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Effect of Two Biostimulants and a Synthetic Fungicide on Anthracnose (*Colletotrichum* sp) and Yield of Four Hybrid Clones and One Local Variety of Yam (*Dioscorea alata*) in Central Côte d'Ivoire

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Abstract

Yam is an important food crop for the people of Côte d'Ivoire. However, it is subject to several parasitic attacks. The most damaging of which is anthracnose, caused by *Colletotrichum gloeosporioides*. In some varieties and regions, yield losses can be 80% or more. This study evaluated the effect of two biostimulants and a synthetic fungicide on anthracnose and yield in four yam hybrid clones and a local cultivar. The plant material used was yam hybrid clones CNRAiga15/00020. TDa01/00002. TDa01/00012. CNRAiga15/00028 and local variety Ma01. Two biostimulants [solution A "biofungicide" and OCIBIO 5% (essential oil)] and a synthetic fungicide (mancozeb) were used for the different treatments. Observations were made between the second and sixth month after planting. The incidence and severity of anthracnose on developing plants and yield at harvest were observed. The results showed that yam clones were susceptible to anthracnose, with an incidence rate of 100%. But with low severity rates, except for the control plants. Hybrid clones CNRAiga15/00020, CNRAiga15/00028 and TDa01/00012 showed no significant difference between treatments (T2, T3 and T4). A significant difference between treatments was observed for the hybrid clone TDa01/00002 with a severity score of 2. Yield parameters were not significantly different between treatments. T4 with OCIBIO 5% showed some efficacy against yam anthracnose.

Keywords

Bio-Stimulants, Anthracnose, Hybrid Clones, Incidence, Severity

1. Introduction

Yam is the most cultivated in an area of 7.7 million hectares worldwide with a production of 74.8 million tonnes in 2020 [1]. This crop is the staple food of more than 500 million people. Especially in tropical areas and throughout the world [2]. It is the fourth most important root and tuber crop in the world after potato, cassava and sweet potato. The main production area is West Africa, which accounts for almost 93% of world production. Nigeria is the leading yam producer producing 48 million tonnes of the world total. Most of which is for local consumption. Followed by Ghana (8.9 million) and Côte d'Ivoire (7.6 million) [1]. In terms of volume, Yam is the leading food crop in Côte d'Ivoire well ahead of cassava and plantain [3]. Yam is grown throughout the country, although the main production areas are in the centre and northern part of the country [4]. *Dioscorea alata* Linné of Asian origin and *Dioscorea rotundata* Linné of African origin are the most widely cultivated species in Côte d'Ivoire and are of great economic importance nationally. However, production is dominated by *D. alata* (55% - 60% of total yam production) [5]. This commodity can be consumed in several forms: boiled, pounded, steamed, fried or stewed [3]. Despite the economic, socio-cultural and nutritional importance of yam cultivation, it faces biotic and abiotic constraints that tend to reduce tuber yields. The national yield of this crop is about 8 to 12 T/ha, which is lower than the expected potential yield of 65 T/ha [6].

Among the biotic constraints, a disease anthracnose caused by *Colletotrichum gloeosporioides* is the main pathology of *D. alata* yam. It is the most economically damaging of all *D. alata* yam diseases [7]. The yam species *D. alata* is reported to be the most susceptible to the disease [8]. In *D. alata*, yield losses due to the disease reach 80% or more in some cultivars and regions [9]. It is manifested by damage to the aerial organs of yam plants [9]. The disease begins with brown punctate spots, lighter in the center which gradually darken to form necroses on the leaves [10].

Without effective fungicides to control this disease, without the risk of residues in the tubers, yield losses could reach 100%. The loss of income for farmers would be a real problem. The search for bio-pesticides that are effective against yam anthracnose and that can be made available to farmers is one of the challenges facing scientific research in Côte d'Ivoire. As it is important to assess the real effectiveness of this product. It is therefore necessary to find an organic product that is effective against yam anthracnose, without the risk of residues being deposited in the tubers. The aim of this study is to use bio stimulants to effectively control *D. alata* yam anthracnose.

