

Endomycorrhizal Status of Some Herbaceous Weeds in Cocoa Farming in the Department of Lakota, Côte d'Ivoire

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How to cite this paper: Cherif, M., Anguiby, B.L.A., Guinagui, N.B., Konan, A.O.V., Bomisso, E.L., Yeo, G., Kouadio, E.Y.G. and Aké, S. (2025) Endomycorrhizal Status of Some Herbaceous Weeds in Cocoa Farming in the Department of Lakota, Côte d'Ivoire. *Agricultural Sciences*, 16, 1130-1143.

<https://doi.org/10.4236/as.2025.1610065>

Received: June 2, 2025

Accepted: October 6, 2025

Published: October 28, 2025

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Abstract

Currently in Côte d'Ivoire, the management of herbaceous weeds on cocoa plantations systematically involves chemical weeding, the negative effects of which contrast greatly with sustainable production. The objective of this study is to contribute to the sustainability of cocoa production through a better consideration of herbaceous weeds. To achieve this, the dominant weeds in the cocoa plantations of three localities in Lakota, Côte d'Ivoire (DJELEBOUE, GNAKPALILIE and DIEKOLILIE) as well as soil samples were collected. After identification of weeds using the Picture This version 3.33 and PlantNet version 3.11.1 applications, the roots of the weeds were coloured with ink blue and their colonisation frequencies and intensities were evaluated. In addition, the spores of arbuscular mycorrhizal fungi were isolated by wet sieving and then counted. The identification made it possible to record 14 species of herbaceous plants divided into 12 families, with the predominance of Euphorbiaceae and Poaceae. With the exception of two species, they are all mycorrhizal with intensities ranging from 0.73% to 61.70%. In addition, an average density of 4.30 g⁻¹ spores of soil was recorded in cocoa farms. It appears from this study that the maintenance of certain mycotrophic and less aggressive herbaceous weeds such as *Parietaria officinalis* could lead to the sustainability of cocoa farms.

Keywords

Mycorrhizae, Weed, Cocoa Farming, Côte d'Ivoire

1. Introduction

The cocoa tree (*Theobroma cacao* L.) is a tropical plant of the Malvaceae family whose cultivation is for the production of beans mainly intended for the chocolate industry. In Côte d'Ivoire, cocoa cultivation is of paramount importance as it is the pillar of the Ivorian economy. Indeed, cocoa accounts for 30% of the gross domestic product (GDP), more than 50% of export earnings, and 2/3 of the direct or indirect jobs of the Ivorian population [1]. In addition, with an estimated production of 2.23 million tonnes during the 2021-2022 season, the country is the leading producer of marketable cocoa [2].

In cocoa farming, a good yield implies the application of phytosanitary products [3]. As a result, in addition to fertilizers, a wide range of pesticides are used to reduce the impact of biotic constraints [4]. Among these factors, weeds require special attention as they are one of the main biotic constraints behind the decline in crop system production [5]. Indeed, weeds have a negative impact on cocoa production depending on whether they compete with cocoa trees for water and mineral resources in the soil [5] or are vectors of pathogens [3] [6]. To curb the proliferation of these sometimes very aggressive weeds, chemical or mechanical weeding is carried out by producers [7]. However, with the arduousness of manual weeding combined with the scarcity of labour, the use of synthetic herbicides is becoming more and more widespread and is even the preferred means of weed control for producers [8]. However, several studies agree that the repeated use of herbicides, in addition to being harmful to crops, negatively affects human and animal health and the environment [9]. For example, N'guessan *et al.* [10] recorded a very high content of glyphosate and cadmium in giant African snails belonging to the genus *Achatina*, which are nevertheless very popular with African populations and those of Côte d'Ivoire in particular. Blé *et al.* [4] also indicated that in the Soubré area, Côte d'Ivoire, the pesticides used have a negative impact on soil water. With regard to the health of cocoa farmers, non-compliance with good practices for the use of herbicides due to their low level of education associated with the dangerousness of the active ingredients, Glyphosate and Paraquat, recommended herbicides, constitute real risks [11].

