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The Abundance of peatland fungi mikoriza arbuskula (FMA) for palm oil plantation in East Kotawaringin Regency

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This study aims to determine the abundance of peat lands Fungi Mikoriza Arbuskula (FMA) for oil palm plantations, conducted in 4 (four) locations in East Kotawaringin Regency. Roots and Soil sampling in a composite five (5) point of soil sampling at a depth of 20 cm in each location as well as replicates. The sample weight of each point is 500 gr, so the total soil sample for each observation plot is 2.500 gr. Soil samples for each point in a plot are mixed in one place until they are homogeneous to represent a plot of observation, then taken 100 g per point. The research findings show the structure of infection that forms the FMA structure in the form of hyphae and vesicles, whereas the FMA structure in the form of arbuscular is not found. The highest spore density (100 g of peat soil) occurs on peat land for palm oil plantations with a planting age of less than 4 years (320,40), The lowest spore density in natural peat forest (152,20). The identification results of FMA spores find twelve (12) species of the genus Glomus sp FMA Spores. The genus Glomus sp FMA Spores is the only type of FMA spore, either in natural peat forests and peat lands for oil palm plantations of various ages. The highest average abundance of Genus Glomus sp FMA spores (100 g of peat soil) on peat land for palm oil plantations with less than 4 years of planting age (112,80) while the lowest average on peat land for palm oil plantations with planting age greater than 10 years (47,40). The highest relative abundance of Genus Glomus sp FMA Spore (100 g peat soil) is in natural peat forest (42,64%), and the lowest average on peatland for palm oil plantations with the planting age over than 10 years (22,48 %). This study recommends the conversion of peatland for oil palm plantations which in its management keep prioritizing the sustainability of peat ecosystems by taking into account the physical, chemical and biological characteristics of peat soil.

Keywords: Mycorrhizae, Peatlands, Oil Palm, Identification, Structure, Abundance.

INTRODUCTION

The conversion of peatlands into palm oil plantations is faced with challenges related to the physical, chemical and biological properties inherent in peat, this is categorized as a global problem. The problem of peat soil lies in the high acidity and lack of mineral supplies needed for growth, so it is feared that it will produce unfavorable results for oil palm plants. Deru, et al., (2019); Liimatainenet al., (2018); Mi et al., (2018); Smith et al., (2018); Riddleet al., (2018); Khaled&Fawy (2011); Glaseret al., (2002); and Sarwar et al., (2008); stated that the chemical properties of peat soil can be improved through fertilization, if physical improvement in peat soil is sufficient. The giving of normal nutrients to peat soil will not be enough to stimulate the activity of soil microorganisms because microorganisms

such as mycorrhizae fungi also need an appropriate physical environment which usually leads to a neutral reaction.

Mycorrhizae is a symbiotic association between plant roots and fungi (Hoeksema, et al., 2018; Neuenkamp et al., 2018; Cosme ett al., 2018; Jiang, et al., 2018; Akiyamaet al., 2005; Hajoeningtijas, 2012; Smith & Gianinazzi, 1998, and Johnson et al., 1997), can be symbiotic with more than 80% of plants including palm oil (Phosri et al., 2010). These fungi contribute to the nutrient cycle (Sasli and Ruliansyah, 2012) and increase plant resistance to environmental conditions that are less supportive for plant growth, such as drought (Hapsoh et al., 2006), and acid (Rohyadi, 2008), contaminated with heavy metals (Bhaduri and Fulekar, 2012) and can protect plants from pathogens (Budi and May, 2013). Mycorrhizae not only develop in well-drained soils, but also in flooded soils. Even in very poor environments or environments contaminated with hazardous waste, mycorrhizae fungi can still show its existence (Usman et al., 2018; Kong, 2017; Harms et al., 2011; Chaudhry et al., 2005, and Khan et al., 2000). One form of environment that reflects such conditions can be found in histosol soil types or more commonly called peat soils (Hanafiah, 2004).

