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Rhizospheric Soil Fungi from Several Food Crops in Purwokerto

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Abstract. There are several interaction patterns of fungus that live in the rhizosphere of plants, i.e. mutualism, commensalism, saprophytism, and parasitism. The objective of this study was to determine the genera of microscopic fungus collected from the rhizosphere of groundnut, mays plant, and rice field. In addition, the diversity of soil fungi of those three plants was considered. Research method applied was survey with purposive sampling. The rhizospheric soil sample was obtained from research locations of groundnut, mays plants, and rice field in Purwokerto area. The soil samples were taken from 1-10 cm deep. Next they were isolated, and then they were purified. Furthermore, the obtained data of fungus collection were then analysed descriptively, and also described based on their macro and micro morphology. Then, the collected fungus were identified by using identification manual for fungus. The result showed that fifteen (15) isolates were found in three different rhizosphere of ground nut, mays plants, and rice field. The description is as follow: *Aspergillus*-1, *Aspergillus*-2, *Penicillium*-1, *Penicillium*-2, *Mycophyta*, *Aureobasidia*, *Cylindrocarpon*, *Mucor*-1, *Mucor*-2, *Chaetomium*, *Gliocladium*, *Trichoderma*-1, *Trichoderma*-2, *Pleurophragmium*, and *Gonytrichum*. Finally, the diversity Index (H') noted from rhizosphere of groundnut plants was 0.312; mays was 0.195; and (H') of rice field was 0.124.

1. Introduction

As a tropical country with high rainfall and sunshine throughout the year, Indonesia is known to have high biodiversity, either plants, animals and microorganisms. The biodiversity has each potential, and many have not been explored by researchers, especially for microorganisms that need accurate determination, diversity and potential. One of the microbes that has a lot of potential is the fungus that occupies the soil around the roots of plants (rhizosphere). In the rhizosphere of different plants, different types of fungi were found. Fungi that occupy the rhizosphere of plants have a pattern of interaction with plant roots which can be mutualism, commensalism, saprophytism, and parasitism. The mutualistic fungi provide benefits to host plants, such as improving growth rates, resistance to pests, diseases and drought. The close relationship between the two also allows for the transfer of genetic material between them [1]. Saprophytic fungi play a role in overhauling complex compounds in nature. The existence of the filament structure lead the fungus to penetrate the substrate by using its hyphae. Fungi have a high enzymatic ability in decomposing organic compounds including lignin and cellulose compounds [2]. The presence of fungi plays a major role in maintaining the continuity of the cycle of various materials, especially carbon, nitrogen, and phosphorus [3]. Therefore, fungus directly plays a role in maintaining the level of fertility and the balance of the soil ecosystem.



Food crops which are also hosts of fungi contribute to the survival of fungi in their root systems. The root secretes exudate into the root region which can be used by fungi to metabolize. Exudates released by plant roots are: α -Alanine, Glutamic Acid, Aspartic Acid, Cystine, Glycine and Tyrosine [4]. The interaction between mushrooms and food plants certainly affects the diversity of the fungus in its root area.

Microorganisms that can live in the rhizosphere are very suitable to apply as biological control agents since the rhizosphere is the main area where plant roots are open to attack by pathogens. If there are antagonistic microorganisms in this area, the pathogen will deal with these antagonistic microorganisms as they spread and infect the roots. This is called natural obstacles. These antagonistic microorganisms have the potential to be developed as biological control agents, including *Trichoderma* spp., *Penicillium* spp and *Aspergillus* spp, which are commonly found in soils, grow quickly and are antagonistic to other fungi. The antagonistic mechanism of the fungus occurs by means of competition, mycoparasitic, and antibiosis [5]. The presence of fungi in the rhizosphere of several types of food crops will be different because the type of fungus is influenced by different host plants. Therefore, exploration of microscopic fungal genera and their biodiversity in the rhizosphere of several food crops, namely ground nuts, mays and rice.

2. Methods

2.1. Rhizosphere Soil Sampling

Soil sample of Rhizosphere was taken from the location of food crops (ground nuts, mays, and rice) in Purwokerto region. Rhizosphere soil samples were taken from 1-10 cm depth. Soil sampling was carried out using a Purposive Random Sampling Method.

2.2. Isolation of the Rhizospheric Fungi

Soil samples were diluted to 10^{-6} . Then in dilutions 10^{-5} and 10^{-6} they are isolated on Potato Dextrose Agar (PDA) medium through spread plate method and incubated for 1-5 x 24 hours. After the fungal colony grow, purification and counting of the fungal colonies.

2.3. Mushroom identification

Mushroom identification was carried out macroscopically and microscopically. The results of isolation of fungi in the form of pure culture, was determined based on its microscopic morphology using a binocular microscope. While in determining the fungal genus, key determination of [6]; [7] were elaborated as the reference.

