



The Effect of Pruning and Growing Media Composition on Arbuscular Mycorrhizal Propagation

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ABSTRACT. The symbiosis of arbuscular mycorrhizal fungi with plants is the oldest symbiosis in the world. The obligate nature of mycorrhizal fungi in symbiotic relationships is believed to underlie its enduring presence to this day. The application of arbuscular mycorrhizal fungi (AMF) based fertilizers has become increasingly common. Spores are the most important organs that determine the quality of AMF-based fertilizers, along with AMF colonization in the roots. The production of AMF spores is greatly influenced by plant physiology, including photosynthesis processes affected by canopy abundance, as well as the type of planting medium, which determines plant root growth and AMF habitat. This study was conducted to investigate the effect of pruning and planting medium composition on AMF infection and spore production in AMF propagation using sorghum as the host plant. The pruning levels tested were at one, two, and three months after germination, and thereafter, each plant was maintained for 136 days after planting (DAP). The tested planting medium composition was the ratio between zeolite and compost in five different compositions: (v/v) 1) 100:0, 2) 90:10, 3) 70:30, and 4) 50:50. The chemical characteristic of compost used was contained 15.5% C, 1.49% N, 2.54% P₂O₅, 1.49% K₂O, pH 6.6, and some microelements such as Cr, Fe, Zn, and Mn at 5.2 ppm, 5961 ppm, 294 ppm, and 199 ppm, respectively. The Sukabumi's zeolite used had a size of 2-3 mm. Propagation of AM fungal spores was carried out using polybags containing 10 kg of medium according to the treatment. Plant maintenance was carried out by alternating between watering with water and Johnson nutrient solution. Watering was performed until the plants were 120 days old, and subsequently, no watering was done until 136 days after planting. The observed parameters were the development of AMF spore numbers until a 4.5-month incubation period. The results showed that pruning time can affect spore production if the plant has reached a certain vegetative age, which is three months for sorghum. Additionally, adding 10% compost to zeolite medium can enhance spore formation, even though sorghum growth may not be at its maximum in this medium composition.

Keywords : *compost, zeolite, AMF spore, AMF colonization*

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) tend to make associations with 85% of plant families and play a significant role in the sustainability of an ecosystem. Several research have proven that the symbiosis with AMF can support plant growth in suboptimal soils, reduce the negative impacts of pathogens (Jung et al., 2012), and improve plant water status and water use efficiency (Chitarra et al., 2016). As a result, the use of AMF-based fertilizers is increasingly being adopted. Therefore, in the midst of global warming, the application of AMF-based fertilizers at present is a crucial measure. The main companies dealing with mycorrhizal products are present in Europe, Asia, North, and Latin America in the regional context. However, it is often encountered that obtaining inoculum can be challenging. One determinant of inoculum quality is the quantity of spores, although infective propagules, based on the latest agricultural regulations, are a required parameter.

In the symbiosis, AM fungi receive photosynthates from the host plant (10–20%), while in return, these fungi enhance nutrient and water absorption for the host plant (Khaliq et al., 2022). Nonetheless, from this, the process of plant's photosynthesis will influence the fungus ability to form spores. Shoot pruning is one way to halt vegetative growth of plants and subsequently stimulate them to enter the generative process. However, excessive pruning can reduce the canopy's capacity to produce sugars and induce pathogenic infections, as well as AMF infections. With pruning, one consequence experienced by plants is a hindrance or reduction in photosynthesis. Nonetheless,

photosynthesis will affect the produced photosynthates, thereby also influencing the transfer of photosynthates to AMF. However, the extent of the influence of pruning on AMF spore production has not been widely reported.

Furthermore, plant growth is greatly influenced by root development. The composition of the planting medium significantly affects plant root growth. The addition of sand can enhance medium aeration and porosity, but it cannot retain nutrients. It is suspected that this will also influence the development of the association between AMF and plant roots. This study aims to investigate the extent of the influence of pruning and planting medium composition on the development of AMF spore formation.

