EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 7
Issue: 5
48-53
Year: 2020

SEQUENTIAL PATHOLOGICAL SCALES OF KERATINOPHILIC FUNGI ISOLATED FROM GEOPHILIC SOIL SAMPLES

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Article Received on 28/02/2020 **Article Revised on 20/03/2020** **Article Accepted on 10/04/2020**

ABSTRACT
Keratinophilic fungi have been reported as one of the principal cause of cutaneous and subcutaneous mycoses among individuals living in developing countries. This study was conducted to evaluate the pathological scale of keratinophilic fungi isolated from different soil in Uli community, Ihiala L.G.A, Anambra state in immunocompetent and immunocompromised albino mice. A total of 30 soil samples were collected randomly from poultry farm, goat farm and garden soil and screened for the presence of keratinophilic fungi using hair baiting technique. The isolates obtained were characterized and identified using their macroscopic and microscopic characteristics. The pathological scale of the isolates was assessed by administering the broth culture of the isolates topically on the albino mice and this was observed for period of 3 months. *Trichophyton megninii, Trichophyton mentagrophytes, Trichophyton rubrum, Microsporum nanum, Microsporum audouinii and Microsporum gypseum* were isolated from the soil samples, and the organisms were detected mostly in goat farms and least from poultry farm. *T. mentagrophytes* recorded significantly ($p < 0.05$) the highest occurrence skin of the infected mice, which were mild, moderate or severe depending on the alopecia or scaling nature and presence of inflammatory crust, and this features were seen most among the mice infected with *Trichophyton mentagrophytes* and those infected with *T. rubrum.* This study has revealed the presence of Keratinophilic fungi in the studied soil samples.The isolates showed more obvious pathological features among the infected immunocompromised mice than in the infected immunocompetent mice, of which *T. mentagrophyte* and *T. rubrum* proved to be most pathogenic.

INTRODUCTION
Nature has provided planet earth with a variety of beneficial organisms. Keratinophilic fungi are one of the nature's gifts which have the ability to decompose even the hardest substance like keratin.

According to Richa and Neeraj (2004), Keratinophilic fungi are group of fungi that colonies different keratinous substrates such as hairs nail skin and degrade them into low molecular weight component. These fungi generally parasitized the skin of man and other animals. They obtained their food by degrading keratinous substances and cause various types of skin diseases in man including animals. They are classified into three categories based on their natural habitats: Anthrophilic, when human beings are their natural host, zoophilic, when animals are their natural host and geophilic, when they inhibit soil (Rahul and Rajak, 2003). They are generally referred to as dermatophytes and of three genra: *Epidermophyton*, infects skin and nail, *Trichophyton*, infects hair, skin and nails, *Microsporum*, infects hair and skin. . However, the occurrence of keratinophilic fungi in soil is not only causes diseases in man and animals but these are also responsible in the management of keratinoceous substances in soil due to their degradation activities. Keratinophilic fungi are soil inhabitants usually present in all types of soil and grow as saprophytes by utilizing certain substances present in the soil. Their occurrence and distribution is directly depends upon the existing climatic and edaphic

conditions and amount of keratin substances present. The production of different enzymes by keratinophilic fungi is of immense value for their successful survival and subsequent hydrolysis of keratin. These species produces extracellular enzymes, which are useful in eco-ethical technology such as for de-haring of skin and feather degradation which remove the contaminants from soil. Most keratinophilic fungi have pathogenic potential. Hence, these fungi have attracted the attention of dermatologists due to their association with human mycoses. They are known to cause superfial infection (mycoses) of keratinized tissues (skin, hair and nails) of humans. These fungi are found worldwide and infection is acquired by contact with infected humans or animals, or from exposure to contaminated soil or fomites (e.g., combs, brushes). Dermatophytes do not cause invasive disease except in immune compromised hosts. The clinical disease attributable to dermatophytes varies by organism, site of infection, and host immunologic responses.

Specific organisms usually cause dermatophyte infections in particular parts of the body, dermatophyte diseases are usually classified according to site of infection as Tinea barbae (beard), Tinea capitis (scalp and hair), Tinea corporis (bald skin), Tinea cruris (groin), Tinea manuum (hand), Tinea pedis (feet), and Tinea unguium (nails) also called onchomycosis (Richa and Neeraj, 2004). Exposure to dermatophytes does not always lead to the establishment of the infection. Some individuals appear to be more predisposed than others, possibly related to the type of immune response mounted by the individual. In recent years, keratinophilic fungi have been receiving considerable concern and studies on isolation and distribution of keratinophilic fungi from soil as investigated throughout the world. However, we have not found a report on the pathological effects of keratinophilic fungi. Therefore, this present work was undertaken to evaluate the pathological effects of keratinophilic fungi isolated from soil samples.

