EXPLORATION AND IDENTIFICATION OF THE INDIGENOUS ARBUSCULAR MYCORRHIZAE FUNGI (AMF) IN THE RHIZOSPHERE OF CITRONELLA (*ANDROPOGON NARDUS* L.) IN THE DRY LAND REGIONS IN WEST SUMATRA PROVINCE, INDONESIA

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Abstract

Climate change has an impact on the transition function of the wetland to dry land. This shift led to the increased of the dry land area with less potential for food crops and horticulture. Citronella plants have been identified as one of lemongrass crops that able to survive in the dry land area. However, the resulting yield to produce citronella oil is still very low at 0.8 to 1.0%. In this regard, Arbuscular Mycorrhizae Fungi (AMF) was indicated to be able to increase the absorption of nutrients and water in many plants in the dry land regions. The type of AMF that compatible enough with citronella plant was not identified yet. The purpose of this study was to determine the number and diversity of AMF in the rhizosphere of indigenous citronella (lemongrass). The study was conducted from August to November 2015 in three regions of cultivation of citronella using survey methods and wet screening. The study found four important AMF geniuses in the study site, there are Glomus, Acaulospora Gigaspora, and Sclerocystis.

Keywords : Climate change, indigenous mycorrhizae, rhizosphere, Citronella plants, and dry land

Introduction

Climate changes have created an impact on the shifting of the wetland become dry. The case study of [1] in *Singkarak* lake region has indicated the occurrence of an increased number of wetlands to dry lands. The changes of rainfall pattern in this region have increased the uncertainty of water availability for paddy in the rain-fed rice fields. Since more than the last 20 years, farmers in this region are facing the uncertainty of wet months in the case site. One of their natural reservoirs (called *Talago Janik*) did not able to keep water for long to support even one season of paddy in their rainfed areas.

There is one crop that was identified to be potential for this dry region, that is, citronella scented (*Andropogon nardus* L.). According to [2], this lemongrass scent is the family of *Gramineae* that able to produce essential oil. In the world trade, the citronella essential oil from Indonesia is well known as Citronella Oil of Java. The most widely cultivated in Indonesia is *Mahapengiri* type. This type contains volatile oil of high quality that is 80-97% geraniol and 30-45% citronella.

The agribusinesses of citronella are economically feasible because the essential oils from this crop are used for many industries, are medical, cosmetics and perfumes industries. The human need for these industrial products is increasing every year. However, the main challenge for the cultivation of this crop in the dry land region is its low yielding capacity in producing the essential oils from their leaves. The potential capacity of citronella lemongrass to produce essential oils is only around 0.8 to 1.0%, while from another kind of crops like *Nilam* is quite high for around 2.0 to 4.2% [3], from clove for around 8.6% [4], and from lime leaves is for around 13.4 % [5].

Dry land is one of the marginal lands that have a lot of limitations in providing nutrients for plant growth. According to [6], dry land has high soil acidity with average pH of less than 4.50, high Al saturation, deficient elements of phosphorus (P) because they bind tightly to the aluminium (Al), poor nutrient content, especially P, K, Ca, and Mg, and low organic matter content. Land with high Al saturation can influence the growth of the plant. [7] describes that a high Al content in the soil can bind P element, so it can not be absorbed by plant roots. Land that had a low pH (acidic) adversely affects the availability of nutrients for plants. It may also influence the development of the root system of plants. The capacity of these influenced roots to uptake nutrients and water will then decreased eventually.

According to [8], the dry land area on this earth will increase annually due to the changes of climatic condition, especially as the impact of El Nino as a climatic anomaly. El Nino phenomenon leads to a prolonged drought where the intensity of rainfall is relatively below normal. A long period of El Nino may reduce the availability of water in the soil for long, then cause to become relatively dry. Based on the analysis of [9], the El Nino events in the period of 1981-1999 have changed around large numbers (1,002,055 ha) of wetland areas to become dry land areas. Moreover,[10] has also identified that the El Nino events in the period of 1999-2002 have changed around 167 150 ha of wetland to dry land area. Likewise, the study results of [1] in the region of West Sumatra, especially in the area of the Lake Singkarak, have also identified such changes of land condition from wetlands to dry land. It is found that in the area Simawang there has been a fast increase of dry land, from only 628 Ha in 2000, becomes around 1,078 Ha in 2008.