MATERIALS AND METHODS

Preparation of Planting Materials and Media. The experiment was conducted in a greenhouse from late November to April 2022. The materials used in this experiment were 2-3 mm zeolite from Sukabumi, sorghum seeds, compost, Johnson nutrient solution, and polybags measuring 40x40 cm. Each polybag was supplemented with AMF inoculum ranging from 55 g to 1 kg, depending on the mycorrhizal population content. If the spore count was high (>50 spores/g), then 55 g of inoculum was added to each polybag for 10 kg-sized polybags. If the spore count in the inoculum was below 50 spores/g, then 1 kg of inoculum was added per polybag. Each polybag was planted with 32 sorghum seeds in 16 holes. There were 12 treatment combinations, consisting of 4 planting medium compositions and 3 pruning times (Table 1). Each treatment was replicated 10 times, resulting in a total of 120 experimental units.

Table 1. Treatment assessed in the experiment

No	Treatment	Planting medium composition (v/v,%)		Pruning plant age (month)
		Zeolite	Compost	
1.	A1	100	0	1
2.	A2	100	0	2
3.	A3	100	0	3
4.	B1	50	50	1
5.	B2	50	50	2
6.	B3	50	50	3
7.	C1	70	30	1
8.	C2	70	30	2
9.	C3	70	30	3
10.	D1	90	10	1
11.	D2	90	10	2
12.	D3	90	10	3

Plant Maintenance and Observations. After pruning in accordance with the treatment, the plant's maintenance continues until harvest, which is at 136 days after planting (DAP). Irrigation is done alternately between water and the Johnson Nutrient Solution (JNS) until 120 DAP. The JNS solution contains macro elements (l) consisting of 101.10 g of KNO_3 , 236.16 g of $\text{Ca}(\text{NO}_3)_2$, 115.08 g of $\text{NH}_4\text{H}_2\text{PO}_4$, and 246.49 g of MgSO_4 . The micro elements (l) contain 3.728 g of KCl , 1.546 g of H_3BO_3 , 0.396 g of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.575 g of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.125 g of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, and $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, as well as 24.649 g of Fe-EDTA. The JNS solution is administered in volumes of 400 ml per polybag with different compositions depending on the plant's age (Table 2). In addition to watering, plant maintenance also included measures to prevent pest and disease infestations.



Table 2. Watering time and Johnson nutrient composition given

Plant age (weeks) after germination	Johnson nutrient solution (JNS) composition
2	1/6 concentration of JNS (without P)
2-4	1/4 concentration of JNS, 1/6 P
4-6	1/4 concentration of JNS, 1/6 P
6-8	1/2 concentration of JNS, 1/4 P
> 8	1/2 concentration of JNS, 1/4 P

Observation of Plant Growth and CMA Development. Observations of sorghum plant growth, as the host plant for AMF, were conducted on all plants within one polybag (16 plants) of 3 polybags out of a total of 10 polybags. Observations were made regarding the height of the canopy and the number of leaves at 30, 60, and 90 days after planting (DAP).

Sampling for spore count observations was performed at 30, 60, 90, 120, and 136 DAP. Samples for 30, 60, and 90 DAP consisted of planting medium taken from the root area, amounting to 100 grams, and composite samples were obtained from 10 polybags for replication. The sample taken at 136 DAP, which also served as the harvest time for the AMF culture, involved removing all the medium from the polybag and thoroughly mixing all the medium within the polybag. This sample was a composite of 10 replicated polybags, mixed into one sample. Subsequently, a wet sieving process was conducted to obtain the spore count using the wet sieving method. A sample of 25 g was weighed, then water was added up to 100 mL. The mixture was stirred with a stirring rod to release the spores from the zeolite for approximately 1 minute. The sample was then left to settle until the sediment had sunk. In the next step, the sample was filtered using a multi-level sieve with coarse mesh at the top and fine mesh at the bottom, followed by a slow rinse with tap water. The filtrate from the fine mesh was transferred to filter paper in a funnel, and the filter paper was placed in a Petri dish. The observation of spore count was conducted using a stereo microscope.