2. Materials and Method

2.1. Materials

2.1.1. Study Site

The study was conducted at the Food Crops Research Station (FCRS) of the Centre National de Recherche Agronomique (CNRA) in Bouaké. It is located at latitude 7˚69' N. longitude 5˚03' W and altitude 376 m [11]. It is characterized by a humid tropical climate with four seasons [12], including a long dry season (November to February), a long rainy season (March to June), a short dry season (July to August) and a short rainy season (September to October). The soils have a sandy-clay texture, ferrolitic gravelly, moderately saturated and shallow [13]. The average temperature is 25.73˚C with an average annual rainfall of 1200 mm (**Figure 1**) and an annual sunshine duration of 2200 hours [14].

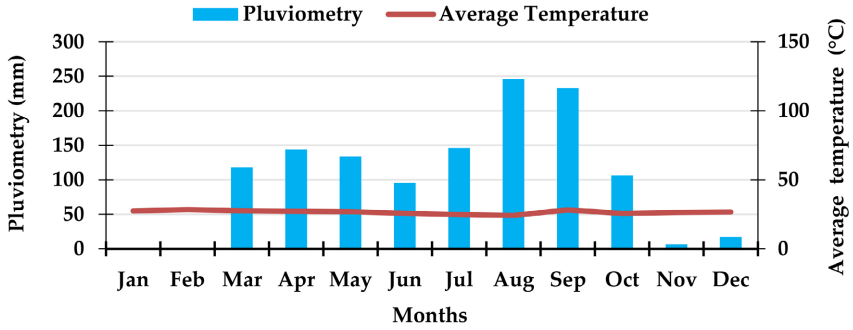

Figure 1. Umbrothermal diagram of the study area.

2.1.2. Plant Material

The plant material used for this experiment consisted of five yam clones of the *Dioscorea alata* species from the CNRA genetic resources collection. These are four hybrid clones: CNRAiga15/00020, TDa01/00002, TDa01/00012, CNRAiga15/00028 and a local variety Ma01. They were used mainly for yielding, culinary quality, fine texture and organoleptic quality.

2.1.3. Technical Equipment

The technical equipment consisted of a tractor to a gyro crusher. Then a disc plough was used for gyro crushing and ploughing the experimental plots. A tape measure (decameter), planting rope and string were used to measure the dimensions of the experimental plots and plant spacing hoes, machetes and bamboo were used to make mounds, cut stakes and stake yam plants respectively. A sprayer was also used to treat the plants. Data collection equipment consisted of a notepad, Pencil, eraser, digital camera and a portable tablet.

2.1.4. Products Used

Several products were used in the experiment including two biostimulants [a solution A. an essential oil (OCIBIO 5%)] and a synthetic fungicide Ivory (Mancozeb), Solution A (alkylate. sulphonate + ethoxylated fatty alcohols + alkylbenzene sulphonate and coconut diethanolamine), OCIBIO 5% (Ocimum gratissi-

mum essential oil + water + terpenes) and Ivory (Mancozeb).

2.2. Methods

2.2.1. Experimental Design

The trial was designed as a split-plot experiment with two factors. The primary factor was the hybrid clones, and the secondary factor was the treatments. A plot of 61 m long and 7 m wide was laid out and divided into three (3) replicates (blocks) separated by 2 m. *i.e.* an area of 427 m^2. Each replicate consisted of five (5) elementary plots spaced 1.5 m apart. Each elementary plot consists of two (2) rows of three (3) mounds, *i.e.* six (6) plants spaced 1m apart. This gives a total of 360 plants in the experimental plot.