MATERIALS AND METHODS

Study Area: The soil samples were collected at Umuoma, Uli town, Ihiala L.G.A. Anambra State. The town is located at $5^0$47'0" North latitude and $6^0$52'0" East longitude on the world map. Uli town shared common boundary with the following towns: Amaofuo (formerly a village in Uli town), Ihiala, Amorka, Ubulu, Ozara, Egbuoma and Ohakpu. Uli town has a land mass area of 99 square meter (256 kilometer square). It has a temperature range of 30 ± 2^0C, 78% humidity and 03m/s wind speed. There are nine (9) major communities in Uli town which includes:Umuoma, Eziama, Umuaku, Umuchima, Ndikokwu, Ndiakuotikpo and Aluora. The anthropological activities of people in this town are farming, fishery, hunting, business activities and other minor administrative activities

Collection of Samples, Handling and Transportation: A total of 30samples comprising of 10 samples each from poultry farms, goat farms and gardens were collected randomly from Uli community in Ihiala L.G.A., Anambra State. The samples were collected from the superficial layer of soil at a depth not exceeding 3-5cm with sterile stainless spoon in sterile polyethylene bags, brought to Microbiology laboratory, Department of Microbiology, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli Campus within 4 h of collection to ensure maximum recovery of the organism.

Isolation and Purification of Keratinophilic Fungi: The keratinophilic fungi were isolated using hair baiting technique. This was done by filling sterile Petri dishes (30 x 3 mm) with 10 g of the soil samples, defatted hair samples were added and then moistened with sterile distilled water. These dishes were incubated at room temperature and examined daily for fungal growth for a period of 3 weeks. After observing the mycelial growth on the baits, the colonies generated from the plates were sub cultured on sabouraud dextrose agar (SDA) medium incorporated with chloramphincol (50 mg /l) for inhibition of bacteria growth and cycloheximide (5 ml / 500 ml) for other fungi inhibition and incubated at room temperature for 7 days. After 7 day, the mycelial growth on the SDA media was sub cultured again on SDA media in order to get a pure culture (Hashe *et al*., 2014).

Identification of the Isolates

- **Macroscopy:** The colonies were carefully examined. The rate of growth, consistency, colour and texture of the surface growth, nature of the reverse side and other peculiar features of the colonies were noted (Aziz and Seema, 2015).
- **Preparation and microscopic examination of needle mount**: A drop of Lactophenol cotton blue (LCB) solution was placed on the centre of a clean grease free slide. A fragment of the colony was placed in the drop and teased with two straight needles. A cover slip was placed over the preparation and pressed by tapping gently with a blunt-ended object (pencil eraser). Excess fluid from the outside of the cover slip was wiped with tissue paper and the slide was passed through a flame to warm it (care was taken not to overheat the set up). This treatment also removed the air bubbles that were trapped underneath the cover slip and facilitated staining of fungal elements. The slide was examined under compound light digital microscope using low power objective (10), followed by high-power objective (40). This revealed the nature of the hyphae, conidia and other diagnostic features (St-Germain and Summerbell, 2000). Fungal atlas aided the quick confermation of the suspected fungal organism (Aziz and Seema, 2015).
- **Slide culture technique**: Riddel's method as described by St-Germain and Summerbell (2000) was used. A filter paper was cut and placed on the bottom of the petri dish. Two slides were crossed over each other on top of the filter paper and the

filter paper was moistened. The set-up was sterilized by autoclaving at 121^0C for 15 minutes. Approximately one centimeter square agar block was cut from already prepared Potato Dextrose Agar (PDA) and placed on the intersection of the two slides. The four edges of the agar block were inoculated with the test organisms. It was then covered with sterile cover slip and incubated at room temperature for 7-10 days. After 10 days of growth, the cover slip was removed and inverted over a slide containing a drop of Lactophenol cotton blue (LCB). The agar block was removed and discarded. A drop of LCB was also placed on top of the adherent colony on the slide and covered with sterile cover slip. The edges of the cover slip were sealed with nail polish to prevent evapouration of the stain. The slides were examined under the microscope. The isolates were identified using standard descriptions given by St-Germain and Summerbell, (2000) and Aziz and Seema, (2015).

