

# MUSHROOM ACTIVITY TEST RHIZOPUSSTOLONIFER IN BREAD, RICE AND TEMPE THAT CAUSE DIGESTIVE DISORDERS

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## ABSTRACT

Rhizopusstoloniferis a fungus which is a phylum zygomycota ordo mucorales. The characteristic feature of this fungus is that it has hyphae that form rhizoids that stick to the substrate. Another characteristic of this fungus is that it has cenocytic hyphae. Digestive disorders are gastritis, the client has complaints of heartburn, nausea, vomiting, and no appetite. eat. The researcher also found that the nursing diagnosis established by the nurse was not in accordance with the patient's complaints. Nurses on duty in the internal medicine room said that they often have difficulty in establishing nursing diagnoses that are in accordance with the nursing process. This study aims to identify the morphological structure of micro fungi (mold) using a microscope.

The method used is the experimental method of a scientific research in which the researcher manipulates and controls one or more independent variables and observes the related variables to find the variations that arise along with the manipulation of the independent variables. This experiment aims to examine the possibility of causality by applying one or more treatment conditions to one or more experimental groups and comparing the results with one or more untreated control groups.

The results showed that Mushroomrhizopusstolonifer in bread, rice and tempeh can cause digestive disorders.

**Keywords:** mushroom rhizopusstolonifer, bread, mushroom, rice

## INTRODUCTION

Indonesia is one of the hot and humid tropical countries, if the cleanliness is not maintained properly it can cause the air to be polluted by organic or non-organic compounds, which can cause disruption of human health by microorganisms such as bacteria, viruses and fungi. Diseases caused by fungi can be classified into aspergillosis, candidiasis, coccidioidomycosis, histoplasmosis. Although in particular the case is not much revealed. Fungi can grow in tropical climates because the climatic conditions in Indonesia, which has a warm and humid

tropical climate, strongly support the growth of fungi [1].

The role of microbiology in the pharmaceutical field is quite large. By studying microbiology, pharmacists can make drugs that can prevent, treat or eradicate microorganisms that cause disease, for example with the discovery of anti-biotic chemotherapy that can combat microorganisms that cause infection. In addition, there are penicillin, streptomycin and chloramphenicol which are antibiotics produced through specific microorganisms that are stimulated to grow so that they can become life-saving drugs.

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Bread is a food that is made from flour. According to Kusuma, the high starch content in flour is then hydrolyzed into simple sugars by fungi, while simple sugars are the main nutrients for bread. Fungi have an important role in the process of making or spoiling bread. The types of fungi that are commonly found in bread spoilage are *Rhizopus stolonifer*, *penicillium sp*, *mucor sp* and *geotrichum sp* and there can also be *Aspergillus sp* and others [9].

Mushrooms are low-level plants that do not have green substances, to live fungi act as saprophytic parasites. Mushrooms live in a variety of environments, but most of them live in humid places with optimal temperatures ranging from 22oC to 35oC, maximum temperatures ranging from 27oC to 29oC, and minimum temperatures around 5oC. however, many fungi live on organisms or the remains of organisms in the sea or in fresh water. Fungi can also live in an acidic environment [18].

*Rhizopus sp* is a genus of saprophytic fungi that are common in plants and specialized in animal parasites. This mold is very important in the food industry as a producer of various enzymes amylase, protease, pectinase and lipase, this mold in food plays a role in the fermentation process or the process of spoilage in food. *Rhizopus sp* is often found on soil, rotting fruit and plants [18].

Meanwhile, microbiology itself has a meaning, namely the study of small organisms (less than 0.1 m in diameter) that cannot be seen with the naked eye and can only be seen with the aid of a microscope.

These creatures, called micro-organisms or microorganisms, are everywhere. Some of them are useful for human life, but many are also harmful to humans, which can cause various kinds of diseases, such as infections.

In this experiment, what we are doing is observing the morphology of the

fungus, while mushrooms are living things that cannot make their own food, therefore they are saprophytic or parasitic. Mushrooms are often found in moist places and are rich in organic substances, for example as we find in bread that has passed its production period, tempeh that has rotted and also rice that has gone stale.

The mushrooms that we observe are fungi that decompose our food so that the food rots, but after we observe the mushrooms it turns out that they can make organic matter decompose and do not fill our nature with organic waste. And mushrooms have also been widely used for food fermentation, for example in tempe which uses *Saccharomyces cereviceae*.

