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Effect of Plant-derived Biofertilizers (Azolla filiculoides and Tithonia diversifolia) on Growth and Yield Parameters of Lokpa Yam (Dioscorea cayenensis-rotundata) Grown on Distric Plinthic Ferralsol in the Forest Zone of Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NKR, KAG and GN designed the study, carried out the experimental protocol and determined the village of M'Bayakoffikro as the study site. Authors TLZ and SD contributed to the selection of Lokpa yam seeds (Dioscorea cayenensis-rotundata) and to the collection of biofertilizers of plant origin (Azolla filiculoides and Tithonia diversifolia) and author BS carried out the treatment statistics. All authors read and approved the interpretation of the study results and the final manuscript.

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ABSTRACT

Aims: The study aims to assess the fertilizing potential of *Azolla filiculoides* and *Tithonia diversifolia* leaves on Lokpa (*D-rotundata*) yam cultivation in the forest zone of Côte d'Ivoire.

Place and Duration of Study: The study took place over 9 months (April-December) in 2022 at M'Bayakoffikro in the Daloa department of central-western Côte d'Ivoire.

Methodology: After clearing a soil fallow classified Distric plinthic ferralsol, microplots in randomized Fisher blocks of 2 replicates each with five treatments (*Azolla filiculoides*-AZ; *Tithonia diversifolia* with fresh biomass-TDF; *Tithonia diversifolia* with decomposed biomass-TDD; *Azolla filiculoides* + *Tithonia diversifolia* with fresh biomass-AZ+TDF and *Azolla filiculoides* + *Tithonia diversifolia* with decomposed biomass-AZ+TDD) and a control without fertilizer were delimited in which,10 mounds were levelled for sowing yam tuber pieces. Before sowing the tubers, the mounds were opened by hand and 0.5kg of each formulated fertilizer was ploughed in and then resealed. After 14 days of fertilization, the mounds were reopened and a single seedling was buried 20cm deep in each mound from top to bottom, followed by 300ml of water and then resealed. After germination, growth and production parameters were evaluated and the data obtained were subjected to an analysis of variance with Statistica 7.1 software at the 5% threshold.

Results: The different biofertilizer formulations had a significant effect on all growth and yield parameters of lokpa yam (*D*-rotundata). However, the highest values for both growth and yield parameters compared with other fertilizers at all measurement times and at maturity were obtained with *Azolla filiculoides* combining decomposed *Tithonia diversifolia* leaves on the one hand and fresh *Tithonia diversifolia* leaves on the other.

Conclusion: Improving soil productivity in forest areas by finding reliable and effective alternatives to mineral fertilizers is a necessity, and the combination of *Azolla filiculoides* and *Tithonia diversifolia* could be a good alternative for sustainable, environmentally-friendly agriculture.

Keywords: Biofertilizers; Lokpa yam (D-rotundata); agromorphological parameters; yield; Côte d'Ivoire forest zone.

1. INTRODUCTION

Yam cultivation contributes to the food security of 300 million people in tropical countries, and its nutritional value varies according to variety. Yam is a staple food for many populations in West Africa [1]. In Côte d'Ivoire, among cultivated food crops, yam occupies a prominent place [2] with an annual production of 7.148 million tonnes [3-4]. This position is justified not only by favorable soil and climate conditions, but also by the cultural fact that yams are the staple food of certain ethnic groups. However, yam production falls short of consumer expectations [2]. Indeed, the growth of yam plants is severely limited by natural conditions linked to climate change, soil depletion in one or more essential nutrients, population growth and increasingly recurrent land tenure problems [5], followed by cultivation practices involving continuous exploitation of the land without any significant input [6]. Faced with these constraints, and with a view to meeting the challenge of food production to satisfy the population's consumption needs, the use of chemical fertilizers has very often been proposed as one of the solutions for meeting crop nutrient requirements, because of their immediate beneficial effect on productivity [7]. However, the lack of knowledge of reasoned fertilization makes it difficult to use mineral fertilizers to compensate for and generally correct the low level of soil fertility for crop nutrition [8-9]. Moreover, their high cost makes them almost inaccessible to growers [10]. Hence the need for organic, natural fertilizers capable of raising or maintaining soil fertility while preserving its ecological balance could prove to be imperative. Scientific studies have shown that organic fertilizers can improve soil structure and enrich it with nutrients to counter soil exhaustion [11-12]. In such a context, the use of organic fertilizers of plant origin, in particular, Azolla filiculoides and Tithonia diversifolia would be a good substitute for chemical fertilizers [13-14] and offer new prospects for improving soil fertility and proper plant development [15]. However, the effects of Azolla filiculoides and Tihtonia diversifolia in different formulations on the agromorphological parameters of cultivated plants, particularly yams, have been little studied. Thus, the main objective of this work is to contribute to the improvement of yam growth and yield through the use of locally available plant-based biofertilizers, notably Azolla filiculoides and Tihtonia diversifolia in the forest zone of Côte d'Ivoire.