2.4. Number Individual Conversions

Individual counts obtained on petri dishes were completed by counting the number of colonies or individuals in each dilution. The individual obtained was determined by multiplying the number of colonies formed by the dilution factor. If there are the same individuals in different plates, the multiplication results are averaged [8].

2.5. Biodiversity Index, Evenness Index Fungus ([9]; [10])

The diversity of microscopic fungi was calculated using the Shannon-Wiener (H'), formula is as follows:

$$H' = -\sum p_i \ln p_i$$

H' = Shannon-Wiener diversity index

$p_i = n_i / N$ (proportion of the number of i -type individuals with the total number of individuals)

n_i = number of individuals (isolates) in the i -th species

N = total number of individuals (isolates)

Criteria:

$H' < 1$ = Low diversity

$H' 1-3$ = Moderate diversity

$H' > 3$ = High diversity

The microscopic fungus evenness index evenness formula:

$$E = \frac{H'}{\ln S}$$

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E = Evenness Index

H' = Diversity Index of Shannon-Wiener

S = Number of species

Criteria

$E \leq 0.4$ 14 Low evenness

$E 0.4 - 0.6$ = Moderate Diversity

$E \geq 0.6$ = High Diversity

The following is Margalef's Species Richness Index of the microscopic fungi:

$$DMg = \frac{S-1}{\ln N}$$

DMg = Margalef's Species Richness Index

N = Number of Individual

S = Number of species/genera

Criteria

$DMg < 3.25$ = Low Species Richness

$DMg 3.5 - 5$ = Moderate species richness

$DMg > 5$ = High species richness

2.6. Soil Texture Analysis Test:

Soil texture analysis test was carried out at the Soil Laboratory of Agriculture Faculty, UNSOED. The method used was the pipette method [11].

2.7. Determination of soil C/N ratio :

Determination of the C/N ratio is carried out in the Soil Chemistry laboratory. Determination of Organic Carbon levels is done based on the method of [12], while the measurement of total Nitrogen content using the Kjeldahl distillation apparatus [13].

2.8. Data analysis

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The data of fungi obtained were analyzed qualitative descriptive and quantitative. Qualitative descriptive analysis was used to determine the genera and morphological characteristics of microscopic fungi. Quantitative analysis is used to determine the biodiversity index of microscopic fungi on soil of food crops.

3. Results And Discussion

The results of fungal isolation obtained from the rhizosphere soil samples of some food crops are as follows: From the groundnut rhizosphere, 11 (eleven) fungi isolates were found, i.e 9 (nine) genera: *Aspergillus* 2 (two) isolates, *Mycothypha*, *Aureobasidium*, *Penicillium*, *Cylindrocarpon*, *Mucor* 2 (two) isolates, *Trichoderma*, *Chaetomium*, and *Gliocladium*.

From the rhizosphere of mays plants, 9 (nine) fungi isolates were found e.a of 6 (six) genera. The genera are: *Mucor* 2 (two) isolates, *Penicillium* 2 (two) isolates, *Pleuropragmium*, *Trichoderma* 2 (two) isolates, *Aspergillus*, and *Gonytrichum*.

From the rhizosphere of rice field, 4 (four) fungal isolates were found consisting of 4 (four) genera. These genera are: *Mucor*, *Aureobasidium*, *Penicillium*, and *Trichoderma*.

The distribution of the observed rhizospheric fungi found were various. Groundnut plant rhizosphere showed the highest variation followed with maize plant rhizosphere and rice plant rhizosphere. They were 11 (eleven) isolates, 9 (nine), and 4 (four) isolates, respectively included in 9 (nine) genera, 6 (six) genera, and 4 (four) genera, as presented in Table 1.

Table 1. Distribution of Microscopic Mushroom Genera in Rhizosphere 3 (three) of Food Crop Plants

No.	Isolate/ genera	Rhizosphere groundnut	Rhizosphere maize	Rhizosphere rice
1	<i>Aspergillus</i> 1	•		
2	<i>Mycotypha</i>	•		
3	<i>Aureobasidium</i>	•		•
4	<i>Penicillium</i> 1	•	•	•
5	<i>Cylindrocarpon</i>	•	•	
6	<i>Mucor</i> 1	•	•	
7	<i>Aspergillus</i> 2	•	•	
8	<i>Trichoderma</i> 1	•	•	•
9	<i>Chaetomium</i>	•		
10	<i>Mucor</i> 2	•	•	•
11	<i>Gliocladium</i>	•		
12	<i>Penicillium</i> 2		•	
13	<i>Pleuropragmium</i>		•	
14	<i>Trichoderma</i> 2		•	
15	<i>Gonytrichum</i>		•	