Therefore, by doing this practicum we can learn the parts of mushrooms that are in foods such as tempeh, bread, and rice that we have observed, and will really help us to make better use of these mushrooms.

Food storage is the end of the production process, after the bread is cooked and then cooled for several hours. Bread is a perishable food with a shelf life of 3-4 days. Bread spoilage is caused by the breakdown of protein and starch, directly bread spoilage is caused by spoilage microorganisms. During storage, bread will suffer some damage if it is stored for too long and is not stored in the right place. Bread spoilage includes physical damage to bread such as texture hardening, mold growth, and rancidity.

The most common molds found in bread were *Rhizopus stolonifer*, *Penicillium expansum*, *P. Stoloniferum*, *Aspergillus niger*, *Neurosporasitophila*, *Mucor sp* and *Geothricum sp*. This mold growth comes from the air during bread cooling, handling, packaging or from cutting tools. Bread spoilage during storage is spoilage and rancidity. Rotten bread is characterized by an unpleasant odor and taste, darker and stickier crumbs,

reddish or dark red skin on the bread. Rancidity in bread is caused by the breakdown of fat or oil, resulting in an unpleasant taste and odor. The amount of damage that occurs depends on the length or time a food is stored. A longer time will cause more damage, except for cheese, wine, whiskey and others that are not damaged during aging [7].

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The fungus *Rhizopus* sp is a fungus that belongs to the phylum zygomycota ordo mucorales. The characteristic of this fungus is that it has hyphae that form rhizoids that stick to the substrate while other characteristics of this fungus have cenocytic hyphae, therefore this fungus is not insulated. Stolons or micelles of this fungus *Rhizopus* sp spread over the substrate because the hyphae of this fungus are vegetative. The fungus *Rhizopus* sp produces asexually and produces stemmed sporangiphores. Sporangiphores are separated from hyphae from other hyphae by a wall like septa. One species of this fungus is the fungus *Rhizopus* sp stolonifer which is found on stale bread [16].

The term fungus or fungi is always associated with a disease. Because it is still poorly understood by the general public. Some fungi are beneficial, some are detrimental. Fungi play an important

role in our daily life. Because it is able to recycle elements in nature that are needed for other life [8].

*Rhizopus* sp has colonies that are whitish to gray-brown to yellowish-brown. Rhizoid of the fungus is brown, branching and opposite in direction to the sporangiophores can arise directly from the stolons without the presence of rhizoids. Sporangiphores can be single or in groups sometimes resemble forks, spiny walls, dark brown color to blackish brown with a diameter of 50-200 m.

The columella is in the form of culture age, and reaches a height of approximately 10 mm. The stolon is smooth or slightly rough-walled and almost colorless, the sporangiospores of this fungus are round or not, usually polyginal in shape, have lines on the surface and are about 4-10 m long. Chlamidospores are spherical, with a diameter of 10-35 m or elliptical in shape and measure (8-130) x (16-24) m.

The role of fungi in our daily lives, among others, can be mentioned in agriculture and plantations causing diseases in economic crops such as rice, corn, potatoes, coffee, tea, cocoa, coconut and rubber, in the forestry sector they damage wood and their processed products. In fact, it is needed in land burial, in the pharmaceutical field, fungi are used to produce various enzymes and certain organic acid compounds, in the medical field a number of fungi are indeed pathogenic for humans, including causing allergies and dermatomycosis, in the public health field, fungal spores in the air cause air pollution which, when Inhaled causes coughing and allergies besides it is also known that fungi can damage the environment, petroleum paints, paper, and textiles [8].

Fungi are microorganisms without chlorophyll, in the form of hyphae or single cells, eukaryotic, cell walls made of

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chitin or cellulose, reproduce sexually and asexually.

Most of the body of a fungus consists of threads called hyphae that are interconnected in a kind of net, namely mycelium. Mycelium can be distinguished into vegetative mycelium which functions to absorb nutrients from the environment and fertile mycelium which functions in reproduction [8].

Fungi can be found on various substrates, both in the land, water and air environment. It is not difficult to find fungi in nature, because the vegetative part, which is generally a mycelium, is white and easily visible on decaying substrates. Conidia or fruiting bodies can have colors (red, black, orange, yellow, cream, white, gray, brown, bluish and so on). On leaves, stems of paper, textiles, leather and others. The fruiting bodies of fungi are more conspicuous because they can be seen directly with the naked eye, while the vegetative mycelium that absorbs food can only be seen using a microscope [8].

