano, G. (2015). Keratinophilic fungi in soils of parks of Corrientes city, Argentina. *Revista Iberoamericana de Micologia*, 33(1), 7-12.

Sharma, R. and Rajak, R. (2003). Keratinophilic fungi: Nature's keratin degrading machines *Resonance.* 8.28-40.

Singh, LA, Mishra, R. and Kushwaha, K. (2009). Dermatophytes, related keratinophilic and opportunistic fungi in indoor dust of houses and hospitals. *Indian Journal of Medical Microbiology.* 27 (7): 242-246.

Tarun, K. K., Anima, S., Vishnu, S. and Seema, B. (2020). A study on the prevalence of keratinophilic biota of semi-arid region Rajasthan, India. *Journal of King Saud University - Science* 32: 1014-1020.

Vanbreuseghem, R. (1952) Technique biologique pour l' Isolement des dermatophytes du sol. Annales de la Societe belge de *medicine tropicale,* 32, 173-178.