International Journal of Research in Engineering and Science (IJRES)
ISSN (Online): 2320-9364, ISSN (Print): 2320-9356
www.ijres.org Volume 4 Issue 7 ǁ July. 2016 ǁ PP. 25-32

The Isolation and Identification of Fungi from the Soil in Gardens of Cabbage Were Contaminated with Pesticide Residues in Subdistrict Modoinding

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Abstract: Application of pesticides in the garden cabbage can cause negative effects harmful to the environment and living things. The objectives of this research were to get species of fungi from the soil in the gardens of cabbage contaminated with pesticide residues in Subdistrict Modoinding. Isolation of fungi using dilution plate method with serial dilutions of 10^{-2} to 10^{-5} on Potato Dextrose Agar (PDA). Of the five soil treatments in the gardens of cabbage obtained 76 isolates of the fungus. Isolates of soil fungi that were successfully identified macroscopically and microscopically. These fungal isolates included in 13 families and 22 species. Fungal species according to the following families: Endomycetaceae (*Geotrichum candidum*), Trichocomaceae (*Penicillium citrinum*, *Aspergillus fumigatus*, *Aspergillus nidulans*, *Paecilomyces lilacinus*, *Aspergillus* foot cell, *Aspergillus sydowii*, *Aspergillus flavus*, *Aspergillus terreus* and *Aspergillus niger*), Sordariaceae (*Chrysonilia sitophila*), Mucoraceae (*Mucor hiemalis*), Pleurostomataceae (*Pleurostmophora richardsiae*), Hypocreaceae (*Gliocladium virens*), Pythiaceae (*Phytophthora infestans*), Chaetomiaceae (*Humicola fuscoatra*), Eremomycetaceae (*Arthrographis cuboidea*), incertae sedis (*Scytalidium lignicola*), Bionectriaceae (*Gliocladium roseum*) and Arthrodermataceae (*Microsporum audouinii*). Retrieved four species with the fastest growing colonies are from Family Sordariaceae (*Chrysonilia sitophila*), Family Endomycetaceae (*Geotrichum candidum*), Family Trichocomaceae (*Penicillium citrinum* (IJ2) and Family Trichocomaceae (*Aspergillus fumigatus*)(IJ3).

Keywords : *Aspergillus fumigatus*, cabbage, *Chrysonilia sitophila*, isolation, *Penicillium citrinum*

I. INTRODUCTION

Cabbage is one of the horticultural type of vegetables are important because they have the economic and social value that is high enough often used as one of the main sources of livelihood of farmers in order to increase the income and standard of living. In addition, cabbage is a group of horticultural crops that are needed by the food consumer.. Cabbage is a vegetable crop that is most often damaged by pests and diseases [1]. According to [2], overcoming the impact or by pests and pathogens on farms that have become a serious problem, then humans since time trying to reduce the rate of damage by using a variety of traditional or modern ways

Today pesticides have become one of the important technological input and an integral part of farming cabbage. Surveys conducted in North Sulawesi in 1990 showed that almost all farmers use pesticides to control pests and plant diseases [3]. Besides, can help people in coping with pests and diseases, pesticide application turns giving great influence on other organisms and the environment rather than the target [4].Soil as a growing medium plant does not only consist of abiotic components but also contains large amounts of microorganisms. A wide variety of microbial activity in the soil affects the soil fertility. Side effects of the application of pesticides to control pests and diseases cabbage are the presence of residue in the soil in the long term that can harm soil organisms. The side effects of pesticides containing chemicals not only on non-target organisms, but also including soil microorganisms [5].The more cabbage sprayed with pesticides it will affect the accumulation of residue on the ground. According to Rao [6], pesticides that can not be broken down by soil biota when the user continuously then the residue will accumulate and can cause contaminated soil. Many types of biota and soil microbes useful for the land, if the existence of pesticides disrupt life or activity of soil biota, the fertility of the soil is disturbed. Results of interviews with horticultural farmers in Subdistrict Modoinding in controlling diseases ofcabbage, they use a lot of pesticides. According to them during cultivate cabbage plants, the use of pesticides to control diseases continue to be done as well as with the use of agricultural land have long done. According to them, when land was less fertile cabbage cultivation is carried out logging in the surrounding area of agriculture to be a cabbage garden. Given this reality, one may suspect there is a possibility of contamination

of soil due to continuous use of pesticides, this could be the cause of the growing extent of agricultural land were contaminated pesticides.

