The Study of Dermatophytes and Saprophytes isolated from Stray Cats in Jeli, Kelantan

By

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A report in fulfillment of the requirements for the degree of Bachelor of Applied Science (Animal Husbandry Science) with Honours

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

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Date: **20 JANUARY 2018**

I certify that the report of this final year project entitled "**The Study of Dermatophytes and Saprophytes isolated from Stray Cats in Jeli, Kelantan**" by **NIK NOOR SYAMIMI BINTI ISMAIL**, matric number **F14B0468** has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Animal Husbandry) with Honours, Faculty of Agro-Based Industry, University Malaysia Kelantan.

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The Study of Dermatophytes and Saprophytes from Stray Cats in Jeli, Kelantan

ABSTRACT

Feline or cat is a small mammalian and carnivore. Human valued cat by adopting them as pet and discard them at public places when they are not needed. Stray cats are the animals that independently survive where they find food and shelter by their own. They are free living animal that expose to spore and has high risk of transmission of disease especially fungus. The purpose of this research is to isolate and identify the fungus on stray cat haircoat and to observe the morphological characteristics of the fungi. Swab of cat's hair coat was used as specimen collection and isolated to Sabouraud Dextrose (SDA) agar with addition of chloramphenicol. SDA agar used in the research and emphasize more to its morphological characteristics and microscopic identification. Results from this research are Dermatophytes (P=0.8311) and Saprophytes (P=0.1034) were found as they are the causal of fungus diseases especially in cats by accepting the hypothesis.

Keywords: cat, fungus, stray cats, fur, mould

Kajian terhadap Dermatofit dan Saprofit daripada Kucing Liar di Jeli, Kelantan

ABSTRAK

Feline atau kucing ialah binatang mamalia dan karnivor kecil. Manusia menghargai kucing dengan mengambil sebagai binatang peliharaan dan buang di tempat awam apabila tidak diperlukan. Kucing jalanan adalah binatang bebas yang dapat hidup jika mereka jumpa makanan and tempat berlindung. Kucing liar juga binatang bebas terdedah dengan spora dan berisiko tinggi terhadap penyakit terutamanya kulat (fungus). Tujuan utama kajian ini ialah untuk mengenal pasti flora kulat kucing jalanan sekitar Jeli dan menentukan kekerapan penyakit zoofilik dan zoonotik bawaan kucing dalam populasi kajian. Swab yang diambil daripada bulu kucing dijadikan koleksi spesimen dan isolasikan ke Sabouraud Dextrose (SDA) media agar dengan tambahan Chloramphenicol. SDA media agar digunakan dalam kajian ini untuk menekankan lagi terhadap karakteristik morfologi dan identifikasi mikroskop. Hasil daripada kajian ini ialah Saprofit (P=0.1034) dan Dermatofit (P=0.8311) oleh kerana penyakit fungus biasa yang berlaku pada kucing di samping keputusan hipotesis kajian ini diterima.

Kata kunci: kucing, fungus, kucing jalanan, bulu, kulat

TABLE OF CONTENTS

LIST OF TABLES

NO. **PAGE**

LIST OF FIGURES

NO. **PAGE**

LIST OF ABBREVIATIONS AND SYMBOLS

SDA	Sabouraud Dextrose Agar
UV	Ultraviolet
^{o}C	Degree Celsius
sp/spp	Species
AAT	Animal-Assisted Therapy
mg	milligram
kg	kilogram
L	litre
ml	mililitre
%	percent
LCBS	Lactophenol Cotton Blue Stain
UMK	Universiti Malaysia Kelantan
FIAT	Fakulti Industri Asas Tani / Agro Based Industry Faculty
PCR	Polymerase Chain Reaction
ELISA	Enzyme-Linked Immnuno Assaat
(ITS)	internal transcribed spacers
BSC	Biological Safety Cabinet

CHAPTER 1

INTRODUCTION

Mycoses are fungal infection of fungi organism that present in environment, which is primary cause if from soil whether by breath, eating and direct, and indirect contacts to the fungal elements. The fungi will infest the host that is weak or immunosuppressed. The infection also can spread in the host by infecting multiple organ system of the host. Cat is one from the domestic animals that are often affected such as ringworm and Sporotrichosis. Examples of the other domestic animals that also affected are dogs, cattle, sheep, goat, horses, pigs and farmed crocodiles. For instance, pathogenic fungi diseases are Histoplasmosis, Coccidioidomycosis, Blastomycosis, Cryptococcosis, Rhinosporidiosis, Candidiasis, Aspergillosis, Zygomycosis, Phaeohyphomycosis, Hyalohyphomycosis, and Oomycosis (Pythiosis And Lagenidiosis) (Taboada, n.d.).

Bryan, Finola, Marie, Ann, & Dores (2013) summarized that fungus that involves the use of keratin in host for growth is dermatophytes. They tend to attack at outer layer skin, nails, claws and hair or animal and human. One of common infection from cat or feline is ringworm which causes round and circular typical lesions to the skin. Dermatophyte is classified in genus Arthroderma, phylum of Ascomycota. It is Deuteromycota (Fungi Imperfecti) which are Microsporum, Trichophyton and Epidermophyton that in genera of anamorphic (asexual or imperfect state). In addition it also included in genera teleomorphic (perfect or sexual) state for special cases.

1.1 Problem Statement

The study research related to fungal study is lack in Malaysia and mostly of the fungal research are not related to cat but related to others such as soils, plants and insects only available.

The journals viable used for this study mainly from the year of 1990's which already more than 5 years from the year of this study been done plus they were from outside from Malaysia. Hence, the older version and of journals were used to make as references.

1.2 Hypothesis

1. Saprophytes

   H_0: All stray cat's samples collected have the growth of Saprophytes in the media agar.

   H_1: All stray cat's samples collected do not have the growth of Saprophytes in the media agar.

2. Dermatophytes

   H_0: All stray cat's samples collected have the growth of Dermatophytes in the media agar.

   H_1: All stray cat's samples collected do not have the growth of Dermatophytes in the media agar.

1.3 Objectives

1. To isolate and identify the fungus on stray cat haircoat.
2. To observe the morphological characteristics of the fungi of isolated fungus from the stray cat fur.

1.4 Scope of study

The scope of this study is to isolate fungi from stray cat's coat fur and determine the morphology characteristics of grown fungi from the samples. A number of samples will be collected from stray cats by depending on accessibility of laboratory's materials and milestone duration of the research as the aim to achieve the objectives to this research. This study can be further by the other related field of study such as zoonotic disease, animal disease and public health since fungus have the species of zoonosis which can cause disease to animal and human.

