

Microfungi rhizo-phylosphere, endomycorrhizae of banana (*Musa paradisiaca* L.) in karangwangi countryside, south cianjur, west java Indonesia

Abstract

Fermented banana “sale pisang” are hold potential home industry in Karangwangi countryside, in South Cianjur. However, recently sale pisang’s production is affected significantly by the pathogenic fungi. Research about microfungi rhizo-phylosphere and endomycorrhizae of banana plant (*Musa paradisiaca* L.) in West Java aims to identify this reason. Preliminary survey to research locations using explorative method, sampling soils around roots, and leaves. Microfungi culture and counting were conducted by dilution technique and Total Plate Count (TPC) methods. Fungi identification was performed with Moist Chamber Technique based on morphology. While plant determination surround of banana plant, infection rate and colouring of endomycorrhizae were conducted according to the standard protocol described elsewhere.

In this study, 11 species of microfungi were identified, namely *Acremonium* sp., *Cladosporium* sp., *Colletotrichum* sp., *Curvularia* sp., *Fusarium* sp., *Monilia* sp., *Nigrospora* sp., *Penicillium* sp1., *Penicillium* sp2., *Phytophthora* sp. and *Trichoderma* sp. Eventually Endomycorrhizae species were *Gigaspora* sp. and *Acaulospora* sp. Infection rate of plants surrounding banana plant were varied for *Mimosa pudica*, *Cyperus rotundus*, *Stachytarpheta jamaicensis*, *Cyperus kyllingia*, *Melinis minutiflora*, *Erechtites hieracifolia*, *Arachis hypogaea*, *Momordica charantia*, *Euphorbia hyrta*, *Amaranthus gracilis*, *Manihot utilissima*, *Ageratum conyzoides*, *Emilia sonchifolia*, *Widelia triloba*, *Amaranthus spinosus* and *Solanum nigrum* as much as 20, 20, 20, 40, 60, 80, 100, 20, 60, 40, 100, 40, 40, 40, 40 and 20%, respectively. This species-specific rate of infection is discussed in order to obtain insight how the diversity of microfungi may affect mycorrhizal infection in banana plants.

Keywords: banana, endomycorrhizae, phyllosphere, microfungi, musa paradisiacal, rhizosphere

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Introduction

Banana is a common fruit consumed by the Indonesian people and is known for its high nutrition content. The contained nutrients in banana is beneficial in improving the health and tissue regeneration due to its relatively high content of calcium, nitrogen and phosphorus. To add more variant in banana’s product, the Indonesian community process the bananas to sale banana (here after named: Sale pisang). Sale pisang is a product that was processed using traditional fermentation techniques, i.e. by keeping bananas in holes under the ground and dried or smoked using firewood.

One of the famous sale pisang products is from Cianjur. Although the product was well-known, yet the availability of raw materials becomes an obstacle in producing sale pisang. According to the information obtained from one of the producers of bananas, few years ago in the village of Karangwangi, Cianjur, West Java, there were plenty of banana trees with more than 13 varieties that were uprooted (personal communication). It was suspected that the lifting of the banana trees were caused by pathogenic microorganisms that attacked the banana plants, thus the sale pisang’s production decreased significantly.¹ Therefore, the producers have to buy bananas from other areas. Of course this situation hampered the economic activities of karangwangi community, thus, it is important to conduct an investigation to determine the cause.

Roots and leaves are parts of plants that can be infected by microorganisms. Microorganisms that infect plants are normally from the group of fungi since its Haustoria facilitate the process of infection. Several fungi that inhabit the surface of roots and plants in the form of mycelia are from the genus *Fusarium*, *Mucor*, and *Aspergillus*. Besides, there are also a group of fungi that can be symbiotic with the plant root, namely mycorrhiza.

