Isolation and Identification of Pigment-Producing Microfungi from Selected Terrestrial Habitats in University of Southern Mindanao

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Cristel Jade B. Lumawig, Elma G. Sepelagio, Bryan Lloyd P. Bretaña
Department of Biological Sciences, College of Arts and Sciences, University of Southern Mindanao, Kabacan, North Cotabato, Philippines
cjblumawig@usm.edu.ph

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Abstract - *The study was conducted to isolate and identify pigment-producing microfungi from the soils of three different habitats found in University of Southern Mindanao, Kabacan, North Cotabato, Philippines. This study also qualitatively characterized the pigment extracts produced by each identified microfungal isolates using paper chromatography. Soil analysis was also conducted. There were a total of 30 colonies present in the Sabouraud dextrose agar plates after 7 days. For preliminary studies, five culturally and morphologically distinct pigment-producing microfungi were selected and isolated. These were identified as Penicillium sp., Aspergillus sp., Fusarium sp., and Curvularia sp. while one isolate was characterized as non-sporulating mold. Fungal pigment development was also observed everyday for 7 days. The final pigments produced by the five isolates after incubation at 30°C were red, green, orange, brown and pink, respectively. Of the five pigment extracts, the red pigment from Penicillium sp. exhibited the most concentrated and intense coloration which can be a good source of organic pigment for industrial application. The pigment extracts were qualitatively analyzed using paper chromatography employing acetone as the solvent system and Whatman no. 42 as the chromatogram. Penicillium sp. produced four distinct colored bands, non-sporulating mold produced two colored bands, Fusarium sp. and Curvularia sp. had one colored band while Aspergillus sp. did not produce any band in the chromatogram.*

Keywords: *Biopigments, microfungi, paper chromatography, colorants.*

INTRODUCTION

Colors are compounds which give significant visual properties into marketable products [1]. Its application in food, textile and cosmetic industries has become the basis of consumer preference in purchasing goods [2]. The continuous demand for broad spectrum of hues eventually resulted in the invention and production of synthetic colorants which have physical properties of good stability and coloring ability [3]. In addition, artificial synthetic colorants are economically efficient and technically advance thereby aids the slow pigment productivity from plant origin [4,5].

Currently used synthetic colorants are almost exclusively from fossil oils which are non-renewable and impose several hazardous effects not only in the environment but as well as in the health of factory workers and consumers [6,7]. Furthermore, artificial synthetic colorants have been proven to be carcinogenic to human and their waste product contributes to the increase in environmental pollution [4,6,8]. This problem has triggered increased interest to search more sources of natural pigments [5]. Thus, according to Mapari et al. [9], searching for renewable and environmentally friendly resources for the production of colorants is an urgent need.

Bio-pigments are pigments produced by living organisms. They can be obtained from several sources like plants and microorganisms [10,11]. For instance, natural pigments for industrial use are largely from plant extracts which imposes several disadvantages like slow productivity and instability. Pigments from microorganisms however have potential in different applications due to their natural color and safety in use. They also have numerous clinical benefits like antioxidant, anticancer, antibiotic, immunosuppressive, treatment for diabetes mellitus

and many more [3]. In addition, pigment producing microorganism is of advantage over other sources for they can grow rapidly in cheap media culture as they produce controllable and predictable amounts of pigments [12,13]. Hence, microbial pigments can be considered as promising alternatives for synthetic dyes used for food and other industrial products.

Pigment- producing microorganisms can be isolated, cultured and purified from various environmental sources such as water bodies, soil, plants, insects and animals [14]. Soil microbial communities are among the most complex and diverse microorganisms that are significantly the source of molecules with biotechnological importance such as microbial pigments that are used as natural colorants [15]. In nature, specifically in terrestrial habitats, there is a wide range of
color-rich and pigment-producing microorganisms that offers a considerable scope for commercial production [16].

However, the shades of color available in the market are still at its limitation [10]. This study aims to serve as basic information in terms of finding more plausible ranges of pigments from different soil conditions which have possible economic importance.

Generally, the study aimed to isolate and identify pigment-producing microfungi from three selected terrestrial habitats in University of Southern Mindanao, Kabacan, Cotabato. Specifically, it aimed to: 1) isolate pigment-producing microfungi from three selected terrestrial habitats; 2) identify and characterize isolated pigment-producing microfungi through cultural and morphological examination and 3) qualitatively characterize pigments produced by the isolates using paper chromatography.