The use of pesticides to eradicate pests or specific diseases often sterilize soil ecosystem, so that bacteria and fungi in the soil decreased population. Effect of pesticides on microorganisms quite serious because nitrogen mineralization and nitrification. The problems above has yet to be resolved, while the land on which agricultural crops growing media and soil ecosystem biodiversity inhabitants bear a heavy burden because it has become a pollutant accumulation of pesticide residues.

To determine the species of fungus in the soil in the gardens of cabbage were contaminated with pesticides is necessary to isolate and identify fungi species because many unknown quantities and species. The number species of fungi have been known until now only about 69,000 of the estimated 1.5 million species exist in the world. It is certain that Indonesia is very rich diversity of plant and animal diversity also has a fungus which is very high considering the environment humid and tropical climate that supports the growth of fungi [7].

II. MATERIALS AND METHODS

2.1 Time and Place of Research

This research was conducted in the central areas of cabbage plants are widely used pesticides in the Modoiding Subdistrict, South Minahasa Regency for 12 months. Isolation and identification of soil fungi was conducted in the Laboratory of Microbiology, Faculty of Agriculture, University of Sam Ratulangi, and Laboratory of Microbiology, Faculty of Mathematics and Natural Sciences, University of Manado..

2.2 Research Procedure

Soil samples were taken in the area of cabbage with a plot area of 10 x 10 square meters in which there are five subplots (beds) as a sampling. The size of each subplot is 90 cm wide and 10 meters long. Sampling was done every subplot. Soil samples from plots that had been treated were taken as much as 1 kg at a depth of 0-30 cm. Soil treatment as follows:

A. Cabbage plants sprayed with insecticide of organophosphate class
B. Cabbage plants are sprayed with insecticide class of diphenyl (botanical insecticide)
C. Cabbage plants are sprayed with insecticide, carbamate group
D. Cabbage plants are sprayed with *Bacillus turingiensis* ((microbiological insecticide))
E. Control

Each treatment beds in the planting area of vegetables cabbage, taken soil samples as much as 5 to 5 treatments are composited together.

Isolation of fungi used dilution plate method. The method used is based on the methods used by Humaidi, *et al* [8]. The method is done by taking samples of the soil as much as 1 gram and was added to 99 ml of sterile distilled water and shaken until homogeneous. Next take 1ml suspension of land at 10^{-2} was added to test tubes containing 9 ml sterile distilled water, then shaken until a homogeneous suspension is obtained a 10^{-3} dilution. In the same way made further dilution to 10^{-7}. In the treatment of A-E, dilutions used there 4 dilutions namely: 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. The next step is to pour 10 ml PDA in each petri dish. To avoid the growth of bacteria is necessary to add anti-biotic Streptomysin 0.1 g /1L media PDA and neomycin 0.01 g / 1 L of PDA. The next step took 1ml suspension of the fungus from each serial dilution using a sterile pipette and put into a petri dish which contains a PDA before it solidifies. Then the culture is incubated at room temperature for 7 days [8]. Isolates of the fungus is then isolated further to be observed under miskroscope and identified by matching the characteristics of the fungus obtained from observations with reference to the book Pictorial Atlas of Soil and Seed Fungi [9], the book Illustrated Genera of Imperfect Fungi [10], the book Compendium of Soil Fungi identification Work [11], The Introduction of Tropical Fungus General [12], and Introduction to Food-Borne Fungi [13]. Isolates were identified macroscopically (to see the shape of the colony) and microscopic (see form conidia, spores / sporangium / sporangia, conidiophores, phialides, metula and hyphae) after 7 days-old culture.

III. RESULTS AND DISCUSSION

A total of 76 fungal isolates were obtained from 5 soil treatments in the area of cabbage that consists of treatment A: 17 isolates, treatment B: 15 isolates, treatment C: 16 isolates, treatment D: 14 isolates and treatment E: 14 isolates. The fungi identification results have been obtained 13 families and 22 species (Table 3.1).

