

Effect of plant-derived biofertilizers (*Azolla filiculoides* and *Tithonia diversifolia*) on growth and yield parameters of Lokpayam (*Dioscorea cayenensis-rotundata*) grown on Districplinthic Ferralsol in the forest zone of Côte d'Ivoire

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 Districplinthic 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 0.5 kg 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 20 cm deep in each mound from top to bottom, followed by 300 ml 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 Lokpayam (*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, Lokpayam (*D-rotundata*), agromorphological parameters, yield, Côte d'Ivoire forest zone.

1. INTRODUCTION

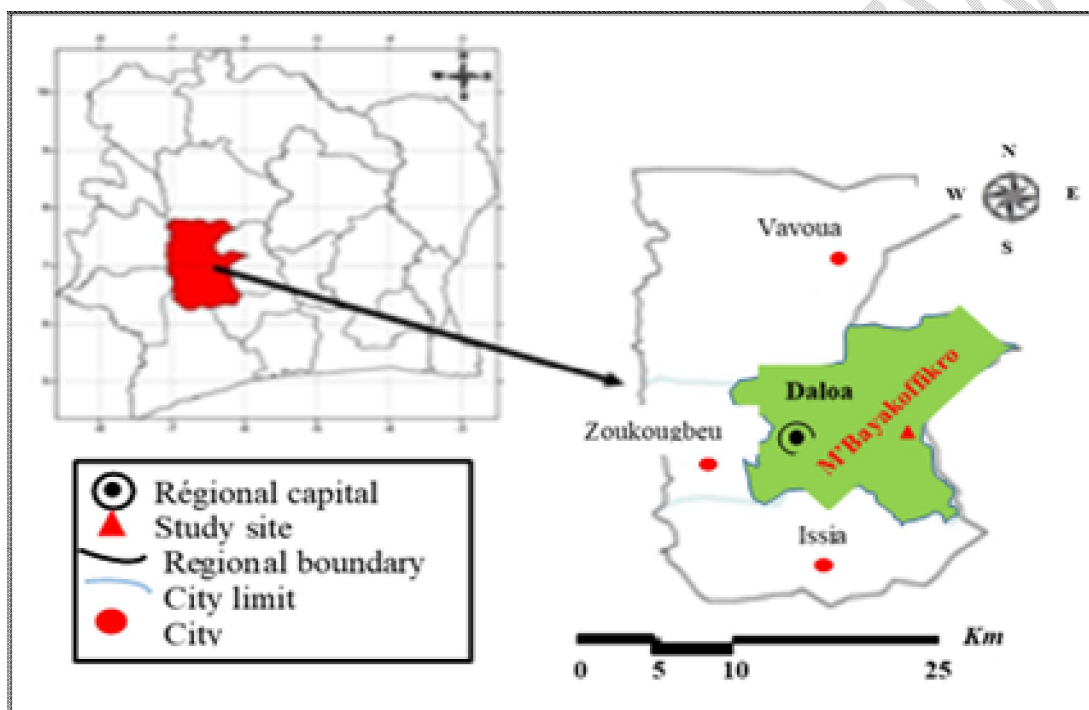
Yam cultivation contributes to the food security of 300 million people in tropical countries, and its nutritional value varies according to variety. Yam is a staple food for many populations in West Africa [1]. In Côte d'Ivoire, among cultivated food crops, yam occupies a prominent place [2] with an 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. 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 *Tithonia 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 *Tihonia 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 (Figure 1) between 6°53'58" N latitude and 6°26'32" 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 (Figure 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.