Preparation of Keratinophilic Fungi

The inoculum was prepared by flooding the surface of the agar plates with sterile normal saline (0.85% NaCl). The normal saline was prepared by weighing 0.85 g of NaCl in 100 ml of water and then sterilized. After flooding the plates, a sterile spatula was used to scrap the surface of the plate which contains the fungi mycelium under aseptic condition and the resultant suspension was collected, and then homogenized by adding more normal saline to it. The suspension was filtered using a sterile filter paper to remove the fungi hyphae and the filtrate which contains the test organisms (keratinophilic fungi) was collected. Then the test organisms were standardized using 0.5 McFarland standards. The 0.5 McFarland standard was prepared by weighing 0.6 ml of 1% $BaCl_2$ "H_2O and 99.4 ml of 1% concentration H_2SO_4.

EXPERIMENTAL DESIGN

Procurement of albino mice: A total of 52 mice of mixed sex obtained from an animal keeping house in Awka, Anambra State were used for the study. They were housed in thoroughly cleaned and disinfected wooden cages and provided with feeds and water prior to infection.

Inoculation into the mice: The mice were grouped into three; A, B, and C .Groups A and B comprises six sub groups each and each sub group contained four mice for each isolate. Group C contained only four mice that were administered topically with 0.5 ml of normal saline as control group. The immune systems of group B were suppressed with 0.05 ml of hydrocortisone acetate and after 24 h were administered topically with broth culture of each of the isolates using swab stick. The group C is the control group. The mice were allowed for a period of three (3) months (Aziz and Seema, 2015).

Cross examination of the morphologies of the skin of the mice: The adult albino mice of mixed sex from each group were randomly selected and observed for the pathological scale of keratinophilic fungi till the end of the experiment. Pathological signs associated with their skin such as alopecia, erythema, scaling, crust formation, inflammation and ulceration were noted and recorded (Aziz and Seema, 2015).

Statistical Analysis: The data obtained from this study was presented in tables, figures and percentages. The significance of the study was carried out using one way Analysis of Variance (ANOVA) at 95% confidence level (Iheukwumere *et al*., 2018).

RESULTS

The study revealed the presence of Keratinophilic fungi in fourteen (14) samples out of the thirty (30) soil samples from poultry farm, goat farm and garden, with the soil sample collected from goat farm showing the highest occurrence of Keratinophilic fungi whereas soil sample from poultry farm showed the lowest occurrence of Keratinophilic fungi.

The study revealed the Macroscopic and microscopic characteristics of *Trichophyton megninii, T. mentagrophytes, T. rubrum, Microsporum audounii, M. gypseum* and *M. nanum*. The Keratinophilic fungi isolates exhibited varying Macroscopic and microscopic characteristics. *T. megninii, T. mentagrophytes* and *M. nanum*showed moderate rapid growth whereas the growth rate of *T. rubrum* was slow to moderate rapid. *M. audounii* showed slow growth rate whereas the growth rate of *M. gypseum* was rapid. The isolates exhibited similar texture in petri dish except *T. rubrum* and *M. audounii* that were fluffy and cottony respectively. There was variation in their surface and reverse colours in Sabouraud Dextrose Agar (SDA) plate. *T. megninii, T. mentagrophytes* and *T. rubrum* produced numerous pyriformed microconidia and few pencil-shaped macroconidia. *T. mentagrophytes* produced spiral hyphae. *M. gypseum* and *M. nanum* produced few club-shaped microconidia whereas *M. audounii* produced terminal chlamydospores. *M. gypseum* and *M. nanum* produced numerous rough, thin walled macroconidia which was ethptical in formal and ovoid in the latter. *M. audounii* showed spindle-shaped, sparsely echinulated macroconidia which was poorly developed.

The study showed the occurrences of Keratinophilic fungi from the soil samples collected from poultry farm, goat farm and garden. *T. mentagrophytes* significantly ($p<0.05$) recorded the highest occurrences whereas the occurrences of *M. nanum* was the least. The soil sample collected from goat farm significantly ($p< 0.05$) recorded the highest occurrences of Keratinophilic fungi whereas the soil sample from the poultry farm showed the least occurrences. The occurrences of *T. mentagrophytes* was most from the soil samples collected from poultry farms and goat farms whereas *T. rubrum* recorded the highest occurrences in the soil samples collected from the garden.

