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The Dynamics of The CMA Spores Population In Mass Propagation of Two Greenhouses

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ABSTRACT. The dynamics of the cma spores population in mass propagation of two greenhouses. The production of arbuscular mycorrhizal fungi (AMF) propagules is highly necessary for AMF-based biofertilizer production. This research was conducted to ustudy the dynamics of spore populations in mass propagation of AMF propagules in two greenhouses. AMF propagule propagation was carried out in vitro using sorghum plants as host plants grown in 5 kg capacity polybags totaling 200 polybags in all. The results showed that the reactions of both greenhouse H and greenhouse I media was acidic. There was a dynamics of AM fungi spore populations in those both green houses. Regrowth of sorghum plants (up to ratoon 2) failed to increase the density of AMF spore populations in both ratoon 1 and ratoon 2, and there was even a decrease in AMF spore populations at some incubation days. Drying of media could decrease or increase spore density in propagation in greenhouse H, while agitation to increase aeration couldn't enhance spore populations. In greenhouse I, neither agitation nor drying could increase the number of AMF spores. The highest spore population obtained from greenhouse H with a total propagation period of 49 weeks (12 months 1 week, ratoon 2) was observed at the beginning of maintenance of the second plant (ratoon 1), reaching 8.3 spores g-1, while for greenhouse I with a longer total time of 53 weeks (13 months 1 week, ratoon 2), the highest spore population was at the end of drying in the maintenance of the third plant (ratoon 2), reaching 6.7 spores g-1.

Keywords : *AM fungi propagation, spore population, pot culture.*

INTRODUCTION

Fertilizers are essential tools in agricultural production, especially after the "Green Revolution", where fertilization is often carried out at high doses, particularly with inorganic fertilizers known as intensification (Kalamula et al., 2022). This is driven by the increasing global food demand in line with population growth. However, intensification often overlooks the impact of agricultural chemicals on natural ecosystem functions. Soil quality degradation and disruption of natural ecosystem functions are real threats to environmental sustainability; furthermore, they could pose warnings to future food security. To address these issues, the implementation of sustainable agriculture, utilizing environmentally friendly approaches in all farming practices, is a better alternative.

Arbuscular mycorrhizal (AM) fungi form symbiotic relationships with the majority of terrestrial plants and fungi from Glomeromycotina (Spatafora et al., 2016). This mutualistic symbiosis is based on nutrient exchange: arbuscular mycorrhizal fungi (AMF) receive up to 20% of the photosynthesis-fixed carbon from plants, and in return, they provide mineral nutrients (Bago et al., 2000; Smith and Smith, 2011). On the other hand, fungi absorb these nutrients from the soil through extraradical hyphal networks and release them into root cortex cells via tree- like hyphal structures called arbuscules (Luginbuehl and Oldroyd, 2017). Among symbiotic microorganisms, besides Rhizobium, AM symbiosis is extensively studied. The role of AM symbiosis with plants includes enhancing nutrient uptake, improving soil structure, and promoting plant growth and health (1). The availability and effectiveness of

AM inoculants are crucial for the success and functionality of the symbiosis (Kalamula et al., 2022; Kameoka and Gutjahr, 2022).

Fertilizer based on AMF is produced by propagating propagules in the form of spores and infected roots or mycelium. The main constraint behind propagule propagation or mass production techniques of AMF is their obligate nature, alongside the impossibility of identifying AMF species at the early stages of development.

Although AMF can produce spores without symbiosis with plants, they have limited nutrient reserves without symbiosis, so they lack sufficient nutrients to produce new and mature spores indefinitely. Thus, host plants play a crucial role in the life cycle of AMF as they facilitate spore formation and development, and their survival. Nevertheless, many techniques have been developed in recent decades for mass production of AMF. Some well-known methods in propagating for mass production of AMF are substrate-based production systems, substrate- free production systems, and in vitro production systems. This research aims to understand the dynamics of spore populations in the production of AMF propagules for substrate-based biofertilizer production in two greenhouses.

MATERIALS AND METHODS

Propagation of AM Fungi in Green house H. A total of 1000 kg of AMF-based biofertilizer products (from 40 sacks, Bayah zeolite) were placed into 5 kg polybags, resulting in 200 polybags. Subsequently, sorghum seeds, 10 seeds each, were planted in each polybag on April 23, 2022. Sorghum plants were maintained with nutrient watering (Johnson and NPK). Johnson nutrients were watered every 2 days according to the plant's age, while NPK 15-9-20 was applied at a concentration of 10 g per 100 liters sprayed onto the growing media, totaling 250 ml per month. After the plants were 3.5 months old (until the first week of August), they were dried for 2 weeks until the third week of August and then pruned.