The benefits of mycorrhizae fungi are clearly seen if the soil conditions are poor in nutrients or dry conditions, whereas in fertile soil conditions the role of fungi is not so real (Setiadi, 2001; Lakitan, 2003). Mycorrhizae is important for the resilience of an ecosystem, the stability of plants, and the maintenance and diversity of plants and increasing crop productivity (Moreira et al.,2007*)*. At least there are five benefits of mycorrhizae for the development of plants that host them, namely increasing nutrient absorption from the soil as a biological barrier to root pathogen infections, increasing host resistance to drought, increasing growth booster hormones, and ensuring the implementation of biogeochemical cycles. In this symbiotic relationship, fungi get nutritional benefits (carbohydrates and other growth substances) for their life needs from plant roots ((cosme et al., 2018; Gerz et al., 2018; Menzel et al., 2018; Hoeksema et al., 2018; Brundrett & Tedersoo, 2018; and Lambers, 2008; Noli et al., 2011).

Fungi mikoriza arbuskula (FMA) has four functional roles, namely: (1) bioprocessor; able to act as a pump and live pipe because it can help plants to absorb nutrients and water from locations that are not reached by the hair roots; (2) bioprotector or life shield because it can protect plants from biotic (pathogens, pests, and weeds) and abiotic (temperature, soil density, and heavy metals) stress; (3) bioactivator because it is proven to be able to help increase carbon deposits in the rhizosphere thus increase microorganism activity to run biogeochemical processes; and (4) bioagregator because it is proven to be able to increase soil aggregation (Nobre et al., 2018; Mallmannet al., 2018; Barros et al., 2018; Back et al., 2018; Hidayatet al., 2018, and Nusantara et al.,2012).

Mycorrhizae fungi get the supply of reduced carbon provided by plants. Plants get the benefits obtained from mycorrhizae fungi, in the form of: (1) the roots of mycorrhizae stimulate nutrient uptake and water from the soil because external mycelia can explore a wider range of land compared to non-mycorrhizal roots; (2) fungi absorb low concentrations of nutrients more efficiently than roots that are not mycorrhizal; and (3) fungal hyphae produces various hydrophilic enzyme that releases nitrogen and phosphorus from organic compounds that were previously unavailable to plants (Handayanto and Hairiah, 2007).

The conversion of peat forest areas to the management of oil palm plantations will certainly lead to changes in the ecosystem of peat soils due to differences in treatment than before. Certain changes in ecosystems will also have an impact on the physical and chemical properties of peat soils which will eventually lead to the development of microorganisms such as Fungi Mikoriza Arbuskula (FMA). The purpose of this study was to determine the abundance of peatlands Fungi Mikoriza Arbuskula for oil palm plantations.

MATERIALS AND METHODS

Location and Time of Research

The study was conducted in East Kotawaringin Regency, Central Kalimantan Province with the determination of 4 (four) research locations, respectively namely : (1) Natural peat swamp forest in Kota Besi Subdistrict (112^0 41' 35.73" BT, 2^0 22' 0.57" LS), (2) Peatlands for oil palm plantations with a planting age less than 4 years in Parenggean Subdistrict (112^0 42' 27.53" BT 2^0 6' 28.54" LS); (3) Peatlands for oil palm plantations with a planting age between 4-10 years in Cempaga Subdistrict (112^0 54' 42.58" BT 2^0 16' 10.71" LS); and (4) Peatland for oil palm plantations with a planting

age more than 10 years in Sub-district of Mentawa Baru Ketapang (112^0 42' 27.53" BT 2^0 6' 28.54" LS).

Analysis of peat soil was carried out at the Soil Laboratory of the Faculty of Agriculture, Brawijaya University and University of Palangka Raya, while the analysis of Fungi Mikoriza Arbuskula (FMA) was carried out in the Laboratory of Plant Disease / Hama Penyakit Tanaman (HPT), Faculty of Agriculture, Brawijaya University. The time of research starting from preparation to data processing is carried out for 10 (ten) months, starting from June 2017 to March 2018.

Materials and Tools

The material used is a sugar solution (glucose) 60%, a solution of *Polyvinyl Alcohol Lactic acid Glycerol* (PVLG) as a spore preservative and *Melzer's* solution as a coloring agent, aquadest, clear nail paint, tap water, plastic strap, plastic bag and label paper. Tools for soil sampling land are hoes or shovels, while laboratory testing equipment are a set of 600 µm , 250 µm, 106 µm, 53 µm, and 38 µm multilevel sieve, 1000 mL goblet, 100 erlenmeyer bottles, petri dishes, spore tweezers, glass slide, glass covers, stereo microscopes, compounds microscope, tea filters, tweezers, analytical scales, hot plates, scissors, digital cameras, and calculators.