The pathological scale of the studied Keratinophilic fungi revealed that the Immunocompromised albino mice were significantly ($p< 0.05$) infected compared to Immunocompetent mice. Among the Immunocompetent mice, *M. nanum* and *T. megniniis*howed zero infection whereas the maximum infection was recorded among those infected by *T. rubrum* which showed moderate infection. Severe infection was recorded among those Immunocompromised mice infected by *T. rubrum* whereas *M. gypseum* and *T. mentagrophytes* showed moderate to severe infection.

Table 1: Soil samples that was positive for keratinophilic fungi.

Soil source	Positive sample (%)	Negative sample (%)	Total (%)
Poultry farm	2(20.00)	8(80.00)	10(100.00)
Goat farm	8(80.00)	2(20.00)	10(100.00)
Garden	4(40.00)	6(60.00)	10(100.00)
Total	14(46.67)	16(53.33)	30(100.00)

Table 2: Macroscopic and microscopic characteristics of the isolates.

Isolates	Growth rate	Texture	Surface colour	Reversed colour	Macroconidia	Microconidia
Trichophyton megnini	Moderate rapid	Powdery	Pale pink	Red	Pencil-shaped	Pyriform to round shape. Numerous
Trichophyton mentagrophytes	Moderate rapid	Powdery velvety	White to creamy-tan	Reddish-brown	Club-shaped. Few	Pyriform to round shape. Numerous with spiral hyphae
Trichophyton rubrum	Slow to moderate rapid	Fluffy	White to pale pink	Wine red	Few smooth walled pencil-shaped	Pyriform in shape. Numerous
Microsporum audouinii	Slow	Cottony	Grayish-white	Rose-brown	Thick and spindle shaped, sparsely echinulated and poorly developed	Absent. Presence of terminal chlamydospores
Microsporum gypseum	Rapid	Powdery	White to buff	Red	Numerous rough, thin walled and ethptical	Club-shaped. Few
Microsporum nanum	Moderate rapid	Powdery	White to yellow	Red-brown	Ovoid shaped, numerous	Club-shaped. Few

Table 3: Occurrences of the isolates in the studied soil samples.

Isolate	Poultry farm (%)	N= 10	Goat farm (%)	Total (%)
M.audounii	1(1.85)	4(7.41)	2(3.70)	7(12.96)
M.gypseum	2(3.70)	5(9.26)	4(7.41	11(20.37)
T.rubrum	3(5.56)	4(7.41)	5(9.26)	12(22.22)
T.mentagrophytes	5(9.26)	11(20.37)	1(1.85)	17(31.48)
T.megninii	0(0.00)	4(7.41)	0(0.00)	4(7.41)
M.nanum	0(0.00)	2(3.70)	1(1.85)	3(5.56)
Total	11(20.37)	30(55.56)	13(24.07)	54(100.00)

Table 4: Pathological scales of the tested Keratinophilic fungi in Immunocompetent and Immunocompromised albino mice.

	Immunocompetent			Immunocompromised		
Isolate	Number of mice	Infection ratio	Extent of infection	Number of mice	Infection ratio	Extent of infection
Microsporum audouinii	4	¼	Mild	4	2/4	Moderate
Microsporum gypseum	4	¼	Mild	4	¾	Moderate to severe
Microsporum nanum	4	0/4	Nil	4	¼	Mild
Trichophyton rubrum	4	2/4	Moderate	4	4/4	Severe
Trichophyton mentagrophytes	4	¼	Mild	4	4/4	Moderate to severe
Trichophyton megninii	4	0/4	Nil	4	2/4	Moderate to severe

Mild – Alopecia or scaling, Moderate – Slight inflammatory crust, Severe – Inflammatory crust

DISCUSSION

The presence of the Keratinophilic fungi *T. megninii, T. mentagrophytes, T. rubrum, M. audounii, M. nanum* and *M. gypseum* in the studied samples supported the findings of many researchers (Pratyoosh *et al.*, 2003; Anbu *et al.*, 2004; Ogbonna and Pugh, 2007 and Ganaie *et al*., 2010). Richa and Neerja, (2015), reported the occurrence of Keratinophilic fungi *T. mentagrophytes, M. gypseum* and *M. audounii* in different soil samples collected from different area of animals' habitat including goat farms and poultry farm.