1.5 Significance of study

The numbers of stray cats population in Jeli is increasing as the human population is spreading also. There is no any study research on cat's fungus around Jeli thus this is an opportunity to apply the fungi study on cat particularly stray cats health by knowing the cause of main fungus causing type of disease especially the zoonosis type which also can affect the public health. This study can provide valueable information to environmental organization of spreading disease and can help for further research of zoonosis in Malaysia. In the other way, this study is a novel finding of study on mycology field of research towards feline in Malaysia and this is one of the steps to gain more interest of other researchers to contribute in mycology area.

1.6 Limitation of study

There were few limitations faced during the research. Firstly, there are lacks of morphological characteristic references (images) on fungus grow on media such as books available in library UMK and Internet sources. So this research only involves morphological characteristic and microscopic identification and population data analysis.

The samples of strays cats might give consequences to handler if not take precautions in hygienic and wear proper attire during sample collection or research handling in laboratory because the cats have high possibility to spread contagious disease such as Sporotrichosis or Ringworm. The sample might contain fungus flora and some fungi are zoonotic. Fungus can be spread by direct contact between animals and humans especially skin or even worse in air by fungi flora (Welsh, 2003).

Lastly, the laboratory's equipments on biosecurity not following the Standard Operation Procedure that specialized for fungi research to applied in Universiti Malaysia Kelantan (UMK) Jeli Campus. Here still not upgraded its systems of laboratory and did not have special equipments specifically for fungi research uses such as incubators, microscopes and Biological Safety Cabinet Class III. This is because the spores from fungus can be spread to human and dangerous to the laboratory surroundings if not apply the strict practices on handling the fungus specimens. This research was cancelled after a week incubated at UMK Jeli Campus as the Head of Biosecurity of Faculty Agro Based (FIAT) seized all the incubated agar media plates and needed to continue the research at UMK Kota by using the Veterinar Faculty's (FPV) laboratory. The visits to the FPV laboratory were able to manage for once a week. Therefore, the daily observation of fungus growth was not able to be done.

CHAPTER 2

LITERATURE REVIEW

2.1 Cat

Cat can be found anywhere in this world other than these countries Antarctica, Australia, New Zealand, Madagascar and Japan. When there is a population, there is cat. Authorities have recognized few genera of Felidae which are 18 genera and 36 species. But felid of big cats (tiger, lion, leopard & etc.) are friendless but for small felids, they are domesticated to be social to human and by their anatomy of toughening hyoid bone where small cats cannot roar as big cats (Etnyre, Lande & Mckenna, 2011). The colony of urban stray cats depends on food and shelter given by the environment of the animals lived and the behaviour of the cats progression by social system from the area they lived (Natoli, 1985).

Cats also used in human therapy treatment as an article of Animal-assisted therapy with farm animals for persons with psychiatric disorder: effects on self-efficacy, coping ability and quality of life, a randomized controlled. A trial by Berget, Ekeberg, & O Braastad, (2008) claimed that Animal-Assisted Therapy (AAT) to treat patient of mental disorders with animals such as cats is efficient in handling ability for the psychiatric. The patients were treated for 6 months by self-efficacy by different behaviours such as patting, brushing, looking after, feeding, and communicate with the cats (Berget, Ekeberg, & Braastad, 2008). In other research appealed that AAT can help the loneliness of resident for long term facilities therapy, 45 residents with varied background joined the research and results showed that they longing to their previous pets as they were needed half an hour, three times in a week spent time with the pets.

This longing to cats presented the reduced of resident's loneliness and was effective (Banks & Banks, 2002).

In addition, S. Hartwell (2003) claimed in an article that cat fur is a business. The fur is sold in some countries such as in United Kingdom and Europe Union countries as cat fur is not an illegal to sell and often mistaken as artificial fur by the customers. The writer also stated that China is the biggest importer or producer as in China they even have cat farms specialized for fur productions and meat productions (Hartwell, 2003).

2.2 Fungus

Khosravi & Mahmoudi, (2003) found that the common saprophytic fungi isolated were *Penicillium* spp., *Aspergillus* spp., *Altenaria* spp., *Mucor* spp. and *Clasdosporium* spp. *Acremonium* spp., *Candida* spp., *Cephalotrichum* spp., *Chrisosporium* spp., *Geotrichum* spp, *Gliocladium* spp., *Malassezia* spp., *Microsporum gypseum* spp., *Paecilomyces* spp., *Rhinocladiella* spp., *Rhizopus* spp., *Scopolariopsis* spp., and *Trichoderma* spp. While for dermatophytes fungus is *Microsporum canis* with result of 26 cats were positive and only two cats showed physical infection on their skin or fur while the others just as carrier and healthy.

A research was made in the Pacific coastal of USA, which was divided into four regions. The regions are Region 1 (San Francisco bay area, CA), Region 2 (Los Angeles basin, CA), Region 3 (Seattle metropolitan area, WA) and Region 4 (Sacramento valley, CA). Dermatophytes were discovered as 11 cats with dermatophytes and the rest with saprophytes; 97 cats. Ringworm or *M. canis* and *Trichophyton mentagrophytes* were discovered for dermatophytes infections which in region 1, region 2 and region 4. The rests were *Penicillium* spp, *Scopulariopsis* spp.,

Aspergillus spp., *Chrysosporium* spp., *Cladosporium* spp. and *Alternaria* spp. (Boyanowski, Ihrke, Moriello, & Kass, 2000).

Besides, in city of Mexico and Nezahualcoyotl have showed result with 67 cats out of 100 cats that positive with Keratinophilic fungi. This research used method of seasonal where they collect samples from period of September 1996 to April 1997. Fungi organisms that growth were *Chrysosporium* spp., *T. terrestre* spp., *M. gypseum* spp. and *M. canis* spp.. The most total of fungi strains was *C.* spp. Data from the research stated that samples they collected were from breed animals and stray animal and eventually, both are equally affected with the same fungi (Guzman Chavez, Segundo Zaragoza, Cervantes Olivares, & Tapia Perez, 2000).

Two populations from different area in USA which were from northern (cold climate) and another one from southern (warm climate) of US collected with total 150 cats samples. The observations showed fungi dermatophytes (*T. rubrum* spp. and *Epidermophyton* spp.) were known more collective in cats for northern. In other hand of southern, dermatophytes (*T. verrucosum* spp*., M. canis* spp*., T. mentahrophytes* spp.) were significantly more common in cats (Boyanowski, Ihrke, Morielle & Kass, 2000).

2.2.1 Saprophytes

Saprophytic fungi are common infection to cats but dermatophytes is a necessity concern to the cat owners, veterinarians, and doctors as it can attack to cats that has immunosuppressed or immunocompromised. There are some factors for infections to occur such as geographical location and living conditions of animal living. (Boyanowski et al., 2000).

Lappin, (2001) explained that uncommon fungus mycobacterial infections are *Mycobacterium fortuitum* spp *Mycobacterium chelonae* spp*., Mycobacterium phlei* spp*., and Mycobacterium smegmatis* spp. These saprophytes depend on animal's immunocompetent and feline are easier to get this infection rather than other species and even to human as this infection is deliberately not a zoonotic.

