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Screening four trap plants for the multiplication of indigenous Arbuscular Mycorrhiza Fungi in Daloa, Côte d'Ivoire

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Abstract **Original Research Article**

Arbuscular mycorrhizal fungi (AMF) are valuable biological resources for developing indigenous inocula adapted to local agroecological conditions. This study evaluated the suitability of maize (*Zea mays* L.), cowpea (*Vigna unguiculata* L.), onion (*Allium cepa* L.), and lettuce (*Lactuca sativa* L.) as trap plants for the multiplication of indigenous AMF resources, with a view to subsequent inoculum production. These plant species were grown in the presence of an indigenous inoculum, while non-inoculated plants served as controls. Root colonization frequency and intensity were assessed at 45 and 75 days after sowing (DAS), and AMF spore density in the substrate was determined at 75 DAS. At 45 DAS, all inoculated plants exhibited root colonization, whereas no colonization was detected in the controls. Colonization frequency did not differ significantly among plant species, while colonization intensity was significantly higher in cowpea than in onion. At 75 DAS, neither colonization frequency nor intensity differed significantly among the four species. AMF spores were detected in the substrate of all inoculated pots, with no significant differences in spore density among plant species. Across trap plants, mean colonization frequency increased significantly from 14.19% at 45 DAS to 58.75% at 75 DAS, while mean colonization intensity increased from 2.78% to 21.13%. Neither the trap-plant effect nor the trap plant × time interaction was significant. Overall, all four species supported the establishment and development of indigenous AMF colonization and were associated with detectable AMF spores after 75 days. However, no species showed a clear advantage over the others. Maize, cowpea, onion, and lettuce can therefore be considered potential trap plants for the multiplication of indigenous AMF resources prior to inoculum production. Further studies should characterize AMF community diversity before and after trap culture and assess the effectiveness of the resulting inocula.

Keywords: Arbuscular mycorrhizal fungi; Trap plants; Indigenous inoculum; Root colonization; Spore density.

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INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) constitute a major component of the microbiota of agricultural soils and establish symbiotic associations with the majority of terrestrial plant species (Smith & Read, 1996). Through their extraradical mycelial networks, they extend the soil exploration zone beyond that accessible to the root system alone and contribute to the acquisition of poorly mobile mineral nutrients, particularly phosphorus. Mycorrhizal symbiosis therefore plays an important role in plant mineral nutrition and functioning, while also contributing to several ecological processes related to nutrient cycling and soil health (Zhang *et al.,* 2024).

The agronomic importance of AMF, however, extends beyond the improvement of phosphorus nutrition. Mycorrhizal symbiosis can also contribute to plant growth and performance under environmental conditions characterized by various abiotic stresses. Generally, AMF are increasingly considered as microorganisms with great potential to support the transition toward more sustainable agricultural systems,

due to their potential effects on crop nutrition, stress tolerance, and several functions related to soil biological functioning (Díaz-Urbano *et al.,* 2023; Zhang *et al.,* 2024).

These properties have promoted the development of AMF-based inoculants intended to enhance or restore the mycorrhizal potential of soils and promote the establishment of mycorrhizal symbiosis in crops. The production of mycorrhizal inoculum is therefore an important step between the identification of mycorrhizal resources with agronomic potential and their use in agricultural systems. Several propagation approaches have been developed, including substrate-based pot systems, hydroponic and aeroponic systems, and root organ cultures. Among these approaches, pot culture using a host plant remains a conventional and widely used method for multiplying AMF from soil samples containing indigenous propagules (Pandit *et al.,* 2025; Tenzin *et al.,* 2022).

This dependence on a host plant is directly related to the particular biology of AMF, which are obligate biotrophic fungi (Bago & Bécard, 2002). Their development and multiplication depend closely on the establishment of symbiosis with living plant tissues, which provide, among other resources, the carbon compounds required to complete their life cycle. Therefore, the propagation of AMF from natural samples generally relies on establishing symbiosis with a mycotrophic plant that enables the fungi to colonize roots and produce new propagules. In trap-culture systems, the host plant is used to promote the amplification of propagules present in the initial inoculum, which may consist of a mixture of soil and root fragments collected from natural habitats (Pandit *et al.,* 2025; Tenzin *et al.,* 2022).