The mechanisms of fungal infections in plants were varied, depend on several factors such as the organ and constituent layers of the plant itself. However, in general pathogenic fungal infection include spore attachment, germination and plant surface recognition, pre-penetration processes, appressorium formation and penetration.²

Several types of fungi which were known as the cause of anthracnose disease banana leaves, were *Colletotrichum* spp., *Mycosphaerella musicola* or *Pseudocercospora musae* that caused the yellow Sigatoka disease, *Phyllacora musicola* that caused black cross disease, *Pseudocercospora fijiensis* that caused black Sigatoka and black leaf streak, *Cladosporium musae* that caused Cladosporium speckle, *Cordana cordana* that caused leaf spot musae, *Curvularia* sp., *Phyllosticta* spp., *Hendersonia toruloides* and *Helminthosporium* that caused leaf spot and *Fusarium oxysporum* that caused Panama disease and fusarium wilt. While fungi that caused disease in banana roots are *Pythium* spp which caused rotten roots. Mycorrhizal symbiosis could

give benefit in plants by improving plant's growth and increasing plant's tolerance to multiple diseases. At the time of pre-symbiotic, roots released essential metabolites for the growth of fungi and the process of colonization in the roots. Root exudates are released in the form of sugars, amino acids, proteins, carbon, several lipophilic compounds, flavonoids, and other biomolecules. Exudates that released by hyphae and plant roots influenced the characteristic and also affecting the availability of land and mikrorizosphere. Moreover, previous study also showed a positive correlation between the content of mycorrhizal spores and Sodium-Potassium (N and K content) in the soil. This condition not only affects the infected plants, but also the plants surround it.

The existence of beneficial fungi for plants should be maintained, while the growth of harmful fungi should be suppressed by conducting further studies on biofungicide. The purpose of this research was to determine the species of fungi that were occurred on the leaves and around the plants' roots along with mycorrhizal infection surrounds the banana plants.

Materials and methods

Study area

The research conducted in Karangwangi village, Cianjur, West Java. Preliminary survey of the site was held randomly through the unhealthy banana tree. Unhealthy banana trees were marked by their leaves's changing color from green to yellow, breakage stalk and plant withering.

Procedures

Materials: Tools: autoclave, stirring rod, glass beaker, spray bottle, film bottle, Bunsen, petri bowls, cutter, erlenmeyer, measuring cups, heater, incubators, preparat and cover glass, cotton, gauze pads, HVS paper, filter paper, label, luxmeter, magnetic stirrer, micropipette, microscope, mortar, analytical balance, ose, wood clamp, pintips, pipette, tweezers, plastic sample, plastic wrap, tube rack, shovel, soil tester, test tubes, thermometer, and ice thermos.

Ingredients: Roots seedling, 70% alcohol, distilled water, banana' leaves and rhizosphere soil (*Musa paradisiaca L.*),^{3,4} dry ice, HCL 1%, chloramphenicol. 10% KOH, NaCl physiologic, fuchsin dyes, Potato Dextrose Agar (PDA), methylated spirits, black ink Shaeffer solution and Vaseline.

Fungi rizosfer dan filofser

Sampling rhizosphere and filofser: Soil samples were taken around the root (20-30cm depth) by using shovel and were placed into plastic sample bag then inserted into the iso-thermal bag (16°C).

Leaf samples were taken by cutting the banana leaves that look unhealthy, and were then placed into plastic sample bag and kept in the iso-thermal bag (16°C).

Physical data measurement

Physical data measurements were performed for instance the light intensity, soil moisture, soil pH, humidity and air temperature. In addition, the observation on the characteristics of soil texture and soil color was also conducted.

Soil sample dilution

Soil sample dilution was conducted by stratified dilution up to eight (10-8).

Planting on PDA medium

Planting was conducted by pouring plate technique. Briefly, the ground suspension in the last three-dilutions (10-6, 10-7 and 10-8) were poured into sterile Petri dish. Subsequently, the prepared PDA was then placed into a Petri dish, homogenized and incubated at room temperature for 72hours.

As for leaves samples, approximately 1gram of these samples were cut, then washed with alcohol, rinsed with sterile distilled water and finally drained. Afterwards, leaves samples were placed on a petri dish containing PDA frozen medium, then were incubated at room temperature for 72hours.

Fungal colonies purification

Growing fungi were purified by taking hyphae of a colony, then inoculated into PDA medium in a petri dish and incubated for 72hours at room temperature. When its pure, culture in the streak should be tilted and incubated for 72hours at room temperature.