As said by researcher Riita, (1983) fungus of Saprobes is the main fungi will grow in first isolation of media when researcher used to study the animal doubted infested with dermatophytes. The unknown fungi isolated by living creature of healthy such as animal or human can have probability of pathogenic if the the creature lack of immune systems (Aho, 1983; Khosravi & Mahmoudi, 2003). In the common species from saprophytes can be isolated easily from cats are Penicillium, Aspergillus, Altemaria, Mucor and Cladosporium spp. (Khosravi, 1996).

There are many ways cat can get fungi such as *Aspergillus* spp. from their consuming diet, *Alternaria* spp., *Chrysosporium* spp., *Ulocladium* spp., *Fusarium* spp., and *Mucor* spp., from its surroundings, and for *Penicillium* spp., they can obtain from the soils (Ilhan et al., 2016).

2.2.2 Dermatophytes

Dermatophytoses are strong mycology living creature which gives effect mycoses of dermatophytes. They take over the keratized tissues area of patient's and don't care whether human or animals. There are three types of dermatophytes which are mainly caused invasion to human only, human to animal and animal to human with different species. Dermatophytes comprise of four genera, which are Microsporum, Trichophyton, Arthroderma and Epidermophyton with Microsporum spp. where commonly affecting pets animal such as cats, dogs, horses and livestocks. The usual

finding from previous observations, 80% of cat that have no lesion and with lesion and dogs with skin lesion caused by dermatophytes (Claudia Cafarchia et al., 2013).

There are three groups of basic dermatophyte types which are Antropophilic; fungus infection in human that can transfer to human too but very rare case of transmission human to animal such as *M. audouinii,* Geophilic; dermatophytes that infest human and animals such as *M. gypseum,* and *T. terrestre,* and Zoophilic; those found in animals, but transmitted to other or humans (M. canis, *T. mentagrophytes*). Zoophilic or zoonotic dermatophytes which transferred from different species to another species like animal disease to human are the cause of lesions on human such as Ringworm, and Sporotrichosis (Bernardo, Guerra, & Martins, 2005).

Aho, (1983) has done a study on dermatophytes where the samples taken from hair of animals in Finland. Two cats of isolated were positive with dermatophytes of *Microsporum canis* and *Tricophyton terrestre* which has fungal species that is zoonosis to human dermatophytosis. The other animals that affected also were three dogs, one horse, two guinea pigs and one rabbit of both dermatophytes. The cats were from house pet and from the research, out of 61 cats tested, 13 were positive with *Microsporum canis.*

Research journal by Nita and et al. (2016), they suspected Persian cat with long hair coat was carrier of *M. canis* infection. But through the research, there was no dermatophytes detected. The samples he took was from a catteries located at Sao Paulo with total 61 of healthy cats. Differ in England research, total of positive dermatophytes were 9 cats from 139 cats. The rest with 130 cats were negative for the result. Fungi of isolated were *M. canis, T. mentagrophytes, T. terrestre* and *Malassezia pachydermatiss.* The researchers said the spread might because of rodent transmission as the cats has history catching rats in the Britain city

2.3 Implication of fungi on cat

Previous study by Refai, Marouf, Abuelala, & Sayed El-Ahl, (2016) reported fungal diseases of cats from dermatophytes. As we know dermatophytes mostly are zoophilic and zoonotic, so they can spread disease and bring concern to animal health and public health. First, Ringworm; which cause by *Microsporum canis, M. gypseum, Trichophyton mentagrophytes, M. persicolor, T. verrucosum* and *T. quinckeanum*. The author stated the transmission of ringworm depend on factors such as direct contact zoophilic, contaminated things used by animal and airborne spore transmission. Second, Candidiosis which is from yeast infection commonly at animal's genital and gastrointestinal tracts. Examples of Candidiosis in feline are *Candida albicans*, Candida sp., and *Candida parapsilosis*. Third, Cryptococcosis also can affect the cat. It is an infection from *Cryptococcus neofromans* and *Cryptococcus gattii* which can visible at nasal form, lymph nodes, ocular, central nervous system and etc. Fourth is Malassezia disease. The most easily to be found is Malassezia pachydermatis which is a yeast infection full biotope of yeast on the animal skin and ear. Fifth, Sporotrichosis disease in cat is caused by *Sporothrix schenckii* where this disease can cause outbreak if not in control such as in Brazil before.

2.4 Fungus identification

2.4.1 Morphological characteristics

The conventional method of mycology identification which still be used until today to see the morphological physical appearance of fungi is by see its charateristics of macroscopic and microscopic structures available which can be seen virtually by our eyes (Malinovschi, Kocsubé, Galgóczy, Somogyvári, & Vágvölgyi, 2009).

In the other Website from University of Adelaide handled by Dr David Ellis, (n.d.), there are six ways to observe a culture characteristics as a key to steps of morphological characters. They are the apparent texture, surface topography, surface coloration, contrary colour, the growth rate and growth temperature.

2.4.2 Antifungal assay

For a suspected dermatophyte sample can use broth micro dilution assay by using anfungal agents of fluconazole, ketoconazole, itraconazole, miconazole, griseofulvin and amphotericin-B to see the range of susceptibility alongside with Dermatophyte Test Medium (DTM) tubes by changes of colour after incubate the DTM at 28°C for three weeks. Other than that, MIC or minimal inhibitory concentration is another way to test antifungal susceptibility form of isolated dermatophytes which uses six of antifungal agents (Cox, 2010; Debnath, Mitra, Kumar, & Samanta, 2016).

2.4.3 Polymerase Chain Reaction

Gabriela *et al*,(2009) did a research on dermatophyes of *M. canis* and *T. tonsurans* as these two pathogens are zoonotic and can bring serious infection to patient plus the sizes of these two dermatophytes are significantly not same. The research was verified by the gene region of internal transcribed spacers (ITS) sequenced in the research using PCR of usual contrast primer. Many PCR techniques have been done for dermatophytes research especially for human but lack for animal research where the analysis of animal required to use only certain genetic markers available by previous (Claudia Cafarchia et al., 2013).

2.4.4 ELISA test

Enzyme-Linked Immunosorbent Assay (ELISA) is an assessment of using antibodies and colour changes to identify a substance of certain sample tests. The test is an accurate result where it is a good precautions steps towards the infection but required skills and long time to have the result. The blood samples were taken by seeing its difference in IgG-specific levels whether positive or negative result of fungus species of *M. canis.* While for Western Blot technique identifying the *M. canis* by banding of 13 bands and 50 kDa protein (Santana et al., 2017).