These spacious dry land are potentially enough to be used on economically valuable agricultural land. However, there are not all types of plants can be cultivated in this dry land area. Horticulture and some food crops are usually not economically feasible because mainly the required a lot of water for their life cycle.

Cultivation of citronella is a good choice dry land area [2]. Citronella plants have also the potential capacity to reduce erosion when they growth in the slope area [11]. A research of [12] has also explained that the citronella plant can be intercropped with cacao plants in the dry land and slope area (around 30%). These citronella plants can reduce the erosion in the slope area because they are able to tie up the topsoil structure with their root system. The damage of cacao fruit may also decrease compare to the area without citronella. The yields of Cocoa crops in intercropped with citronella increased by 40% compare to cocoa crop without citronella.

Further investigation of cultivating citronella are still important especially related to the capability of citronella leaves to be extracted become an essential citronella oil. Since the dry land has many limitations especially in terms of its availability of nutrients, there is a potential way to increase the root capacity to absorb water and nutrient in the soil, especially by increasing the living connection between the root and the microorganism in the soil. Such microorganisms contained in the rhizosphere of plant roots can be bacteria and fungi.

The bacteria funds in the rhizosphere of plants are called rhizobacteria. Rhizobacteria is an aggressive group of beneficial bacteria, which usually colonized in the rhizosphere (the thin soil layer between 1-2 mm around the root zone). According to [13] rhizobacteria can act as Plant Growth Promoting Rhizobacteria (PGPR). Rhizobacteria can only act as PGPR, they cannot take distant nutrients and other nutrients that cannot be absorbed by the plant roots.

Other microorganisms in the root rhizosphere of plants are fungi. Fungi that are often utilized are fungi that are capable to symbiosis with plant roots. The symbiosis of root with fungi may increase the capacity of root to absorb nutrients and water. Fungi have hyphae that can grow and develop in the soil. This symbiosis is presumed to help the growth and development of citronella in the dry land that has less water in the soil. The symbiosis of fungi and plant roots known as Mycorrhiza. The mycorrhiza found in many agricultural crops is known as the Arbuscular Mycorrhizal Fungi (AMF)

The AMF may serve as bio-stimulants for plants [14] because this microorganism has increased the capacity of the plant in absorbing nutrients from the soil and increase its resistance from abiotic stresses [15]. As bio-stimulants, this AMF can reduce the use of external fertilizer without reducing the availability of nutrients for plants [16]. While [17] describes that AMF can act as bio-fertilizers to increase the capacity of root to uptake phosphate nutrients for plants. According to [18], AMF can also encourage the development of glomalin that able to improve the physical properties of soil.

The AMF symbiosis in citronella plants in the dry land area is expected to increase the capacity of its root to absorb nutrients and water. According to [19] and [20], the AMF is a symbiotic soil fungus with plant roots. These kind of fungi are obligate because they can not grow and reproduce when they did not in symbiosis with the host plant. The type of the host plant and the type of AMF determine the symbiotic form between root and fungi. Each plant will have specific kind of AMF that can infect its root system.

The roots of plants will emit exudate that can stimulate AMF spores to germinate. When the exudate does not match with the AMF spores, the spores will not germinate to form hyphae, and then the root infection will not occur. The symbiosis between fungi and plant roots will not be formed. The suitable AMF type that matches the root system of citronella has not been identified yet. There is no information describing the type of indigenous AMF in the rhizosphere of citronella plants. The study exploration and identification indigenous AMF will be able to provide early information about any kind of AMF that potentially effective to be developed for citronella plants.

The exploration and identification of indigenous AMF in the rhizosphere of such plants will provide a complete information when it is done not on a plantation area. Explorations in one place will not representative of the environmental conditions of a plant because the type and numbers of indigenous AMF are very limited. Aside from that, the length of the cultivation area also needs to be considered, because it would also affect the type and population AMF.

Therefore, the study on exploration and identification of indigenous AMF in the citronella rhizosphere is carried out in three regions in West Sumatera Province; in Nagari Balai Batu Sandaran, Kota Sawah Lunto, in Nagari Laiang, District of Solok and in Nagari Simawang, District of Tanah Datar. Those three areas of citronella plants are different in terms of the length of the cultivation. In Laiang, citronella has been planted for more than 25 years, in Batu Balai Sandara is for about 15 years, and in Simawang, citronella plants were just newly planted. The results of the study [1] mentioned that formerly the citronella area is paddy field area that has experienced drought condition for more than 30 years. This area was no longer economically viable for paddy.