Choosing trap plant is therefore an important consideration for inoculum production. Plant species used as hosts may differ in their ability to establish symbiosis, support root colonization, and promote the production of new fungal propagules. This ability may depend on both host-plant characteristics and those of the AMF community present in the initial inoculum. Host choice may consequently influence spore production following propagation and potentially the composition of the recovered community. Studies using different host plants have shown that spore propagation can vary depending on the identity or combination of host plants used, highlighting the importance of selecting hosts suited to the objectives of AMF multiplication (Tenzin *et al.,* 2022). Trap-plant selection should therefore be considered as a distinct selection step in the development of AMF inoculants.

This issue is particularly important when indigenous AMF communities are being used. Unlike the multiplication of an isolated fungal strain, trap culture of a natural inoculum generally aims to amplify a set of propagules potentially belonging to several taxa. Identifying trap plants capable of effectively supporting the multiplication of an indigenous mycorrhizal resource is therefore an important preliminary step toward the subsequent development of locally adapted inoculants.

Despite the growing interest in the use of AMF as biological resources for sustainable agriculture, the multiplication potential of indigenous communities associated with different plants and agroecosystems remains insufficiently documented in many tropical regions, particularly in West Africa. Likewise, the diversity of AMF associated with trees in tropical forests remains relatively poorly documented, particularly in tropical Africa (Olanipon *et al.,* 2024). The valorization of these indigenous resources in Côte d'Ivoire therefore requires the identification of propagation systems adapted to local mycorrhizal communities. Bété (*Mansonia altissima* A. Chev.) is an indigenous forest tree species in Côte d'Ivoire whose timber has important use value (Kougbo *et al.,* 2023). Exploring its rhizosphere may therefore provide access to indigenous microbial resources that remain poorly characterized. In this context, this study aimed to evaluate the ability of four trap plants to support the multiplication of indigenous AMF from the rhizosphere of Bété, with the objective of identifying promising host plants for future work on the production of indigenous mycorrhizal inoculum.

MATERIALS AND METHODS

Study Area

The field experiment was conducted in a greenhouse located in Jean LOROUGNON GUEDE University of Daloa, Haut-Sassandra Region, in west-central Côte d'Ivoire. Daloa is located at approximately 6°53′58″ N latitude and 6°26′32″ W longitude. The area experiences a humid tropical climate characterized by four alternating seasons, comprising two wet and two dry seasons. Mean temperatures range from 24.65 to 27.75 °C (Adjiri *et al.,* 2019), while the mean annual rainfall is approximately 1,120.4 mm (Ligban *et al.,* 2009).

Sampling and obtaining the Indigenous Inoculum

The study was conducted in Digbapia village, located in the Daloa Department, west-central Côte d'Ivoire. A relatively well-preserved private forest located in the village was selected as the sampling site. Three Bété trees (*Mansonia altissima* A. Chev.) were selected, with a minimum distance of 20 m between trees. For each tree, rhizosphere soil was collected from a depth of 0–20 cm at four sampling points corresponding to the four cardinal directions around the tree. Approximately 1 kg of rhizosphere soil was collected from each plant. Soil samples collected from the three trees were then thoroughly mixed to obtain a single composite Bété rhizosphere soil inoculum.

Trap culture establishment

Plant material

The plant species selected as trap plants were maize (*Zea mays* L.), cowpea (*Vigna unguiculata* L.), onion (*Allium cepa* L.), and lettuce (*Lactuca sativa* L.). Seeds were obtained from Semivoire, except for cowpea, which was purchased from a local market. These species were selected based on their ability to establish arbuscular mycorrhizal associations and their previous use as host plants in studies involving the propagation or evaluation of arbuscular mycorrhizal fungi (Cantrell & Linderman, 2001; Liu & Wang, 2003; Tenzin *et al.,* 2022).

Substrate preparation, Pot filling and seed sowing

A growth substrate consisting of sand and potting soil was prepared for the trap-culture experiment. The two components were mixed at a 2:1 (v/v) ratio, corresponding to two volumes of sand for every one volume of potting soil. Before mixing, the potting soil was sieved to remove coarse materials, whereas the sand was thoroughly washed and subsequently dried to eliminate unwanted particles. The sand and potting soil were then thoroughly homogenized.