2.4.5 Wood's lamp

The Wood's lamp or ultraviolet (UV) lamp can detect prevalence of dermatophytes on animal especially cat an dog fur or haircoat. The UV exposure then can detect by glowing yellow-green colour or fluorescene of dermatophytes such as *M. canis* which caused by tryptophan metabolites from dermatophyte species. But, this UV lamp can not be a main measurement of detection dermatophytes as some of the species which are (*T. mentagrophytes, M. persicolor or M. gypseum*) cannot fluoresce or from effect of topical medication intake (Breu, Guggenbichler, & Wollmann, 2011; C. Cafarchia et al., 2004; Nitta, Teixeira Daniel, Taborda, Santana, & Larsson, 2016; Proverbio et al., 2014).

CHAPTER 3

MATERIAL AND METHODS

3.1 Material

3.1.1 Chemicals, reagents and equipment

Lactophenol cotton blue stain and immersion oil are essential when identifying the cell morphology of fungi under microscope. While Sabouraud Dextrose Agar powder, distilled water and 0.05 g/L chloramphenicol (Sierra, Guillot, Jacob, Bussiéras;, & Chermette, 2000) are the ingredient to make agar media in agar plates.

3.2 Methods

3.2.1 Sampling collection area

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JELI

Figure 3.1

PINGGIRAN UMK, JELI

Figure 3.2

KAMPUNG GEMANG, JELI

Figure 3.3

PASAR JELI, JELI

Figure 3.4

PASAR AYER LANAS, JELI

Figure 3.5

The samples were collected around Jeli because it is located close to with the study research activity such as laboratory, accommodation and study place. Jeli is a district that not a domain place to be discovered yet for study research especially related with fungus study on stray cats. Figures 3.1 (a) to 3.5(e) are the places are public places and many stray cats were discoverable at there. The locations samples

collected were at UMK Campus Jeli (5°44'41.6"N 101°51'50.3"E), Taman Pinggiran UMK (5°44'56.4"N 101°51'51.8"E), Pasar Ayer Lanas (5°47'02.2"N 101°53'17.4"E), Pasar Jeli (5°41'32.9"N 101°50'49.2"E) and Kampung Gemang (5°45'43.8"N 101°51'55.9"E).

3.2.2 Collection of sample

Table 3.1: Table of experimental design

Areas for sample collection	Number of collected samples
UMK Campus Jeli	4
Taman Pinggiran UMK	3
Pasar Ayer Lanas	12
Pasar Jeli	11
Kampung Gemang	2

Before proceed the swabbing, proper attire was needed such as wearing rubber gloves and mask because stray cats are exposed to diseases and they might contagious to human by Zoonotic diseases.

First was to prepare a cat by feed the cat with cat food. This tactic was to restrain and attract the cat from moving and ease the sample collection procedure.

Figure 3.6: The picture of swabbing the cat's head fur.

Next was open the cotton swab and collect the swab specimen using wet swab/cotton bud by rolling the swab lightly back and forth to the main areas as (Khosravi & Mahmoudi, 2003) did in their research by swabbing head area, through the neck surfaces, dorsum, trunk, ventrum, limbs and tail. The swab was placed into the zipper plastic bag and labelled with code reference number. A cotton swab was for a cat and these steps were repeated as cat available during collection. The samples were transferred into refrigerator immediately at 4^{o}C and were kept at least for 3-4 days used as specimen.

3.2.3 Media preparation

This research used Sabouraud Dextrose agar from the brand of OXOID. SDA is a common medium used in growing contaminant fungi. The media is selective agar and more efficient when added 0.5mg/ml chloramphenicol powder type, bottle of 25g brand of Nacalai. Chloramphenicol is an antibacterial which can reduce contamination of bacteria grows on media other than fungus. The media of SDA has pH of 5-6 which suitable for common fungus growth pH and its measurement is 65g of SDA powder for 1L media agar (Bryan, Finola, Marie, Ann, & Dores, 2013). The first step in making media preparation was by measure the medium powder (refered to the side of the

bottle) mix with distilled water by how much wanted to use. Then, the glass bottle was placed on hotplate by stir using magnetic bar and little heat to mix the powder and dH_2O until completely dissolved. After that the mouth of glass bottle were covered with cap and labelled. The bottle was placed in the autoclave basket and loosen its cap before start the autoclave at 121°C for 15 to 20 minutes. Lastly, the glass bottle was taken out from the autoclave and was let cool by the room temperature or rinsed the outer bottle with tap water to half-cool it.

The procedures were done in Biosafety Cabinet Class II (BSCII) as to ensure the security of the handler and environment from unknown fungus of collected specimens. The BSC firstly was closed it sliding sash and opened Ultraviolet (UV) light for 15 minutes and opened the blower for 10-15 minutes for air circulation in the BSC. The surface of workplace inside the BSC was sterilized with 70% ethanol by wiping the surface (ESCO, 2004). Bunsen burner was light on inside the BSC and the materials and equipment needed were placed inside BSC such as agar glass bottle and new empty media plates. The media agar was poured 1/3 amount only from the depth of the agar plate. Then it was covered its lid and let cool or hardened. This process was repeated until the agar poured into new plates. The type of media and date of media made was labeled behind the plate. After the agar cool and hardened, the media plates were placed inverted to prevent condensation and packaged in plastic with labelling of name, date media made, type of media and matric number. The plastic of media plates then placed in refrigerator at temperature of $4^{o}C$ and can be kept and use for one to three months if not contaminated at its surface of media with bacteria or unknown contamination (Tiwari, Hoondal, & Tewari, 2009).

3.2.4 Isolation

The specimens was made sure prepared inside the BSC workplace together with the procedure of preparing the workplace using BSC such as UV light treatment, surface sterilization with 70% ethanol and light on the Bunsen burner . First step was to take out the media plates of SDA out from refrigerator and placed them at the workplace. The back of media plates were labelled first before streaking the specimen on it. The details were name, date, and code of specimen. After that, the zipper bag of the specimen cotton swab was opened and taken out its swab. The swab was streak to the surface the agar plate was streak using medium swab by four rotation streaking technique (Tiwari et al., 2009). The isolation streaking technique was by streak the top one-fourth of the dish plate. The cotton swab specimen was placed back into the zipper bag for back up for any redo of futher experiment. The lid of media plate was covered and Parafilm seal was used to wrap the sides of agar plate to prevent contamination from outside (Sanders, 2012). The agar plate is incubated invert, upside down for 7 days at 37^{o}C (Markey, 2013). Daily observation was done and growth was recorded.

Subculture of selected fungi from isolation culture media plates were made in new agar plates to get a single colony of fungus. Same labelling was done for each new plates. A loop inoculation was used to pick a colony from the isolation culture media plates by touch or placed a piece pick from the isolation plate of colony at the centre of fresh new media plate of SDA. The agar plates were incubated again for 7 days and observed daily for the fungus growth (Hamizah & Hamid, 2015).