2.2.2. Planting Yam Seed

Healthy tubers were used for each clone. They were cut into 300 g seedlings dipped in a fungicide-insecticide bath for 10 to 15 minutes and air-dried for 48 hours. The seedlings were planted in April in the mounds at a depth of 5 to 7 cm [15]. Straw pads were placed over the mound to prevent the sun drying out the seeds and to keep the mound moist.

2.2.3. Treatments Applied to the Plants

Treatments were applied to the aerial part at 3, 5 and 6 months after planting. *i.e.* three repeated treatments. Different treatments were applied:

- Treatment 1 (T1): control;
- Treatment 2 (T2): Solution A: 30 ml for 12 to 15 litres of water;
- Treatment 3 (T3): Ivory (mancozeb: 800 g/kg);
- Treatment 4 (T4): Essential oil (OCIBIO 5%): 1 litre for 60 litres of water.

2.2.4. Data Collection

Observations and measurements were made on the leaves and tubers of various yam plants.

2.2.5. Anthracnose Severity

Anthracnose severity was observed and recorded from one month after planting until leaf senescence. These observations were made on all plants present in each elementary plot. Disease severity refers to the degree of damage (disease symptoms) caused to the plant or part of the plant (leaf stem or tuber). Disease severity scores were assigned to each plant based on observation of the characteristic symptoms of anthracnose. This assessment was made using a scale from 1 to 5 [16]. Healthy plants had a score of 1 and diseased plants had a score ranging from 2 to 5 (**Table 1**). Estimate means anthracnose severity by summing severity scores > 1 in a plot divided by a total number of symptomatic plants.

The severity index (or Symptom Severity Index, SSI) assesses the degree of the attacks (disease). It was estimated to use the following formula:

$$\text{SSI} = \frac{\sum \text{Severity scores} > 1}{\text{Total number of symptomatic plants}}$$

Table 1. Disease symptom severity rating scale [16].

Rating score	Yam Anthracnose Disease Severity Score [scale], (YADS)
1	no visible symptoms of anthracnose disease
2	few anthracnose spots or symptoms on 1 to ~25% of the plant
3	anthracnose symptoms covering ~26 to ~50% of the plant
4	symptom on > 51% of the plant
5	severe necrosis and death of the plant

2.2.6. Yield Parameters

The yield components were measured at harvest time according to the different treatments for each variety. Table 2 shows the different parameters studied.

Table 2. Parameters studied and methods of measurement.

N°	Parameters studied	Codes	Measurement methods
1	Number of small tubers	Nstub	Counting the number of tubers weighing less than 500 g
2	Mass of small tuber (kg)	Mstub	Weigh average small tuber mass on each elementary plot using scale
3	Number of large tubers	Nltub	Counting the number of tubers weighing 500 g or more
4	Mass of large tubers (kg)	Mltub	Weighing the average mass of large tubers on each elementary plot using a scale.
5	Total Number of tubers	Ntub	Calculation of the total number of tubers produced on each elementary plot. This is the sum of the number of tubers obtained during the two harvests. Ntub = Nstub + Nltub + Nseed
6	Total mass of tubers from first and second harvests (kg)	TMtub	Calculation of the average mass of tubers on each microplot. This is the sum of the mass of tubers obtained during the two harvests TMtub = Mstub + Mltub+ Mseed

Nseed: Number of seedlings; Mseed: Mass of seedlings.

2.2.7. Yield Evaluation

The total Yield (Y) of yams was determined for each variety according to the following formula and expressed in T/ha:

$$\text{Yield} = \frac{\text{Total Weight of tubers in tonnes}}{\text{Area of the elementary plot in hectare}}$$

2.2.8. Statistical Processing of the Data

Analyses of variance (ANOVA) were carried out on the yield parameters and severity to compare the means associated with each yam variety. When there was a significant difference, Turkey's homogeneity test was used to identify homogeneous groups at the 5% level. As for the agronomic and health performance, seven (7) parameters were considered. They were analysed by Principal Component Analysis (PCA) and Ascending Hierarchical Classification (ACH). These analyses were used to group the agronomic and health parameters of the four hybrid clones

and local variety yam according to their agronomic and health performance. The analyses were carried out using Statistica 7.1 software.