High-level and low-level fungi have a distinctive feature, namely in the form of a single thread or branching called hyphae. Fungi are eukaryotic organisms that have the following characteristics:

1. Have a spore
2. Produce spores
3. It doesn't have chlorophyll so it doesn't photosynthesize
4. Can reproduce sexually and asexually
5. The filamentous body and cell wall contain chitin, glucan, cellulose and manan [19].

Fungi are divided into two groups: molds and yeasts. Mold is a filamentous fungus or has a mycelium. Yeasts are single-celled and non-filamentous fungi. Fungi are organisms that resemble plants, but have several differences, namely:

1. Does not have chlorophyll,
2. Have cell walls with different compositions,

3. Reproduce by spores,
4. Has no branches, stems, roots and leaves,
5. Does not have a vascular system like plants,
6. It is multicellular and does not have the division of functions of each part as in plants.

Some fungi are parasitic and some are saprophytic. Parasites when meeting their food needs by taking from living things they are carrying. While it is saprophytic if it gets food from inanimate objects and does not harm the object itself. Fungi synthesize proteins by taking carbon and carbohydrate sources (eg glucose, sucrose or maltose), nitrogen sources from organic or inorganic materials, and minerals from their substrates. There are also some fungi that can synthesize vitamins needed for their own growth and reproduction. But there are also those that cannot synthesize themselves, so they must obtain them from substrates, such as thiamin and biotin.

Multicellular fungi or molds have mycelia or filaments and their growth in foodstuffs is easy to see, which is like cotton. The growth of the fungus is initially white, but when it has produced spores it will form a variety of colors depending on the type of mold. Microscopic or macroscopic appearance properties are used for the identification and classification of molds [19].

Molds can be divided into two groups based on the structure of hyphae, namely non-insulated or nonseptate hyphae and insulated or septate hyphae which divide hyphae into segments, where each segment has one or more nuclei. The insulating wall in molds is called a septum that is not tightly closed so that the cytoplasm can still freely move from one space to another. The septate molds are mainly the class Ascomycetes. While the non-septate molds are the Phycomycetes

class. The non-septate molds are essentially scattered along the septa [19].

Both unpretentious mushrooms and mushrooms with a high level of body have a distinctive feature, namely in the form of a single branching thread called mycelium. Or in the form of a collection of threads that can become one. Only the yeast group has a single cell body. The second characteristic is that fungi do not have chlorophyll, so their lives are forced to be heterotrophs. This trait reinforces the notion that fungi are a continuation of bacteria in evolution [4].

The fungi group includes more than 55000 species; this number is far more than the bacterial species. There is no unified overall opinion about the classification of taxonomists. Bacteria and fungi are groups of plants whose bodies do not have differentiation. Therefore, it is called a thallophyte plant, a complete thallophyta that does not contain chlorophyll [4].

Some fungi, although saprophytic, can also invade living hosts and thrive there as parasites. As parasites they cause disease in plants and animals, including humans. However, among about 500,000 species of fungi, only about 100 are pathogenic to humans. Infection mortality by fungi other than skin diseases is very high. This may be due to a late or incorrect diagnosis during the course of the disease or to the unavailability of antibiotics. Medically appropriate non-toxic antibiotics.

Many pathogenic fungi, such as *Histoplasma Capsulatum*, cause histoplasmosis (mycotic infection of the reticuloendothelial system covering many organs). Can also live as a saprophyte, such functions show dimorphism, meaning they can exist in a unicellular form like yeast or in a clear (filamentous) form like molds. The yeast phase occurs when the organism lives as a parasite or pathogen in the tissue, while the mold forms when the organism is a saprophyte

in the soil or in a laboratory medium. Laboratory identification for pathogenic fungi often depends on whether or not this dimorphism is demonstrated [14].

Fungi can survive in unfavorable environmental conditions compared to other less capable microorganisms. For example, yeasts and molds can grow in a substrate or medium containing a sugar concentration that inhibits most bacteria. Similarly, molds and yeasts generally can withstand more acidic conditions than most other microbes [14].

The factors that affect the growth of fungi are as follows, in general, the growth of fungi is influenced by [17].