Despite the pressure exerted by these weeds on crops, Touré *et al.* [12] stressed that the presence of certain species does not necessarily imply the existence of a potential danger to agrosystems and may even be beneficial for the crop. Based on this observation, studies have been carried out to identify weeds and at the same time their degree of infestation in order to achieve integrated management [13] [14]. This is how Amba *et al.* [15] classified the weeds found in a cocoa plantation in Ivory Coast into non-aggressive, slightly aggressive and very aggressive species

in order to optimize the use of herbicides. This research fits perfectly into the current development programs of agricultural countries such as Ivory Coast where a compromise must be found between agricultural production and environmental protection [16] [17]. Indeed, the new environmental policy placed under the aegis of the Ministry of Health, Environment and Sustainable Development (MINSEDD) advocates more sustainable cropping systems, with low use of synthetic inputs that are more favourable to the protection of the environment and the safeguarding of biodiversity. Thus, the systematic elimination of all herbaceous flora by these chemical treatments is therefore an obstacle to sustainable cocoa production, especially since some have symbiotic relationships with arbuscular mycorrhizal fungi (AMF) which are favourable to plant growth [14]. Mycorrhizal symbiosis is a symbiotic relationship between fungi and plant roots from which they exchange nutrients [18]. Through this mutually beneficial association, the fungus, thanks to its network of extra-root hyphae that extends well beyond the soil area explored by the roots, improves the mineral nutrition of plants [19]. In addition, the mycelium of arbuscular mycorrhizal fungi often connects two or more plant individuals of the same or different species establishing arbuscular mycorrhizal networks [20]. This results in a long-distance transfer of nutrients through this network that promotes plant coexistence [21]. Compared to the cocoa tree, in addition to being 23% mycorrhizal [22], it benefits from a significant effect of this symbiosis on its growth [23].

In relation to herbaceous weeds, some of them, because they are highly mycotrophic, are likely to promote the population of arbuscular mycorrhizal fungi [24]. In addition, some weeds, in this case Fabaceae, have the ability to fix atmospheric nitrogen and fertilize the soil thanks to the presence of root nodules [25]. Just like the practice of agroforestry, whose benefits on the sustainability of cocoa production have been documented in in-depth studies [26], the management of herbaceous plants could also be considered [8].

This study aims to contribute to the sustainability of cocoa production in Côte d'Ivoire through a reasoned management of herbaceous weeds. Specifically, the aim was to: (i) identify the dominant herbaceous weeds on cocoa plantations, (ii) determine the physicochemical parameters of the soils of these plantations, (iii) assess the level of endomycorrhizal colonization of dominant herbaceous weeds, and (iv) quantify the spore density of arbuscular mycorrhizal fungi in soils collected under herbaceous plants.

2. Material and Methods

2.1. Study Sites

This study was carried out in the LOH-DJIBOUA region, a cocoa-producing area of Côte d'Ivoire. The cocoa plantations selected are located in three localities of the city of Lakota: GNAKPALILIE, DIEKOLILIE and DJELEBOUE (**Figure 1**). The geographical coordinates provided by the GPS as well as the areas of the plantations chosen are displayed in **Table 1**.

2.2. Hardware

Hardware material consists of the “Picture This Identifier version 3.33” and “PlantNet version 3.11.1” applications for Android phones available on the Google Play download platform. These applications make it possible to identify plants simply by photographing them with a smartphone.