The results of this study will provide information about the diversity and the population of AMF species that exists in the rhizosphere of citronella plants. The diversity of AMF species can be the basis for subsequent research on the potential capacity of indigenous FMA to be developed as a source of biological inoculants and as environmentally friendly fertilizers in the cultivation of citronella in the dry land area. This study aims to determine the number and diversity of indigenous AMF in the rhizosphere of citronella plants.

Materials and method

A. Study Site and Sampling

Soil samples were collected from three cultivation regions of citronella, are Laiang, District of Solok (around 25 years' citronella planted area), Balai Batu Sandaran, Sawahlunto Municipality (around 10 years' citronella planted area) and Simawang, District of Tanah Datar (newly planted area with lemongrass 6 months).

Fig. 1. Sampling sites (I) Balai Batu Sandaran, Sawahlunto (II) Laiang, Solok, and (III) Simawang, Batusangkar [21]

Table 1. Information About Location Of The Sampling Population Of Amf (Latitude And Altitude)

No.	Location	Elevation	Geographic position
1	Laiang, Distric of Solok	480 m asl	100,32 E – 0,32 S
2	Balai Batu Sandaran, Kota Sawahlunto	785 m asl	100,47 E – 0,46 S
3.	Simawang, District of Tanah Datar	600 m asl	100,28 E -- 0,28 S

Soil samples from each area of citronella plantation, have been taken at five points randomly from planting plots of around 20m × 20m, for about 500g of 0-20cm from ground level. These soil samples from those five points were then mixed and stirred until homogeneous.

B. Extraction of AMF Spores

About 50g of those homogenous and dried soil samples were then extracted by using wet sieving [22]. Those samples were mixed with 200ml of water and then filtered using a multi-filter-rise from the size of 500µm, 300µm, 106µm, and 45µm. The distillate samples of 45µm are placed into the centrifuge tube and added with 60% glucose, then centrifuged at the speed of 2,500rpm for 3 minutes. The result of centrifuged solution is poured into the 45µm filter and then rinsed with water. The remaining supernatant solutions in this filter are then placed into the 150ml beaker.

C. The Calculation of the Number of Spores

The supernatant solution is pipetted into a 50ml beaker, and 1 ml of this solution is inserted into a Petri dish. The number of spores was then observed and counted by using a binocular microscope with a magnification of 40x. The number of spores is calculated by using the formula:

$$\frac{A}{B} = \frac{X}{Y} \qquad (1)$$

Where:
A = Number of observed spores in 1 ml volume of sample
B = volume of sample, 1 ml
X = Number of observed spores in 50 ml volume of sample
Y = volume of the sample, 50 ml

D. Frequency of the Presence of Spores

The frequency of the presence of spores was carried by observing the presence of spores in the surrounding area of citronella plants. When the spores of indigenous AMF were present in all the planting area then the frequency of the presence of spores are 100%.

E. Identification of the morphology of spores

The identification of the morphological types of spores is determined by a method developed by [23] through the observation of color, size, and shape of spores using a microscopic binocular with a magnification of 100x.

F. Identification of Root Colonization Percentage

To identify the colonization of roots, this study uses root staining technique based on the method developed by [24]. Fresh root samples of 0.5 – 2.0mm have been taken randomly. Those roots samples were washed and cleaned, and then soaked into a 10% KOH for 24 hours, then continued soaked with 1% HCl for 10 minutes. After that, the roots samples were strained with lacto glycerol trypan blue for 1 hour. Colored roots samples are then chosen randomly and cut it for about 1 cm length as many as 10 pieces. All of those pieces of roots are arranged over the glass object and then closed with a cover glass. Those colored roots samples were then observed by using a binocular microscope with a magnification of 100 x. This study used a method of [25] to calculate the colonization intensity of those roots samples.

Results and Discussion

A. The Type, Numbers, and Frequency of Spores

This study found 4 genus and 7 species of the AMF in the rhizosphere in three areas of lemongrass plantation in West Sumatra (Table 2), consists of *Glomus* (2 species), *Acaulospora* (3 species), *Gigaspora* (1 species) and *Sclerocystis* (1 species).