The subsequent growth (ratoon) was maintained as the second cycle. The second maintenance period lasted for 3.5 months until the second week of December, followed by a 2-week drying period until the fourth week of December, and then pruned.

Next, the media was stirred, and maintenance continued for another 3.5 months (January - second week of April) with a 3-week drying period until the fourth week of April, followed by pruning. The propagation process of AMF propagules in RK H is schematically illustrated as follows:

3,5 bln 2 mg		3,5 bln 2 mg			3,5 bulan		3 mg
Planting	Dry Pruning	Maintenance II	Dry Pruning	Stirring	Maintenance III	Dry Pruning	

Figure 1. Schematic description of the propagation process in Green house H

Samples for spore count calculation started from the beginning of the drying period of the second cycle until the end of the drying period of the third planting cycle, with the following details:

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Table 1. Sampling time for analysis of CMA spore populations from GH H

No	Time sampling	Notes
1.	12 December 22	Maintenance II, before drying
2.	28 December 22	Maintenance II, end drying
3.	9 January 23	Maintenance III, 1 week old
4.	23 January 23	Maintenance III, 3 weeks old
5.	13 February 23	Maintenance III, 6 weeks old
6.	27 February23	Maintenance III, 8 weeks old
7.	13 March23	Maintenance III, 10 weeks old
8.	27 March 23	Maintenance III, 12 weeks old
9.	4 April 23	Maintenance III, 13 weeks old (initial drying)
10.	27 April 23	Maintenance III, end drying
11.	5 May 23	Maintenance III, end drying

Propagation of AM Fungi in Green house I. A total of 1000 kg of AMF-based biofertilizer products (from 40 sacks, Bayah zeolite) were placed into 5 kg polybags, resulting in 200 polybags. Subsequently, sorghum seeds, 10 seeds each, were planted in each polybag on May 9, 2022. Sorghum plants were maintained with nutrient watering (Johnson and NPK). Johnson nutrients were watered every 2 days according to the plant's age, while NPK 15-9-20 was applied at a concentration of 10 g per 100 liters sprayed onto the growing media, totaling 250 ml per month. After the plants were 4.5 months old (until the third week of September), they were dried for 2 weeks until the first week of September, and then the tops were pruned.

The subsequent growth (ratoon) was maintained as the second cycle. At this stage, plant maintenance lasted for 3.5 months from the first week of October to the third week of January, with a 2-week drying period until the first week of February, followed by pruning of the tops. Next, the media was stirred, and maintenance continued for another 3.5 months (from the first week of February to the third week of May) with a 0.5-month drying period until the first week of June, followed by pruning of the tops. The propagation process of AMF propagules in RK I is schematically illustrated as follows:

Figure 2. Schematic description of the propagation process in Green house I

Samples for spore count calculation started from the beginning of maintenance periode, 10 weeks old up to the end of drying period of the second cycle until the end of the drying period of the third planting cycle, with the following details:

Table 2. Sampling time for analysis of CMA spore populations of Green house I

No	Days sampling	Notes
1.	12 December 22	Periode II, 10 weeks old
2.	28 December 22	Periode II, 12 weeks old
3.	9 January 23	Periode II, 14 weeks old
4.	23 January 23	Periode II, before drying
5.	13 February23	Periode II, the end drying
6.	27 February 23	Periode III, 4 weeks old
7.	13 March 23	Periode III, 6 weeks old
8.	27 March 23	Periode III, 8 weeks old
9.	4 April 23	Periode III, 9 weeks old
10.	27 April 23	Periode III, 12 weeks old
11.	5 May 23	Periode III, 13 weeks old
12.	23 May 23	Periode III, before drying
13.	7 June 23	Periode III, the end drying

RESULTS AND DISCUSSION

Propagation of AM fungi in Green house H

Soil reaction influences root development and thus affects the development of AMF in its symbiosis with sorghum. Soil analysis results showed a soil reaction ranging from 5-6, with an average of 5.8. The favorable soil reaction range is between (5.85-6.64) for fungi and supports spore formation, especially for Glomus, Scutellospora, Entrophospora, and Archaeospora, but negatively affects the spore formation of Acaulospora and Gigaspora (Muckongo et al., 2023). Muchane et al. (2012) reported that beneficial AMF development occurs at pH 5.51-6.67, while Dobo et al. (2016) conveyed that in agricultural soil, the pH range suitable for increasing AMF spore formation is 6.18-6.28. However, Acaulospora spp. are expected to thrive in acidic conditions because

Acaulosporaceae are tolerant to acidic tropical soil (Temegne et al., 2017; Bagyaraj et al., 2014). Acaulospora laevis dominates in soils with low pH and germinates well at pH 4-5, while Gigaspora heterogama from tropical soils shows variable germination (8-78%) under the same environmental conditions (de Novais et al., 2013). From these results, it appears that the soil reaction conditions are close to the ideal pH for AMF, especially for Acaulospora spp. and Gigaspora, whose propagules were propagated in this experiment.