The occurrence of Keratinophilic fungi in the studied soil samples supported the findings of (Sure and Ghosh, 2000; Dixit and Kushwaha, 2001; Sarangi and Ghosh, 2001; Deshmukh, 2004). Samples from goat farm recorded the highest occurrences of Keratinophilic fungi and this agrees with the findings of (Harish, *et al*., 2004; and Richa and Neerja, 2015) and disagrees with the findings of (Deshmukh and Agrawal, 2003) and Deshmukh and Verekar, 2006). Richa and Neerja, (2015) reported the highest occurrences of Keratinophilic fungi *T. mentagrophytes, T. rubrum, M. audounii* and *M. gypseum* in soil samples collected from goat farm and garden. The highest occurrence of *T. mentagrophytes* in soil samples from poultry and goat farms corroborated with the findings of (Anbu et al., 2004; Ogbonna and Pugh, 2007 and Richa and Neerja, 2015). Jain and Sharma, (2011) reported the highest occurrence of *T. rubrum* from poultry and goat farms. Tambekar *et al*., (2007) reported the highest occurrence of T. rubrum in soil samples from garden, and this is in line with the findings of this study.

The pronounced pathological scale exhibited by the Keratinophilic fungi supported the findings of (Ogbonna and Pugh, 2007 and Al-Shimaa *et al*., 2015). The Immunocompromised mice were mostly infected in the present study. This could be due to their impaired immune system, which makes the immune system of the mice unable to fight infection adequately. This is in line with the report of (Bowman *et al*., 2007; Vincent *et al.*, 2010 and Claire *et al*., 2016). Ramos *et al*., (2010) also reported that host with compromised immune system are more susceptible to infections, which might be more severe than when compared to an Immunocompetent host.

The severity of infection exhibited by *T. mentagrophytes* and *T. rubrum* in the studied Immunocompromised albino mice agrees with the findings of (Anbu *et al.*, 2004; Santwana, 2007 and Claire *et al*., 2016), but disagrees with the findings of (Mark *et al*., 2015 and Theresa *et al*., 2019). Ran *et al*., (2003) also reported that *T. rubrum* causes severe infection.

CONCLUSION

This study has shown the presence of *T. mentagrophytes, M. audouni, M. gypseum, T. rubrum, T. megninii,* and *M. nanum,* of which *T. mentagrophytes* was mostly encountered in the studied samples. The isolates showed significant pathological scale, which was more pronounced in immunocomprised mice.

REFERENCES

1. Al–Shimaa, M.A., Ouf, S.A., Tarek, A.A. and Eltahlawi, S.M. Dermatophytes and other associated fungi in patients attending to some hospitals in Egypt. *Brazilian Journal of Microbiology*, 2015; 46(3): 799–805.
2. Aziz, A.I. and Seema, C.E. Microbioilogical and physicochemical qualities of selected commercial poultry feeds in Akure, Nigeria. *Journal of Biological Sciences*, 2015; 7(6): 981–984
3. Anbu, P., Hilda, A. and Gopinath, S.C. Keratinophilic fungi of poultry farm and feather dumping soil in Tamil Nadu, India. *Mycopathologia*, 2004; 158(3): 303–309.
4. Bowman, M.R., Pare, J.A., Sigler, l., Naeser, J.P., Sladky, K.K., Hanley, C.S., Helmer, P., Phillips, L.A.,Brower, A. and Porter, R. Deep fungal dermatitis in three inland bearded dragons (*pogona vitticeps*) caused by *chrysosporium* anamorph of *nannizziopsis vriesii. Medical Mycology*, 2007; 45: 371–376.
5. Claire, R., Roderick, H., Olivier, C., Nicolas, D., Anne, P., Olivier, L. and Fanny, L. Severe dermatophytosis and acquired or innate immunodeficiency: A review. *Journal of Fungi*, 2016; 2(1): 4–9.
6. Deshmukh, S.K. keratinophilic fungi on feathers of pigeon in maharashtra, India. *Mycoses*, 2004b ; 47(5–6): 213–215.
7. Deshmukh, S.K. and Agrawal, S.C. Prevalence of dermatophytes and other keratinophilic fungi in soils of Madhya Pradesh, India. *Mycoses*, 2003; 26(11): 574–577.
8. Deshmukh, S.K. and Verekar, S.A. The occurrence of dermatophytes and other keratinophilic fungi from the soil. *Czech Mycology*, 2006; 58(2): 117–124.
9. Dixit, A.K. and Kushwaha, R.K.S. The occurrence of keratinophilic fungi on indian birds. *Folia Microbiologica*, 2001; 36(4): 383–386.
10. Ganaie, M.A., Sooh, S., Rizvi, G. and Khan, T.A. Isolation and identification of keratinophilic fungi from different soil samples in Ihansi City, India *Plant Pathology Journal*, 2010; 9(4): 194–197.
11. Guy, G. Identifying Filamentous Fungi: A Clinical Laboratory Handbook, Second Edition. Star publication, Belmont, California, 2006; 78–82.
12. Harshe, C.G., Soni, S. and Kharl, W. A preliminary study on the occurrence of keratinophilic fungi in soils of Jamaica. *Revista do Instituto de Medicina Tropical de Sao*, 2014.
13. Jain, N. and Sharma, M. Distribution of dermatophytes and other related fungi in Jaipur city with particular reference to soil pH. *Mycoses*, 2011; 54(1): 52–58.