2. METHODOLOGY

2.1 Study Area

The study took place in M'Bayakoffikro in the Daloa department and Haut Sassandra region in west-central Côte d'Ivoire (Fig. 1) between 6°53'58" N latitude and 6°26'32"W W longitude [16]. The climate is transitional humid tropical, with bimodal rainfall varying between 1,200 and 1,600 mm/year [17]. The average annual temperature is between 24 and 25°C and the average relative humidity is around 70% [18]. Vegetation cover is highly heterogeneous, varying gradually from semi-deciduous rainforest to pre-forest savannah. The region's soils are based on vast granitic massifs, metamorphic and schistose rocks. They are represented as a Distric plinthic ferralsol complex, which overall

has good agricultural suitability for all crop types [19].

2.2 Plant Material

The plant material used is an early yam variety known under the vernacular name of Lokpa, belonging to the *Dioscorea cayenensis-rotundata species* (Fig. 2). The choice of this early variety was guided by its ease of adaptation to different pedoclimates and by its ability to be grown from the first rains (March-April), so that the tubers can be harvested very early and made available on local markets from December-January for the end-of-year festivities. It is also highly appreciated in all culinary forms.

2.3 Biofertilizer Materials

The biofertilizers used are of plant origin, consisting of the aquatic fern *Azolla filiculoides* (Fig. 3a), which colonizes our ponds [21], and the fresh biomass (fresh leaves and soft branches) of *Tithonia diversifolia* (Fig. 3b), which grows on fallow land or sunny open spaces along roadsides [22]. The choice of these biofertilizers is justified by the fact that they are characterized by a high productivity of nitrogenous substances, and therefore by their ability to fertilize and improve soil texture. They are locally available and improve soil nutrient potential by providing plants with physiologically assimilable nutrients for growth, development and production.

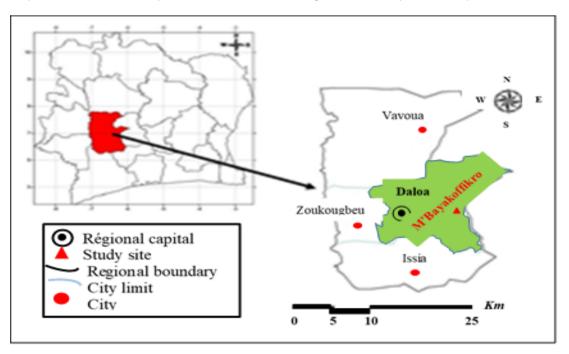


Fig. 1. Location of study site, [20]. modified

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Fig. 2. D. rotundata yam tuber with germ



Tithonia diversifolia plant

Fig. 3. Harvested plant fertilizers

2.4 Preparation of **Biofertilizer Treatments**

Preparation of the Azolla filiculoides treatments involved harvesting 2kg of fresh Azolla filiculoides plant from a growing medium at the Université Jean Lorougnon Guédé. This quantity of aquatic plant material was divided into 2 batches of 1kg each, constituting a fertilizing formula named Azolla filiculoides-AZ.

As for the fresh biomass of Tithonia diversifolia, 2kg of the plant were also harvested from the surrounding fallow land in the study area. The fresh biomass harvested was also divided into 2 batches of 1kg. The first 1kg batch of fresh biomass was chopped to form a fertilizer formula named: Tithonia diversifolia with fresh biomass-TDF.

The second 1 kg batch of fresh biomass was placed in bags and sprayed with water to be preserved and decomposed after 14 days, thus forming another fertilizer formula also named: Tithonia diversifolia with decomposed biomass-TDD.

A total, 3 fertilizers formulas have been composed:

- Azolla filiculoides fertilizer (AZ);
- *Tithonia diversifolia* fresh biomass fertilizer (TDF);
- Tithonia diversifolia fertilizer with decomposed biomass (TDD).