Table 2. The Existence, Spores Types And Frequency Of FMA Presence In Three Study Sites

No	Genus of AMF	Location		
		Balai Batu Sandaran	Laiang	Simawang
1	*Glomus* sp 1	+	+	+
2	*Glomus* sp 2	+	+	+
3	*Acaulospora* sp 1	+	+	+
4	*Acaulospora* sp 2	+	+	-
5	*Acaulospora sp* 3	+	+	-
6	*Gigaspora sp 1*	-	+	-
7	*Sclerocystis sp 1*	+	+	+

+) Spores presences, -) No spores presences

The frequencies of spores' presence of each species in all three locations of citronella crops are not similar. There are four species with existence frequency of 100%, i.e. *Glomus* sp1 and sp2, Acaulospora sp1 and Sclerocystis sp1, and they are all presences in those three locations. Based on this frequency analysis, the existences of those four species are very potential to be developed as a source of inoculants for citronella plants. Further investigation of the potentiality of those four species will be conducted in the next study step.

Species of those four AMF spores were allegedly has a level of conformity with the root system of citronella plant. It is mainly because it was found in all areas of cultivation. Even in new crop areas in Simawang, all four species have been able to symbiosis with the root system of citronella.

Suitability of those four species is thought to fit with exudates produced by the root system of citronella. Exudates produced from citronella plant were able to stimulate the spores to germinate and grow to form hyphae. This type of AMF can grow and multiply in the rhizosphere of citronella plants. According to [26], the exudates of plant root can affect the germination of spores and the growth of hyphae. [27} mentioned that the root exudates produce a signal that can be responded by the AMF, if there is a match then the spores will germinate and grow to form hyphae. The numbers of spores of all species in Laiang are much higher than those found in the other two locations (Fig. 2). There are one species was found only in Laiang, that is, *Gigaspora* sp1.

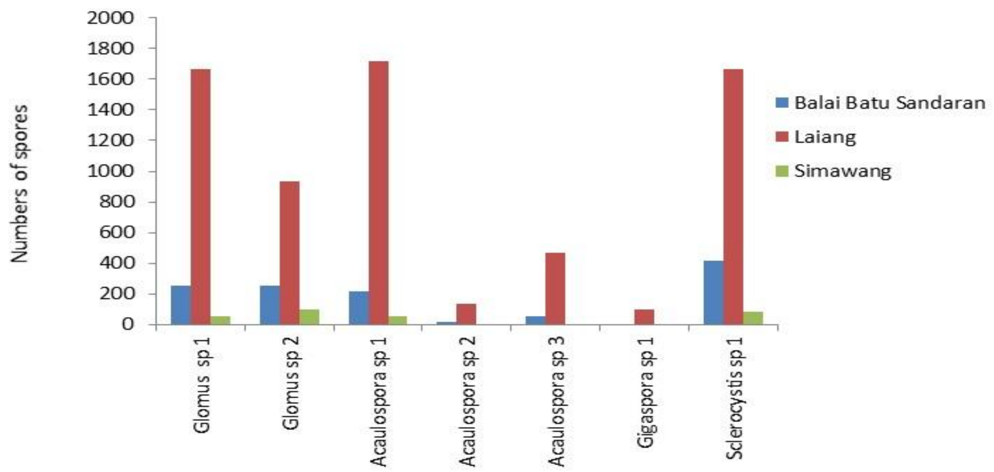


Fig 2. Type and number of spores on three locations of citronella in West Sumatra

It was found that the numbers of AMF type and the numbers of AMF spores in Laing are much bigger than in Simawang. This is significantly related to the length of cultivation of citronella plants in that two location, where citronella in Laiang has been there for more than 25 years, while in Simawang are just relatively new. Both areas are planted as a monoculture citronella. The population of AMF in the rhizosphere grows over the time of citronella plants. Similar facts were also found in rubber plants, where the numbers of AMF population is much bigger in the old rubber plants compare to the young rubber plant [28].