Plot Making

Observation plots sized 20 m x 20 m are made according to the ICRAF method (Ervayenri et al., 1999). Determination of observation plots was conducted randomly as many as 3 (three) plots and 5 (five) soil sampling points in each location. Total of soil sampling amounted to 60 points.

Soil Sampling

Composite roots and soil sampling on 5 (five) points as replicates in each plot at a depth of 20 cm. The weight of the soil taken every point is 500 gr, so the total soil samples taken for each plot are 2.500 gr. Soil samples for each point in a plot are mixed in one place until they are homogeneous to represent one observation plot, then taken 100 g per point or 500 gr for each plot.

Spore Extraction

FMA spore extraction using a wet filter technique and continued with centrifugation technique. The working procedure of wet filter technique was first carried out by mixing 50 g of soil samples with 200-300 ml of water and stirring until the soil grains were destroyed. Then filtered in a set of 600 µm , 250 µm, 106 µm, 53 µm, and 38 µm multilevel sieve sequentially from top to bottom. From the upper sieve sprayed with tap water to facilitate the escape of filter material. Then the upper sieve is removed and the second filter/ sieve is sprayed again with tap water. After the second filter/ sieve is removed a number of residual soil left in the bottom filter is transferred into the centrifuge tube.

The making of spore microscope slide set using the Melzer's coloring agent. FMA spores obtained from extraction after calculated the numbers then placed in Melzer's solution. Then the spores are broken carefully by pressing the cover glass of the slide using the end of the stick. The discoloration of the spores in Melzer's solution is one of the determinants indicator of existing spore types.

Spore Isolation

Spore isolation was done in order to separate the spores from the soil samples so that the characteristics of the FMA spores and the amount can be known. Isolation of FMA spores was conducted by wet filter pouring technique (Pacioni, 1992 in Brundrett et al., 1996) dan dilanjutkan dengan teknik sentrifugasi (Brundrett et al., 1996). 50 g of soil is dissolved in 500 ml of water and stirred for ± 15 minutes until the soil grains were destroyed. Furthermore, the soil suspension was filtered with a multilevel sieve sized 600 µm, 250 µm, 106 µm, 53 µm, and 38 µm using running water until clear. The filter/ sieve results then transferred to a centrifuge tube and centrifuged for 3 minutes at 2.500 rpm.

Supernatant the result of centrifuge was removed, and the deposit was resuspended with a 60% glucose solution and centrifuged again for 1 minute at 2.500 rpm. The remaining deposits in the filter/ sieve were poured into a petri dish and then observed under a binocular microscope for calculating the spore density and the making of a microscope slide set to identify the existing FMA spores. The spores are harvested by filtering the supernatant using a 38 µm sieve.

Identification of Spores

Identification of FMA spores was carried out by observing the structure of FMA spores, diversity of FMA spores, and spore morphology, including: shape, size, and color of spores and spore reaction after *Melzer's* solution was dropped. Observation of spore morphology was

carried out using a binocular microscope based on the state of the spores on a slide and also based on observations of the structure of the FMA (intraradical hyphae, vesicles, and arbuscular). The foundation of identification is a guidebook of "*Manual for The Identification of VA Mychorhizal Fungi*" (Schenk and Ferez, 1990). Identification of spores is carried out until the determination of the spore genus. Spores placed on microscope slide set, then dripped with Melzer's solution. Spores are destroyed to see the reaction between lipids inside the spore with Melzer's solution. Spores are taken with spore tweezers and transferred to the glass of microscope slide set in the same shape and color, then respectively given one drop of PVLG preservative and Melzer's coloring by leaving a place for the label. the glass of microscope slide set was covered with a slip cover with glue on each side using nail polish.

Statistical analysis

Data analysis was conducted by descriptive method and assisted by figures, graphs, and tables. The abundance of FMA is described sequentially as follows: (1) Structure of FMA, (2) Diversity of FMA Spores, (3) FMA Density, (4) FMA Spore Abundance; and (5) Relative Abundance of FMA Spores.