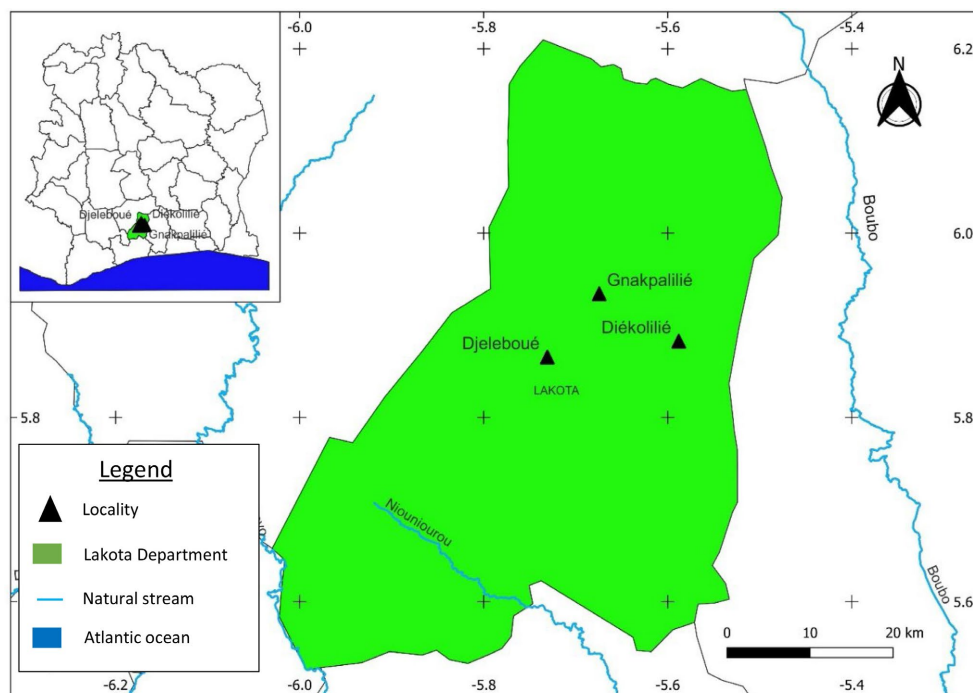


Figure 1. Location of the villages chosen for this study.

Table 1. Characteristics of the cocoa farms covered by the study.

| Locality (village) | Geographic coordinates | Surface area (ha) |
|--------------------|-----------------------------|-------------------|
| DJELEBOUE | 05° 51.952 N, 005° 43.860 W | 2 |
| DIEKOLILIE | 05° 52.967 N, 005° 35.290 W | 1 |
| GNAKPALILIE | 05° 56.064 N, 005° 40.473 W | 1 |

2.3. Sampling

The data collection was carried out in three cocoa plantations located in the villages of DJELEBOUE, GNAKPALILIE and DIEKOLILIE in the month of May 2022. In each field, a sampling device consisting of two quadrats (1 m × 1 m) at least 100 m apart was set up. For the same orchard, the species with a strong dominance in both areas (quadrats) were chosen. Then, a plant was removed using a hoe in order to remove the stump while keeping the root system intact (Figure 2). For soil sampling, five soil samples were collected per quadrat at a depth of 0 to 20 cm. These elementary samples were then mixed to obtain a composite sample per quadrat.



Figure 2. Collected whole plant and soil samples put in bags.

2.4. Identification of Herbaceous Weeds

All the plant species collected in the different plantations were identified using the *Plantnet* and *Picture This* applications. It involved taking a photo and importing it into the applications afterwards. The latter, through comparisons to plants already listed in available databases, indicate the name. These identifications were made for some species directly in the fields when conditions permitted, or once they arrived at the laboratory.

2.5. Determination of the Physicochemical Parameters of Soil Samples

The parameters evaluated were pH, organic carbon (CO), nitrogen (N), exchangeable phosphorus (P_2O_5), potassium (K), cation exchange capacity (CEC), moisture, clay. The texture was deduced from the clay content. These analyses were carried out with the Agrocates Scanner which uses near-infrared reflection spectroscopy (NIR).

2.6. Assessment of Mycorrhizal Colonization of Weeds

The collected roots, washed and cut into 1 cm fragments were stained according to the method of Phillips and Hayman [27]. The colouring solution was prepared from fountain pen ink. The mycorrhization indices of mycorrhization frequency and intensity were determined using the scoring grid of Trouvelot *et al.* [28].