The resulting substrate was sterilized in an autoclave at 121°C for 1 h, and the sterilization procedure was repeated after a 24-h interval. This repeated sterilization was intended to minimize the presence of indigenous microbial propagules that could potentially interfere with the experimental treatments.

After cooling, the sterilized substrate was used to fill the pots. Each pot was filled with 1.8 L of substrate, to which approximately 200 g of the Bété rhizosphere soil inoculum were added.

Maize, cowpea, onion and lettuce seeds were subsequently sown in the pots and covered with approximately 0.2 L of sterilized substrate.

Experimental design and culture condition

The experiment was conducted using a completely randomized design with four treatments corresponding to each plant material: lettuce, maize, onion and cowpea. A non-inoculated treatment for controlling culture conditions were also used. Each treatment consisted of four replicates. A total of 20 pots were used. The seedlings were watered once every two days and maintained under greenhouse conditions for 75 days.

Data collection

The trap plants were compared based on three mycorrhizal parameters: root mycorrhizal frequency and intensity, assessed at 45 and 75 days after sowing (DAS), and spore density, determined at 75 DAS.

- ***Assessment of mycorrhizal colonization***

At 75 DAS, a root sample was collected from each trap-culture pot in the greenhouse. The roots were carefully rinsed under running tap water to remove substrate particles and then cut into approximately 1-cm-long segments. The root segments were subsequently placed in small, labeled bags and prepared for microscopic observation following the method described by (Phillips & Hayman, 1970).

First, the roots were immersed in a 10% KOH solution and heated in a water bath at 90°C for 30 min to soften the tissues and remove cellular contents. The roots were then thoroughly rinsed with running tap water, drained, and immersed in a 1% HCl solution maintained at 80°C for 15 min for acidification. They were subsequently rinsed again with running tap water.

The roots were then stained with a 0.05% Trypan blue solution and heated in a water bath at 80°C for 30 min. Finally, the roots were thoroughly rinsed and mounted on microscope slides in 50% glycerol for microscopic observation. The prepared slides were allowed to stand for at least 24 h before microscopic examination.

Mycorrhizal colonization was subsequently assessed according to the method described by Trouvelot *et al.,* (1986). Mycorrhizal frequency (F) and mycorrhizal intensity (I) were calculated using the following formulas:

Mycorrhizal frequency (F):
F (%) = (number of colonized root fragments × 100) / total number of root fragments examined
Mycorrhizal intensity (I):
I (%) = $(95n_5 + 70n_4 + 30n_3 + 5n_2 + n_1)$ / total number of root fragments examined

where n_1 to n_5 represent the numbers of root fragments assigned to the corresponding colonization classes:

- Class 0: no mycorrhizal colonization
- Class 1: 1–4% of the root fragment colonized
- Class 2: 5–29% colonization
- Class 3: 30–69% colonization
- Class 4: 70–95% colonization
- Class 5: ≥95% colonization

- ***AMF spores 'density determination***

The density of AMF spores in the trap-culture soil was determined using wet sieving followed by sucrose centrifugation, based on the procedure of Gerdemann & Nicolson, (1963). For each pot, 50 g of air-dried soil were placed in a 2-L beaker and mixed thoroughly with 1 L of water. The resulting suspension was sequentially passed through sieves with mesh openings of 500, 125, 90, and 40 µm. The fraction retained on the 500-µm sieve, consisting predominantly of coarse soil particles and organic debris, was discarded.

The fractions collected from the 125-, 90-, and 40-µm sieves were pooled for subsequent processing.

The pooled material retained on the 125-, 90-, and 40-µm sieves was first centrifuged at 2,000 rpm for 5 min. After centrifugation, the supernatant was discarded, and the resulting sediment was resuspended in a 50% (w/v) sucrose solution. The suspension was then centrifuged a second time at 2,000 rpm for 1 min. Following this second centrifugation, the supernatant containing the AMF spores was carefully recovered and filtered to collect the spores, which were subsequently examined and counted under a stereomicroscope according to the method recommended by the International Culture Collection of Vesicular and Arbuscular Mycorrhizal Fungi (INVAM, 2026). This procedure was used to estimate AMF spores' density in soil under cowpea, maize, onion and lettuce plants for each pot culture.