Observation of AMF infection was carried out on root samples taken during the medium dismantling process, which coincided with the AMF harvest time at 136 DAP. The entire root system was chopped, and random samples of roots were taken for AMF infection observation. In the observation of AMF colonization, plant roots were rinsed with flowing water and cut into 1 cm sections. Subsequently, the roots were treated with 2.5% KOH at 90°C for 45 minutes, followed by a rinse with water. After incubation, the KOH solution was discarded, and the roots were rinsed with flowing water. Alkaline H₂O₂ was added, and the mixture was heated again at 90°C for 45 minutes, followed by another rinse with water and overnight soaking in 1% HCl. Staining was then carried out with acidic trypan blue (0.05% of trypan blue in lactoglycerol) and incubated at 90°C for 10 minutes. The staining solution was discarded, and the roots were rinsed with flowing water while adding 5 mL of lactoglycerol (Phillips and Hayman, 1970). The stained roots were arranged on a glass slide and observed using a binocular microscope for the presence of internal hyphae, external hyphae, vesicles, or arbuscules at a magnification of 40x.

RESULTS AND DISCUSSION

Performance of Sorghum Plants

The plants were incubated for 136 days (4.5 months). The performance of the sorghum plants varied for each treatment (Figure 1). Vegetatively, it can be observed that the addition of compost resulted in better sorghum plant growth or performance, with leaves appearing greener and wider, and plant height significantly taller compared to sorghum planted with 100% zeolite. The highest sorghum growth at 90 DAP was observed in the medium with a ratio of 70:30 (zeolite: compost). This result

indicates that the addition of compost can influence the growth of sorghum plants. However, there exists an optimum amount of compost to achieve optimal sorghum growth. In this experiment, based on the performance of sorghum plants, it is shown that adding compost up to 30% is the optimal dosage compared to 10% and 50% (Figure 1).



Figure 1. Performance of sorghum plants in each planting medium treatment at 30 DAP (before the first pruning at one month)

The number of leaves increased in observations at 30, 60, and 90 DAP regardless of the 1, 2, and 3-month pruning treatments. The very rapid leaf growth is suspected to be the cause of the absence of a significant difference in leaf count between the 1, 2, and 3-month pruning treatments. The number of leaves varied among the tested treatments; however, the highest leaf count was exhibited by sorghum plants in medium C (70:30), especially in the 2-month pruning treatment. The highest increase occurred on day 120 after planting. In this treatment, it means that the leaf count is recorded as 60 DAP after pruning. However, after 90 days, there was no more pruning, so the plant with the highest leaf count performance is plant C2.

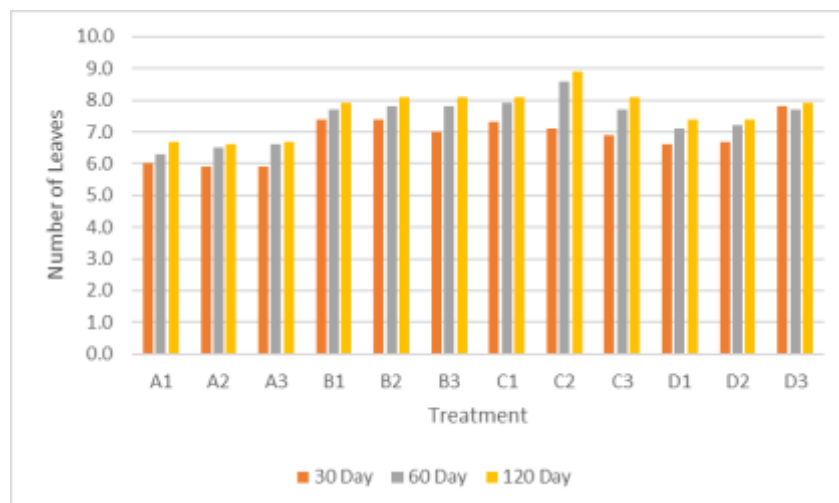


Figure 2. Development of the number of leaves of sorghum plants in each treatment 120 DAP

The plant height in medium A (zeolite only) increased in observations at 30, 60, and 90 DAP, even with 1, 2, and 3-month pruning treatments. Meanwhile, in medium B, pruning actually resulted in a decrease in plant height in observations at 30, 60, and 90 DAP. In medium C, the 2-month pruning produced the highest plant height, especially at the 90 DAP observation. On the other hand, in medium D, plant height showed slight differences in the 1, 2, and 3-month pruning treatments at 30, 60, and 90 DAP. Nevertheless, after 90 days, there was no more pruning, so the plant with the best plant height performance is plant C2.