2.7. Study of the Spore Density of Soil Arbuscular Mycorrhizal Fungi Collected in Cocoa Fields

To assess the density of the spores, they were first extracted from soil samples and then counted. Spore extraction was carried out by the wet sieving method of Gerdeman and Nicolson [29]. 100 g of dry soil was suspended in 500 ml of tap water and the supernatant was passed through a series of four decreasing mesh sieves (710 μm , 500 μm , 90 μm and 45 μm). The rejection of the last three sieves was centrifuged for 7 min at 2500 rpm in the presence of a 70% sucrose solution. The

supernatant was poured onto the 45 µm aperture mesh sieve and then washed. The spores were collected in a petri dish using a wash bottle. The spore count was carried out using a MOTIC binocular magnifying glass by successive sampling of small quantities of the spore solution. Spore density was expressed as the number of spores contained in 1 g of dry soil. For one field, it was the average of five replicates.

2.8. Statistical Analyses

All the data collected was analyzed with version 4.1.2 of the free software R. The analysis of variance (ANOVA) was performed on the means of the different quantitative variables. In case of significant difference, the Turkey mean separation test at the 5% probability threshold was used to separate the means.

3. Results

3.1. Dominant Weeds in the Cocoa Fields of the Surveyed Localities

Table 2 shows the dominant weed species in the localities of DJELEBOUE, GNAKPALILIE and DIEKOLILIE. In total, there are 14 species divided into 14 genera and 12 families. Thus, with the exception of *Thaumatococcus daniellii* and *Spigelia anthelmia*, which were found in the fields of both the localities of DIEKOLILIE and GNAKPALILIE, the others were specific to a locality. In addition, the Euphorbiaceae and Poaceae with two species each were the most observed.

Table 2. List of species observed in the different plantations.

| N° | Species | Genres | Families | Locations | | |
|----|------------------------------------|-----------------------|----------------|-----------|-------------|------------|
| | | | | DJELEBOUE | GNAKPALILIE | DIEKOLILIE |
| 1 | <i>Chamaesyce hypericifolia</i> | <i>Chamaesyce</i> | Euphorbiaceae | | × | |
| 2 | <i>Croton hirtus</i> | <i>Croton</i> | Euphorbiaceae | × | | |
| 3 | <i>Cyperus difformis</i> | <i>Cyperus</i> | Cyperaceae | | | × |
| 4 | <i>Damnacanthus indicus</i> | <i>Damnacanthus</i> | Rubiaceae | | | × |
| 5 | <i>Heterostemma alatum</i> | <i>Heterostemma</i> | Apocynaceae | | | × |
| 6 | <i>Panicum capillare</i> | <i>Panicum</i> | Poaceae | | × | |
| 7 | <i>Rottboellia cochinchinensis</i> | <i>Rottboellia</i> | Poaceae | × | | |
| 8 | <i>Peperomia rotundifolia</i> | <i>Peperomia</i> | Piperaceae | × | | |
| 9 | <i>Phyllanthus amarus</i> | <i>Phyllanthus</i> | Phyllanthaceae | | × | |
| 10 | <i>Pollia japonica</i> | <i>Pollia</i> | Commelinaceae | | | × |
| 11 | <i>Polygonum aviculare</i> | <i>Polygonum</i> | Polygonacées | | | × |
| 12 | <i>Parietaria officinalis</i> | <i>Parietaria</i> | Urticaceae | | × | |
| 13 | <i>Spigelia anthelmia</i> | <i>Spigelia</i> | Loganiaceae | × | × | |
| 14 | <i>Thaumatococcus daniellii</i> | <i>Thaumatococcus</i> | Marantaceae | × | × | |

Note: ×: presence of the species in the locality.