3. Results

3.1. Effect of Different Bio Stimulants and Synthetic Fungicide on Anthracnose Severity in Yam Hybrid Clones and Local Yam Varieties

- **Hybrid clone CNRAiga15/00020**

The anthracnose severity index as a function of treatment for the hybrid clone CNRAiga15/00020 ranged from 2 to 2.11. Statistical analysis of treatments showed no significant difference ($p > 0.05$) between treatments for clone CNRAiga15/00020 (Figure 2(A)).

- **Hybrid clone TDa01/00012**

The anthracnose severity index for hybrid clone TDa01/00012 ranged from 1.96 to 2.11. The lowest severity indices were observed with treatments T2, T3 and T4 (with a mean of 2.01, 1.98 and 1.96 respectively). The highest severity index was observed in control T1 (with a mean of 2.11). Statistical analysis of the treatments showed a significant difference ($p < 0.05$) between treatments for the hybrid clone TDa01/00012. This resulted in two homogeneous groups. The first group includes only treatment T1, while the second group is made up of treatments T2, T3 and T4 (Figure 2(B)).

- **Local variety MA01**

The anthracnose severity index for the local variety MA01 ranged from 2.54 to 2.86. The lowest severity index was observed in treatments T2 and T4 (with a mean of 2.52 and 2.54, respectively), while the highest severity index was observed in treatment T1 (2.86). Statistical analysis of the data showed a significant difference ($p < 0.05$) between the treatments for variety MA01. These treatments were divided into three homogeneous groups. The first group included only treatment T1, the second group included treatments T2 and T4, and the third group included only treatment T3 (Figure 2(C)).

- **Hybrid clone TDa01/00002**

Figure 3(D) shows the severity of anthracnose as a function of treatments for the hybrid clone TDa01/00002. The severity index for the hybrid clone TDa01/00012 varied between treatments. Low severity was observed for the T4 treatment (with a mean of 1.96) and higher severity for the T1 control (with a control mean of 2.15). Statistical analysis of the treatments showed a significant difference ($p < 0.05$) between treatments for the hybrid clone TDa01/00012. This resulted in three homogeneous groups. The first group included only treatment T1, the second group included treatments T2 and T3, and the third group included only treatment T4.

- **Hybrid clone CNRAiga15/00028**

The results for mean anthracnose severity as a function of treatment are shown in Figure 2(E). Analysis of variance showed no significant differences ($p > 0.05$) between treatments for the hybrid clone CNRAiga15/00020.