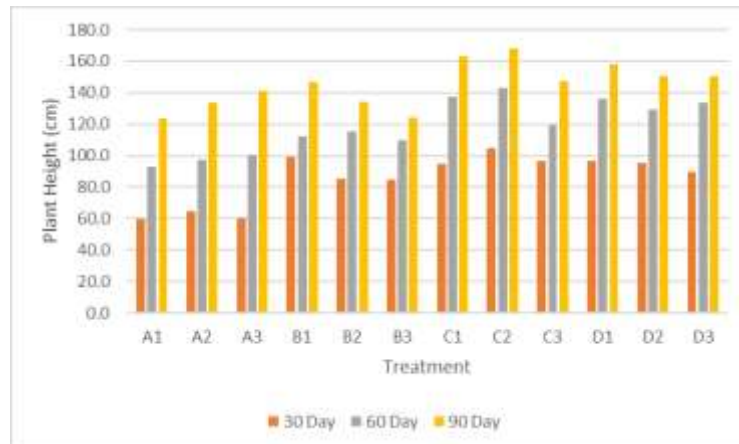


Figure 3. Development of the height of sorghum plants in each treatment

Moisture Content of Planting Medium

Observations of water content in the planting medium from day 104 to day 136 are presented in Figure 4. In general, the water content decreases with increasing incubation time consistently from day 104 to 136. However, medium composition B contains higher water content compared to the other media. Meanwhile, medium A has the lowest water content compared to the other media. This is likely due to zeolite being a material that is less capable of retaining water compared to compost. This result aligns with the water content in media D, C, and B, which increases as the proportion of zeolite decreases and the proportion of compost increases in the medium. The decrease in water content until day 127 does not show a significant difference, even though nutrient and water irrigation was stopped at 120 DAP. Nevertheless, the cessation of irrigation at 120 DAP reduced the water content in the planting medium with a similar trend among the tested treatments. The decrease in water content in the medium at 136 DAP is very significant compared to the water content at 127 DAP.

The influence of canopy pruning on the water content of the medium does not seem to have a significant effect. This result is interesting, as with pruning, the water requirements of sorghum plants decrease, so it is expected that the water in the 3-month pruned medium would be higher compared to those pruned at 1 or 2 months. However, the higher water content in plants pruned for 3 months compared to those pruned for 2 and 1 month is not significantly different. (Figure 4)

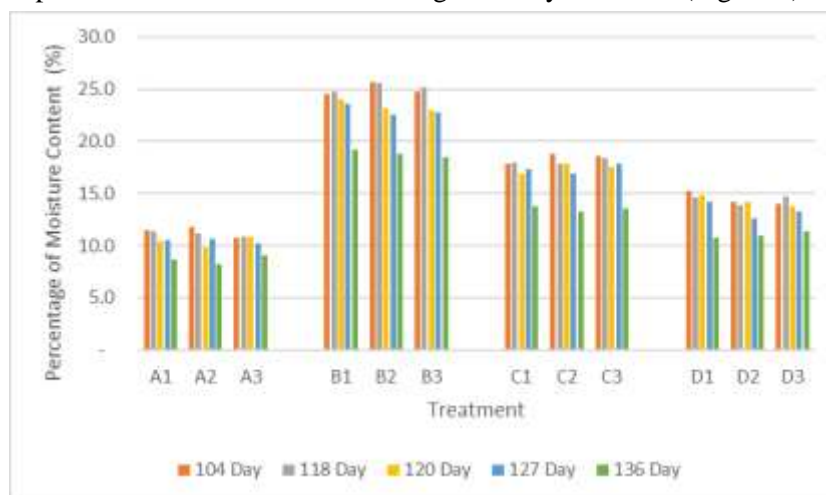


Figure 4. The Influence of Single Factors and Their Interaction on the Development of Moisture Content from Day 104 to 136 After Planting