RESULTS AND DISCUSSION

Structure of Fungi Mikoriza Arbuskula (FMA)

Fungi Mikoriza Arbuskula (FMA) in general it has several structures to survive inside plant roots and in the soil. Research by Puspitasari et al., (2016), Proborini et al., (2013), Indriani et al., (2011), Saidi et al., (2014), and Dewi (2007) stated that the main structures of FMA are arbuscular, vesicles, external hyphae, and spores. The association between FMA and a plant can be identified by the presence or absence of infection in the root with the discovery of arbuscular, vesicle, external hyphae.

Figure 1, findings of this study namely infections in the roots of oil palm plants found in the structure of infection that forms the structure of FMA in the form of hyphae and vesicles, while the FMA structure in the form of arbuscular is not found. Branched hyphae like the letter H is the characteristic of Glomus. The germinated spores then extend the hyphae into the plant roots and swell to form spores (right). Vesicles located at the tips of the hyphae, shaped like a bag and distend. FMA colonization indicated by the presence of structures such as hyphae, arbuscular, and vesicles is significantly affected by plantation processing systems. The findings of previous research conducted by Nobre et al., (2018); Tuheteru et al., (2017); Costa et al., (2016); Kivlin et al., (2011); Cruz (2015); Lara-Pérez etal. (2014), and Corryanti et al.(2007); that the presence of FMA spores is influenced by environmental factors and host plants.

Fungi Mikoriza Arbuskula (FMA) Spores Density

Spore density is the number of spores in 100 grams of peat soil. The average of Spore Density (100 g) in each study location can be seen in Table 1.

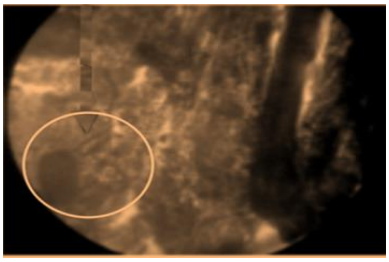
The research findings show that the average density of spores (100 g of peat soil) sequentially is the highest in peatlands for palm oil plantations with a planting age less than 4 years (320,40), palm oil plants aged between 4-10 years (276,20), and followed by palm oil plants with the aged over than 10 years (211,20). The lowest occured in natural peat forests (152,20).

Forest conservation for agricultural land will reduce the diversity of species and number of propagules of fungi due to changes in plant species, amount of organic matter produced, nutrients, and soil structure. Multi-species forest transformed into monoculture forest with a uniform age greatly influences the number and diversity of mycorrhizae (Gilbertet al., 2016; Setiadi, 2001; and Meijer & Govers, 2007). FMA spore density is influenced by the management system (Higo et al.,2013). The highest number and type of FMA in the Tetragastris sp. plant rhizosphere. aged 5 years (Herre et al., 2007).

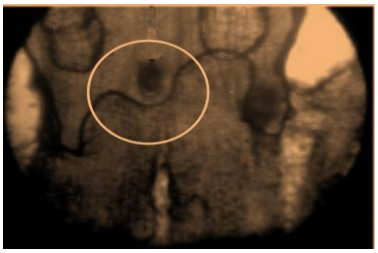
Fungi Mikoriza Arbuskula (FMA) Spore Diversity

The diversity of FMA spores is the result of identification of FMA spores to the genus level by looking at the morphological characteristics of spores that have been preserved with *polyvinil alcohol lactid acid glycerol* (PVLG). Identification of FMA spores is conducted through microscope documentation with 40x magnification.

Figure 2, identification results showed 12 (twelve) species of genus Glomus sp. FMA spores which has types and characteristics that are not the same in each genus. FMA spores of the genus Glomus sp is the only type of FMA spore, either in natural peat forests and peatlands for palm oil plantations of various ages.