From Table 3.1 shows that the most dominant species of fungi growing in four serial dilutions (10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) were from Family Trichocomaceae with nine isolates among other things: *Penicillium citrinum* (IJ2), *Paecilomyces lilacinus* (IJ6), *Aspergillus fumigatus* (IJ3), *Aspergillus nidulans* (IJ4), *Aspergillus* foot cell (IJ9), *Aspergillus sydowii* (IJ10), *Aspergillus flavus* (IJ13), *Aspergillus terreus* (IJ17), and *Aspergillus*

niger (IJ18). Members of the Trichocomaceae most commonly found in soil samples from the gardens of cabbage may be caused by pesticides and fertilizers used continuously by farmers over the years so as to kill microorganisms, especially soil fungi found on land cabbage plant. As stated by Nasahi [14], the use of pesticides over the years (± 15 years) can reduce the diversity of soil organisms. Fungi in the rhizosphere that can survive only fungi that have high adaptability or fungi that have experienced coevolution. Of the nine species of the family Trichocomaceae, *Aspergillus fumigatus* (IJ3) (Fig. 3.1 and 3.2) were dominant and had the fastest growth.

TABLE 3.1
Results Identification of Fungal Isolates from Five Treatments in the Gardens Of Cabbage

Isolates	Fungi	Family	Species
BI1, DI1	IJ1	Endomycetaceae	*Geotrichum candidum*
CI2	IJ2	Trichocomaceae	*Penicillium citrinum*
AI3, BI3, CI3, DI3, EI3	IJ3	Trichocomaceae	*Aspergillus fumigatus*
AI4, BI4	IJ4	Trichocomaceae	*Aspergillus nidulans*
AI5, BI5, CI5, DI5, EI5	IJ5	Sordariaceae	*Chrysonilia sitophila*
AI6, BI6, CI6, DI6,EI6	IJ6	Trichocomaceae	*Paecilomyces lilacinus*
AI7, BI7, CI7, DI7, EI7	IJ7	Mucoraceae	*Mucor hiemalis*
AI8, BI8, CI8, DI8, EI8	IJ8	Pleurostomataceae	*Pleurostomophora richardsiae*
AI9, BI9, CI9, DI9	IJ9	Trichocomaceae	*Aspergillus foot cell*
AI10, BI10, CI10, DI10, EI10	IJ10	Trichocomaceae	*Aspergillus sydowii*
AI11, BI11, CI11, DI11, EI11	IJ11	Hypocreaceae	*Gliocladium virens*
AI12, BI12, CI12, EI12	IJ12	Pythiaceae	*Phytophthora infestans*
AI13, BI13, CI13, DI13, EI13	IJ13	Trichocomaceae	*Aspergillus flavus*
AI14, BI14, CI14, DI14, EI14	IJ14	Chaetomiaceae	*Humicola phialophoroides*
AI15, BI15, CI15, DI15, EI15	IJ15	Nectriaceae	*Fusarium oxysporum*
AI16, B16, CI16, DI16, EI16	IJ16	Chaetomiaceae	*Humicola fuscoatra*
AI17, CI17	IJ17	Trichocomaceae	*Aspergillus terreus*
CI18, DI18	IJ18	Trichocomaceae	*Aspergilus niger*
EI19	IJ19	Eremomycetaceae	*Arthrographis cuboidea*
EI20	IJ20	Incertae sedis	*Scytalidium lignicola*
AI21	IJ21	Bionectriaceae	*Gliocladium roseum*
AI22	IJ22	Arthrodermataceae	*Microsporum audouinii*

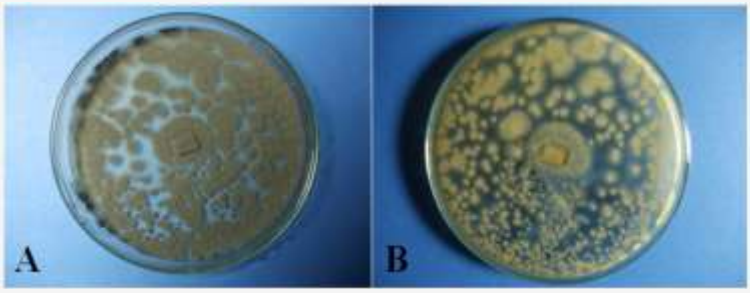


Figure 3.1. Microscopic Morphological Shape *Aspergillus Fumigatus* (Ij3) During Incubation Seven Days (A: Front, B: Reverse Of Colony)
Source: Personal Photos