Identification of fungi through moist chamber methods

Fungi identification was performed by using a sterile Petri disc in which covered with filter paper, also slide and cover glass. Briefly, PDA was dripped on the glass objects, when the jelly was frozen, one side of the glass object was cut to instill fungal hyphae that have been purified. This cut then smeared with Vaseline on the three side of the glass cover and was placed above the fungus which has been planted. Afterwards, distilled water was dripped on the filter paper, hence the atmosphere became moist then incubated for 72hours at room temperature. After growing, the fungi were observed under a microscope with a magnification of 400 x and 1000x. Fungi identification was performed based on its morphology by referring to the guide book "Introduction to Food Borne Fungi" by Robert A. Samson in 1981 and "Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species by Tsuneo Watanabe in 1987.

Endomycorrhizae

The transect preparation: Three transects were made by using a rope, each 15meters long, and transects were made randomly. Then, each 5meter quadratic plot was created by the size of 1x1meters in zigzag.

Sampling around banana plants: Sampling plants and roots were taken by using shovel. Samples were taken from the plants that are taller than 30cm. They were cut, then inserted into a plastic bag and labeled.

Plants identification: Plants were identified according to the morphology and determinant key.

Endomycorrhiza infection observation

Observation on endomycorrhizal infection was carried on by the staining method. Briefly, roots were cleaned until no soil attached, then were cut into pieces (1cm), soaked with 70% alcohol in a bottle. The roots were subsequently removed from the alcohol and soaked into 10% KOH then heated in water with temperature of 90-100°C in 10minutes (if the roots were hard, heating time was more than 10minutes). Furthermore, roots were rinsed with water and soaked again into the Shaeffer black ink dye and HCL 5% (1ml ink % and 19ml of HCL 5%) then heated for 5minutes. Roots were arranged on the slide glass as many as five pieces, where each plant species required

three glass objects. Subsequently, each preparat were given Fuchsin dye fucshin or HCl/Vinegar 5% solution with water (1:1), covered with a cover glass and observed directly under the microscope. The endomycorrhiza structure in form of external hyphae, internal hyphae, vesicles and arbuscular was observed.

Data analysis

Calculation of fungi colonies

Colonies of fungi that grow were calculated by Total Plate Count method.

Calculation of endomycorrhizal infections

Calculation in endomycorrhizal infection at the root was conducted by the conversion of a number of infected roots (hyphae internal/vesicles/arbuscular) divided by the number of roots observed.

Classification of infection was determined based on the classification of infection of Mycorrhizal Institute of Research and Development, United States Development of Agriculture (USDA) as follows:

- I. Class 1, when the colonization was 0-5% (very low, +).

- II. Class 2, when the colonization was 6-25% (low, ++).

- III. Class 3, when the colonization was 26-50% (moderate, +++).

- IV. Class 4, when the colonization was 51-75% (high, ++++).

- V. Class 5, when the colonization was 76-100% (very high +++++).

Results and discussion

Physical data

Research on micro fungi rhizo-filosfer was done in two weeks i.e. two days soil sampling and nine days of isolation up to identification. Soil sampling and leaves were conducted at point 1 and point 2, where the fallen bananas due to pathogenic microorganisms were reported. The sample of banana trees were taken from young leaves which turn to yellow and fragile branches that are suspected as the indicator of disease development.

The physical data was measured as supporting parameters that were taken at Point 1 and 2 with two distinct points (Table 1A & 1B).

In addition, the physical data sampling plants which were infected by mycorrhizal is shown in the Table 1.

Table 1A & 1B Physical data of soil sampling and leaves

Physical Conditions Measured	Point 1	Point 2
Soil temperature (°C)	30	31
Soil humidity (%)	87	85
Soil pH	7	7
Height (mdpl)	125	203

Measured physical conditions	Point 1	Point 2
	a	b
Air Temperature (°C)	30	31
Humidity (%)	74	51
Soil humidity (%)	100	50
Soil pH	6,4	6.6
Light intensity (lux)	87148	94457
The physical condition of the soil	Clay, wet, dense and sticky. Brownish-black.	Slightly pebbly soil. Grayish brown.
	a	a
	b	b
	29	28
	58	60
	50	48
	6.6	6.6
	87273	70511
	Fine soil, slightly pebbly. Dark brown.	Gravel. Soil, Brown.