External hyphae on *FMA*

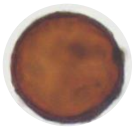


Vesicles on *FMA*

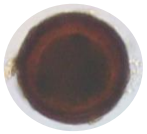
Figure 1. The structure of FMA external hyphae and FMA vesicles

Table 1. FMA Spore Density (100 grams)

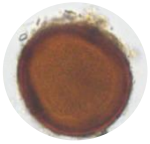
Peatlands	Replicate					Amount (100 g)	Average±SD
	I	II	III	IV	V		
Natural peat forest	178	123	149	153	158	761	152,20±17,68
Palm oil plants aged less than 4 years	315	322	301	327	337	1.602	320,40±12,06
Palm oil plants aged between 4-10 years	286	271	264	270	290	1.381	276,20±10,01
Palm oil plants aged above 10 years	208	211	204	217	216	1.056	211,20±4,87



Glomus sp 1

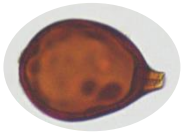


Glomus sp 2

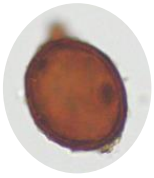


Glomus sp 3

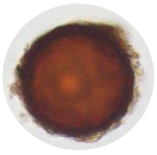
Glomus sp 4



Glomus sp 5

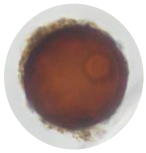


Glomus sp 6



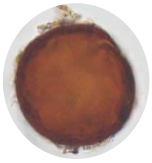
Glomus sp 7

Glomus sp 8

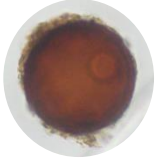


Glomus sp 9

Glomus sp 10



Glomus sp 11



Glomus sp 12

Figure 2. Types of Genus Glomus sp. FMA Spores

Table 2:Spore Morphology of genus *Glomus sp*. Fungi Mikoriza Arbuskula (FMA) (100 g)

Genus identification	Spore Characteristic of *Fungi Mikoriza Arbuskula (FMA)*						Reaction of Melzer's.
	Color	Shape	Size (μm)	Wall Thickness	Surface Texture	Hifa's stalk	
Glomus sp 1	Brown	Oval round	152,15	2 layers 3,4 μm	Fine	None	Unreacted
Glomus sp 2	Dark brown	Oval round	150,22	2 layers 3,1 μm	Very coarse	None	Unreacted
Glomus sp 3	Brown	Oval round	152,02	2 layers 3,7 μm	Coarsely speckled	Clots	Unreacted
Glomus sp 4	Brown	Oval	151,45	2 layers 2,7 μm	Fine	Straight	Unreacted
Glomus sp 5	Brown	Oval	149,65	2 layers 3,2 μm	Very coarse speckled	Straight	Unreacted
Glomus sp 6	Brown	Oval	148,24	2 layers 2,9 μm	Coarsely speckled	None	Unreacted
Glomus sp 7	Brown	Round	147,38	2 layers 4,2 μm	Coarsely speckled	None	Unreacted
Glomus sp 8	Dark brown	Round	142,20	2 layers 4,5 μm	Finely speckled	None	Unreacted
Glomus sp 9	Dark brown	Round	144,37	2 layers 5,5 μm	Very coarse speckled	Clots	Unreacted
Glomus sp 10	Dark brown	Round	134,31	2 layers 3,5 μm	Coarsely speckled	None	Unreacted
Glomus sp 11	Light brown	Oval round	139,22	2 layers 5,3 μm	Very fine	Straight	Unreacted
Glomus sp 12	Brown	Oval round	132,17	2 layers	Finely speckled	Straight	Unreacted

This is in line with the research of Cahyani et al., (2014) which showed *Glomus* was the dominant FMA spore compared to *Acaulospora* and *Gigaspora* in Alluvial soil in Pamekasan Madura Regency.

Glomus is a type of FMA that has a fairly good adaptability to the environment either in acidic and neutral conditions, so its existence tends to be more dominant compared to other genus (Delvian, 2006). *Glomus* is a genus that dominates agricultural land, and has a higher resistance to environmental stress compared to other genus. *Glomus* has a fairly high level of adaptation to various environmental conditions and has a wide distribution (Shi et al., 2007). This genus can develop at a pH of less than 5.00 to neutral, and on the texture of sandy clay loam to clay soil. Spore Morphology of genus *Glomus sp*. Fungi Mikoriza Arbuskula (FMA) (100 g) in Table 2.

Glomus sp. is the mycorrhizae genus from the family of *Glomeraceae*. *Glomus sp*. is a genus that has the highest diversity of others. Some characteristics of Genus Glomus sp. Fungi Mikoriza Arbuskula (FMA) Spore namely the spores are formed individually or in pairs in the non gametangium hyphae terminal which is undifferentiated in the sporocarp. At adulthood, the spores are separated from adhesive hyphae by a septum. Spores in the form of *globose, sub-globose, ovoid, or obovoid* with spore walls consisting of more than one layer, hyaline to yellow colored, brownish red, brown and black, sized between 20-400 μm (Morton, 2014).