This study only focused on the isolation and identification of carotenoid and carotenoid associated pigments up to the lowest possible classification within the three selected habitats such as grass field, vermicompost and dragon fruit rhizosphere in University of Southern Mindanao, Kabacan, North Cotabato. Collection of soil samples which may contain potential pigment-producing fungi was conducted in three selected terrestrial habitats from University of Southern Mindanao, Kabacan, North Cotabato namely, Department of Animal Science Vermiculture project for the vermicompost soil and University of Southern Mindanao Agricultural Research Center (USMARC) for dragon fruit rhizosphere and grass field.

Isolation and identification of pigment-producing microfungi was conducted at the Microbiology Laboratory, Department of Biological Sciences, College of Arts and Sciences, University of Southern Mindanao, Kabacan, North Cotabato from June 2017 to April 2018.

MATERIALS AND METHOD

Study Area

Random sampling was applied during the collection of samples. Soil samples were obtained from University of Southern Mindanao Kabacan, Cotabato specifically in University of Southern Mindanao Agricultural Research Center (USMARC) and Department of Animal Science Vermiculture Project.

Preparation of Glassware and other Materials

Glassware were washed using detergent soap and were rinsed with distilled water. These were air-dried, wrapped with paper, and sterilized in the oven for 2 hours at 180˚C.

Preparation of Media

The culture medium used in this study was Sabouraud Dextrose Agar (SDA).The dehydrated culture media was prepared following the procedure indicated by the manufacturer. Sabouraud Dextrose Agar (SDA) was sterilized in the pressure cooker for 15 minutes at 121˚C at 15 psi. After sterilization, medium was poured in sterile Petri dish (20 ml/plate) under the laminar flow hood until solidify. The petri plates were then kept inside the refrigerator until used.

Collection of Soil Samples

Soil samples were collected from three selected terrestrial habitats in University of Southern Mindanao Kabacan, North Cotabato namely grass field, vermicompost plot and dragon fruit orchard. Soil samples of organic garden soil and dragon fruit rhizosphere were collected at University of Southern Mindanao Research Center (USMARC) and the vermicompost soil were obtained from the Department of Animal Science Vermiculture Project. Soil samples were taken using a sterilized garden shovel with an estimated depth from the upper portion of the soil. At least 2 kilograms of soil samples were taken in each site for laboratory and soil analysis. Soil samples were placed in a sterile zip-locked bag, labeled and stored at 4˚C.

Soil Analysis

At least 1 kilogram of each soil sample was air-dried and subjected to analysis. Soil samples were submitted in the Regional Soil Laboratory DA-AmRes, Amas, Kidapawan City. Soils were analyzed for its pH, total Nitrogen, available Phosphorus and Potassium content. Physico-chemical analysis of soil samples were summarized in Appendix Table 1.

Enrichment and Isolation of Pigment-Producing Microfungi

Ten grams of each soil samples were subjected to enrichment and isolation of pigment-producing microfungi. Prior to serial dilution, soils with different labels were thoroughly mixed. From the composite samples of each soil, 5 grams of soil sample plus 500mL distilled water were mixed in the Erlenmeyer flask. The samples was serially diluted in sterile saline blank solution, 1.0 mL of sample was transferred aseptically in 9 mL solution hence 10^{-4}, 10^{-5} and 10^{-6} dilution were used. Exactly 0.1mL of sample was spread over the surfaces of Sabouraud Dextrose Agar (SDA) [17]. The inoculated selective agar medium was incubated at room temperature for 3 to 7 days. Colonies which produced pigments were fished out of the medium sub-cultured and purified using the same selective agar medium.

Morphological and Cultural Characterization of Fungi

Colonial morphology of the pure culture of each fungus was determined. The colonies were characterized according to their pigment produced, margin, elevation, texture and consistency on Sabouraud Dextrose Agar (SDA) [17].

For microscopic examination, one to two drops of Lactophenol cotton blue stain was place on a clean glass slide. Using a sterilized inoculating needle, small part of fungal colonies was picked from the medium and was placed in the stained slide. Then, this was teased and covered with a cover slip. The slides were observed under microscope using high powered objective lens. The morphology was determined by isolates' manifestation of cellular characteristics like hyphae and spores.

Identification of Fungal Isolates

Fungal isolates cannot be distinguished by colonial morphological identification alone. Therefore, isolates have been subjected to microscopic examination [18]. Microscopic characteristics of fungal hyphae, conidial head and spores were observed. Photographs were taken for documentation. The presumptive identification was confirmed by a mycologist.