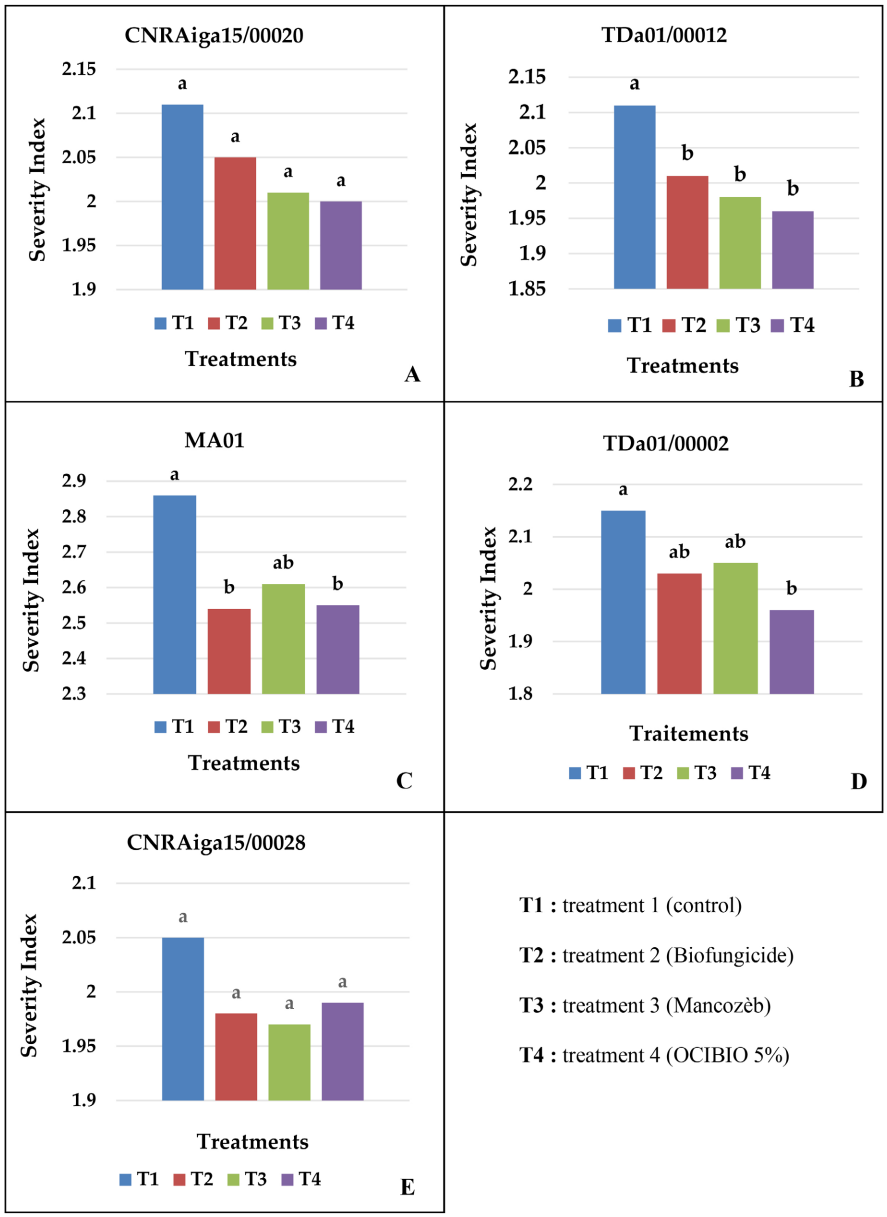

Figure 2. Severity Index of anthracnose in the different hybrids clone and the local yam variety as a function of treatments T1, T2, T3 and T4.

3.2. Effect of Different Bio Stimulants and Synthetic Fungicide on Tuber Yield of Each Hybrid Clone and Local Yam Variety

The probability associated with the effect of the treatments on the average fresh tuber yield of the varieties CNRAiga15/00020, CNRAiga15/00028, TDa01/00012 and MA01 is below the threshold probability (5%). In other words, biostimulants and synthetic fungicides have no significant effect on the average tuber yield. **Figure 4** shows the average tuber yield of CNRAiga15/00020, TDa01/00012, MA01 and CNRAiga15/00028 (**Figure 3(A)-(C)** and **Figure 3(E)**). TDa01/00002 showed a significant difference ($p < 0.05$). Three homogeneous groups were obtained. The

first group consisted of treatments T1 and T3 with an average yield of 44.17 t/ha and 40.21 t/ha, respectively. The second and third groups consisted of treatments T4 and T2, respectively. The average yield for treatment T4 was 35.21 t/ha, followed by 28.33 t/ha for treatment T2.