Statistical analysis

Statistical analyses were performed using STATISTICA version 7.1. Mycorrhizal colonization frequency and intensity were analyzed separately at each sampling time (45 and 75 days after sowing) to assess differences among trap plants. When the assumptions for parametric analysis were met, one-way analysis of variance (ANOVA) was used to compare trap plants. When these assumptions were not met, differences among trap plants were assessed using the Kruskal–Wallis test, followed by pairwise comparisons when the overall test was significant.

To assess temporal changes in mycorrhizal colonization, frequency and intensity measured at 45 and 75 days after sowing were analyzed separately using repeated-measures analysis of variance (ANOVA), with sampling time as the within-subject factor and trap plant as the between-subject factor. The effects of sampling time, trap plant, and their interaction (time × trap plant) were tested.

Spore density measured at 75 days after sowing was compared among trap plants using the Kruskal–Wallis test. Statistical significance was set at $p < 0.05$. Results are presented as means ± standard deviation (SD).

RESULTS

Mycorrhizal colonization frequency and intensity at 45 Days after sowing

At 45 days after sowing (DAS), all inoculated plants exhibited root mycorrhizal colonization (Figure 1), whereas no colonization was detected in the uninoculated control plants. Mycorrhizal colonization frequency ranged from 10.0% in lettuce to 20.0% in cowpea, with intermediates values of 11.75% in onion and 15% in maize. However, mycorrhizal colonization frequency did not differ significantly among the inoculated trap plants.

At 45 days after sowing (DAS), mycorrhizal colonization intensity differed significantly among the trap plants. The lowest colonization intensity was recorded in onion, whereas the highest value was observed in cowpea. Cowpea showed significantly higher colonization intensity than onion, while maize and lettuce did not differ significantly from either cowpea or onion (**Error! Reference source not found.**).

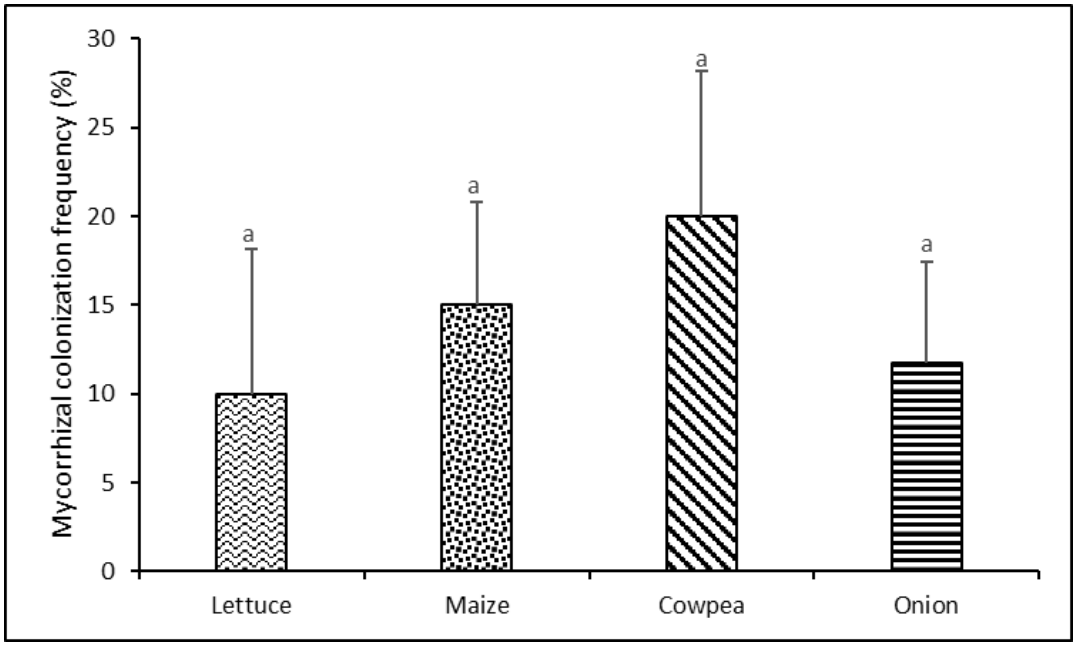

Figure 1: Mycorrhizal colonization frequency of Lettuce, Maize, Cowpea and Onion roots at 45 Days After Sowing Bar graphs following the same letter in each graph are not significantly different at the 5% significance level