3.2. Physicochemical Parameters of Soil Samples

Table 3 presents the physical and chemical parameters of soils by location. The analysis showed that, with the exception of humidity, these parameters varied significantly with the locality. Soils at the localities showed roughly the same trend for pH, organic carbon, exchangeable phosphorus, and cation exchange capacity. Indeed, these parameters were significantly higher in the localities of DIEKOLILIE and GNAPKALILIE. Nitrogen was significantly higher at DIEKOLILIE compared to DJELEBOUE. With regard to potassium, the average separation test led to the division of the localities into three groups with in decreasing order of content GNAPKALILIE, DIEKOLILIE and DJELEBOUE. In terms of texture, it was sandy-silty, sandy-clay and clayey respectively with DJELEBOUE, DIEKOLILIE and GNAPKALILIE based on the richness of clay in the soils.

Table 3. Physico-chemical elements of soils according to the localities.

| Locations | pH | CO (g/Kg) | N (g/Kg) | P ₂ O ₅ (mg/Kg) | K (mmol+/Kg) | CEC (mmol+/Kg) | Soil moisture (%) | Clay (%) | Texture |
|-----------------|-----------------------|----------------------|----------------------|---------------------------------------|------------------------|-----------------------|----------------------|-----------------------|-------------|
| DJELEBOUE | 5.67 ± 0.07 b | 8.80 ± 0.15 b | 0.80 ± 0.00 b | 11.33 ± 0.33 b | 1.10 ± 0.06 c | 16.00 ± 0.00 b | 2.27 ± 0.16 a | 12.00 ± 0.58 c | Sandy-silty |
| DIEKOLILIE | 6.00 ± 0.03 a | 16.60 ± 2.59 a | 1.57 ± 0.22 a | 13.67 ± 0.33 a | 1.63 ± 0.09 b | 50.00 ± 10.15 a | 3.03 ± 0.12 a | 32.00 ± 4.73 b | Sandy-clay |
| GNAPKALILIE | 6.13 ± 0.06 a | 16.53 ± 0.24 a | 1.33 ± 0.03 ab | 11.00 ± 0.00 a | 2.83 ± 0.07 a | 51.33 ± 1.20 a | 2.93 ± 0.13 a | 63.33 ± 0.88 a | Clayey |
| Average | 5.93 ± 0.03 | 13.98 ± 1.50 | 1.23 ± 0.13 | 12.00 ± 0.44 | 1.86 ± 0.26 | 39,11 ± 6.49 | 2.74 ± 2.93 | 35.78 ± 7.60 | |
| Test Statistics | F= 31.20; p = 0.00 | F= 8.90; p = 0.01 | F= 9.47; p = 0.01 | F= 28.50; p = 0.00 | F= 152.00; p = 0.00 | F= 11.52; p = 0.00 | F= 4.46; p = 0.06 | F= 85.67; p = 0.00 | |

Values followed by the same letter in the same column are not significantly different at the 5% threshold according to the Tukey Test ($p \leq 0.05$). The values are the mean and ± Standard error.

3.3. Intensity and Frequency of Mycorrhization of Herbaceous Plants

Observation of the roots after staining revealed an endomycorrhization of the majority of the dominant weeds in the prospected fields (**Figure 3**). Thus, with the exception of *Pollia japonica* and *Cyperus difformis*, traces of endomycorrhizal symbiosis were observed in the roots of the other twelve species, *i.e.* 85.71%. Specifically, in DJELEBOUE, all species have been mycorrhizal (**Table 4**). *Croton hirtus* was the least mycorrhizal (2.11%) and *Rottboellia cochinchinensis* the most mycorrhizae. At GNAPKALILIE too, all species have been endomycorrhizaled. In this locality, although the frequencies ranged from 66.67 to 100%, the variation was not significant (**Table 5**). In terms of intensity, the weakest mycorrhizal species was *Thaumatococcus daniellii* with a value of 4.81%. The species *Parietaria officinalis* was the most heavily mycorrhizal. At DIEKOLILIE, where three of the five species identified show traces of symbiosis, the frequency of mycorrhization was 100% in *Damnacanthus indicus* and *Heterostemma alatum* (**Table 6**). The species *Polygonum aviculare* with a mycorrhization intensity of 0.73% was the weakest mycorrhizal.