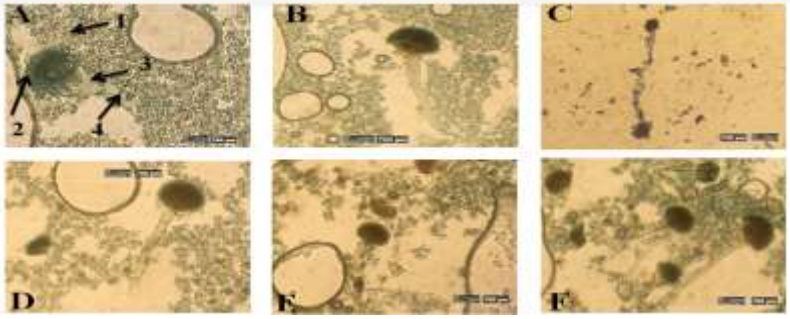


Figure 3.2. Microscopic Forms of *Aspergillus Fumigatus* (A: Phialides Over 2/3 of the Vesicles and Parallel to the Axis Conidiophore, Conidia (Arrow 1), Phialide (Arrow 2), Vesicule (Arrow 3), Conidiophore (Arrow 4); BC: Long Conidiophores; D - F: Some of *A. Fumigatus* at 40x Magnification. (Source: Personal Photos)

Morphological characteristics of fungi macroscopically and microscopically matched with identification book Compendium of Soil Fungi [11], The Introduction of the Common Tropical Fungus [12], and Introduction to Food-Borne Fungi [13], IJ3 isolates (*Aspergillus fumigatus*) was included in fungi kingdom, phylum Ascomycota Class eurotiomycetes, Oeder Eurotiales, Family Trichocomaceae and Genus *Aspergillus* [15]. The observation of macroscopic and microscopic (based on Figure 3.1 and 3.2) show the morphological characteristics of the macroscopic as follows: the colony surface with a texture like velvet (felty), colonies that grow on the medium PDA has a color that varies according to the time of incubation, at day -3 greenish-white colored colonies, the 5th day of dark green and growing on the 7th day green gray (rather dark) color. While the colony reverse color was yellowish white to pale yellow middle section, conidia round with a dark green color and uneven walled. The results of this are also the same as that of the Samingan [16], the colony diameter of 1.8 cm, yellow, light green middle part, texture felty, the edges of colonies pale yellow, not flat. According to Baker and Bennett [17]; according to [18], *Aspergillus* growing on agar plates (eg potato dextrose agar (PDA), Czapek agar (CZA), Sabouraud dextrose agar (SDA), and malt extract agar (MEA), *Aspergillus* species usually produce colonies colored (depending on the species, regardless of the media used the color will be different).

Identification *A. fumigatus* was based mainly on the morphology of conidia and conidiophores [19]. In this study, *A. fumigatus* has conidia round and half round green and elongated conidial head (columnar), conidiophores with long stems are smooth, transparent and white (these results differ from the results of [12] which describes *A. fumigatus* has konidiosfor short), septate and branching hyphae, vesicles wide, there is a gourd-shaped phialide 2/3 of a vesicle formed parallel to sumbuh conidiophores. The results obtained in accordance with the description of *A. fumigatus* by [20], *A. fumigatus* has the color green conidia, conidial heads columnar, phialides directly attached to the vesicle and conidia hemispherical to spherical shaped with size of 2.5 - 3 µm. This result is also the same as that of the [16], hyphae hyaline with diameter ± 6 µm, aspergillauniseriate, conidiophores hyaline and about 8µm in diameter, length phialide about 5 µm, conidia globus and 5 x 5 µm in diameter, and approximately 22.5 µm in diameter vesicle.

A. fumigatus have long stalks (conidiophores), conidiophores septate or nonseptate arising from foot cells, at the end of the conidiophores appear a bubble, out of this bubble appears sterigma, on sterigma appears konidium-konidium sequentially arranged like a string of pearls that form supports large head (vesicles). These vesicles contained in the spores are arranged like a chain [21]. While according to [12] that the colony of *A. fumigatus* dark green, because the pigment contained in conidia. Columnar-shaped head is typical conidia, conidiophores short, smooth-walled and green. Club-shaped vesicles with a diameter of 20-30 µm and coarse walled like thorns.