14. Iheukwumere, I.H., Olusola, T.O. and Chude, C. Molecular characterization and diversity of enteric bacteria isolated from chicken feeds. *Journal of Natural Sciences Research*, 2018; 8: 21–33.
15. Mark, T.W., Nicole, B., James, G. and Stephen, W.B. Biology and Diseases of Mice, Third Edition. Elsevier incorporation, California, 2015; 43–149.
16. Ogbonna, C.I.C. and Pugh, G.J.F. Keratinophilic fungi from Nigerian soil. *Mycopathologia*, 2007; 99(2): 115–118.
17. Pratyoosh, S., Shukla, C.B., Naveen, K. and Amritash, S. Isolation and characterization of a dermatophyte, *Microsporum gypseum* from poultry farm soils of Rewa (Madhya Pradesh), India. *Pakistan Journal of Biological Sciences*, 2003; 6(6): 622–625.
18. Rahul, S. and Rajak, R.C. Keratinophilic fungi: nature's keratin degrading machines. *Resonance*, 2003; 8(9): 28–40.
19. Ramos, E.M., Lima, C.M., Schechtman, R.C., Trope, B.M. and Carneiro, S. Superficial mycoses in immunodepressed patients (AIDS). *Clinical Dermatology*, 2010; 28: 215–217.
20. Ran, N.P., Hila, E., Gerald, P. and Itzhack, P. Deep infection by *Trichophyton rubrum* in an immunocompromised patient. *Journal of Clinical Microbiology*, 2003; 41(1): 5298–5301.
21. Richa, S. and Neeraj, C. Isolation of keratinophilic fungi from soil samples of agricultural fields of Saharanpur (u.p), India. *International Journal of Current Microbiology and Applied Sciences*, 2015; 4(7): 229–237.
22. Santwana, V. Current spectrum of dermatophytosis in a tertiary care hospital of North India. A 6–year clinic mycological study. *Journal of Medical Science and Clinical Research*, 2007; 5: 19488–19494.
23. Sarangi, S. and Ghosh, G.R. Survey of keratinophilic fungi inhabiting *passer domesticus* in two districts of Orissa, India. *Mycopathologia*, 2001; 114: 109–116.
24. St–Germain, G. and Summerbell, R. Identifying fungi. A clinical laboratory handbook, Second Edition. Star publishing company, Korea, 2010; 71–84.
25. Sure, B. and Ghosh, G.R. Keratinophilic fungi from orissa, India, ii: isolations from feathers of wild birds and domestic fowls. *Sabouraudia*, 2000; 18(4): 275–280.
26. Tambekar, D.H., Mendhe, S.N. and Gulhane, S.R. Incidence of dermatophytes and other keratinolytic fungi in the soil of Amravati (India). *Trends in Applied Sciences Research*, 2007; 2: 545–548.
27. Theresa, B., Agnes, F., Candy, G., Silke, U., Tilo, H., Pietro, N., Christoph, G.B. and Wieland, S. Trichophyton benhamiae and *T.mentagrophytes* target guinea pigs in a mixed small animal stock. *Medical Mycology Case Reports*, 2019; 23: 37–42.
28. Vincent, C.M., Richard, K., Francisco, M.M., Duane, R.H. and Camille, N.K. Disseminated dermatophytes in a patient with hereditary hemochromatosis and hepatic cirrhosis: case report and review of the literature. *Medical Mycology*, 2010; 48(3): 518–527.