AMF Infection in Sorghum Plant Roots

Observations of root infection were conducted when the plants were 4.5 months old (136 DAP) (Figure 5). The results showed that the percentage of AMF infection varied among the tested treatments (43 - 76%). Treatment D1 with a medium consisting of 90% zeolite and 10% compost, pruned at 1 month, resulted in the highest percentage of AMF infection, reaching 76%. Meanwhile, B1 with a composition of 50% zeolite and 50% compost pruned at 1 month, and C3 with a composition of 70% zeolite and 30% compost, pruned at 3 months, yielded the lowest percentages (43%). This result seems not entirely consistent with spore population. Generally, media D, B, and C produced higher spore counts compared to medium A.

Sorghum plants pruned for 2 months, during the observation of AMF infection, were in the vegetative phase, and even plants pruned for 1 month were already in the maximum vegetative phase. Meanwhile, plants pruned for 3 months, AMF infection was observed when the plants were in the vegetative phase (46 days). This result may be the reason for the low percentage of AMF colonization, especially in plants pruned for 3 months in media containing 50% and 30% compost. Yung et al. (2018) suggested that there is variation in AMF infection influenced by the plant growth stage. AMF colonization in seedling-stage plants is significantly lower compared to plants in the generative or mature phase. However, the dominance and composition of the AMF community were not affected by compost application, and the effect of each AMF type varied in response to compost application

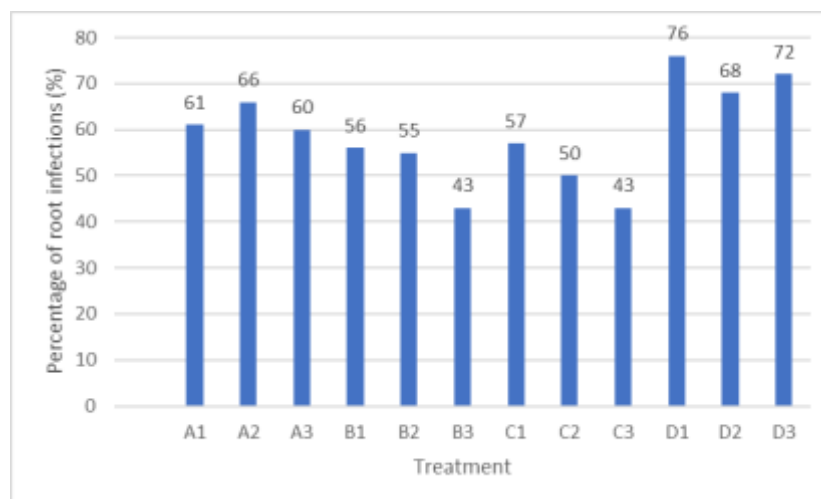


Figure 5. AMF Infection in Sorghum Roots at Harvest (136 DAP)

Development of AMF Spore Count

In general, the AMF spore count increases with extended incubation time. However, a noticeable spike in spore count was observed between 30 to 60 days of incubation for all medium compositions (Figure 6). The significant increase in spore count from 30 DAP to 60 DAP is quite intriguing. If infection occurs within the first week, then it takes less than 60 days for spore formation. This result indicates that the 60-day period is crucial for spore propagation.