3.2.5 Incubation

Incubation is a machine where giving optimum conditions to the microorganism to grow via in-vitro. The growth of fungal depends on species but in general and short

incubation duration is 5 days to 2 weeks at 25ºC (Bosshard, 2011). The agar plates are best to stack 2-3 layers only as each plates required oxygen exposure from its outer as dermatophytes are aerobic (Bryan et al., 2013; Sanders, 2012). For the isolation, this research was done using incubation temperature of 37ºC as specialized dermatophytes are able to grow well at temperature of 37ºC (Bryan et al., 2013).

3.2.6 Identification

3.2.6.1 Fungus morphological characterization

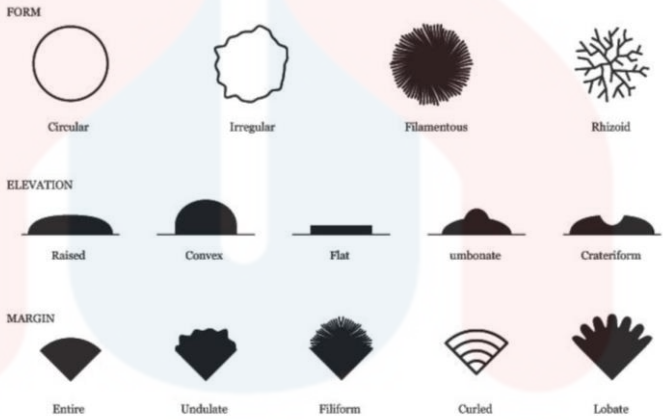

Figure 3.7: The fungus colony morphology chart (Microbiology Online, 2015)

The observation features used in classification and identification of fungi on media plates was form, elevation, and margin of fungus grow on media agar plate as shown in the Figure of 3.2.6.

3.2.6.2 Microscopic identification

The slide observation was performed by aid of wet mount Lactophenol cotton blue stain (LPCB) as it can give microscopic examination in expressions of hyphae, macroconidia and microconidia (Khosravi, 1996; Moriello, 1990; Seker & Dogan,

2011). The steps were the new glass slide added with a drop of Lactophenol cotton blue brand of Merck. By using inoculating needle or loop, a bit of fungi colony was picked and mixed at the drop of stain. The cover slip was placed over the stain using forceps to cover the stain and the slide was examined under compound microscope.

CHAPTER 4

RESULT & DISCUSSION

4.1 The cats number collected for samples

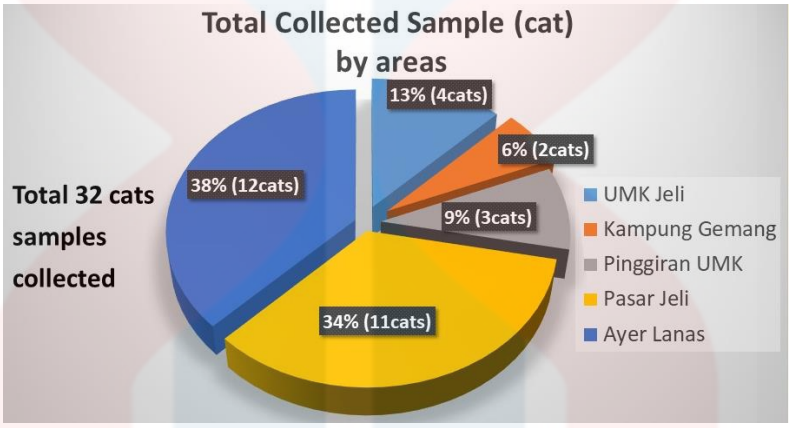

Figure 4.1: Pie chart of percentage samples collected by different areas.

Referring to Table 4.1 Pie chart of collected sample, total of cats collected were 32 cats. The most highest frequency cats in those 4 areas was in Pasar Ayer Lanas which contribute 38% out from 100% with 12 cats were swabbed. Next second highest cat area collected was from Pasar Jeli as much as 34% with 11 cats succesful collected specimens. After that followed by UMK Jeli with 4 cats which is taking 13% out of all and continue with Pinggiran UMK that resulting percentage of 9% with 3 cats. Last area involved was in Kampung Gemang where able to collect 2 cats only with 6%.

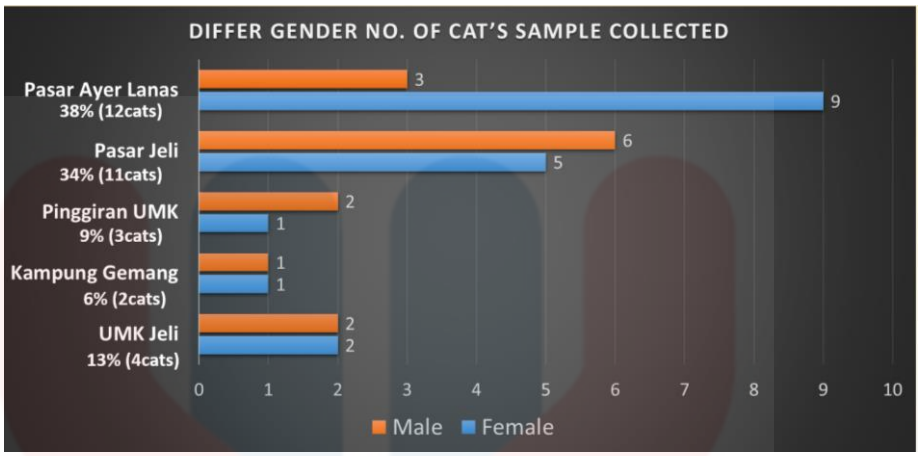

Figure 4.2: The collection of samples by differentiate the cat gender on areas collected.

Figure 4.2 showed the most collected samples for female was from the area of Pasar Ayer Lanas, then Pasar Jeli, UMK Jeli, and similar number with a cat for both places were from Kg. Gemang and Pinggiran UMK. Contrary with male cats, the highest was from area of Pasar Jeli, then Pasar Ayer Lanas, same amount of cats of two at place of UMK Jeli and Pinggiran UMK and lastly with one cat at the Kg. Gemang.

4.2 The number of fungus species present at five different places.

Table 4.2 : The area of sample collected by the fungus species

AREA SAMPLE COLLECTED	*Aspergillus flavus*	*Aspergillus fumigatus*	*Aspergillus nidulans*	*Aspergillus niger*	*Aspergillus terreus*	*Candida albicans*	*Cocidiodes immitis*	*Cunninghamella berthollletiae*	*Epidermophyton floccosum*	*Trichophytan rubrum*
PINGGIRAN UMK	**3**	0	0	0	0	0	1	0	0	2
UMK JELI	**3**	1	1	0	1	0	0	0	1	**3**
PASAR JELI	7	3	0	2	1	4	0	0	0	5
PASAR AYER LANAS	1	0	0	**2**	0	0	1	1	0	**2**
KG. GEMANG	**2**	0	0	**2**	0	0	0	0	0	1

Table 4.2 directly above was recorded after a week incubation of subculture fungus media. This table can shown the number of species presented from which place. At Pinggiran UMK, the most highest frequency of fungus was *A. flavus*. Next, for UMK Jeli, the highest was *A. flavus* and *T. rubrum*. In Pasar Jeli, the highest of fungus number growth from the sample collected was *A. flavus*. After that, for Pasar Ayer Lanas, *A. niger* and *T. rubrum* recorded same amount of fungus media by two. Lastly, Kampung Gemang area has highest frequency of *A. flavus* and *A. niger*.