Table 3. The pH of the media in AM Fungal propagation in Green House H

Sample	pH
GH-H1	6
GH-H1	6
GH-H2	6
GH-H2	6
GH-H3	6
GH-H3	6
GH-H4	6
GH-H4	6
GH-H5	5
GH-H5	5

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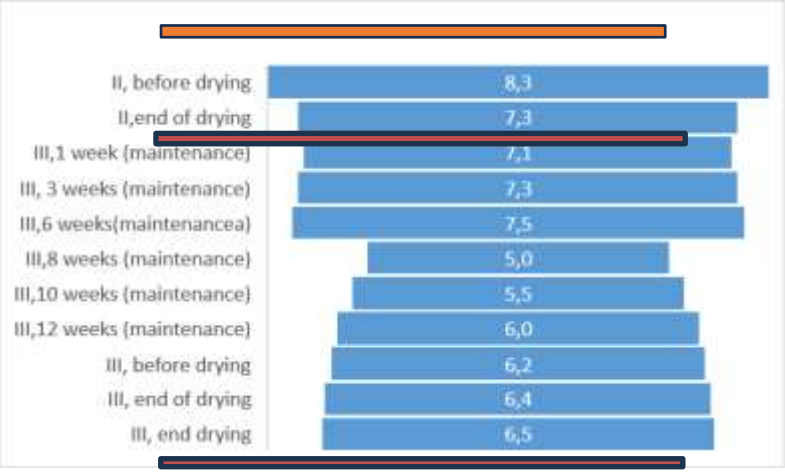

Figure 3. Dynamic of spore population (per g media) in Green house H

The results of spore count in RK H showed that after the drying period of the second cycle, the spore population decreased. At the end of the drying period, stirring was performed, gradually increasing the spore population until the 6th week of plant growth in the third maintenance cycle. This result indicates that aeration provided through medium stirring gradually increases the spore population. However, during the third maintenance cycle, the spore count decreased and reached its lowest point of 5 spores g-1 when the plants were 8 weeks old in the third maintenance cycle. This decrease is suspected to be caused by parasitism against AMF spores. Sreenivasa and Bagyaraj (1989) stated that in pot cultures, the application of captan and carbofuran significantly suppressed contaminant fungi and nematodes. The influence can vary depending on the formulation, dosage, soil type, environmental conditions, and AMF and contaminant species (Jansa et al., 2006). Furthermore, the decrease in spore count in this study appears to be slightly different from the results of in vitro spore propagation (Ghorui et al., 2023). In that study, spore production initially showed gradual growth until day 27 (4 weeks), then increased significantly until day 76 (11 weeks) when it stabilized (Costa et al., 2023). Differences in culture methods may account for this discrepancy. Additionally, in vitro methods, the limitation of sucrose is suspected to enhance the number or formation of irregular spores of AMF Rhizophagus irreguler. This was also suggested by Dalpe et al. (2005). In this study, propagation was conducted in vivo, so continuous photosynthesis would supply sugar compounds to fungi, inhibiting spore formation. Moreover, it may also be due to differences in AMF species. In this study, the AMF inoculum consisted of a mixture of Acaulospora, Gigaspora, Glomus, and Entrophospora. Muckongo et al. (2023) reported that the response of each genus differs, especially regarding organic matter content, N, pH, silt, Mg, and Na. Nevertheless, the spore count of AMF gradually increased and peaked at the end of the drying period in the third planting cycle, reaching 6.5 spores g-1.

Based on these results, it seems that the addition of plant maintenance periods or ratoon plants cannot increase the AMF spore population. Spores produced from plants during the second maintenance cycle originated from plants in the first maintenance, and similarly, spores produced from the third maintenance (Ratoon 2) originated from ratoon 1 and the first maintenance of plants. Based on this, spores produced during the third maintenance originated from spores that had undergone more propagation than spores produced from plants during the second maintenance. Decreased AMF sporulation after several cycles is also

reported elsewhere. This may be due to the loss of viability, meaning they are not suitable for propagation. This result differs from Stutz & Morton (1996), who observed higher spore production in the third cycle. However, this activity demonstrated that host plants can maintain the viability of spores or propagules in general, as evidenced by the presence of a viable spore population until the end of plant maintenance.