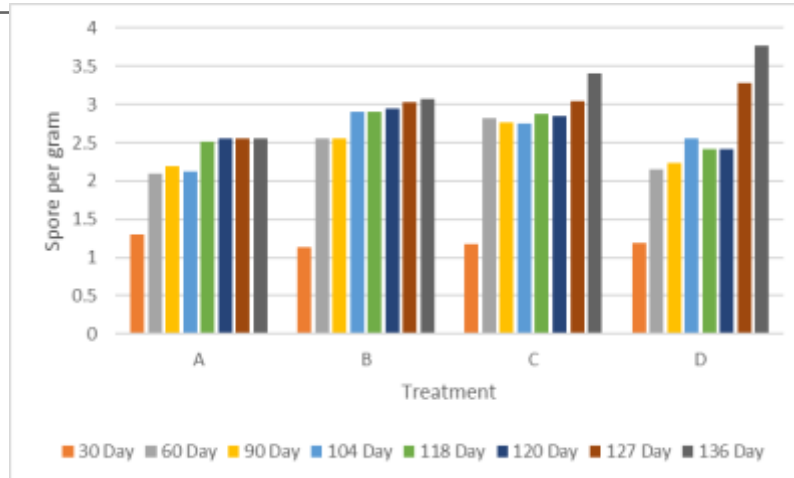


Figure 6. The Influence of Medium Composition on Spore Count

From 60 to 127 DAP (67 days), there was also an increase in spore count, although not as pronounced as before. It can be said that the rate of spore increase during the period from 60 to 127 days was not as high as from 30 to 60 days. However, for media C and D, there was an increase in spore count at 136 DAP compared to 127 DAP (9 days). Medium D and C contain 90% and 70% zeolite respectively, and in these media, there is already compost in proportions of 10% and 30%. Looking at the medium's water content, in 127-136 DAP, the plants have entered the generative phase and no watering was done for 1 to 2 weeks, resulting in a decrease in water content in the planting medium, ranging from 11-13 (D) and 14-18 (C). This result indicates that zeolite is needed for spore propagation, but the addition of compost in a certain amount can induce spore propagation, and drying can enhance the spore formation rate. This is especially true for the combination of zeolite with compost in the ratios D and C.

The high spore count in media D and C is presumed to be related to the plant's, including the roots, need for micro environmental conditions in the medium, including nutrients, temperature, and aeration, which in turn affect the propagation of AMF spores. Nutrients, especially in compost, are needed for spore propagation, although the required amount of nutrients is specific. Yang et al. (2018) reported that moderate (22.5 Mg/ha) and high (45 Mg/ha) levels of compost addition significantly increased AM root colonization and extraradical hyphal (ERH) density compared with control, whereas a low (11.5 Mg/ha) level of compost addition did not cause a significant increase in AM root colonization and ERH density. AM fungal spore density was significantly enhanced by all the compost rates compared with control. However, the research results indicate that high levels of P in the medium actually inhibit AMF infection in plant roots. The decrease in water content at 136 days also seems to induce AMF spore propagation, as indicated by a significant increase in spore count at 136 DAP compared to 127 DAP. Although media with compositions D and C do not contain the highest water content, this moisture level is sufficient for spore propagation, especially from the early stages to 136 DAP. Soil texture variation had a significant impact on AMF root colonization and spore production, as a mixture of sand and clay, with clay variation from 20-43%, was found to favor both parameters. These data suggest that soil texture variation and nutrient solution concentration can significantly improve AMF spore production and Sorghum symbiotic performances.

The influence of pruning shows that the highest increase in spore count occurred from 30 days to 60 days, regardless of whether pruning was done for 1, 2, or 3 months (Figure 7). This result indicates that 1-month pruning can induce an increase in spore count after 30 days of incubation. In the subsequent incubation period, up to 127 DAP (97 days after pruning), plants pruned for 1 month only showed a slight increase in spore count, and even the spore count decreased during the observation at 136 DAP (106 days after pruning).