Numbers of colony and types of hizosphere fungi and filosfer

Number of colonies and types of fungi in rhizosphere and filosfer samples of bananas in point 1 and point 2 along with its calculation was shown in Table 3 & 4. Based on the results from isolation and

identification, there are 7 species of microfungi in rhizosphere samples were identified (Table 2), namely *Cladosporium sp.*, *Phytophthora sp.*, *Acremonium sp.*, *Penicillium sp2.*, *Monilia sp.*, *Trichoderma sp.* and *Colletotrichum sp.* and 6 species on filosfer samples (Table 3), i.e. *Curvularia sp.*, *Phytophthora sp.*, *Acremonium sp.*, *Penicillium sp1.*, *Fusarium sp.* and *Nigrospora sp.*

Table 2 Number of colonies and types of rhizosphere fungi in banana

No.	Name of species	Numbers of colony fungi rizosfer											
		Point 1			Point 2								
		a			b			a			b		
		10 ⁻⁶	10 ⁻⁷	10 ⁻⁸	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸
1	<i>Cladosporium</i> sp.											2	
2	<i>Phytophthora</i> sp.	21	0	1			4						
3	<i>Acremonium</i> sp.				3	4	4				1	2	0
4	<i>Penicillium</i> sp2.					2							
5	<i>Monilia</i> sp.								6	7			
6	<i>Trichoderma</i> sp.						2						
7	<i>Colletotrichum</i> sp.						2						
Total Colony		21	0	1	3	6	4	8	6	7	3	2	0
CFU g ⁻¹		60.5x10 ⁶			154.33x10 ⁶			256x10 ⁶			11.5x10 ⁶		

Table 3 Types of fungi of banana filiosfer

No.	Name of species	Numbers of colony fungi filiosfer			
		Point 1		Point 2	
		a	b	a	b
1	<i>Curvularia</i> sp.	✓	✓		
2	<i>Phytophthora</i> sp.	✓	✓		
3	<i>Acremonium</i> sp.				✓
4	<i>Penicillium</i> sp1.				✓
5	<i>Fusarium</i> sp.	✓			
6	<i>Nigrospora</i> sp.	✓	✓		

Table 4 Types of plants infected with mycorrhizae

Transects	No.	Plants name	Endomycorrhizae structure	Infections	Root infections	
Point I	T1, P1	1	<i>Cyperus rotundus</i>	Internal hyphae	1:05	20% (++)
	T1, P2	1	<i>Phyllanthus amarus</i>	Internal, External hyphae	2:05	40% (+++)
		2	<i>Stachyatarphaeta jamaicensis</i>	Internal hyphae	1:05	20% (++)
	T1, P3	1	<i>Cynodon</i> sp.	Internal, External hyphae	1:05	20% (++)
	T2, P1	1	<i>Stachyatarphaeta jamaicensis</i>	Internal hyphae	1:05	20% (+++)
		2	<i>Amaranthus gracilis</i>	Internal, Eksternal hyphae	2:05	40% (++)
	T2, P2	1	<i>Vernonia cinerea</i>	Internal hyphae	1:05	20% (++)
	T2, P3	1	<i>Ipomoea aquatica</i>	Internal hyphae	1:05	20% (++)
	T3, P1	1	<i>Echinochloa crus-galli</i>	Internal Vesikula hyphae	3:05	60% (++++)
	T3, P2	1	<i>Phyllantus urinaria</i>	Internal hyphae	2:05	40% (+++)
	T3, P3	1	<i>Kaempferia galanga</i>	No endomikoriza found	0:05	0% (+)

Plant species infected by mycorrhizae

In this study, infection rate on point 1 and 2 was ranged from 20 to 100%. From both points, *Endomycorrhizae species* obtained from *Gigaspora* sp. and *Acaulospora* sp. with 20% of infection rate was observed in *Mimosa pudica*, *Cyperus rotundus*, *Stachyatarpheta jamaicensis*, *Cyperus kyllingia*, *Melinis minutiflora*, *Erechtites hieracifolia*, *Arachis hypogaea*, *Momordica charantia*, *Euphorbia hyrta*, *Amaranthus gracilis*, *Manihot utilissima*, *Ageratum conyzoides*, *Emilia sonchifolia*, *Widelia triloba*, *Amaranthus spynosus* and *Solanum nigrum*.