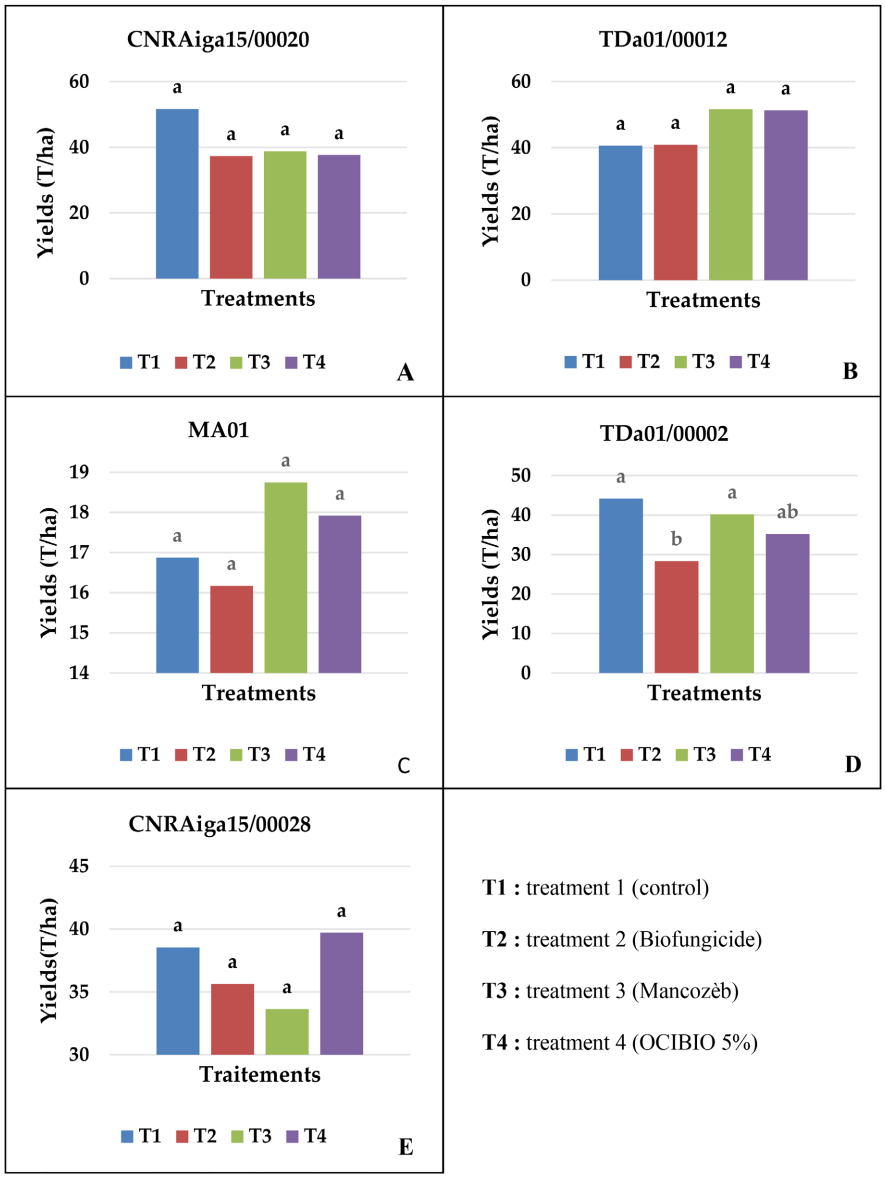

Figure 3. An average yield of fresh tubers of the yam hybrid clones and local cultivar according to the treatments.

3.3. Effects of Various Bio Stimulants and Synthetic Fungicide on Agronomic and Heath Performance

- **Hybrid clone CNRAiga15/00020**

Figure 4(A) shows the correlation circle after principal component analysis of yield parameters and anthracnose severity on variety CNRAiga15/00020. The analysis shows that yield (T/ha), Mltub and TMtub were strongly positively cor-

related with anthracnose severity. In contrast, Nltub, TNtub, Nstub and Mstub were negatively correlated with anthracnose severity on dimension 1 (factor 1). Figure 4(A) explains 93.9% of the information on the first two axes of the analysis.