The distribution of fungi in the soil was influenced by several factors including temperature, humidity, pH, soil texture, and availability of soil nutrients. According to [14], that the distribution size of organisms is often influenced by the abundance and characteristics of soil organic matter content, climatic conditions, vegetation surface, and soil texture. The results of observations of humidity in the rhizosphere of groundnut plants have a range of 50%, corn plants 67%, and rice plants 65%. Environmental temperature in the three types of plants ranged from 25°C-27°C. Soil pH in the three types of plants is the same: 7. The environmental factors of the soil sampling site did not show a significant difference, so that the difference in the spread of soil fungus in the rhizosphere of the three plant species observed was not absolute due to the influence of these factors.

The results of soil texture analysis in the three rhizosphere of the plant show that the rhizosphere of groundnut plants belongs to the clayey clay class, while the rhizosphere of maize and rice plants belong to the clay class soil. Soil texture of the rhizosphere of the three types of food plants observed was not the major factor causing differences in the fungal distribution.

Variation in the fungal distribution in the three rhizosphere of food crops is expected to be due to the influence of exudates produced by the roots of each plant. According to [15], that plant roots provide organic material which generally stimulates microbial growth. According to [16], plants play an important role in determining microbial diversity in the rhizosphere. Plant roots cause rhizosphere physical and chemical changes that will affect microbial diversity in and around the rhizosphere. Root exudate will select to invite or fight certain microbes. Many plants have genetic traits for tolerance or resistance to microbial invasion in the rhizosphere. Plant varieties determine rhizosphere microbial diversity.

Fungal biodiversity in food crop soil shows the results that can be categorized into 3 parameters as listed in Table 2.

Table 2. Species richness, biodiversity index and evenness index of rhizospheric fungi of some food crops.

	Groundnut	Mays	Rice
S	11	9	4
N	73.28	309.71	15.01
DMg	0,89	0,63	0,31
H'	0,31	0,19	0,12
E	0,13	0,08	0,08

10 number of isolates

N = total number of individuals

DMg = Margalef's species richness index

H' = biodiversity index

E = evenness index (Evenness)

The results shown in Table 2 indicates the highest Margalef species richness (DMg) was found in groundnut soil at 0.89, while the lowest was found in rice fields at 0.31. Therefore it can be seen that species richness in the three food crop soil in Purwokerto is classified in the low criteria (DMG<3.50). According to Maguran [17], the criteria for species richness is low when DMG <3.50; 3.50 <DMg <5.00, the species richness is moderate category; if DMg> 5.00 the species richness is high. Species wealth is the number of certain species found in a selected place.

The highest average index value (E) was obtained in the groundnut plant area of 0.13; while the lowest evenness index was obtained in maize fields at 0.08. This means that in the three fields studied are included in the category of low level of evenness. According to [18], the level of evenness is high if E> or = 0.60; moderate level of evenness if E ranges from 0.40 to 0.60 and is low if E <or = 0.40.

The results of the calculation of diversity index (H') note that the highest value is found in the groundnut crop land that is equal to 0.31 and followed with the maize plant area e.a 0.19. Furthermore, the lowest biodiversity index value was found in rice fields at 0.12. Of the three lands included in the category of low diversity. According to [9], if the diversity index value (H') <1 is included in the low diversity category. Margalef species richness (DMg) and Evenness Index (E) are components of the diversity of species or genera (H') in a community. Maguran (2011) [17] states that the diversity index value (H') is related to species richness at a particular location. The higher the value of H', the higher the diversity of species, productivity, and stability of the ecosystem.

The value of the Margalef Richness Index, the Evenness Index and the biodiversity index (H') on groundnut rhizosphere show higher yields compared to other two fields. This is presumably because in the rhizosphere of peanut plants there are more diverse sources of nutrition available than the other two plants rhizosphere due to the nature of groundnut roots which are able to associate with N₂-inhibiting bacteria. Furthermore, the results of the analysis of the C/N ratio in groundnut rhizosphere soil showed 5.80; maize 7,13; and rice plant rhizosphere 11.10. Analysis result showed that the highest amount of organic N in groundnut rhizosphere.

4. Conclusion

Based of the three food crop lands in Purwokerto there were 15 isolates of fungi included in 11 genera namely *Aspergillus*, *Penicillium*, *Mycotypha*, *Aureobasidium*, *Cylindrocarpon*, *Mucor*, *Chaetomium*, *Glyocladium*, *Trichoderma*, *Pleuropragmium* and *Gonytrichum*. Diversity index for groundnut 0.31; maize 0,19 and rice 0,12.

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