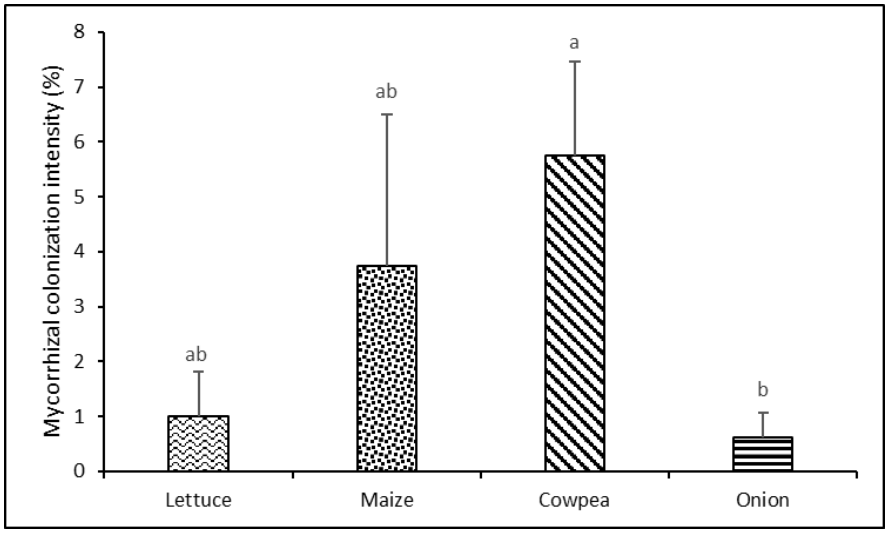

Figure 2: Mycorrhizal colonization intensity of Lettuce, Maize, Cowpea and Onion roots at 45 Days After Sowing Bar graphs following the same letter in each graph are not significantly different at the 5% significance level

Mycorrhizal colonization and spore density at 75 days after sowing

At 75 days after sowing (DAS), all inoculated plants exhibited root mycorrhizal colonization (**Error! Reference source not found.**), whereas no colonization was detected in the uninoculated control plants. Mycorrhizal colonization frequency was 57.5% in lettuce and cowpea, 52.5% in onion, and 67.5% in maize. However, colonization frequency did not differ significantly among the inoculated trap plants.

Similarly, mycorrhizal colonization intensity did not differ significantly among the trap plants. Colonization intensity was 25.30% in onion, 11.55% in cowpea, 23.85% in maize, and 23.82% in lettuce.

At 75 DAS, AMF spores were detected in all inoculated trap-culture pots, whereas no spores were observed in the uninoculated control pots. Spore density did not differ significantly among the trap plants, with mean densities of 37.25, 87.25, 149.25, and 142.25 spores per 50 g of soil in onion, cowpea, maize, and lettuce, respectively.

Table I: Lettuce, Maize, Cowpea and Onion Root colonization frequency and intensity and AMF spores'density 75 DAS

Within columns, means following the same letter are not significantly different at the 5% significance level.

Trap cultures	Root colonization frequency (%)	Root colonization intensity (%)	AMF spores' density
Lettuce	57.5±43.49 a	23.825±30.64 a	37.25±37.01 a
Maize	67.5±39.48 a	23.85±29.77 a	87.25±110.34 a
Cowpea	57.5±35 a	11.55±9.62 a	149.25±203.38 a
Onion	52.5±40.31 a	25.3±26.68 a	142.25±103.64 a

Temporal dynamics of mycorrhizal colonization

Mycorrhizal frequency

Mycorrhizal colonization frequency increased significantly over time, rising from 14.19% at 45 DAS to 58.75% at 75 DAS ($F(1,12) = 17.181$, $p = 0.00136$; Table II). In contrast, the main effect of trap culture was not significant ($F(3,12) = 0.207$, $p = 0.890$), and the time × trap plant interaction was also not significant ($F(3,12) = 0.098$, $p = 0.960$). These results indicate that mycorrhizal colonization frequency increased significantly between 45 and 75 DAS, with a similar temporal pattern across the four trap cultures.