A cultivated land with only one crop for a long time period will encourage the proliferation of certain types of AMF that have compatibility with the plant rhizosphere. The presence of several kinds of AMF growing in the rhizosphere of citronella shows that there is a match between the AMF and the root of citronella. According to [29], the AMF has formed a symbiosis mutualistic between fungi and plant. The AMF utilize the host plants as a food source of exudate form of carbohydrates and other elements released by plant roots. While the plant itself got its supply of nutrients and water taken by the AMF. The AMF has an external net of hyphae that able to grow and thrive in the soil. The external hyphae absorb nutrients and water and then transfer them to internal hyphae contained in plant root tissues. The AMF will provide nutrients and water to plants through the arbuscular formed in cortical tissue in the roots. This mechanism will take place continuously, as long as the symbiosis between them still exists.

Small numbers of spores found in the newly planted of citronella plant in Simawang are allegedly because of the rhizosphere condition is not stable yet. The soil in the newly cultivated land need to takes the time to form a stable ecology in the rhizosphere. The steady state rhizosphere will encourage the proliferation and development of the AMF, increased the diversity and the population density of AMF. According to [30], the disturbed land will encourage succession, affecting the amount of population diversity of AMF. The succession process will also affect the effectiveness of the symbiosis between the AMF and the plant roots. Meanwhile, according to [31], the ability of a type of AMF living in one specific environment are largely determined by the adaptation capacity of this type of the local environment. The results of the study [32] also explain the existence of a type FMA will be affected by the changes of their ecological environment.

The citronella plants in Simawang is just about 6 months. Previously, the land was just a dryland overgrown by grass. A few months prior to planting of citronella, the land is relatively very dry during a long dry season. A simple preparation of land has resulted in the imbalance of the ecological system in the root zone and increased the water loss in topsoil. The low availability of water has possibly affected the development of the AMF in the root zone, reducing the diversity and population of AMF in the rhizosphere. According to [33] the diversity and density of AMF spores in the soil are determined by the activity of the previous crop cultivation. Moreover, according to [34], the precipitation may have also a direct impact on the diversity and population of AMF.

B. Morphological Characteristics of Spore

This study found that the morphological characteristics of those all AMF in the rhizosphere of citronella plants are different each other (Fig. 3.).

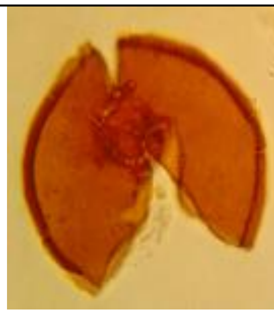
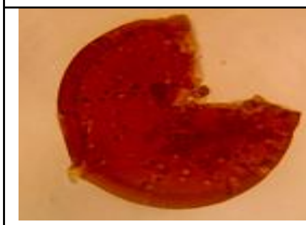
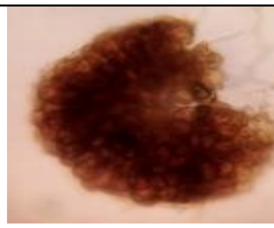
Type of AMF	Notes of identification	Type of AMF	Notes of identification
 *Glomus sp 1*	The spherical shape of spores; do not react with Melzer, pass through the sieve of 300µm; the color is light yellow (jonquil); smooth spore surface, two-layered spore wall, the outer layer is thicker.	 *Acaulospora sp 3*	Spores oval and rounded shape; react with Melzer; pass through the sieve of 300µm; tawny-colored; surface rather smooth; thick spore wall.
 *Glomus sp 2*	Oval shape of spores; do not react with Melzer; pass through the sieve of 300µm; the color is light yellow (jonquil); smooth spore surface; two-layered spore wall; the outer layer is thicker	 *Gigaspora sp 1*	Spores slightly rounded shape; reacts with Melzer; pass through the sieve of 300µm; tawny-colored; surface rather smooth; thick spore wall; there is a bulbous suspensor
 *Acaulospora sp 1*	Spores are rounded shape; reacted with Melzer; pass through the sieve of 300µm; tawny-colored; slightly rough surface; two-layered spore wall	 *Sclerocystis sp 1*	Spores are formed from the center of hyphae; does not react with Melzer; pass through the sieve of 300µm, dark blackish brown; a surface; coated walls of spores are clustered.
 *Acaulospora sp 2*	The Oval shape of spores; reacts with Melzer; pass through the sieve of 300µm; tawny-colored, somewhat smooth surface; two-layered spore wall.		