These fertilizers formulations were used to compose 5 main treatments compared to a control as follows:

- the control with no fertilizer;
- Azolla filiculoides fertilizer-AZ;
- Tithonia diversifolia fresh biomass fertilizer-TDF
- Tithonia diversifolia fertilizer with decomposed biomass-TDD;
- Azolla filiculoides + Tithonia diversifolia fertilizer with fresh biomass-AZ+TDF;
- Azolla filiculoides + Tithonia diversifolia fertilizer with decomposed biomass-AZ+TDD.

2.5 Obtaining Yam Tuber Seeds

The Lokpa yam tuber seeds selected were mature, healthy, whole yam tubers from the previous season purchased on the market and ready to germinate. These tubers were fragmented into pieces of around 30g, then soaked in a solution made from kitchen ash of around 150g in 8L of water for disinfection. After 20 minutes, the fragments were removed and dried in the shade, ready to be sown in previously prepared mounds.

2.6 Experimental Set-up and Maintenance

After clearing a 200 m² (20 m x 10 m) plot of fallow land over 10 years old with a machete and clearing it of plant debris, the trial was conducted using a randomized complete block design with two replicates separated by a 1.5 m aisle. In each repetition, five (06) microplots measuring 4 m x 1.5 m and spaced 1 m apart were distributed. In each elementary plot, 10 levelled ridges 30 cm high and 50 cm wide at the base were made at a regular spacing of 1m x 1m. Two weeks before sowing the prepared vam tuber pieces, the mounds were opened by hand and 0.5 kg of each fertilizer formulation (AZ, TDF, TDD, AZ+TDF and AZ+TDD) was applied to each mound and then closed. All these applications were compared to a blank control with no fertilizer formulation, according to the experimental set-up below (Fig. 4). After 14 days, the mounds were reopened to sow Lokpa yam tuber fragments. A single fragment was sown at a depth of 20 cm in each mound from the top to the base, followed by the addition of 300ml of water and then closed again. After germination of the yam tuber fragments, stakes were installed. This enabled the yam stalks to be lifted, not only to expose a larger leaf surface to the light, but also to facilitate weeding without damaging the stalks.

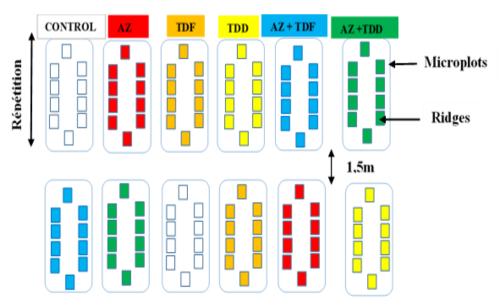


Fig. 4. Experimental set-up

2.7 Data Collection

Data were collected every seven (07) days after germination (DAG). Measurement times were T1 (14DAG), T2 (21DAG), T3 (28DAG), T4 (35DAG), T5 (42DAG) and physiological maturity after 9 months. Growth parameters measured on yam plants included:

- neck diameter: The diameter of the stem neck was measured with a caliper in centimetres.
- number of leaves on the creeper stem: Leaves were counted from the first leaf to the last true leaf. The last true leaf is marked to facilitate subsequent counting.
- the number of branches on the stem: The number of branches on the main vine stem is counted.
- length of main stem: The length of the main stem was measured in centimetres (cm) using a tape measure from the surface of the mounds to the plant apex.

Yield parameters were measured in terms of:

- length and diameter of yam tubers using a tape measure
- weight of yam tubers.

2.8 Data Analysis

The data collected were entered into Excel 2013. These data were subjected to a one-factor analysis of variance for each variety using STATISTICA 7.1 software at the 5% threshold. When a difference was found to be significant (p < 0.05) for a characteristic, the analysis of variance was completed by Fisher's LSD test at the 5% threshold to determine homogeneous groups of means.