1. Water requirement, some fungi require very little water to grow than bacteria,
2. Growth temperature, molds and yeasts grow optimally at room temperature, namely at temperatures ranging from 25-30°C, but there are several types of fungi that can grow at 35-37°C-37°C and higher temperatures, for example, *Aspergillus sp*,
3. Oxygen and pH, oxygen and pH are factors that affect the growth of fungi because every fungus requires oxygen, it would be better if the conditions were acidic or the pH was low because the fungus itself is aerobic,
4. Substrate or media, in general, mushrooms can take advantage of various components in both simple and complex foods. Fungi can make hydrolytic enzymes such as amylase, proteinase, and lipase. Therefore, fungi can grow on foods that contain starch, protein, and lipids in them,
5. Inhibitory component, some fungi can secrete components that inhibit other organisms, these components are antibiotics. There are several other components that are mycostatic, namely inhibiting the growth of fungi or fungicidal, namely killing fungi.

Classification of fungi is a grouping of fungi based on their kinship. According to

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Darnetty, until now it has not been perfect and often changes, there are still many differences of opinion regarding this classification due to differences in interpretation and incomplete data regarding the structure, development, and physio. Stated that each fungus belongs to the same category which is distinguished by the type of spore, hyphal morphology and the fruiting body emergence cycle will be followed by the formation of other parts such as stems and mushroom caps to become perfect mushrooms this fungal cycle can be seen in the Macroscopic Fungus Life Cycle.

Fungi are a grouping of fungi based on their kinship, the classification and naming of fungi until now has not been perfect and often fluctuate or there are still many differences of opinion about the classification. This difference of opinion is caused by differences in interpretation and data that are still incomplete on development, physiology and DNA analysis results from fungi, each fungus belongs to the same category which is distinguished by spore type, hyphae morphology and cycle 16 following the formation of other parts such as stalks and hoods. Fungus is a grouping of fungi based on the classification and naming of fungi that change or are not stable, because of their sexuality. This group is Oomycota, Zygomycota, Deutromycota, Ascommycota, and Basidomycota [12]. the morphology of hyphae and cycles 16 follow the formation of other parts such as the stalk and hood of this fungus can be seen in. fungi are a grouping of fungi based on the classification and naming of fungi that change or are not stable, because of their sexuality. This group is Oomycota, Zygomycota, Deutromycota, Ascommycota, and Basidomycota, the morphology of hyphae and cycles 16 follow the formation of other parts such as the stalk and hood of this fungus can be seen in [12]. Fungi are a grouping of fungi

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1. Oomycota, the division Oomycota is also known as the water molds because most members of this division live in water. Oomycota are commonly found in all places, both fresh water and sea water, especially in estuaries, rivers, ponds or shallow lakes near riverbanks or near the coast, while the majority of Oomycota fungi that live on land are facultative parasites or special parasites on vascular plants.

The main characteristic of the division Oomycota fungi is that in their reproduction process this fungus will produce oospores and zoospores with 2 flagella. One flagellum is long, hairy (whiplash), and points forward, while the other is short, plain (insel) and points backward. Oomycota have a morphology similar to fungi and also obtain nutrients by absorption, but this does not make oomycota have a close relationship with true fungi. Oomycota have a close relationship with algae so that Oomycota are included in the kingdom Stramenopila [3].

2. Zygomycota, zygomycota consists of two classes, namely Trichomycetes and Zygomycetes. Zygomycetes are saprophytic or haustorial, or non-haustorial parasitic in animals, plants and fungi. Trichomycetes are symbionts in the gut, or sometimes around the anal region of the arthropod, which attach to the host cell by means of a cellular or noncellular grip or holdfast.

A distinctive feature of the division Zygomycota is that the fungi in this division produce thick-walled zygospores in sexual reproduction and in asexual reproduction, producing sporangium which is generally round in

shape, formed on specialized fertile hyphae called sporangiophores. The sporangium contains sporangiospores. There are also species with small sporangium that are formed simultaneously, called sporangiola [8].