In point 1, the highest infection was obtained in *Echinochloa crusgalli*, as much as 60%. In contrast, infection was absent in *Kaempferia galangal*.

In point 2, the highest mycorrhizal infection (100%) was observed in *Arachis hypogaea* and *Manihot, utilissima*. Whereas, the lowest infection (20%) was obtained in *Mimosa pudica*, *Cyperus rotundus*, *Stachyatarphaeta jamaicensis*, *Momordica charantia* and *Solanum nigrum*.

Table Continued.....

Transects	No.	Plants name	Endomycorrhizae structure	Infections	Root infections	
Point 2	1	<i>Mimosa pudica</i>	Internal hyphae	1:05	20% (++)	
	T1, P1	2	<i>Cyperus rotundus</i>	Internal hyphae	1:05	20% (++)
		3	<i>Stachyatarphaeta jamaicensis</i>	Internal hyphae	1:05	20% (++)
	T1, P2	1	<i>Cyperus kyllingia</i>	Internal, External hyphae	2:05	40% (+++)
		2	<i>Phaseolus vulgaris</i>	Internal, External hyphae	1:05	20% (++)
	T1, P3	1	<i>Melinis minutiflora</i>	Internal, External hyphae	3:05	60% (++++)
		2	<i>Erechtites hieracifolia</i>	Internal, External, Arbuscular hyphae	4:05	80% (+++++)
	T2, P1	1	<i>Arachis hypogaea</i>	Internal Vesikula hyphae	5:05	100% (+++++)
		2	<i>Momordica charantia</i>	Internal hyphae	1:05	20% (++)
	T2, P2	1	<i>Euphorbia hirta</i>	Internal, External hyphae	3:05	60% (++++)
		2	<i>Amaranthus gracilis</i>	External hyphae	2:05	40% (+++)
		1	<i>Manihot utilissima</i>	Internal, External, Arbuscular hyphae	5:05	100% (+++++)
	T3, P1	2	<i>Ageratum sonchifolia</i>	Internal, External, Arbuscular hyphae	2:05	40% (+++)
		3	<i>Emilia sonchifolia</i>	Internal, External hyphae	2:05	40% (+++)
	T3, P2	1	<i>Widelia triloba</i>	Internal hyphae	2:05	40% (+++)
		2	<i>Amaranthus spynosus</i>	Internal hyphae	2:05	40% (+++)
	T3, P3	1	<i>Solanum nigrum</i>	Internal Vesikula hyphae	1:05	20% (++)

In the present study, from banana leaves (*Musa paradisiaca L.*) which were allegedly contained pathogenic microorganisms; 3 species (*Curvularia sp.*, *Phytophthora sp.*, and *Nigrospora sp.*) were observed with most widely types of microorganisms as shown in point 1b, whereas the other leaf samples only showed 2 species of microorganisms. Several environmental factors such as humidity, temperature, light intensity and the condition of the banana plant itself can affect either the existence of microorganisms which are pathogenic or the beneficial microorganisms.

TPC results showed that the highest number of microfungi was obtained in sample point 2a (256x106 CFU g⁻¹) with the highest number of species (*Phytophthora sp.*, *Monilia sp.*, *Trichoderma sp.* and *Colletotrichum sp.*). In contrast, the fewest number of microfungi was obtained in sample point 2b (11.5x106 CFU g⁻¹) with only 2 microfungi species (*Cladosporium sp.* and *Acremonium sp.*). These differences may be caused by the physical factors and soil conditions. Even though the physical conditions at the point 2a and b was not different, but the soil conditions were different. The soil in point 2a has a smooth texture, pebbly and the ground color was dark brown, meanwhile in point 2b was pebbly with light brown color.