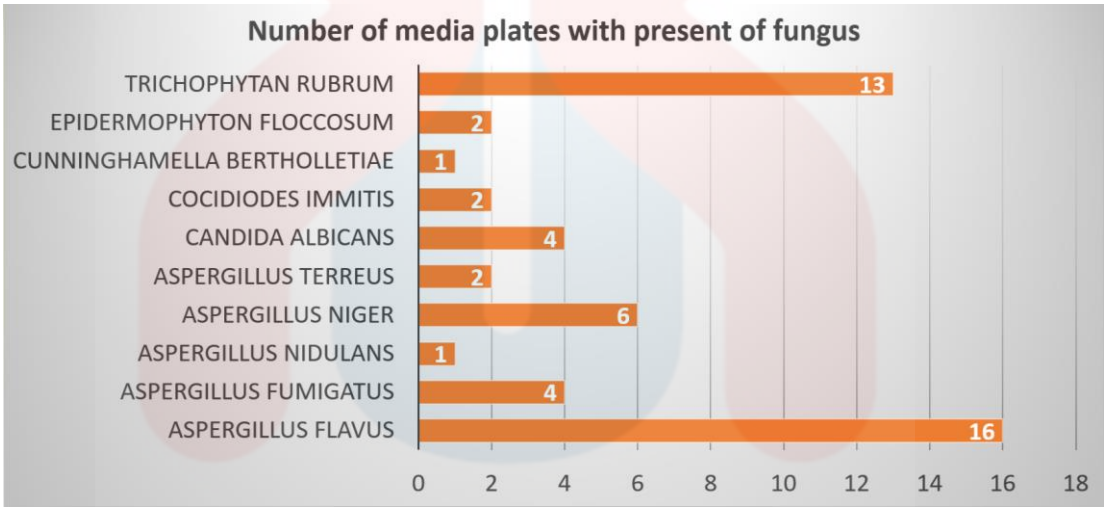

Figure 4.3 The observation result of media plates by different fungus species.

Table of 4.3 displayed the list of fungus species detected by observing its morphological features by qualititive. The presence of those listed fungus showed the rate of regularity have on selected cats taken their samples for this research in Jeli's area.

Aspergillus spp. is one from Saprophytic fungi and from the Table 4.7, *Aspergillus* spp. was the most number of fungi presented on the subculture agar plates. The highest number was from *A. clavatus (16), A. niger* (6). *A. fumigatus (4), and A. nidulans (1).* The differentiation of the *Aspergillus* spp. was done mostly by see it

feature colour present range from orange, yellow and green. While the shape of it where mostly of it has the form of irregular, elevation of raised and margin of curled or undulate.

Next is the *Candida albicans* which also from Saprophytic fungus. From the study, *Candida albicans* have growth of four media plate out from 32 plates. The feature of it on agar plate were creamy and shiny, the form were irregular, the elevation were raised and margin were undulate.

Lastly is the dermatophytes. Dermatophytes conquer the areas of Pasar Jeli with the second highest rate of fungus present out of dermatophytes. *Trichophytan rubrum* can cause the disease of Ringworm to skin of animal and human as they are zoonotic. It can produced inflammatory reaction and lesion to the skin. Common fungal infection for human from *T. rubrum* is athlete's foot, fungal infection of nail & jock itch (ringworm). While for cat, this fungus infection can bring lethal where for kitten, this fungus usually lesion at the area of face and front legs of kittens then the skin will be crusting and interfere the blood and inner flesh of the kitten (Moriello, 1990).

4.3 Fungus isolated and morphological characteristics

All the identification of fungus species growth on media plates were based on comparing the atlas or pictures from using reference source from book of “Atlas of Clinically Important Fungi”. The book has tonnes of images available in the book with macroscopic and microscopic figures provided (Sciortino, 2017).

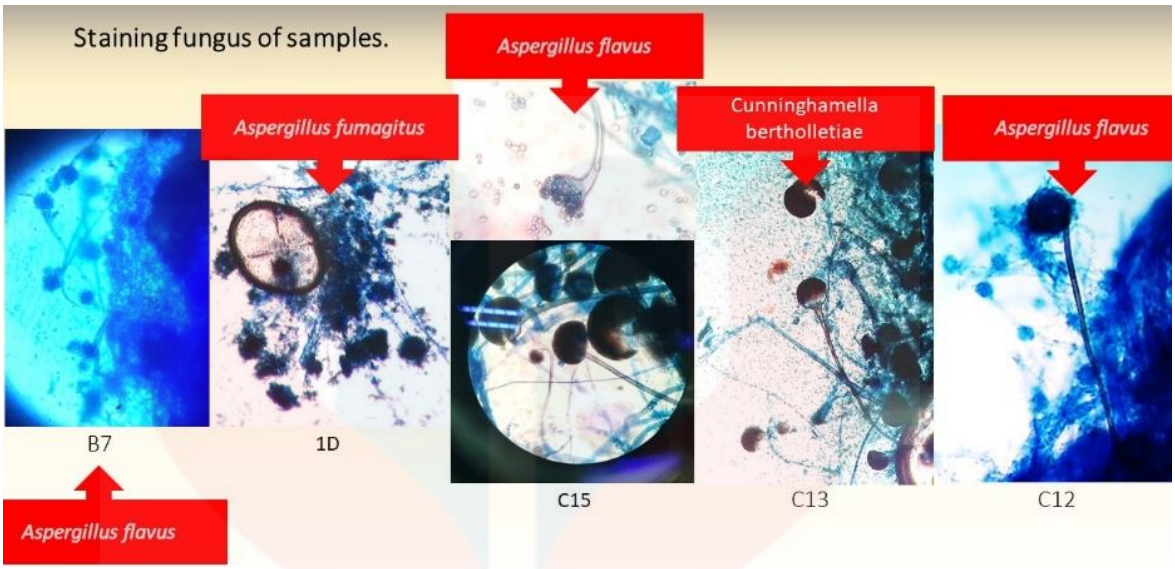

Figure 4.4: The staining of fungus samples.

Above of Figure 4.4 were the pictures of microscopic staining of fungus of four different species of fungus. The picture was not very clear because of the bad condition of microscope. The microscope was limited and only can use for special uses such as this microscope was specialized for mycology uses as fungus equipments can not be shared with other microbiology uses. Thus, the research on labeling the parts of each fungus hyphae, conidia and others were not further in this study.