3. RESULTS

3.1 Effect of Fertilizer Formulas on Lokpa Yam Growth Parameters

3.1.1 Stem neck diameter

Plant neck diameter of Lokpa yam (Table 1) was significantly (p < 0.05) and variably affected by treatments at measurement times T3 (28DAG), T4 (35DAG) and T5 (42DAG), in contrast to measurement times T1 (14DAG) and T2 (21DAG), which showed insignificant neck diameter values. Explicitly, there were no significant variations in plant collar diameter in the image at measurement times T1 (14DAG)

and T2 (21DAG) for all treatments. On the other hand, the stimulating effect of the treatments was observed from T3 (28DAG) onwards. Thus, treatments AZ+TDD and AZ+TDF significantly (p 0.05) emerged as the best fertilizer < formulations that gave the highest values of crown diameter compared with the other treatments, which showed mixed values. At measurement time T5 (42DAG) the highest collar diameters were recorded with fertilizer formulations AZ+TDD (0.8cm), AZ+TDF (0.72cm), TDD (0.65cm), TDF (0.63cm) and AZ (0.54cm) in descending order.

3.1.2 Main stem length by treatment

Table 2 shows vam main stem length by treatment. We note that treatments significantly (p < 0.05) affected yam main stem length at all measurement times, except for time T1 (14DAG), which displayed non-significant values (p > 0.05). The highest stem lengths were obtained at time (42DAG) respectively T5 with AZ+TDD (279.2cm), AZ+TDF (228cm), TDD (177cm), TDF (151.6cm) and AZ (131.2cm) formulations. Fertilizer formulations AZ+TDD and AZ+TDF increased yam plant length with the highest values compared to the other treatments.

3.1.3 Number of main stem branches

The values for the number of branches on the main stem of yam are shown in Table 3. Yam stem branching number was influenced by treatments at all observation periods. However, the highest values were obtained with AZ+TDD and AZ+TDF fertilizer formulations at all times of measurement. At time T5 (42DAG), the number of stimulated branches was highest for AZ+TDD formulations (13.57), followed by AZ+TDF (10.20) and finally TDD (6.40). The TDF (3.80) and AZ (3.20) formulations had similar branchings, more or less identical to the control.

3.1.4 Number of leaves by treatment

The values for the number of leaves on the yam plant are shown in Table 4. It can be seen that all treatments significantly affected the number of leaves on the yam plant at all observation periods or measurement times from T1 (14DAG) to T4 (35DAG), except for time T5 (42DAG) when the number of leaves stopped increasing regardless of treatment. However, the results in the table show that yam plant leaf numbers were higher under the AZ+TDD and AZ+TDF fertilizer formulations. At time T4 (35DAG) specifically, leaf numbers were 25.44 for AZ+TDD and 20.60 for AZ+TDF.

		Diameter of main stem at collar (cm)			
			Measuring time	;	
Treatments	T1 (14DAG)	T2 (21DAG)	T3 (28DAG)	T4 (35DAG)	T5 (42DAG)
Control	0,34±0,31a	0,45±0,25a	0,56±0,05ab	0,57±0,06ab	0,58±0,06ab
AZ	0,37±0,21a	0,47±0,03a	0,49±0,04b	0,51±0,04b	0,54±0,06b
TDF	0,43±0,24a	0,55±0,08a	0,56±0,06 ^{ab}	0,61±0,07a	0,63±0,5a
TDD	0,55±0,07a	0,56±0,07a	0,6±0,04a	0,64±0,03ac	0,65±0,03ac
AZ+TDF	0,62±0,07a	0,63±0,07a	0,63±0,08a	0,7±0,06c	0,72±0,07c
AZ+TDD	0,65±0,08a	0,68±0,08a	0,70±0,07c	0,75±0,07c	0,8±0,07c
CV	6,43	9,07	3,96	37,13	37,62
P value	0,199	0,233	0,029	0,001	0,002

Table 1. Average crown diameter values according to treatments	Table 1. Average	crown diameter	values accordin	g to treatments
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For each measurement time, the mean values bearing the same letters in the same column are statically identical at the 5% threshold

Table 2. Mean values for main stem length by treatment

		Le	ngth of main ster	n (cm)	
			Measuring tim	е	
Treatments	T1 (14DAG)	T2 (21DAG)	T3 (28DAG)	T4 (35DAG)	T5 (42DAG)
Control	33,8±47,20a	53,8±50,55a	88,4±50,91a	120,2±41,68a	142,4±39,43a
AZ	44,2±32,63a	43±21,38a	80,8±24,61a	110,8±34,69a	131,2±32,49a
TDF	20,8±25,02a	53,6±39,52a	75,6±34,37a	102,8±30,45a	151,6±39,89a
TDD	59,2±7,82a	103,8±18,67b	121,4±28,26ab	157,4±41,93ab	177±42,81ab
AZ+TDF	77,72±25,90a	141,8±30,21b	170,6±51,76ab	207,2±91,83b	228±100,30b
AZ+TDD	80,19±25,90a	180,22 ± 20,2 ^c	200,70±51,76b	236,44±91,83c	279,2±100,30c
CV	7,50	8,86	4,30	33,95	36,12
P value	0,064	0,0006	0,005	0,030	0,043