Propagation of AM fungi in Green house I

Soil medium analysis indicated acidic pH (pH 5). The occurrence of acidic pH encourages Acaulospora spore formation, while for Glomus, Scutellospora, Entrophospora, and Archaeospora, they tend to be optimal at higher pH levels, namely 5.85 – 6.64 (Muhkongo et al., 2023). In this activity, the propagated AMF consisted of a mixture of Acaulospora, Gigaspora, Scutellospora sp, Enterophospora, and Glomus sp. However, individual species density analysis was not conducted in observing the AMF spore count.

Table 4. The pH of the media in Green house I

Sample	pH
GH-I1	5
GH-I1	5
GH-I2	5
GH-I2	5
GH-I3	5
GH-I3	5
GH-I4	5
GH-I4	5
GH-I5	5
GH-I5	5
GH-I6	5
GH-I6	5
GH-I7	5
GH-I7	5
GH-I8	5
GH-I8	5

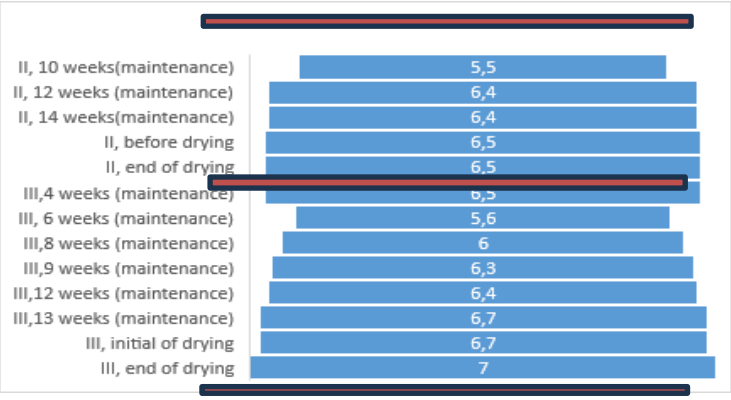

Figure 4. Dynamic of spore population (per g media) in Green house I

In RK H, at the beginning of the analysis, the spore count showed 5.5 spores g-1, and subsequently, the spore count increased to 6.4 spores g-1. During the drying period, there was no increase in spore count. This result seems slightly different from the dynamics of spore

count in RK H. Plant drying during the second planting cycle in RK H actually led to a reduction in spore count but increased during the drying phase of the third planting cycle, whereas in RK I, there was no change in spore count. Theoretically, drying should induce spore formation as the decrease in carbon supply from the host plant to the fungus would increase the spore count. According to Dalpe et al. (2005), the low sucrose content in in vitro AMF propagation stimulates spore formation. The same was suggested by Ghorui et al. (2023). In the research, spore propagation using the root organ culture method (in vitro) showed that vegetative spores of AMF Rhizophagus irregularis were abundant after 60 days, presumably due to the reduced sucrose level in the medium (Srinivasan M, 2014). Rhizophagus irregularis exhibits faster growth when cultured on sucrose-free medium (D'Souza, 2013). Low sucrose concentration consistently correlates with spore germination rates and increased spore formation (Dalpe et al., 2005).

Stirring after the second maintenance was unable to increase the population of AMF spores, and the spore count even decreased 6 weeks after stirring but gradually increased until the end of the third plant's incubation (ratoon 2). This period also marks the beginning of ratoon plant maintenance, but in the subsequent stages, the spore population continued to increase until the plants were 13 weeks old.

Additionally, the spore count from samples in RK H at the beginning, during the second planting cycle, before drying, was 8.3 g-1, while in RK I, it was 6.5 spores g-1. Further analysis of spore count at the end of the experiment showed that the spore propagation results in RK H were higher than the spore count in RK I, although the experimental period in RK I was one month longer than in RK H. This difference may be due to the initial spore population.

However, generally, the spore count produced in this propagation was still low. The low spore count is likely due to the presence of non-symbiotic spores. The life cycle of arbuscular mycorrhizal fungi (AMF; Mucoromycota: Glomeromycotina) is dominated by spores. AMF spores are almost always produced during the symbiotic phase when AMF has formed arbuscules and actively obtains nutrients from the host plant. However, spore formation can also occur in the non-symbiotic phase (secondary spores), although spores formed when not in symbiosis undergo senescence. In other words, AMF can produce spores without symbiosis with plants, but AMF has limited nutrient reserves without symbiosis. Without forming a symbiotic relationship, AMF does not have enough nutrients to produce new and mature spores indefinitely. Thus, host plants play a crucial role in the life cycle of AMF as they facilitate spore formation and development, and therefore, the survival of the AMF generation (Tzng, 2022).

CONCLUSION

The production of propagules, in this case, spores, undergoes dynamics influenced by the plant's life cycle and maintenance. Increasing the maintenance period does not necessarily double the spore count or population, although it can maintain spore viability. Spore propagation is influenced by the origin of the medium, watering, or plant maintenance related to nutrient and water supply.

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