Fig. 3. Morphological characteristics of AMF spore types, isolated from citronella cultivation area (100 x magnification)

There are seven types of AMF spores found in all three areas planting of citronella; three species of Acaulospora (sp 1, 2, and 3), two species of Glomus (sp 1 and 2), and one species Gigaspora sp, and one species of Sclerocystis. The numbers of AMF types in the rhizosphere are different from one to another place. Study of [35] found 9 indigenous AMF types in the rhizosphere of Corn plantation. Three of those nine AMF type are potential for the improvement of physical properties of soil because that three AMF has produced large numbers of glomalin. Here, the research [33] mentioned that the type of plants on the ground would determine the diversity and density of AMF spores.

C.The Intensity of AMF Colony

The AMF from Laiang shows a higher percentage of infection and infection intensity compared to the AMF from other two planting areas (Figure 4). These empirical facts have verified that the length of cultivation has a significant impact on the development of AMF in the rhizosphere of plants. When the fact in Figure 4 is connected with a number of spores in Figure 2, it shows the connection between them, that the number of spores directly proportional to the percentage and intensity of infection AMF

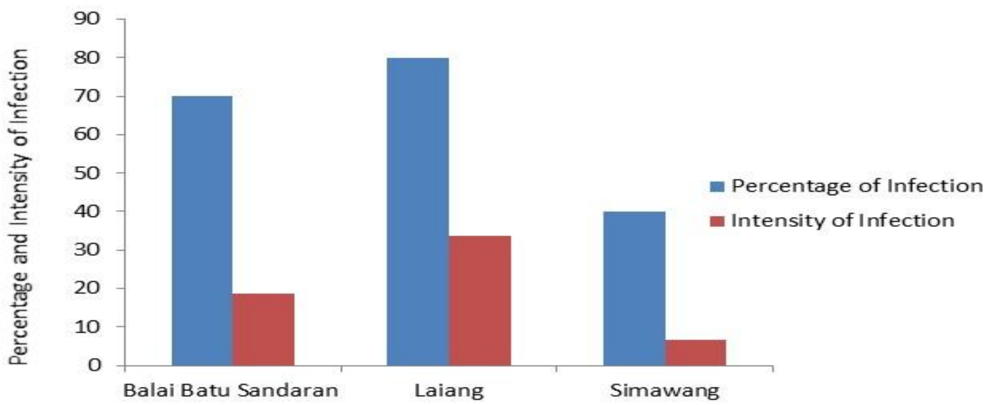


Fig 4. The percentage and the infection intensity of AMF at three locations of citronella plants in West Sumatra

The percentage of infection and the intensity of root infection by the AMF on citronella plant could be influenced by many factors, especially those related to environmental conditions and the length of time of cultivation. According to [36], the successful symbiotic process of AMF with the plant roots is depended on many factors, including the competition and the plant habitat itself. The competition will determine the level of symbiotic microorganisms in the rhizosphere of plant roots. The AMF symbiosis with plant roots is determined by habitat of plants. It also determined by the rotation of crops, because each plant has not similar kind of AMF that able to symbiosis [37].

Another factor affecting the rate of infection and the intensity of the infection percentage FMA is the intensity of disturbance and land preparation. A Higher percentage of infection and intensity of infection in citronella plants in Laiang and in Balai Batu Sandaran because the rhizosphere condition has already stabled. The stable condition of rhizosphere has encouraged better symbiosis process between AMF and the root system of citronella so that it increase the intensity of colonization. While in Simawang the rhizosphere was not stable yet. The ecological conditions in the root zone do not support the proliferation of AMF. This phenomenon led to the low percentage of infection and infection intensity at the FMA plant roots of citronella. Study of [38] has also given a similar indication in the pastureland area. In the undisturbed pastureland, the root of the grass has relatively high infection percentage (almost 100%) of AMF that encourage the higher proliferation of AMF in the rhizosphere. While in the disturbed pastureland have lower infection percentage, for only around 10%.

CONCLUSIONS

There is four genera of AMF were found in the rhizosphere of three cropping area of citronella, namely *Glomus*, *Acaulospora*, *Gigaspora*, and *Sclerocystis*. They are seven species been identified from that four genera; two species of *Glomus*, three species of *Acaulospo*ra, one species of *Gigaspora*, and one species of *Sclerocystis*.

There are fewer spores are found in the dryer cropping area in Simawang compared to the numbers of spores in the other two locations, and there is a positive correlation between the numbers of AMF spores with the percentage and the infection intensity of AMF in the plant roots.

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