For each measurement time, the mean values bearing the same letters in the same column are statically identical at the 5% threshold

Table 3. Average values for number of main stem branches according to treatments
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		Nu	mber of plant br	anches	
			Measuring tin	ne	
Treatments	T1 (14DAG)	T2 (21DAG)	T3 (28DAG)	T4 (35DAG)	T5 (42DAG)
Control	0,00±0, 00a	0,80±1,78a	2,60±2,60a	4,20±3,03a	3,20±1,30a
AZ	0,00±0,00a	0,00±0,00a	0,60±0,89a	2,60±1,67a	3,20±1,30a
TDF	1,00±2,23a	2,00±2,73a	2,00±2,73a	2,40±2,88a	3,80±3,42a
TDD	0,00±0,00a	1,60±2,60a	3,00±3,31a	4,80±4,14a	6,40±5,17ab
AZ+TDF	3,60±2,070b	7,60±2,50b	9,00±3,309b	9,60±3,64b	10,20±3,83b
AZ+TDD	4,48±2,07c	9,78±2,50c	11,56±3,31c	12,78±3,64c	13,57±3,83c
CV	15,58	16,76	11,44	25,02	3,23
P value	0.001	0.0001	0.001	0.012	0.037

For each measurement time, the mean values bearing the same letters in the same column are statically identical at the 5% threshold

3.2 Effect of Fertilizer Formulas on Yield Parameters at Physiological Maturity of Lokpa Yam

3.2.1 Tuber length

Tuber length of Lokpa yam was significantly (p < 0.05) and variably affected by the treatments applied compared with the control

(Table 5). The highest tuber length was observed with the AZ+TDD fertilizer formulation (23cm). The AZ and TDF formulations obtained similar and identical tuber length values around 21cm. Similarly, AZ+TDF and TDD formulations showed similar and identical tuber length values (19cm). The control recorded the lowest tuber length at 13cm.

		Νι	Imber of plant le	aves	
			Measuring tim	e	
Treatments	T1 (14DAG)	T2 (21DAG)	T3 (28DAG)	T4 (35DAG)	T5 (42DAG)
Control	1,00±2,23a	3,80±2,58a	7,40 ±3,5a	10,6±3,71a	14,20±6,18a
AZ	0,80±1,09a	3,40±1,94a	7,40±2,96a	11,00±4,00a	12,00±4,94a
TDF	0,00±0,00a	1,00±2,00a	2,50±3,31a	5,00±3,91a	6,80±5,29a
TDD	1,80±1,64a	3,80±3,49a	7,60±3,20a	12,20±2,94a	16,00±3,39a
AZ+TDF	8,80±4,08b	14,80±6,61b	18,60±9,28b	20,60±11,86b	21,00±13,29a
AZ+TDD	9,56±4,08c	16,26±6,61c	22,18±9,28c	25,44±11,86c	23,33±13,29a
CV	14,58	15,38	12,24	30,82	47,41
P value	0,000	0,000	0,001	0,012	0,069

Table 4. Mean values for number of plant leaves by treatment

For each measurement time, the mean values bearing the same letters in the same column are statically identical at the 5% threshold

Treatments	Harvest yield parameters					
	Tuber length (cm)	Tuber diameter (cm)	Weight (kg)			
Control	14±1,50a	9±17,45a	9,5±1,50a			
AZ	20±0,20b	6±23,02a	11,2±17,22b			
TDF	21±13,10b	8±12,33b	12±17,22b			
TDD	19±11,50ab	12±10,10c	13,7±11,20ab			
AZ+TDF	19±1,15ab	10±0,50ab	15±3,50c			
AZ+TDD	23±3,50c	11±10,50c	17,1±3,50c			
CV	12,17	23,01	17,22			
P value	0,034	0,014	< 0,001			

For each measurement time, the mean values bearing the same letters in the same column are statically identical at the 5% threshold

3.2.2 Tuber diameter

Tuber diameter of Lokpa yam was also significantly (p < 0.05) affected by the treatments applied compared with the control (Table 5). The highest tuber diameters were observed with the fertilizer formulations TDD (12cm) and AZ+TDD (11cm). The AZ+ TDF formulation showed intermediate tuber diameter values (10cm), while the TDF and AZ formulations showed the lowest tuber diameter values (8cm and 6cm respectively).