Table II: Temporal variation in mycorrhizal colonization frequency

Sample time	Root colonization frequency (%)
45 DAS	14.19
75 DAS	58.75

Mycorrhizal intensity

Mycorrhizal colonization intensity increased significantly from 2.78% at 45 DAS to 21.13% at 75 DAS ($F(1,12) = 8.759$, $p = 0.01193$;Table III). Neither the main effect of trap plant nor the time × trap plant interaction was significant). These results indicate that mycorrhizal colonization intensity increased significantly over time, with no significant difference in the temporal pattern of colonization intensity among the four trap cultures.

Table III: Temporal variation in mycorrhizal colonization intensity

Sample time	Root colonization intensity (%)
45 DAS	2.78
75 DAS	21.13

DISCUSSION

Mycorrhizal colonization at 45 days after sowing

At 45 days after sowing (DAS), all inoculated trap plants exhibited root colonization by AMF, whereas no colonization was detected in the non-inoculated control plants. This result indicates the establishment of the mycorrhizal propagules present in the inocula and their ability to colonize the four plant species studied. The capacity of AMF to establish associations with a wide range of plant species is one of the foundations for their use in trap cultures designed to multiply mycorrhizal inocula (Genre *et al.,* 2020; Smith & Read, 2008).

At this stage, colonization frequency did not differ significantly among maize, cowpea, onion, and lettuce, although numerical values ranged from 10.0% in lettuce to 20.0% in cowpea. The absence of a statistically significant difference indicates that the four plants supported AMF establishment to comparable extents at the beginning of the experiment. In contrast, colonization intensity differed significantly among plant species, with the highest value recorded in cowpea and the lowest in onion. This difference between colonization frequency and intensity suggests that, even when several plant species allow the establishment of the symbiosis, the development of colonization within roots may vary among host species.

The greater colonization observed in cowpea may be related to its ability to establish associations with AMF. Legumes are generally considered favorable hosts for AMF, although the extent of colonization depends on several factors, including host characteristics, growing conditions, and interactions with the fungi present in the inoculum (Genre *et al.,* 2020; Smith & Read, 2008). However, the difference between cowpea and onion concerned only colonization intensity and was no longer observed at 75 DAS.

The relatively low level of colonization observed in onion at 45 DAS therefore does not support considering this species as a poor trap plant for AMF. Onion (*Allium cepa* L.) is considered highly responsive to mycorrhizal symbiosis, notably because of its relatively sparse root hair system, and has consequently been widely used in studies of AMF (El-Sherbeny *et al.,* 2022; Galván *et al.,* 2011; Smith *et al.,* 1986). The differences observed at 45 DAS may therefore reflect different rates of establishment and development of mycorrhizal colonization during the first weeks of growth. Indeed, the same chronological age does not necessarily correspond to the same developmental stage among different trap plants. The ontogenetic stage of the host plant can influence AMF colonization, with lower colonization rates having been reported in younger plants than in adult plants (Šmilauer *et al.,* 2021). In the present experiment, onion may therefore have been at a relatively less advanced developmental stage at 45 DAS than maize, cowpea, and lettuce, due to its generally longer cropping cycle. This difference in developmental stage may have contributed to the lower colonization intensity observed in onion at this time point.

Mycorrhizal colonization and spore density at 75 days after sowing

At 75 days after sowing (DAS), mycorrhizal colonization was more developed in all four trap plants. Colonization frequency ranged from 52.5% in onion to 67.5% in maize, with no significant differences among plant species. Likewise, no significant differences were observed in colonization intensity, despite values ranging from 11.55% in cowpea to 25.30% in onion. Thus, the differences in colonization intensity observed between cowpea and onion at 45 DAS were no longer detectable after 75 days of cultivation.