Aspergillus species are naturally present everywhere, especially on food, stale vegetable, in leaf litter or compost piles, is a fungus that is cosmopolitan. Conidia usually found in the air both inside and outside the room and throughout the year. Conidia are spread by wind [22]. *A. fumigatus* produces thousands of conidia [22]. *A. fumigatus* do not have a complex mechanism to release conidia into the air; depends only on the dissemination of environmental disturbance and a strong air currents [19]. According to [23] *A. fumigatus* is a fungus that belongs to a class Ascomycetes easily isolated from the environment air. This species mainly lives as saprobic soil fungi that can be found in the substrate plant, compost, seeds and rotten wood. *A. fumigatus* can be a contaminant in laboratorium. *A. fumigatus* is a fungus that is growing rapidly in about 3 days. *A. fumigatus* is thermophillic, good growth at 45^{o}C and often up to 50 ° C [21]. This capability can be used to distinguish it from other species.

In addition to *A. fumigatus*, the species was also found with the fastest growing colonies with high adaptability are from Family Sordariaceae (*Chrysonilia sitophila*), Family Endomycetaceae (*Geotrichum candidum*) and Family Trichocomaceae (*Penicillium citrinum*). This family has the fastest growth of the colony, where the incubation period of 3 days of the colony already covered petridish. All three isolates were identified in this morphological characteristics and microscopy macroscopically different as presented in Table 3.2.

Observations showed conidia of *P. citrinum* form long chains. Conidia of *P. citrinum* form long chains, divergent or column, globular, elliptical or fusiform, transparent or greenish, with a smooth or corrugated wall [7]. Characteristics *P. Citrinum* in Table 2 have characteristics corresponding to that put forward in Mycology online [24]; according to [10] that the chains of conidia unicellular in production at the end phialide, and konidium youngest are at the very bottom of the chain of conidia. Conidiophores may establish branches in the call metulae. *Penicillium* microscopically has a distinctive shape of conidiophores. Conidiophores emerged upright from the mycelium, often forming sinnemata, and branching approaching its end. The tip has a bunch fialid conidiophores with conidia globus or ovoid-shaped, arranged to form a chain basipetal. *P. citrinum* are microorganisms that are found in isolation arable land. This microorganism is a microorganism that has a relatively rapid growth, as well as have the ability to suppress other microorganisms (competing) [25].

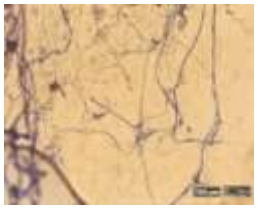
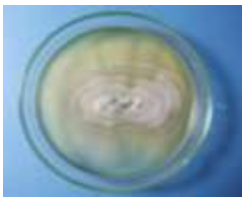
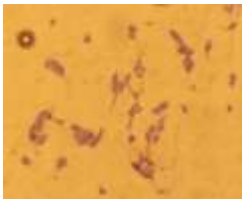
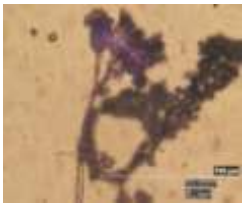
Rhizosphere fungi, such as *Aspergillus* sp., *Fusarium* sp., *Humicola* sp., *Penicillium* sp. is common and has been widely reported to be found on farms planting vegetables and fruits [11, 26, 27, 28, 29, 30]. *P. Citrinum* a typical soil fungus and has been widely reported [26, 27, 28, 31, 32]. According to [33] *Penicillium* sp., *Mucor* sp. and *Trichoderma* sp. is a saprophyte fungus most commonly found in soil. This species is cosmopolitan and very common air contaminants.This species frequently isolated from substrates previously high temperature, such as compost, and has been isolated from forest soil after the event of a fire, the soil salty high, sea, air, palm leaves, wood pulp, straw decomposing municipal waste, nest, feather and droppings of birds, peanuts, wheat, bananas, carrots, cabbage, onions, corn, sorghum, and rotten old palm saved. This species is easily spoil foodstuffs [12].

G. candidum microscopic morphology were found in accordance with Table 3.2 above, the same as found by Yuri [34], where observations on the SAB and PDA media, colonies showing rapid growth, resulting in a white cream colored colonies with a velvety texture, the appearance of this fungus transparent as glass / glass. Colony grows best at temperatures around 25^{o}C to 30^{o}C but growth may be restricted by microscopic identification 37^{o}C. *G. candidum* have hyphae hyaline (clear) and have dichotomy branching hyphae with 7µm - 11 µm in width. Disjunctor cell (cell space between fragments arthroconida to release arthroconidia). Cells can be cylindrical or it may be a long form. Blastoconidia, conidiophores and pseudohyphae are not produced by *Geotrichum* sp. However, these results differ from [35] that states fungus *Geotrichum* sp. has not septate. Hifa hyaline hyphae break into arthropores rectangular and oval, no blastospora generated. Surface colonies creamy and slightly raised and grew very fast in the cup petridish [11].