Extraction of Pigments

Colonial mycelia of the pure culture were carefully scrapped off and were transferred in a test tube with 10mL methanol: distilled water (v/v) solution and was carefully mixed. Thereafter, the mixtures were immersed in a 45 °C water bath for 30 minutes [19]. The next day, mycelial samples were teased using a stirring rod and was vortex for 10 minutes. Pigments were extracted by centrifugation with conditions set as 2000 rpm for 25 minutes. The pellet was discarded and the supernatant was used for qualitative analyses [8].

Paper Chromatography Analysis

Paper chromatography analysis was used to characterize the pigments. In the analysis, Whatman filter paper no.42 was used as a chromatogram and pure acetone as the solvent system. Pigmented supernatant was fixed in uniformed position in the filter paper and a 15mL amount of solvent was added in a 600 mL beaker. The beaker was covered at the top to prevent fast evaporation of the solvent [8].

The presence of the bands in the chromatogram presented by different colors was recorded.

Research Design and Statistical Analysis

The study employed a randomized sampling in the area of interest, each soil sample were subjected to exploratory and soil analysis. Furthermore, the data collected in the study was described by means of descriptive statistics.

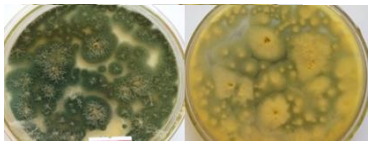
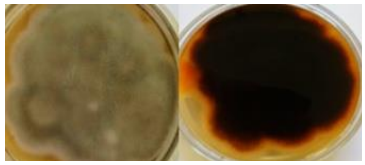
RESULTS AND DISCUSSION

Isolated Pigment-Producing Microfungi

In this study, dilutions were made from each soil samples collected from three sampling sites (Appendix Figure 1). After 7 days of incubation at 30°C, about 30 colonies were present in the Sabouraud dextrose agar (SDA) plates. For preliminary studies, a total of five microfungi which produced different shades of pigment were selected. According to Rajguru et al. [10], colonies that exhibit discrete pigment should be selected and cultured for pigment production. Thus, five isolates were selected as they produced distinct types of pigments specifically red, green, orange, brown and pink. Summary of five pigment-producing fungal colonies is presented in Table 1.

Table 1. Isolated pigment-producing fungi in the Sabouraud Dextrose Agar plates after 7 days of incubation

**Gr1=Garden red isolate 1, Vg1=Vermicompost green isolate 1,*

ISOLATE CODE*	FUNGAL COLONIES ON SABOURAUD AGAR PLATES		PIGMENTS PRODUCED
	Obverse view	Reverse view	
Gr1			Grey and Red
Vg1			Green
Do1			Orange
Gb2			Brown, Gray and Black
Vp2			Pink and Orange

Do1=Dragon fruit isolate 1, Gb2=Garden Brown isolate 2, Vp2= Vermicompost Pink isolate 2

The presence of different pigments exhibited by fungal isolates in Sabouraud dextrose agar plates were used as the basis in considering them for identification and processing. Calvo et al. [20] described that the colors produced by fungi are secondary metabolites that plays an important role in variety of environmental factors like desiccation, exposure to extreme temperatures or in ecological interactions with other organisms. In this study, however, selected fungal isolates were seen to produce pigments independently.

In table 1, the isolates were considered for they have the potential in carotenoid production which is known to be synthesized by many fungal [9]. According to Yabuzaki [21], carotenoid pigments absorb wavelength ranging between 400-500 nanometers that is from color violet to green which causes the compounds to be deeply yellow, orange or red.

Babitha et al. [22] reported that fungi are potent pigment producing microorganisms. In the study conducted by Fouillaud et al. [19], they isolated a total of 41 pigment-producing fungi from marine environment and the dominating colors are in the shades of red, orange, brown and violet. However, Chakraborty et al. [2] isolated only 1 pigment-producing actinobacteria which appears to be in dark pink color sampled from the soils of mangrove forest in India. The current study on the other hand isolated five pigment–producing microfungi from the soils of 3 terrestrial habitats in the University of Southern Mindanao, Kabacan, Cotabato.

Characterization and Identification of Fungal Isolates

The summary of fungal characterization is presented in Table 2. Cultural and morphological characteristics were used to identify the five pigment-producing microfungi from University of Southern Mindanao, Kabacan, Cotabato. The results showed morphologically diverse colonies on Sabouraud Dextrose Agar (SDA) plates.