The Abundance of genus Glomus sp. FMA Spores

The abundance of Genus *Glomus sp FMA* Spores is the number of Genus Glomus sp FMA Spores at 100 grams of peat soil. The average abundance of FMA spores can be seen in the following Table 3.

The highest average abundance of Genus Glomus sp FMA Spores (100 g of peat soil) is located on peatland for palm oil plantations with planting age less than 4 years (112,80), followed by peatlands for palm oil plantations with planting age between 4-10 years (104,10), then natural peat forests (64,20. The lowest average abundance of a Genus Glomus sp FMA Spores (100 g of peat soil) is on peatland for palm oil plantations with the planting age over than10 years (47,40). Plant age greatly affects the FMA population in the soil (Widiastuti, 2006), Symbiosis of FMA and host plants is influenced by soil properties, type and age of host plants (Nusantara, 2011).

The Relative Abundance of Genus *Glomus sp.* FMA Spores

Relative abundance is the percentage comparison between *genus Glomus sp* FMA spores and the total of FMA spores. The average abundance of FMA spores can be seen in the Table 4.

The highest average of relative abundance of genus Glomus sp FMA Spore (100 g peat soil) is in natural peat forest (42,64%), then peatlands for palm oil plantations with planting age between 4-10 years (37,69%), peatland for palm oil plantations with planting age less than 4 years (35,34%). The lowest average is in peat land for palm oil plantations with planting age over than 10 years (22,48%)

Table 3. The Abundance of Genus *Glomus sp.* FMA Spores (100 g)

Peatlands	Replicate					Amount (100 g)	Average±SD
	I	II	III	IV	V		
Natural peat forest	62	59	63	68	69	321	64,20±3,76
Palm oil plants aged less than 4 years	109	111	125	122	97	564	112,80±10,01
Palm oil plants aged between 4-10 years	137	98	102	97	87	521	104,10±17,23
Palm oil plants aged above 10 years	42	48	56	48	43	237	47,40±4,96

Table 4. The Relative Abundance of Genus *Glomus sp.* FMA Spores. (%)

Peatlands	Replicate					Amount (%)	Average± SD
	I	II	III	IV	V		
Natural peat forest	34,83	47,97	42,28	44,44	43,67	213,20	42,64±4,33
Palm oil plants aged less than 4 years	34,60	34,47	41,53	37,31	28,78	176,70	35,34±4,16
Palm oil plants aged between 4-10 years	47,90	36,16	38,64	35,93	29,83	188,45	37,69±5,87
Palm oil plants aged above 10 years	20,19	22,75	27,45	22,12	19,91	112,42	22,48±2,71

CONCLUSION

Planting finds an infectious structure that forms FMA structures in the form of hyphae and vesicles. The highest spore density (100 g of peat soil) is on peatland for palm oil plantations with a planting age less than 4 years, while the lowest in natural peat forest. The identification results found 12 (twelve) species of genus Glomus sp. FMA spores which is the only type of FMA spore found in either natural peat forests and peatlands for palm oil plantations of various ages. The highest average abundance of Genus Glomus sp FMA Spores (100 g of peat soil) is on peatland for palm oil plantations with planting age less than 4 years (112,80), while the lowest is on peatland for palm oil plantations with the planting age over than 10 years. The highest average of relative abundance of genus Glomus sp FMA Spores (100 g peat soil) is in natural peat forest and the lowest is on peatland for palm oil plantations with the planting age over than 10 years.

RECOMMENDATION

This study recommends the conversion of peatland for oil palm plantations which in its management keep prioritizing the sustainability of peat ecosystems by taking into account the physical, chemical and biological characteristics of peat soil.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interes.

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AUTHOR CONTRIBUTIONS

The article is part of the Dissertation of Doctoral and all the authors have contributed: RR conducted experiments, data collection, data analysis and writing manuscript, Prof. S contributed to the experimental design, the determination of the research treatment, Prof. EN and Prof. SP contributes to experimental design, determination of research treatment and review of manuscripts.

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