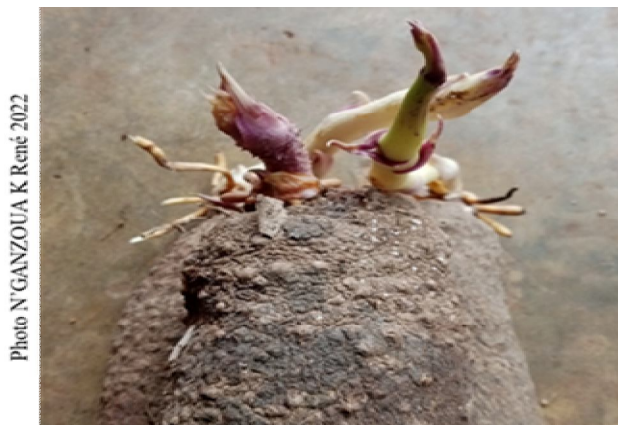


Figure 2: *D. rotundata* yam tuber with germ

2.3 Biofertilizer materials

The biofertilizers used are of plant origin, consisting of the aquatic fern *Azolla filiculoides* (Figure 3a), which colonizes our ponds [21], and the fresh biomass (fresh leaves and soft branches) of *Tithonia diversifolia* (Figure 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.

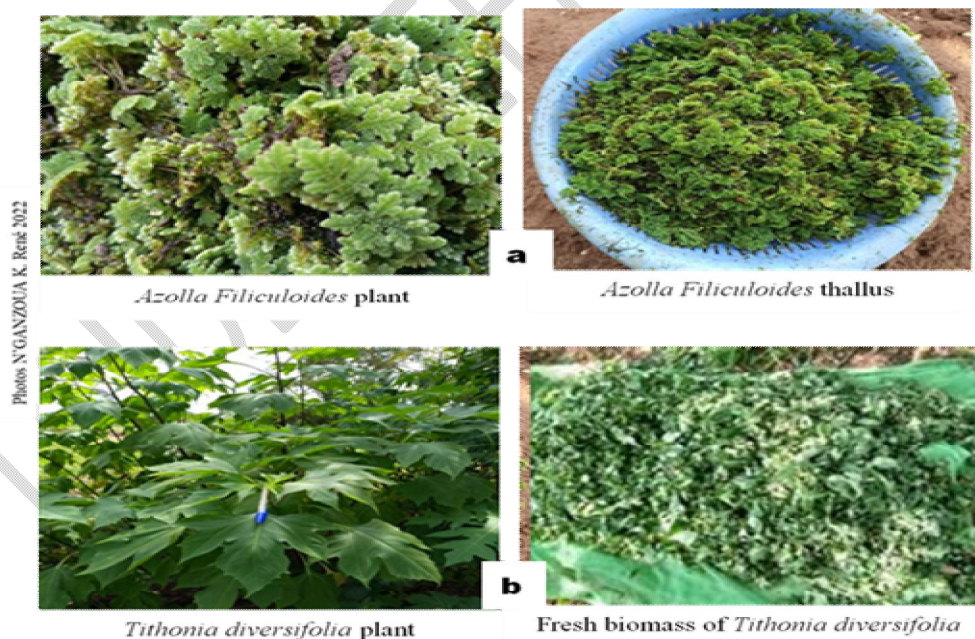


Figure 3: Harvested plant fertilizers

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.

These three fertilizer formulas were used to create the following treatments:

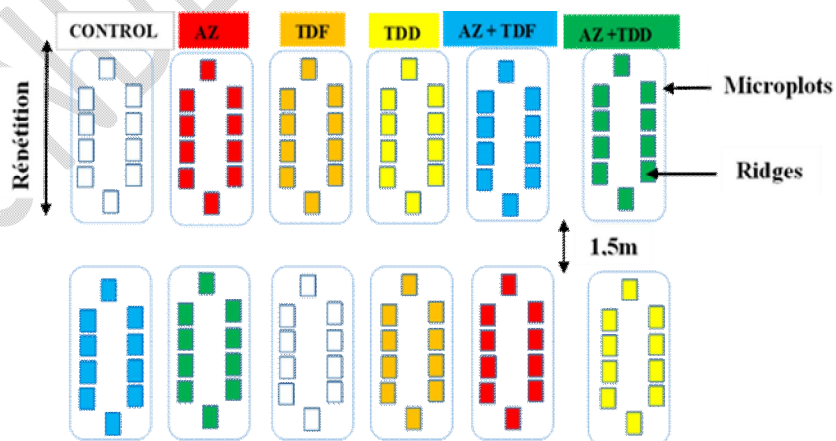
- the control with no fertilizer
- *Azolla filiculoides* fertilizer-AZ;
- *Tithonia diversifolia* fresh biomass fertilizer-TDF
- *Tithonia diversifolia* fertilizer with decomposed biomass-TDD;
- *Azolla filiculoides* + *Tithonia diversifolia* fertilizer with fresh biomass-AZ+TDF;
- *Azolla filiculoides*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

2.5. Obtaining yam tuber seeds

The Lokpayam 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 replication, 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 yam 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 (Figure 4). After 14 days, the mounds were reopened to sow Lokpayam 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.



2.6. Data collection

Figure 4: Experimental set-up

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 tap 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 tap measure
 - weight of yam tubers.

2.7. 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 Lokpayam growth parameters

3.1.1. Stem neck diameter

Plant neck diameter of Lokpayam (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 treatment 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.

Table 1: Average crown diameter values according to treatments

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

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

1.1.2. Main stem length by treatment

Table 2 shows yam 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 T5 (42DAG) respectively 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.

Table 2: Mean values for main stem length by treatment

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

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.

Table 3: Average values for number of main stem branches according to treatments

Treatments	Number of plant branches				
	Measuring time				
	T1 (14DAG)	T2 (21DAG)	T3 (28DAG)	T4 (35DAG)	T5 (42DAG)
Control	0,00±0,00 ^a	0,80±1,78 ^a	2,60±2,60 ^a	4,20±3,03 ^a	3,20±1,30 ^a
AZ	0,00±0,00 ^a	0,00±0,00 ^a	0,60±0,89 ^a	2,60±1,67 ^a	3,20±1,30 ^a
TDF	1,00±2,23 ^a	2,00±2,73 ^a	2,00±2,73 ^a	2,40±2,88 ^a	3,80±3,42 ^a
TDD	0,00±0,00 ^a	1,60±2,60 ^a	3,00±3,31 ^a	4,80±4,14 ^a	6,40±5,17 ^{ab}
AZ+TDF	3,60±2,070 ^b	7,60±2,50 ^b	9,00±3,309 ^b	9,60±3,64 ^b	10,20±3,83 ^b
AZ+TDD	4,48±2,07 ^c	9,78±2,50 ^c	11,56±3,31 ^c	12,78±3,64 ^c	13,57±3,83 ^c
CV	15,58	16,76	11,44	25,02	3,23
<i>P value</i>	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.

3.1.4. 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.

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

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

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 Lokpayam

3.2.1. Tuber length

Tuber length of Lokpayam 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.

3.2.2. Tuber diameter

Tuber diameter of Lokpayam 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.

Table 5: Average values of yield parameters at maturity according to treatments

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

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 significant differences between treatments relating to the different formulations of plant-based 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 and al.[23]. The fertilizing materials used therefore have a satisfactory fertilizing potential. In addition and overall, Lokpayam (*D-rotundata*) was much more affected by the different formulations of plant-based fertilizers combining *Azolla 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 easily-assimilable 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 and 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* and *Tithonia diversifolia* possess mineral elements needed to improve soil fertility for crop nutrition, growth and yield. Indeed, the work carried out by Mucheru-muna and al.[27] and Mucheru-muna and al.[28] as well as that of Thorsmand al.[29] and Jama and 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 act 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 and 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 *Tithonia 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 (*D-rotundata*) yam in the Haut Sassandra forest zone of Côte d'Ivoire. The singular application of each fertilizer on the agro-morphological 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.

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