Table 2. Cultural and morphological characteristics of pigment- producing microfungi from selected terrestrial habitats in University of Southern Mindanao, Kabacan, Cotabato

CHARACTERISTICS	**ISOLATES**				
	***Penicillium* sp.**	***Aspergillus* sp.**	***Fusarium* sp.**	***Curvularia* sp.**	**Non-sporulating mold**
	CULTURAL				
Pigment Produced	Blue-green /Red	Green	Orange	Brown	Pink
Colony Margin	Filiform	Filiform	Filiform	Filiform	Entire
Elevation Consistency	Umbonate Rubbery	Umbonate Powdery	Crateriform Rubbery	Raised Powdery/ Cottony	Convex Powdery/ Cottony
Texture Form	Velvety Irregular	Rough Irregular	Smooth Filamentous	Smooth Irregular	Smooth Circular
Notable Observation	Produces green aerial mycelia and red vegetative hyphae	Produces green mycelia	Colony has slimy texture at the center and cottony in the edge	Grey aerial mycelia and brown to black vegetative structure	Pink filamentous aerial mycelia
	MORPHOLOGICAL				
Aerial Mycelium	+	+	+	+	+
Cell Shape	Spherical /Globose	Spherical/ Globose	Fusiform/ Curved Septated	Septated Ovoid	None observed
Spore	+	+	+	+	-
Notable Observation	Group of phialides terminates ovoid conidia	Globose swelling at the apex and radiates phialides with conidia	Canoe - shape macroconidia with 5-7 septa	Oblong shape macroconidia with 2-4 septa	Septated filamentous vegetative hyphae

To carefully assess the morphological characteristics of the isolates, fungal cultures were culturally and microscopically examined. Colonial characterization was done by recording colony features while microscopic examination was done by employing lactophenol cotton blue staining technique. Brooks et al. [23] stated that macroscopic and microscopic morphology are usually enough to determine the genus of fungal isolates and that the most helpful physical features are the morphology of spores or conidia. In contrast, the statement of Gherbawy and Voigt [24] points out that polyphasic approach involving molecular marker analysis along with phenotypic evaluation is also essential for the identification of fungal isolates.

The Gr1 has a colony which is velvety in texture and appears to be blue-green (obverse) and red (reverse). Microscopically, the conidiophores possess a branching pattern near its apex where a group of phialides are attached radiating small ovoid conidia that are in chains (Figure 1A)

On the other hand, the colony of Vg1 has a rough texture and appears to be green. In the microscopic examination, the conidiophore appears to be simple. A globose swelling at the apex, where short phialides are attached, was also evident (Figure 1B). Thousands of small globose conidia were seen to be in long chains.

Moreover, the colony of Do1 appears to be orange in both obverse and reverse side of the plate. A slender, curved and a canoe-shape macroconidia was observed microscopically (Figure 1C).

Furthermore, the colony of Gb2 is brown in color. A distinct pigment production of yellow to brown color at the bottom of the plate was observed. As observed during the microscopic examination, the brown and oblong shaped macroconidia with at least 2 to 4 septa were held by unbranched conidiophores (Figure 1D). Microconidia are small, globose and typically single celled.

The Vp2 colony appears to be pink in the obverse side of the plate and orange in reverse. During microscopic examination, it was observed that the fungus have branched septated conidiophores that does not radiate to either spores or sporangium (Figure 1E). All five isolates were observed to have colored aerial and vegetative filamentous mycelia.