3. Deutromycota, this group is also called anamorphic fungi, imperfect fungi, conidial fungi, mitosporic fungi, or asexual fungi, and includes 2,600 genera and 15,000 species. Many species that are included in the Deutromycota, after finding their sexual phase (teleomorph), are included in the Ascomycota or into the Basidiomycota. Deutromycota is not a formal taxonomic category. These molds are not a monophyletic unit, but are fungi that do not have a sexual phase.
4. Ascomycota mentions that Ascomycota is also known as sac fungus, this is due to the presence of ascus as a characteristic of the Ascomycota division. Fungi from the division Ascomycota can be found in almost all seasons in various habitats, but there are only a few types of fungi that survive the dry season. Most fungi of the division Ascomycota live on soil or rotting wood and produce large fruiting bodies. The characteristic that distinguishes Ascomycota with fungi from other divisions is the presence of ascus or also called pouch. The mycelium in Ascomycota consists of well-developed hyphae, slender, septate and branched. In the middle there is a small hole or pore. The cell walls of the hyphae in Ascomycota are mostly composed of chitin,
5. Basidiomycota, the Basidiomycota group of fungi is often called a fungus by the layman because many types have large carpus (fruiting bodies) and can be seen with the naked eye. members of the Basidiomycota division known as macroscopic fungi, are a large and important group with a

species number of about 22,000 species. Most of the mushrooms seen in the field or on wood are from this division. Basidiomycota are a group of fungi that have important meaning including species that are both harmful and beneficial [8].

Reproduction and the Life Cycle of Fungi  
Reproduction or reproduction is the formation of new individuals who have the characteristics of their parents. This reproduction aims to maintain the species from extinction. According to the reproduction of fungi are generally divided into two types, namely asexual and sexual. Asexual reproduction does not involve nuclear union, namely by division or bud formation, while sexual reproduction is the union of two fungal nuclei.

Asexually the fungus can grow from a piece of mycelium, but this is rare. Reproduction that generally occurs in fungi is the growth of asexual spores. Fungal asexual spores are produced in large numbers, are small and light in weight, and are resistant to dry conditions. These spores can easily fly in the air and grow into new mycelium elsewhere. There are several types of asexual spores in fungi, namely: conidiospores (singular = conidium, plural=conidia), sporangiospores, arthospora, chlamidospora, blastospores and zoospores. Blastospores are asexual spores formed in yeast, while zoospores are generally found in aquatic fungi [5].

The characteristics of fungi are organisms belonging to the group of fungi, whose members have general characteristics, namely unicellular or single-celled or multicellular (fine threads). Their bodies are composed of thread-like vialine hyphae. Fine threads, eukaryotic (have a nuclear membrane), do not have chlorophyll so they are heterotrophs. That is saprophytic, parasitic and symbiotic, the cell wall is composed

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of chitin. Food reserves are stored in the form of glycogen and protein digestion takes place extracellularly, where food before being absorbed is simplified by extracellular enzymes released from fungal hyphae, having shorter haploid offspring. Unicellular fungi reproduce asexually by forming spores.

### RESEARCH METHODS

#### Tools and materials

1. EMBA (Eosin Methylene Blue Agar) Media, Composition:
  - a. Peptone, 10.0
  - b. Lactose, 10.0
  - c. Eosin, 0.4
  - d. Methylene Blue, 0.0
  - e. So that, 15.0
  - f. DHP, 2.0
2. PDA media composition:
  - a. Potato Infusion: 4.0 (Infusion from 200 gr Potatoes)
  - b. D(+) Glucose: 20.0
  - c. Jelly: 15.0
3. NA (Nutrient Agar), composition every 1000 ml contains:
  1. Beef Extract: 3,
  2. Lactose: 5 gr,
  3. So that: 15 gr,
  4. Distilled water: 1000 ml

The tools used during the practicum are, microscope, object glass and cover glass, round loop, Bunsen, lighter. The materials used during the practicum are; tempeh, bread, and rice that has been overgrown with mushrooms, aquadest [6].

#### Work procedures

1. Take 1 ose of mushrooms on bread, then place it on an object glass, drip with aquadest then cover with a cover glass
2. Observed with a microscope using an objective lens with a magnification of 10x, then with a magnification of 40x. to see the morphology of conidia or spores use an objective lens magnify 100x
3. Note the parts.