There are several types of fungi found in the soil and organic matter, such as *Aspergillus oryzae*, *Chrysonilia sitophila*, Mucor sp., *Rhizopus* sp. and many more varieties of mushrooms that were found [12]; [36]. *C. sitophila* be a source of problems as persistent contaminants in the laboratory and sometimes also reported in foods such as pastries, hazelnuts, nuts, and meat products [37]. Macroscopic and microscopic morphological observation of *C. sitophila* according to Table 3.2 shows the same results with the observations made by [38], which is based on the isolation and identification of macroscopic media PDA *C. sitophila* growing fast (faster growth if a diameter greater than 6 cm), orange and orange whitish middle. Likewise, growth in media MEA (Malt Extract Agar), although the colors are different are all orange. Meanwhile, among other microscopic morphology of hyphae septate, do not have the reproductive structures, have a round or oval conidia with a size of 12.5 µm, a smooth surface and orange, has conidiophores, there are no fialid, Metula and vesicles.

TABLE 3.2
Characteristics of Soil Fungi Morphology on PDA with Rapid Growth

Observations	SORDARIACEAE (*Chrysonilia sitophila)*	ENDOMYCETACEAE (*Geotrichum candidum*)	TRICHOCOMACEAE (*Penicillium citrinum)*
- Colonies on PDA: - Color	Day 3: white as snow Day -5: white brownish Day 7: orange	Day 3: pink Day 5: white and purple Day 7: creamy white with	Day 3: white with the center of the green Day 5: white with the center of the green-white Day 7: gray green
- Colony reverse	White with brown edges	Purple edges	Brownish white
- The colony surface	Smooth	Purple with white middle part blackish brown Velvet and transparent	Velvet
conidia: - Form	+ Round, rectangular and blastoconidia arthroconidia round	+ Arthroconidia spherical and cylindrical, no blastoconidia -	+ Round, rugged and robust form a chain
- conidiophores: - Surface - Color - Branching	+ Smooth, rectangular Purple -	-	+ Smooth, long White transparent (hyaline) +
- Phialid: - Form	-	-	+ Rather large ampuliform like pumpkin
Metula : - Form	-	-	+ Rectangle with an asymmetric structure

			biverticillata / terverticillata
Vesicels - Form	-	-	-
- Additional nature: - Growing Zone - Radial furrows - hyphae - Stolon and Rhizoid - Disjunctor Cells	- - - Septate - +	- - - Septate - +	- - - Septate - +
Pictures of Macroscopic and Microscopic Morphology Colonies	  The incubation period of 7 days	  The incubation period of 7 days	  The incubation period of 7 days

IV. CONCLUSION

1. Results of the isolation and identification of fungi from soil contaminated with pesticides in gardens of cabbage in the Subdistrict Modoinding obtained 76 isolates comprising 13 family and 22 species of soil fungi.
2. The most dominant species grown in four serial dilution (10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) is of a family Trichocomaceae among other things: *Penicillium citrinum* (IJ2), *Paecilomyces lilacinus* (IJ6), *Aspergillus fumigatus* (IJ3), *Aspergillus nidulans* (IJ4), *Aspergillus* foot cell (IJ9), *Aspergillus sydowii* (IJ10), *Aspergillus flavus* (IJ13), *Aspergillus terreus* (IJ17), and *Aspergillus niger* (IJ18) (9 isolates).
3. The species with the fastest growing colonies are from families Sordariaceae (*Chrysonilia sitophila* (IJ5), Family Endomycetaceae (*Geotrichum candidum* (IJ1), Family Trichocomaceae (*Penicillium citrinum* (IJ2) and Family Trichocomaceae (*Aspergillus fumigatus* (IJ3).

ACKNOWLEDGEMENTS

The team of authors would like to thank the Indonesian Directorate General of Higher Education, Ministry of Education and Culture, which has funded this research through Doctoral Dissertation Research Grant Scheme.

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