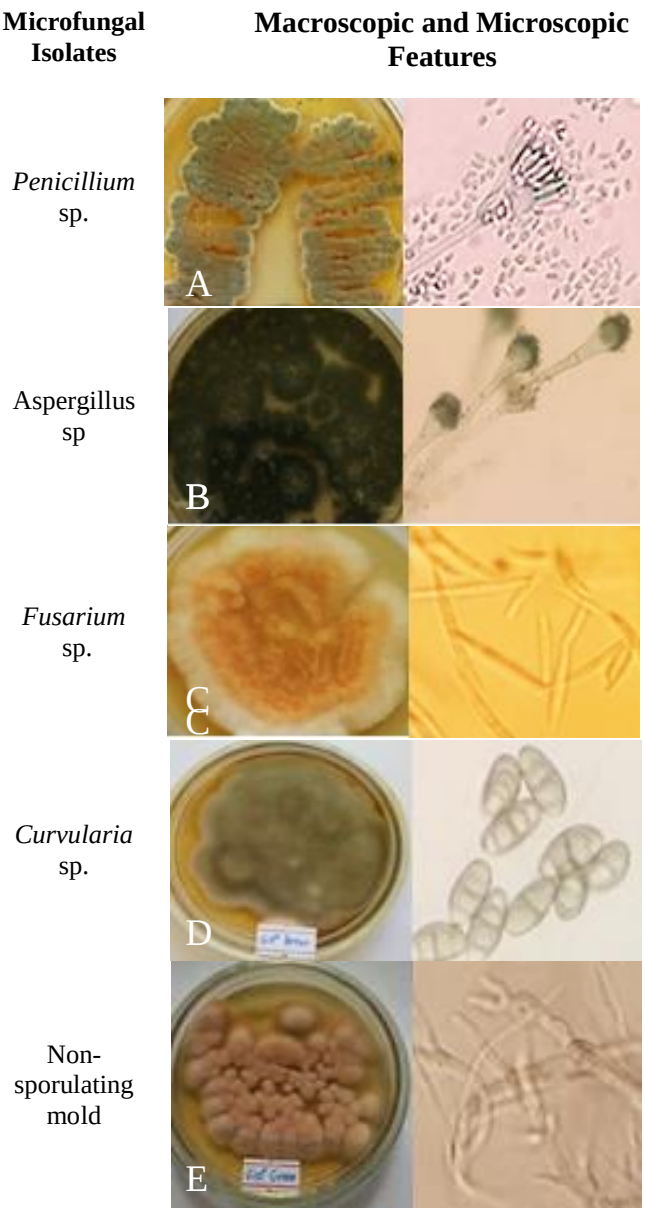


Fig.1.Cultural and microscopic features of fungal isolates observed under 400 x magnifications

Based on the results of the characterization both Gr1 and Vg1 belongs to the family *Trichocomaceae.* The former is under the genus *Penicilium* while the latter is under *Aspergillus.*

Penicillium species are characterized by the presence of conidiophores arising from the mycelium singly or less often in synnemata, branching near the apex and ending in a group of phialides. The conidia are hyaline or brightly colored in mass and mostly globose to ovoid in

shape (Hunter and Barnett, 1972). According to Visagie et al. (2014), conidiophore branching patterns were traditionally used in the classification of *Penicillium* species. The unique brush-like structure of the genus known as penicillus covers all the branching system of the fungi [25]. As stated by Samson et al. [26], the structure provides the basis for the delimitation of the genus, permits distinction, and is also good for inter-specific taxonomy.

In the description of Hunter and Barnett, [27], conidiophores of *Aspergillus* species are upright, simple, terminating in a globose or clavate swelling, and bears or radiate phalides at or from the entire surface of the apex. Conidia are phialosporic, unicellular, dry, smooth, globose, colored in mass and are observed to be in long chains [25].

The above characteristics were notably similar to the characteristics observed in Gr1 and Vg1 (Table 2).

Furthermore, Do1 belongs to the family *Nectariaceae* under the genus *Fusarium* which is characterized by its slender, curved, and septated macroconidia that possesses at least 5-7 septa. As described by Henriques et al. [25] and Barnett and Hunter [27], the macroconidia of the genus are compose of several cells, slightly curved or bent at both ends and are typically in canoe-shaped. However, microconidia are single celled and ovoid in shape.

The results for the characterization of fungal Do1 proposed notable similarities specifically in the shape of the macroconidia which are in canoe-shape, a distinct characteristic of almost all *Fusarium* species.

In addition, Gb2 belongs to the family *Pleosporaceae,* under the genus *Curvularia.* The genus is characterized by an unbranched conidiophore that radiates oblong shape macroconidia with at least 2 to 4 septa. Humber [28] described this genus by their production of sympodial brown conidiophores which are mostly simple. The cells are elongated and transversely septated conidia. In the study conducted by Liang et al. [29], they described that *Curvularia* species produces four-celled conidia which are curved at the third cell from the base. However, Madrid et al. [30] found out that some species with straight conidia are also present.

The isolate Vp2 was classified as member of non-sporulating molds (NSM) which resembles the filamentous basidiomycetes' secondary mycelium. Microscopically, the non- sporulating mold was characterized by the presence of its septated and branching hyphae. According to Pounder et al. [31] identification of fungi usually requires the presence of reproductive structures. Other fungi cannot be fully characterized because molds do not sporulate which makes microscopic identification impossible. Non-sporulating molds (NSM) are defined as molds without reproductive structures and could not be further characterized.