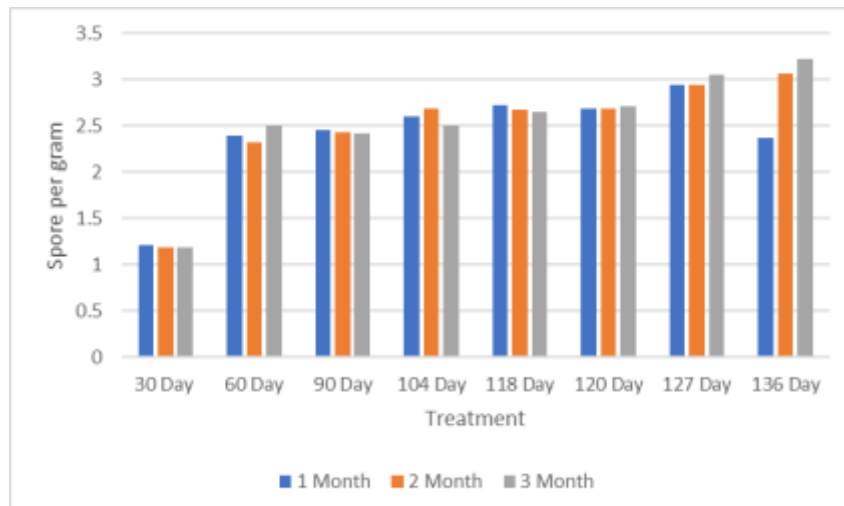


Figure 7. The Influence of Pruning Time on Spore Count from 30 to 136 DAP

Plants in the 2-month pruning treatment showed only a slight increase in spore count 1 month after pruning (observed at 90 DAP). However, there was a continuous increase in spore count in the subsequent incubation period up to 136 DAP. Observations at 136 DAP showed that drying at 120 DAP led to a significant spike in spore count in the plants pruned for 2 months. This indicates that it takes 76 days after pruning to achieve a surge in spore count. Different results were shown by plants pruned for 3 months. In these plants, observations 1 month after pruning indicated an increase in spore count, but the notable surge in spore count was observed during the incubation period from 120 DAP (30 days after pruning) to 136 DAP (46 days after pruning). It is suspected that drying at 120 DAP also induced the surge in spore count in the plants pruned for 3 months.

From these results, it appears that an increase in AMF spore count can be induced by pruning. However, pruning at 1 month did not result in a high spore count. Meanwhile, for plants pruned for 3 months, a shorter time of 46 days was needed to achieve a surge in AMF spore count, whereas for plants pruned for 2 months, a longer time of 76 days was needed. It is suspected that drying can induce a surge in AMF spore count, but this happens when the plant's photosynthate production is already quite high (3-month pruning). This result indicates that maximum vegetative growth is likely needed to obtain a positive pruning effect on increasing AMF spore count. Conversely, if vegetative growth is not yet at its maximum, pruning may actually reduce spore count. Pruning can affect plant physiology, including carbohydrate distribution to the root system, which can influence AMF spore formation. Additionally, it is suspected that pruning at the right time can stimulate the production of growth hormones in plants, which can affect AMF activity, including spore formation. After the first plant cycle, none of the trap culture showed higher spore count than reference spores. However, these results need to be re-evaluated using a larger plant population.

The observation results of spore count as an interaction between medium composition and pruning time are shown in Figure 8. The histogram illustrates that spore count increased up to 136 days of incubation for all treatments. The treatment with a planting medium composition of 90% zeolite and 10% compost with 2-month pruning (D2) produced the highest spore count, reaching 3.85 spores per g at the end of incubation (136 DAP). Meanwhile, medium A2, which only differed in medium composition (100% zeolite), resulted in the lowest spore count (2.45 spores per g). This indicates that medium composition affects the spore count produced. When considering plant performance, particularly leaf count and plant height, treatment C2 appears to have the best performance. It seems that good plant performance does not necessarily correlate with a high count of AMF spores produced. On the other hand, growth stage of host plant has been suggested as another important factor influencing AM fungal hyphal growth and soil AM community composition as AM growth is dependent on the carbon provided by host plant (Dumbrell et al., 2011; Bainard et al., 2012). Several studies indicated that plant growth stage exerted stronger influence in AM fungal biomass, soil AM fungal diversity and community composition than agricultural practices (Tian et al., 2011; Wu et al., 2011; Njeru et al., 2015). For instance, AM fungal spore community and biomass

were mainly affected by growth stage rather than agroecosystem management in an intensively managed maize agroecosystem in North China (Tian et al., 2011). Okiobel et al (2015) reported that Rorison's nutrient solution and soil texture significantly ($P < 0.05$) influenced plant growth, symbiotic and biochemical parameters. Nutrient solution induced significant increase in root colonization (5 to 36 %), and AMF spore production (12 to 23 spores/g of soil). The highest concentration of Rorison's nutrient solution promoted more spore formation, but that was not translated in plant yield. Rombe et al. (2022) reported that the interaction of AMF *Glomus claroideum* and the provision of 2.5 ml AB mix nutrition increased the number of AMF spores by 35 spores/5 g zeolite and root colonization by 69.16%.