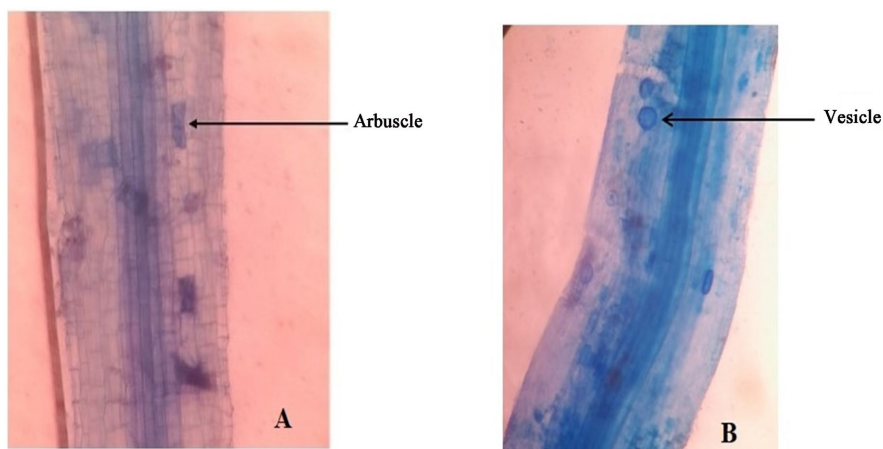


Figure 3. Portions of mycorrhizal weed roots, seen under a microscope ($G \times 100$), (A) *Parietaria Judaica*; (B) *Heterostemma alatum*.

Table 4. Frequencies and intensities of mycorrhization of herbaceous plants found in cocoa plantations at DJELEBOUE.

| Weeds | Frequencies (%) | Intensities (%) |
|------------------------------------|----------------------|---------------------|
| <i>Rottboellia cochinchinensis</i> | 100.00 \pm 0.00 a | 57.69 \pm 3.36 a |
| <i>Croton hirtus</i> | 36.24 \pm 15.42 b | 2.11 \pm 1.45 b |
| <i>Thaumatococcus daniellii</i> | 82.38 \pm 12.95 ab | 18.26 \pm 6.83 b |
| <i>Spigelia anthelmia</i> | 96.30 \pm 3.70 a | 42.83 \pm 11.60 a |
| <i>Peperomia rotundifolia</i> | 77.78 \pm 22.22 ab | 11.81 \pm 5.30 b |

Values followed by the same letter in the same column are not significantly different at the 5% threshold according to the Tukey Test. The values are the mean and \pm Standard error.

Table 5. Frequencies and intensities of mycorrhization of herbaceous plants found in cocoa plantations at GNAKPALILIE.

| Weeds | Frequencies (%) | Intensities (%) |
|---------------------------------|---------------------|---------------------|
| <i>Parietaria judaica</i> | 66.67 \pm 23.13 a | 13.59 \pm 6.28 cd |
| <i>Panicum capillare</i> | 100.00 \pm 0.00 a | 27.68 \pm 4.33 bc |
| <i>Spigelia anthelmia</i> | 100.00 \pm 0.00 a | 16.18 \pm 3.49 cd |
| <i>Phyllanthus amarus</i> | 88.89 \pm 11.11 a | 17.15 \pm 7.27 cd |
| <i>Thaumatococcus daniellii</i> | 55.56 \pm 23.13 a | 4.81 \pm 4.26 d |
| <i>Parietaria officinalis</i> | 96.30 \pm 3.70 a | 61.70 \pm 5.77 a |
| <i>Euphorbia hypericifolia</i> | 100.00 \pm 0.00 a | 37.69 \pm 2.21 b |

Values followed by the same letter in the same column are not significantly different at the 5% threshold according to the Tukey Test. The values are the mean and \pm Standard error.