The isolates were pre-identified using the identification keys of Hunter and Barnett [27], Humber [28]. Also, published journals, articles and reviews of Pounder et al. [31] Vasquez et al. [32] and Henriques et al. [25] were utilized. Pre-identified isolates were confirmed by a mycologist.

Occurrence of Fungal Isolates in Different Sampling Sites

Table 3. Occurrence of five fungal isolates in three different sampling sites.

FUNGAL ISOLATES	SAMPLING SITES		
	Vermicompost	Grass field	Dragon fruit orchard
Pencillium	-	+	-
Aspergillus	+	-	-
Fusarium	-	-	+
Curvularia	_	+	-
Non-sporulating Mold	+	-	-

Isolated microfungi were sampled from different habitats with different conditions and soil

compositions. Table 3 shows the occurrence of microfungi in three different terrestrial habitats in University of Southern Mindanao.

In the data presented in Table 3, *Curvularia* and *Penicillium* species were both present in grass field soil from University of Southern Mindanao Agricultural Research Center (USMARC). The results were similar to the previous findings of Madrid et al. [30] and Visagie et al. [33]. The former found out that *Curvularia* species are grass pathogens and saprobes occurring on plant, dung and soil materials while the latter reported that genus *Penicillium* plays an important role in the decomposition of organic materials and can occur worldwide.

Moreover, *Aspergillus* species and non-sporulating molds (NSM) were isolated from vermicompost soil samples collected from the College of Fisheries and Animal Science (CFAS). In the study of Akmal et al. [34], they considered *Aspergillus* species as saprophytic fungi for it inhabits organic materials and have been found to be abundant in organic compost piles and leaf litters. Whereas in the study conducted by Vasquez et al. [32], they were able to collect fragments of non-sporulating molds from decaying wood materials which may play an important role in the decomposition of lignin.

In addition, *Fusarium* species isolated from the dragon fruit orchard is known to have been isolated from a variety of different plant hosts with broad range of specificity [35]. In the study conducted in Malaysia, they found out that the two *Fusarium* sp. such as *F. proliferatum* and *F. fujikuroi* can occur in red –fleshed dragon fruit (*Hylocereus polyrhizus*) farms causing stem rot disease [36].

Evolution of Fungal Pigment

In the study, five microfungi were observed to produce pigment gradually while noting the changes in pigment quality with respect to the given time of incubation and media composition. Table 5 summarizes the development of fungal pigment from day 1 to 7 at 30 degrees Celsius.

Table 4. Evolution of fungal pigments from day 1 to day 7 under 30 degrees Celsius incubation condition

	FUNGAL ISOLATES				
No. of Days	*Penicillium*	*Aspergillus*	*Fusarium*	*Curvularia*	Non-Sporulating Mold
Day 1	Opaque	White	Opaque Carnation Pink	Opaque White/ Brown	White
Day 2	White	Blue Green	Light Orange	Grey/ Brown to Yellow	Yellow
Day 3	Grey/ Yellow Orange	Green	Yellow Orange	Grey/ Brown to Orange	Yellow Orange
Day 4	Grey/ Red orange	Dark Green	Yellow Orange	Grey/ Brown to Orange	Pink/Yellow
Day 5	Grey/ Red orange	Dark Green	Pink Orange	Grey/ Black to Orange	Pink/ Red Orange
Day 6	Grey/ Red	Dark Green	Pink Orange	Grey/ Black to Orange	Pink/ Red Orange
Day 7	Grey/ Red	Dark Green			

The isolates from *Aspergillus* sp., *Fusarium* sp., and *Curvularia* sp. were observed to produced pigments in the first two days of its growth in the Sabouraud dextrose agar (SDA) plates. On the other hand, isolates from *Penicillium* sp. and non-sporulating mold started to produce pigments on day three. In the research conducted by Dufosse and his colleagues [16], they observed that the production of pigments from *Penicillium*, *Aspergillus*, *Curvularia* and *Fusarium* species started between day 2 and 4. Fouillaud et al. [19] also isolated fungi and found out that the majority of pigment-producing fungi produced pigments after four days of incubation. The presence of usually dark colors in the culture media indicates the potential of fungi for pigment production. These are evidence that supports the result of the current study.