4.4 Data analysis of fungus

Table 4.3 ANOVA Two Way Without Replication on areas studied and fungi species.

ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Rows (area)	19.4	4	4.85	4.386935	**0.005431**	2.633532
Columns (fungi species)	50.8	9	5.644444	5.105528	**0.000182**	2.152607
Within groups	39.8	36	1.105556			
Total	110	49				

Table 4.3 above showed the ANOVA Two Way without replication. The P-value result for areas is 0.005431 and for fungi species is 0.000182. Both hypothesis is

Rejected because P-value is less than 0.05. P-value ≤ α: The differences between some of the means are statistically significant. This means there are no difference relation between area and fungi species available at those 5 areas or the significant difference does exist.

Table 4.4: Percentage positive of saprophytes grown on media agar versus location.

Saprophytes species	**UMK Jeli**	**Kg. Gemang**	**Pggran. UMK**	**Pasar Jeli**	**Pasar Ayer Lanas**	**Percentage of positive (%) out of 32 cats**
Cunninghamella bertholletiae	0	0	0	0	1	3.125
Cocidiodes immitis	0	0	0	0	1	3.125
A. terreus	1	0	0	1	0	6.25
A. niger	0	2	0	2	2	18.75
A. nidulans	1	0	0	0	0	3.125
A. fumigatus	1	0	0	3	0	12.5
A. flavus	3	2	3	7	1	46.875
Candida albicans	0	0	0	4	0	12.5
	6	4	3	17	5	109.375

Table 4.5: ANOVA Two Way Without Replication on Saprophytes versus location.

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	16.25	4	4.0625	2.087156	0.103429	2.641465
Within Groups	68.125	35	1.946429			
Total	84.375	39				

Table 4.5 presented the P-value result for areas is 0.103429. The hypothesis is Accepted because P-value is more than 0.05. P-value > α: The differences between some of the means are not statistically significant. This means there are difference relation between area and Saprophytes fungi available at those 5 areas or the significant difference does not exist.

Table 4.6: Percentage of dermatophytes grown on media agar versus location

Dermatophytes species	UMK Jeli	Kg. Gemang	Pggran. UMK	Pasar Jeli	Pasar Ayer Lanas	Percentage of positive (%) out of 32 cats
Trichophytan rubrum	3	1	2	5	2	40.625
Epidermophyton floccosum	1	0	0	0	0	3.125
	4	1	2	5	2	43.75

Table 4.7: ANOVA Two Way Without Replication on dermatophytes versus location.

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5.4	4	1.35	0.355263	0.831111	5.192168
Within Groups	19	5	3.8			
Total	24.4	9				

Table 4.7 showed the ANOVA Two Way without replication on dermatophytes versus location. The P-value result for areas is 0.831111 where the hypothesis is Accepted because P-value is more than 0.05. P-value > α: The differences between some of the means are not statistically significant and this means there are difference relation between area and Dermatophytes species available at those 5 areas or the significant difference does not exist.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

This study can be concluded that by all cats collected for samples are positively have at least one of Saprophytes and the Dermatophytes present in all areas especially in the area of UMK Jeli which have both type of Dermatophytes of *Trichophytan rubrum and Epidermophyton floccosum.* The Saprophytes does not harmful to living creatures as long it is in low amount of sporulation but contrast to the dermatophytes where if the infection of dermatophytes does exist if the social habits among the cats at those areas to be contact with each other and worst, dermatophytes can be spread to human too and this can has the possibility of affecting the public health also.

Throughout the study, the research has exposed on the processes isolation and identifying of fungus and observation to morphological characteristics of the fungi of isolated fungus which can added another add ons on fungus research in Malaysia especially. The hypothesis of this study were accepted for both hyptheses where the P-value exceed than the alpha value which for Saprophytes; P = 0.103429 and Dermatophytes; P = 0.831111.

The methods and equipments can be improve by using better microscope especially in getting the microscopic observation and structure of morphology each details of fungus parts under microscope such as using Motorised Advanced Microscope with 1000X lens magnification that can give a details picture of the fungi. Next, other improvement from this study to microscopic of fungus is by changing the conventional staining method of using LPCB into other staining such as silver staining

method, Chromic acid-Schiff stain, and periodic acid-Schiff (PAS) stain (Speranza & Fail, 2005).

The further research can use other mycology assays such as using Dermatophyte Test Medium (DTM) media, Enzyme-Linked Immunosorbent Assay (ELISA), DNA or RNA extraction of Polymerase Chain Reaction (PCR) or Pulsed Field Gel Electrophoresis (PFGE), Matrix Laser Desorption Ionization Time-of-flight Mass Spectroscopy (MALDI), and DNA microarray to get more accurate of fungus identification process. By this assays, researchers can create and improve the mycology field such as improving the animal health, public health and novel findings.

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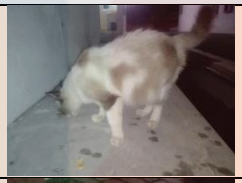
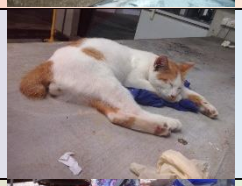
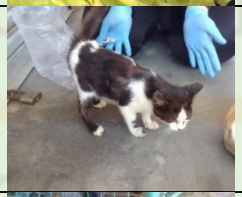
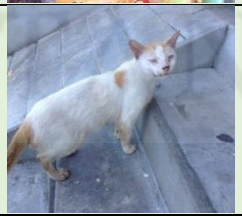
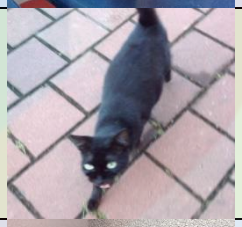
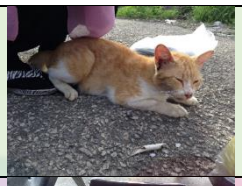
APPENDICES

Table 4.8: The pictorial of cats collected for their samples in four areas in Jeli.