This result suggests that all four plants provided conditions that allowed AMF colonization to continue developing throughout the trap-culture period. The absence of significant differences among plants at 75 DAS is particularly relevant in the context of trap-plant selection, as it indicates that maize, cowpea, onion, and lettuce all supported relatively substantial root colonization after a sufficiently long cultivation period. Liu & Wang, (2003) showed that host-plant selection is an important factor in AMF trap cultures and that several plant species can be used to promote AMF multiplication. In the present study, however, none of the four tested plants differed significantly from the others in terms of colonization levels achieved at 75 DAS.

The presence of spores in all inoculated pots at 75 DAS is also an important finding. It indicates that the trap cultures allowed not only the establishment of root colonization but also the production of new spores in the substrate. Spore densities ranged from 37.25 spores per 50 g of soil in onion to 149.25 spores per 50 g in maize, but these differences were not statistically significant.

AMF sporulation is an important process in trap-culture systems because newly produced spores may contribute to the subsequent multiplication and availability of mycorrhizal propagules. However, spore production depends on the development of the symbiosis and on culture conditions, including host-plant characteristics, cultivation duration, and substrate properties (Biermann & Linderman, 1983; Liu & Wang, 2003). In the present study, the absence of significant differences in spore density among the four trap plants

indicates that all four species supported comparable levels of sporulation under the experimental conditions used.

Temporal dynamics of mycorrhizal colonization

The analysis of temporal dynamics provides an important element for interpreting the results. Mean colonization frequency increased significantly from 14.19% at 45 DAS to 58.75% at 75 DAS. Mean colonization intensity also increased significantly, from 2.78% to 21.13%. These changes indicate that root colonization was not limited to an early establishment of AMF, but progressively developed throughout the experimental period.

This temporal progression is consistent with the dynamics of mycorrhizal associations. Following the initial establishment of contact between fungal propagules and roots, extraradical mycelial growth and the development of intraradical structures may progress as the host plant grows. Consequently, the extent of root colonization may vary with host age and developmental stage (Šmilauer *et al.,* 2021; Smith & Read, 2008). In the present study, the simultaneous increase in colonization frequency and intensity between 45 and 75 DAS therefore reflects a progression in both the extent and degree of root colonization.

The absence of a significant trap-plant effect in the temporal analysis, indicates that, when considered over the entire experimental period, the four plants exhibited comparable overall levels of colonization. Moreover, the absence of a significant time × trap-plant interaction indicates that the progression of colonization between 45 and 75 DAS did not differ significantly among plant species. In other words, the four plants followed a similar temporal pattern of increasing colonization.

These findings highlight the importance of the duration of trap culture for the establishment and development of AMF colonization. At 45 DAS, colonization was still relatively low, whereas at 75 DAS, it was substantially higher and accompanied by detectable spore production in all inoculated pots. Under the conditions of the present experiment, a 75-day cultivation period appeared sufficient to allow well-established root colonization and detectable sporulation in all four plant species studied, consistent with previous trap-culture studies in which substantial AMF colonization and sporulation were obtained after several weeks (60 days) of host-plant cultivation (Djezou *et al.,* 2026).

CONCLUSION

Overall, this study showed that maize, cowpea, onion, and lettuce all supported the establishment and development of AMF root colonization under the experimental conditions investigated. At 75 DAS, AMF spores were detected in the substrate of all inoculated trap-culture pots, providing evidence of sporulation during the trap-culture period. Although spore densities varied numerically among plant species, these differences were not statistically significant. Similarly, the difference in colonization intensity observed between cowpea and onion at 45 DAS was no longer detectable at 75 DAS. Moreover, the significant increases in colonization frequency and intensity over the experimental period, together with the absence of significant effects of trap-plant species and the time × trap-plant interaction, indicate that temporal progression contributed more to the observed colonization dynamics than plant identity.

Thus, none of the four species can be considered superior to the others based on the parameters assessed in this study. Maize, cowpea, onion, and lettuce can therefore all be considered potential trap plants for the multiplication of indigenous mycorrhizal resources under experimental conditions comparable to those investigated here. However, colonization and spore occurrence alone do not provide information on the diversity of AMF communities associated with the trap plants. Further studies comparing the diversity and composition of AMF communities present in the indigenous inocula before and after trap culture would help determine the extent to which the multiplication process influences the diversity of the mycorrhizal resources initially available.

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