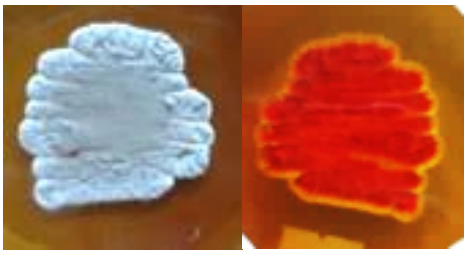
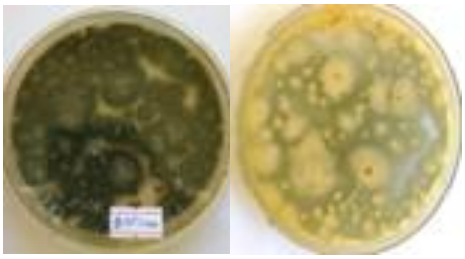
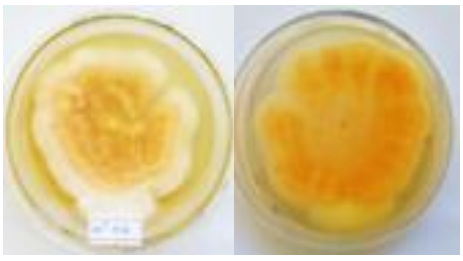
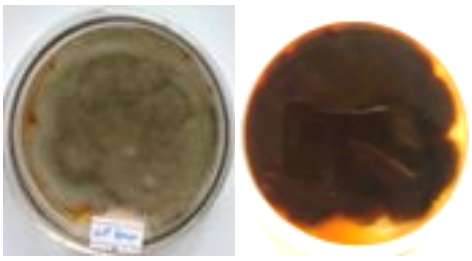
Moreover, Dufosse et al. [16] and Gmoser et al. [37] stated that the most studied filamentous fungi known to produce pigments belongs to genera *Taralomyces*, *Trichoderma*, *Aspergillus*, *Fusarium*, *Monascus*, *Neurispora* and *Penicillium.*

Furthermore, fungal cultures of *Penicillium* sp. and *Curvularia* sp. were seen to produce intense color which was evident in the reverse side of the Sabouraud dextrose agar plates. *Fusarium* sp. and non-sporulating mold however, were observed to produce pigments from pink to orange with lesser intensity in comparison to the two previous fungal cultures. Moreover, *Aspergillus* sp. produces a

green color only in the upper part of the colony and does not yield any pigment in the reverse side of the agar plate.

The composition of the medium (carbon and nitrogen content) as well as the temperature and pH may influence the pigmentation of fungal isolates [38]. The Sabouraud Dextrose Agar contains glucose as carbon source and peptone as nitrogen source for the growth of fungi. According to Shah et al. [39], simple and cheaper media are needed to permit the mass production and commercialization of pigment from fungi. With this, future commercialization of cheap bio-colorants is possible.

Table 5. Extracted pigments from mycelia of seven-day old fungal cultures

FUNGAL ISOLATES	FUNGAL MYCELIUM ON METHANOL:DISTILLED WATER (V/V)	EXTRACTED PIGMENTS
		
		
		
		
		

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The acidic pH of the Sabouraud Dextrose Agar medium with 5.0 pH mimics the soil condition in two sampling sites which are the vermicompost and grass field where pH ranges from 4.3- 5.6, respectively (Appendix Table 1). The pH requirement for pigment production varies on the type of fungi present. Different type of fungi requires different optimum pH value for their growth. The pH value would help in terms of increasing the fungal cell numbers to produce more secondary metabolites [14].

The pigments produced by the five fungal isolates on day 7 were considered to be the final color produced in the study. Fungal mycelia were subjected to pigment extraction.

The fungal mycelia of the isolates were subjected for extraction following the methods used by Fouillaud et al. [19]. Extracted pigments are presented in Table 5. The extracted pigments from five fungi isolates exhibited marked differences in pigment concentration. These pigments are in the shades of red, green, orange, brown and pink.

Among five extracts, the extracted red pigment from genus *Penicillium* showed the highest pigment concentration. A similar finding was described by Sayyed and Majumder [41], where maximum production of pigment was observed in *Penicillium* species. Dikshit and Padmavthi [40] observed that *Penicillium* species are able to tolerate lower temperature but optimum pigment production was seen at 30-32°C which was very similar to the condition set for the growth of the fungal cultures in this study.

Qualitative Characterization of Fungal Pigment

In this study, paper chromatography was used to assess the underlying pigments produced by each fungal isolates. In the results presented in table 6, it indicates that the pigments from different microfungi were multi-component in nature. The findings of Sharma et al. [41] also showed multi-component fungal pigments from species *Curvularia*, *Alternaria* and *Trichoderma* presented by different spots in the chromatogram.