No	PLACE/AREA	CODE	PICTURE	GENDER
1	PINGGIRAN UMK	A5		FEMALE
2	PINGGIRAN UMK	B10		MALE
3	UMK JELI	A1		MALE
4	PASAR JELI	B2		MALE
5	UMK	1D		FEMALE
6	PASAR JELI	C7		MALE
7	PASAR JELI	A6		FEMALE
8	PASAR JELI	B3		FEMALE
9	PASAR JELI	B1		MALE

10	PASAR JELI	B8		MALE
11	UMK	1C		FEMALE
12	PASAR JELI	C3		FEMALE
13	UMK	A2		MALE
14	PASAR JELI	C12		MALE
15	PINGGIRAN UMK	C8		MALE
16	PASAR JELI	B7		FEMALE
17	PASAR JELI	B6		MALE
18	GEMANG	C1		FEMALE
19	GEMANG	C2		MALE

20	PASAR JELI	B4		FEMALE
21	AYER LANAS	C5		FEMALE
22	AYER LANAS	C4		FEMALE
23	AYER LANAS	B9		FEMALE
24	AYER LANAS	C9		FEMALE
25	AYER LANAS	C16		FEMALE
26	AYER LANAS	C10		MALE
27	AYER LANAS	C15		FEMALE
28	AYER LANAS	C14		FEMALE
29	AYER LANAS	C13		FEMALE

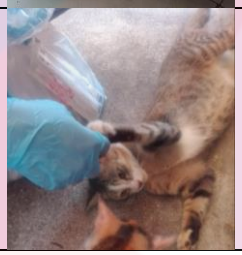
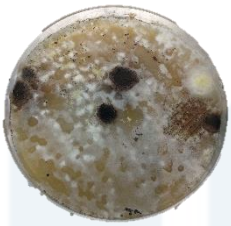
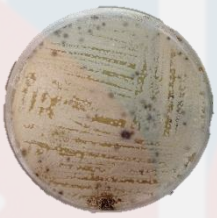
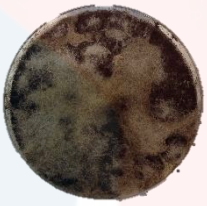
30	AYER LANAS	C6		FEMALE
31	AYER LANAS	C11		MALE
32	AYER LANAS	C17		MALE

Table 4.9: Isolated culture on SDA media from cotton swab of stray cats.

 C12	 C8	 C14	 B1
 A1	 B3	 B10	 A2
 A6	 C11	 C13	 B3
 C17	 B1	 B6	 C21
 B8	 C5	 C15	 1C

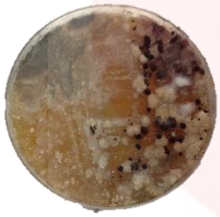
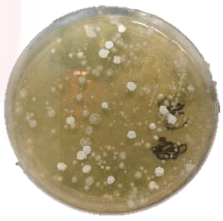
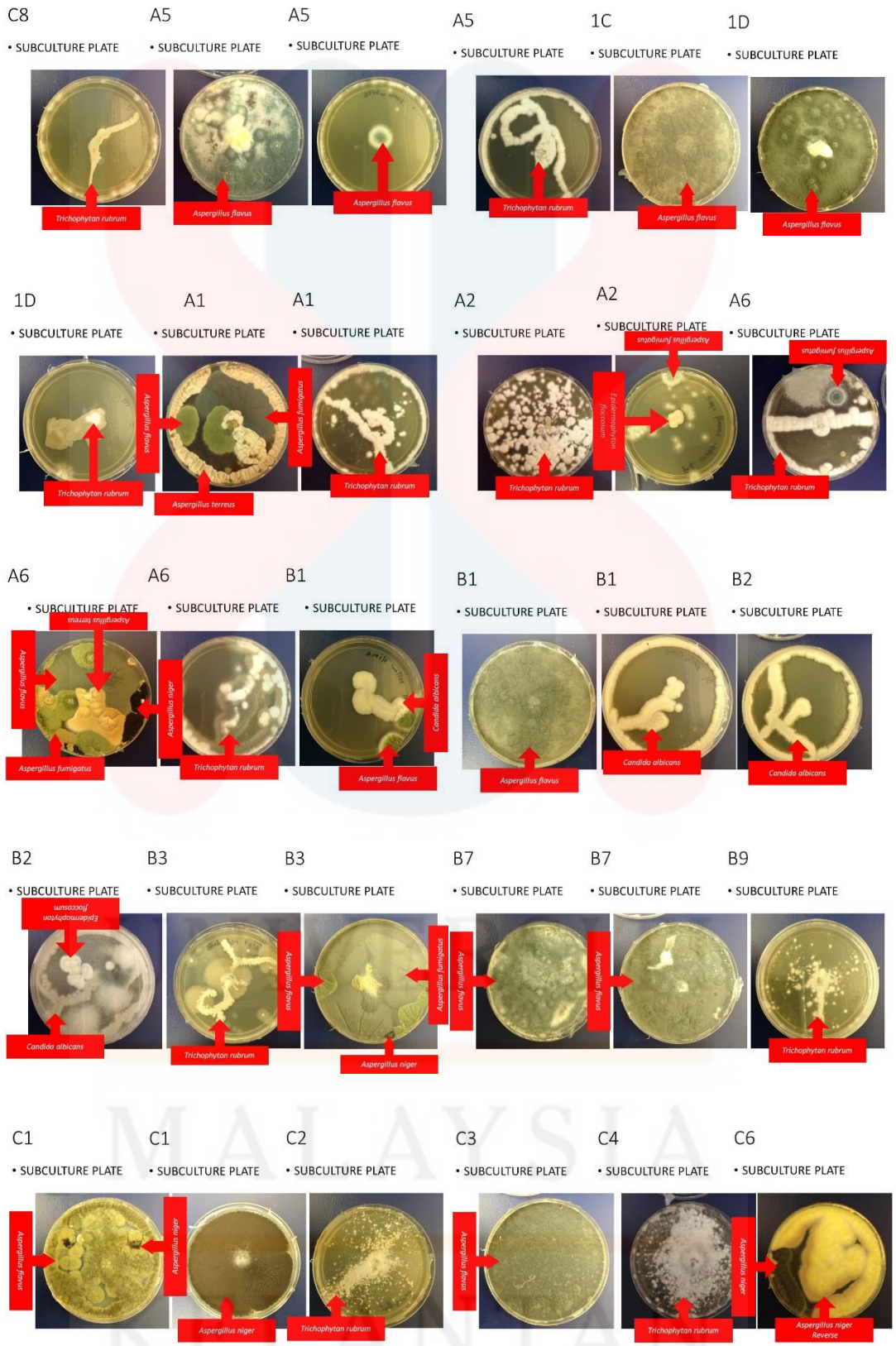
 B2	 C2	 B9	 C3
 1D	 IC	 C7	 C18
 C10	 C2	 C3	 B8
 B4	 C4	 C16	 A2
	 C9	 B7	

Table 4.9.1: The subculture media from isolated culture by selected colony of fungus.

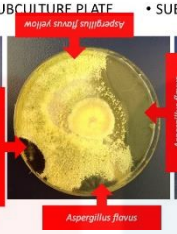


C6

• SUBCULTURE PLATE

C6

• SUBCULTURE PLATE



C7

• SUBCULTURE PLATE

C8

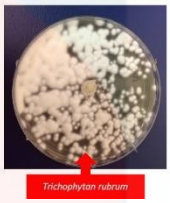
• SUBCULTURE PLATE

C12

• SUBCULTURE PLATE

C17

• SUBCULTURE PLATE



C18

• SUBCULTURE PLATE