The finer the soil grain, the more intense its capability in binding water and nutrients. On the other hand, when the soil is too coarse, the grains cannot hold water and nutrients. The color of the soil also signifies the fertility. For instance, darker soil indicates its high fertility. Hence, this condition may affect the presence of fungi in both soil types. Watanabe states that soil fertility favor the growth of fungi. In contrast, clay in point 1a, the number of fungi colonies were relatively small (60.5x106 CFU g⁻¹), although the soil was fertile soil. Fungi are aerobic and their number declined in high soil moisture. This could be the explanation why the number and fungi species were low. Indeed, when the particles are too dense, the humidity may reach 100% and the aeration was mediocre thus lowering the number and types of fungi. Contradictory, in clay-type soil, actinomycetes can be

abundant. This fact is in accordance with the Public Health England (PHE) (2014), that several actinomycetes were anaerobic. Meaning that the actinomycetes are able to grow dominantly on clay substrate.

The acidity (pH) in study site was neutral (7), indicating the optimum pH for the growth of mycorrhiza and the ongoing of biochemical activity.⁵ The temperature at the study site ranged from 30 to 31°C. This contributes to the presence of mycorrhizae as stated that the optimum temperature for the development of mycorrhiza is about 28-35°C. Indeed, the temperature is positively correlated with the amount of mycorrhizae produced. According to Handayanto and Hairiah, extreme humidity and soil moisture were also create an unfavorable condition for the development of mycorrhizae. Mycorrhiza tend to thrive in an stable humidity and soil moisture. Our measurement revealed that the humidity was around 85%-87%, which is considered as stable humidity.

The role isolated of fungi

Previous studies demonstrated that *Acremonium sp.*, *Cladosporium sp.*, *Colletotrichum sp.*, *Curvularia sp.*, *Fusarium sp.*, *Nigrospora sp.*, *Monilia sp.* and *Phytophthora sp.* are considered as most pathogenic species to plants and attack various organs of plants. *Acremonium sp.* can strike and cause banana crown rotteness. Several *Cladosporium species* are pathogenic in several types of commercially important plants. Genus *Colletotrichum* is spread in tropical and subtropical area and most of the species from this genus causing pathogens in various wooden and herbaceous plants. *Curvularia* is a dematiaceous mold that can infect plants and can be found in the tropical area soil. This genus also appear to cause leaf spot in bananas. *Fusarium* is well known as a plant pathogen.⁶ *Fusarium oxysporum* was found to infect the roots up to pseudostem. The symptoms are such as necrosis of the stem (xylem has a reddish to reddish brown color), rotten root and rhizome, yellowing leaf; withered plants and even causing the death of plants. This fungus can survive in soil for decades although the plants

do not live anymore. *Nigrospora* sp. is the predominant pathogen in banana and the type that normally infects was *N. sphaerica* (Sacc.).

Monilia is a fungus which derives from soil that becomes pathogenic to several plants and widely distributed throughout the world. Genus Phytophthora causing the most parasitic in several plants. Aside from harmful fungi, there are also fungi that beneficial to plants, namely *Penicillium* and *Trichoderma*. Endophytic *Penicillium* sp were found on leaves and roots of *Musa acuminata*. In addition, research revealed that *Trichoderma Viridae Fusarium* sp. can serve as biocontrol which was constitute soil pathogens rhizosphere on banana plants.

Mycorrhizal infections around banana plants

In the present study, it was found that *Gigaspora* sp. and *Acaulospora* sp. that infecting the banana plants, (description).

In point 1, the highest infection was observed in *Echinochloa crusgalli* (60%) and the lowest infection was obtained in *Kaempferia galangal* (0%). As in point 2, the highest theoretical mycorrhizal infection (100%) was found in *Arachis hypogaea* and *Manihot utilissima*, while the lowest infection (20%) was obtained in *Mimosa pudica*, *Cyperus rotundus*, *Stachyatarphaeta jamaicensis*, *Momordica charantia* and *Solanum nigrum*.

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Conflict of interest

The author declares no conflict of interest.

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