The red pigment produced by the fungus *Penicillium* sp. yielded a total of four distinct bands in the shades of red, yellow, pink and orange. The pigment of non sporulating mold which produced pink to red color yielded two distinct bands that are in the shade of pink and yellow. The color produced by *Fusarium* sp. and *Curvularia* sp. yielded one band with distinction, the former produced pink band while the latter produced yellow band. However, the green pigment produced by the genus *Aspergillus* sp. failed to yield any band in the chromatogram.

In the study conducted by Bhardwaj et al [42], the *Penicillium marneffei*a species of *Penicillium* which produces red pigment after analyzed by UV-visible spectroscopy showed three distinct absorption maxima which corresponds to be red, yellow and orange hues. The color maxima produced by *Penicillium marneffei* were highly similar to the bands produced by *Penicillium* sp. in the current study but differs in the method of characterization used.

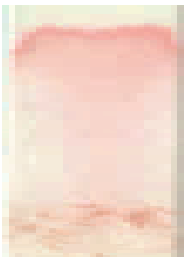
In a similar study conducted by Souza et al. [43], maximum absorption of the supernatant of *Aspergillus* sp., *Penicillium* sp., and *Fusarium* sp. was read at 400nm and corresponds to yellow pigments. These genera are capable of producing anthraquinone and other yellow polyketides [19,43].

In addition, the study showed that *Aspergillus* sp. did not produce bands in the chromatogram which is in contrast to the study of Tanaka et al. [44], who isolated two species of pigment-producing *Aspergillus*, subjected to paper chromatography, which yielded yellow, orange and violet bands. It might be possible that the differences in fungal species, extraction method and solvent system affected the outcome of the two studies. Moreover, Akmal et al. [14] described that *Aspergillus* species can generate small amounts of natural pigment after 8 days of incubation under pH 4 and pH 5.

In the study of Sharma et al. [41], extracted pigment of *Curvularia lunata* produced purple and orange spots on the chromatogram while in the current study the pigments from *Curvularia* sp. exhibited one yellow band in the chromatogram. The dissimilarity between colors present in the chromatogram might be associated to the solvent used. The study used pure acetone while the other study used butanol: glacial acetic acid: distilled water (12:3:5) solution as solvent system.

Currently, there are no solid reports for industrial production of pigments from the five fungal species. The bottleneck is that they are all known to produce mycotoxins yet the ambiguity can be overcome by further studies on the separation and purification of pigments from the five isolates.

Table 6. Results of Paper Chromatography

FUNGI	NUMBER OF BANDS	COLOR OF THE BANDS	BAND IN CHROMATOGRAM*
Penicillium sp.	4	Pink Red Orange Yellow	
Aspergillus sp.	0	none	No band observed
Fusarium sp.	1	Pink	
Curvularia sp.	1	Yellow-Brown	
Non-sporulating mold	2	Pink Yellow	

CONCLUSION AND RECOMMENDATION

Based on the results gathered in this study, it is concluded that pigment-producing microfungi can be isolated from the soils of different terrestrial habitats because fungi are usually saprophytic organisms. Microfungi such as *Penicillium* sp, *Aspergillus* sp., *Fusarium* sp., *Curvularia* sp, and non-sporulating mold were isolated from the soils of University of Southern Mindanao, Kabacan, Cotabato. In this study each fungal isolates produced pigments gradually in 7 days at 30 °C.

Among the five isolates, red pigment from *Penicillium* sp. produced intense pigment in day 4 and yielded four distinct bands in the paper

chromatography analysis confirming the presence of different molecules.

Furthermore, the study proves the capacity of microfungi in producing different shades of pigment that would eventually be beneficial for future research on the production of bio-pigments.

Based on the findings, results and conclusions the following are recommended:

1. Subject isolated fungi to biochemical tests and molecular analysis for the identification up to species level.
2. Apply the method used by Henriques et al. [25] in extracting pigments for qualification of pigment contents.
3. Test the organism pigments for dyeability.
4. Use more advance Chromatographic analysis like Thin Layer Chromatography (TLC) to assess pigment quality.
5. Qualitatively characterize fungal pigments using UV visual spectrophotometer to test the absorbance and try to identify and classify its components.
6. Grow fungal isolates on different culture media under different culture conditions (pH and temperature) to observe different types of metabolites/pigments it will produce.

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