### RESEARCH RESULTS AND DISCUSSION

#### Research result

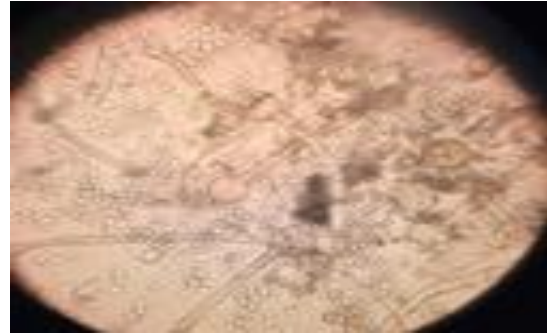


Figure 1. Types of Mushrooms Rhizopusstolonifer

#### DISCUSSION

Fungi are organisms whose cells are true or eukaryotic nuclei, thread-shaped, branched, not chlorophyll, cell walls containing chitin or cellulose or both, heterotrophs, absorptive and most of the body consists of vegetative parts in the form of hyphae and generative parts, namely spores.

Fungi are a group of eukaryotic organisms that make up the world of fungi or regnum fungi. Fungi are generally multicellular (many cells). The characteristics of fungi are different from other organisms in terms of how to eat, body structure, growth, and reproduction. Mushroom body composed of the basic components called hyphae. Hyphae form a network called mycelium. Mycelium arranges pseudo-weave into fruiting bodies. Hyphae are thread-like structures composed of tubular walls [15].

In this practicum, we made observations on bread that had gone stale and visible mushrooms that had grown on the bread, bread molds were greenish and black. Observed in the microscope, this fungus has thin hyphae branching and functions as roots (rhizoids) to attach themselves and absorb the necessary substances from the substrate. Rhizoid is simply a thin filament found in certain fungi and plants and sponges as a place to



attach the body of a plant or organism to the lower layer and is able to absorb nutrients. In addition, there are also sporangiophores (hyphae that stick out into the air and contain many cell nuclei, at the end of which a sporangium is formed (as a spore producer).

In the research that has been carried out with the title of using aquatic microfungi (*Rhizopus stolonifer*) as a bioremediator in the degradation of vegetable oil waste. Stated that the description of *Rhizopus stolonifer* had white colonies at the beginning of growth, then grayish brown. Colonies are shaped like cotton that produces sporangia in large numbers, has long hyphae, not along, has rhizoids, there are stolons that connect a series of ngia spores consisting of 2-5 sporangiophores. The white colonies of *R. stolonifer*, apart from being a contaminant, turned out to have the ability to ferment, for example, ethanol and tobacco. These microfungi can also convert some steroids and synthesize corticoids. Toxic substances were also detected in these micro fungi [2].

The comparison with the research that we have done in the study of degrading vegetable oil waste has white colonies at the beginning of growth, then it is grayish brown and shaped like cotton which produces sporangia in large numbers, has long hyphae, not attached, has rhizoids, there are The stolons that connect the series of ngia spores consisting of 2-5 sporangiophores compared to the results of the research on bread, rice and tempeh have a greenish and black color. Observed in the microscope, this fungus has thin hyphae branching and functions as roots (rhizoids) to adhere and absorb the necessary substances from the substrate.

Micro-fungal *R. stolonifer* can act as a bioremediator in degrading vegetable oil waste and remodel complex organic matter into simpler (inorganic) materials which are then used again as nutrients for

their growth. This was indicated by the decrease in oil concentration (52.38%, 60.06%) and COD (77.78%, 70.27%) by aquatic microfungi *R. stolonifer*. DO decreased at the end of the observation (7.2-0.45mg/L). Temperature and pH fluctuated due to the ongoing decomposition process, ranging from 25.4-27.95°C and 3.665-4.82, respectively.

Turbidity and TDS increased until the end of the observation caused by the effect of the decomposition process that produces simpler materials or inorganic materials. Microfungal biomass *R. stolonifer* increased rapidly at the t4 limit (48 hours) and decreased again at the end of the observation. This indicates that *R. stolonifer* grows optimally at an interval of 48 hours. The addition of biomass beyond the percent cover indicates a bioconversion process of vegetable oil organic matter into microfungi biomass. Thus, the organic matter of vegetable oil is reduced and turned into microfungi biomass, so it can be concluded that the microfungi *R. stolonifer* acts as a bioremediator in degrading vegetable oil waste.

## CONCLUSION

As for the conclusion of this study observed in the microscope, this fungus has a thin hyphae branched and serves as a root (rizoid) to attach itself and absorb the necessary substances from the substrate. Rizoid is simply a thin filemen found in certain fungi and plants and sponges as a place to attach the body of plants or organisms to the lower layer and able to absorb nutrients. In addition, there is also sporangiofor.

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