Table 6. Frequencies and intensities of mycorrhization of herbaceous plants encountered in DIEKOLILIE cocoa plantations.

| Weeds | Frequencies (%) | Intensities (%) |
|-----------------------------|-----------------|-----------------|
| <i>Polygonum aviculare</i> | 22.82 ± 8.34 b | 0.73 ± 0.15 c |
| <i>Damnacanthus indicus</i> | 100.00 ± 0.00 a | 40.08 ± 3.86 b |
| <i>Heterostemma alatum</i> | 100.00 ± 0.00 a | 58.52 ± 4.81 a |
| <i>Cyperus difformis</i> | 0.00 ± 0.00 | 0.00 ± 0.00 |
| <i>Pollia japonica</i> | 0.00 ± 0.00 | 0.00 ± 0.00 |

Values followed by the same letter in the same column are not significantly different at the 5% threshold according to the Tukey Test. The values are the mean and ± Standard error.

3.4. Spore Density of Soils Collected from Cocoa Plantations

The spore density of the different soil samples is represented by the (Figure 4). It ranged from 1.45 to 6.53 spores/g of soil. One-way analysis of variance showed a highly significant difference in spore density across locations ($p < 0.01$). The soils of the fields of DJELEBOUE and GNAKPALILIE with 6.53 ± 1.37 and 4.91 ± 0.92 spores/g of soil, respectively, showed the highest spores density.

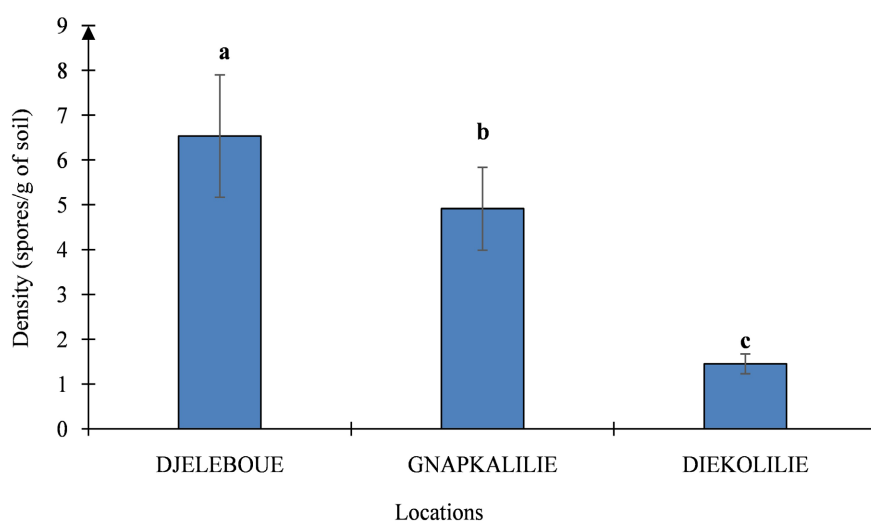


Figure 4. Spore density of arbuscular mycorrhizal fungi isolated from soil samples under from cocoa fields by locality. Mean bar (± Standard error) followed by the same letter in the same column are not significantly different at the 5% threshold according to the Tukey Test.

4. Discussion

In both food and perennial crop plantations [11], several weeds are commonly encountered and these have a negative impact on agricultural production [5] and the establishment of forest plantations [30]. In this study, the prospection made it possible to identify 14 major species in the cocoa fields in the localities of DJELEBOUE, GNAKPALILIE and DIEKOLILIE. These species are divided into

12 families, the most prominent of which are the Poaceae and the Euphorbiaceae. These results are in line with those of Amba *et al.* [15] who reported a high proportion of weeds belonging to the Poacacian family in a cocoa field in Adzopé, Côte d'Ivoire. However, the species judged by Touré *et al.* [12] as well as with high infectivity and highly damaging to crops such as *Chromolaena odorata* and *Euphorbia heterophylla* are absent.