3.2.3 Weights

The weights presented in Table 5 show that the best weights were obtained and significantly so with the AZ+TDD formulations with 17 kg, AZ+TDF with 15 kg, TDD with 13.7 kg, TDF with 12 kg and AZ with 11 kg in descending order compared with the control with 9 kg.

4. DISCUSSION

Analysis of variance of data on yam growth parameters relating to neck diameter, stem

length, number of stem branches and number of leaves, as well as data on yield parameters (tuber length and diameter, weight) showed differences between significant treatments relating to the different formulations of plantbased fertilizers compared with the control without formulation. This difference with the control treatment demonstrates the richness of plant resources as fertilizers and in mineral elements essential for plant growth and development as demonstrated by Ognalaga et al. [23]. The fertilizing materials used therefore have a satisfactory fertilizing potential. In addition and overall, Lokpa yam (D- rotundata) was much more affected by the different formulations of Azolla plant-based fertilizers combining filiculoides with decomposed Tithonia diversifolia leaves on the one hand, and Azolla filiculoides with fresh Tithonia diversifolia leaves on the other, showing the highest values for both growth and yield parameters compared with the other fertilizers at all measurement times and at maturity. These results could be explained by the fact that decomposed fresh Tithonia diversifolia leaves provide the yam with essential and easilyassimilable nutrients for its growth and development, as demonstrated by the work of Salla et al. [24] and Kaho et al. [25]. Furthermore, the decomposition of Azolla filiculoides in the soil takes place gradually and that the plant's uptake of nutrients depends on this to stimulate growth [13]. This seems to show a good synchronization of nutrient release from decomposed Tithonia diversifolia leaves and Azolla filiculoides during its decomposition and their assimilation by the plant. These results corroborate the assertion of Mulaji [9] and that of Cobo et al. [26], who showed that the rate of decomposition of applied organic matter and plant growth were closely linked to the timing of release. Thus, the advantage of plant-based fertilizers on growth and yield parameters could be explained by the fact that Azolla filiculoides Tithonia diversifolia possess mineral and elements needed to improve soil fertility for crop nutrition, growth and yield. Indeed, the work carried out by Mucheru-muna et al. [27] and Mucheru-muna et al. [28] as well as that of Thorsm et al. [29] and Jama et al. [30] in Kenya found that maize yield tripled the following season after Tithonia diversifolia was incorporated into the soil. Also, the work of Kouadio [20] showed the positive role of Azolla filiculoides on rice growth parameters at Vavoua in upper Sassandra. This author recorded increases in rice height, number of tillers and number of rice leaves compared with NPK and the control. These results show that the two plant-derived fertilizers (Azolla filiculoides and Tithonia diversifolia) contain a significant quantity of nitrogen and phosphorus elements, the combination of which acts synergistically to drive plant growth and development [31-32]. In addition, Azolla filiculoides releases minerals gradually, which can ensure that they are available when the plant actually needs them. Nutrients made sufficiently available over time in the soil are efficiently utilized by crop plants [33]. As for Tithonia diversifolia, its contribution to plant growth would be due to the high availability of mineral elements and the improvement of soil physicochemical properties. This argument corroborates Hutomo et al. [34] who say that thanks to its ability to store water, Tithonia diversifolia can increase soil moisture, affecting crop growth.

5. CONCLUSION

The main objective of this work was to contribute to the improvement of yam growth and yield through the use of plant-based biofertilizers, in particular *Azolla filiculoides* and *Tihtonia*

diversifolia in the forest zone of Côte d'Ivoire. At the end of this study, we concluded that formulations of plant-based fertilizers combining Azolla filiculoides with decomposed or fresh Tithonia diversifolia biomass showed great potential for the growth and yield of Lokpa (Drotundata) yam in the Haut Sassandra forest zone of Côte d'Ivoire. The singular application of fertilizer on the agro-morphological each parameters of Lokpa (D-rotundata) yam is not to be overlooked, as its results were as significant but better when combined. These results showed that the two organic fertilizers used could be a good alternative to chemical fertilizers (synthesized fertilizers), which are harmful to the environment and costly to the farmer's purse.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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