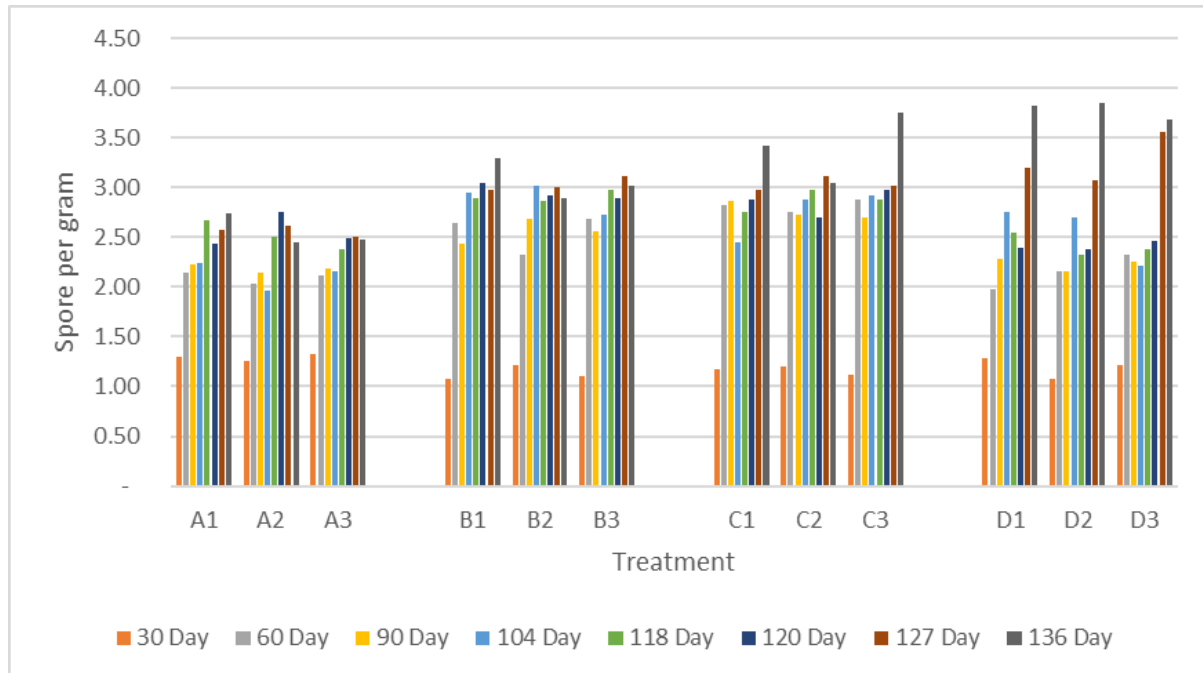


Figure 8. The Influence of the Interaction between Medium Composition and Pruning Time on Spore Count

It is important to note that the effects of pruning and planting medium composition on AMF propagation can vary depending on the type of plant and AMF species involved. Therefore, further research and experiments in the laboratory or field may be needed to gain a deeper insight into this interaction.

CONCLUSION

The experiment results indicate that the composition of the planting medium and pruning time influence the spore count in the propagation of AMF spores. However, pruning time can affect spore production if the plant has reached a certain vegetative age, which is three months for sorghum. Additionally, adding 10% compost to zeolite medium can enhance spore formation, even though sorghum growth may not be at its maximum in this medium composition. Different types of AMF may yield different results. Nevertheless, it is crucial to further examine the spore quality from each tested treatment.

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