The results also revealed the presence of traces of mycorrhizal symbiosis, in this case arbuscles, hyphae, spores and vesicles in the roots of the weeds encountered. These structures observed in the roots of forest trees [31] and edible herbaceous plants [13] and weeds [32] attest to the association of these herbaceous weeds with arbuscular mycorrhizal fungi. In addition, out of 14 species, 12, or 85.71% of herbaceous weeds, are mycorrhizae. This value confirms the writings of Smith *et al.* [33], according to which 80% of plant species accept this symbiosis. The presence of these species could be beneficial to cocoa trees because the mycelial filaments of these non-specific fungi are able to spread in their root system [34]. In addition, the species *Parietaria officinalis*, which has been most intensely mycorrhizal, is not very aggressive [15]. In addition to having enormous medicinal properties, according to Lambion and Amour [35], it would be of great use in agroecology because it could compete with more harmful weeds and reduce the pressure of bed bugs. Indeed, the sciophytic character of this species, associated with its rapid growth, will promote its propagation, thereby reducing the development of other weeds. Furthermore, the results confirm the non-mycorrhizal status of species belonging to the families Commelinaceae (*Polia japonica*) and Cyperaceae (*Cyperus difformis*) established in the literature [36].

The importance of cocoa farming in the Ivorian economy is the result of the convergence of various pedoclimatic and vegetation factors. Thus, the soils of Lakota plantations have this characteristic. However, a dynamic seems to be apparent at the level of these parameters from one locality to another as well as observed for the organic matter content and the pH. Moreover, this result is closely related to that of Amani *et al.* [22]; the latter having obtained physico-chemical parameters that change from one agro-ecological zone to another while remaining above the fertility threshold. Relative to acidic Ph, the values are close to those of Aikpokpodion *et al.* [37]. This acidification of cocoa plantations can also be attributed to a high use of pesticides over a long period of time, as demonstrated by the latter.

In these soils, the average spore density of arbuscular mycorrhizal fungi was 4.29 spores. G-1 with a maximum of 6.53 and a minimum of 1.45. These values are close to those observed by Amani *et al.* [22] in cocoa plantations but significantly lower than that obtained from Voko *et al.* [38] in cassava fields. This difference can be attributed to cultural practices that differ from one crop to another. Indeed, the cultivation of cassava requires fewer phytosanitary treatments compared to the cocoa tree; therefore, soil mycorrhizal fungi are prone to a small decline in their community. Thus, the low population of microorganisms can result

either from the stability that prevails in the environment, such as the forest [39] or also from the use of pesticides [37]. Also, it is only in the cocoa fields of DJELEBOUE and GNAKPALILIE where the spores were more abundant, that the three most mycorrhizal species, namely *Rottboellia cochinchinensis*, *Panicum capillare* and *Parietaria officinalis*, were identified. This suggests that they are favorable to the fertility of the environment. Indeed, according to Oehl *et al.* [40], mycorrhizal fungi are bioindicators of soil fertility.

5. Conclusions

This study was undertaken to evaluate the endomycorrhizal status of the dominant herbaceous weeds in the cocoa plantations of the localities of DJELEBOUE, GNAKPALILIE and DIEKOLILIE. A population of 14 species divided into 12 families has been identified. Of these species, 12 out of 14 are mycorrhizal, *i.e.* 85.71%. In these localities, the average density of arbuscular mycorrhizal fungal spores per gram of soil was 4.30.

At the end of this study, it appears that the benefits inherent in the maintenance of certain mycotrophic and non-aggressive herbaceous weeds such as *Parietaria officinalis* can be considered in two points: (i) improvement of the fertility of cocoa plantations as well as of the mineral and water nutrition of cocoa trees, and (ii) reduction of pressure of invasive herbaceous weeds.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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