- **Hybrid clone TDa01/00012**

Figure 4(B) shows the correlation circle after principal component analysis of yield parameters and anthracnose severity on variety TDa01/00012. The analysis shows that all yield parameters are strongly positively correlated with anthracnose severity on dimension 1. Figure 4(B) explains 96.26% of the information on the first two axes of the analysis.

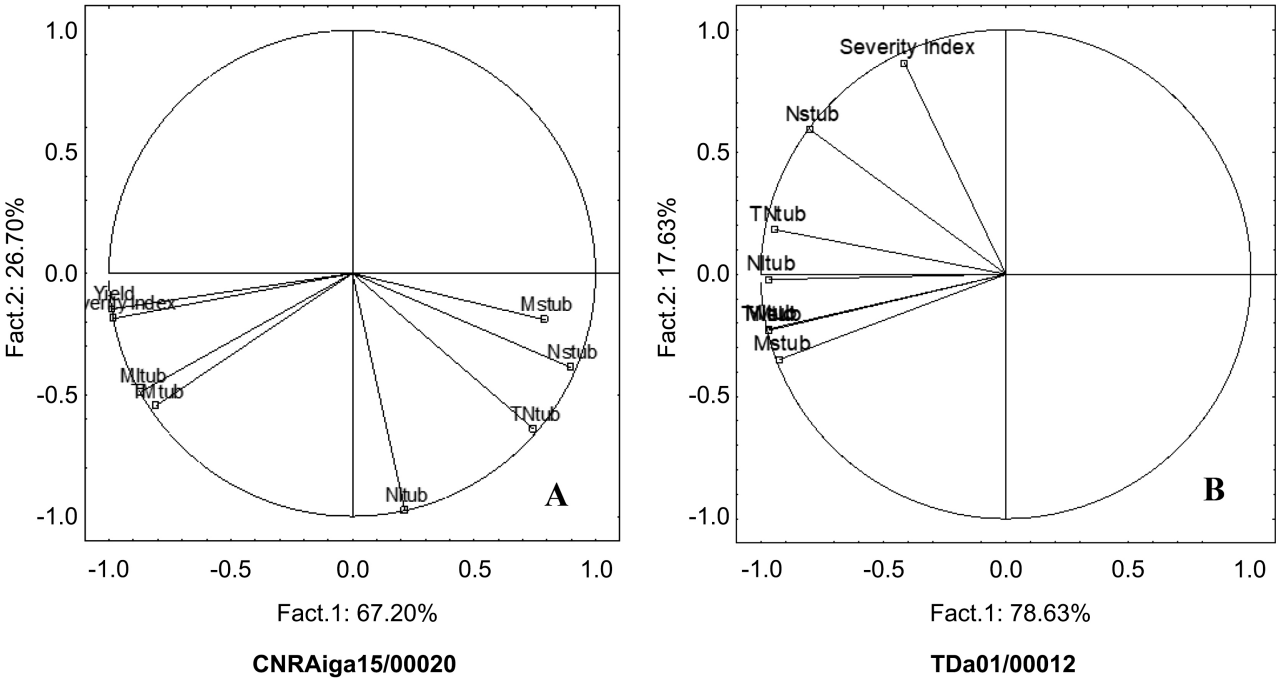

Figure 4. Correlation circle of yam hybrid clones CNRAiga15/00020 and TDa01/00012.

- **Local cultivar MA01**

Figure 5(A) shows the correlation circle after principal component analysis of yield parameters and anthracnose severity for variety MA01. The analysis shows that yield (T/ha), Nstub, TMtub, Ntub, TNtub and Mstub are strongly positively correlated with anthracnose severity. In contrast, Mltub was negatively correlated with anthracnose severity on dimension 1. Figure 5(A) explains 89.57% of the information on the first two axes of the analysis.

- **Hybrid clone TDa01/00002**

Figure 5(B) shows the correlation circle after principal component analysis of yield parameters and anthracnose severity for variety TDa01/00002. The analysis shows that yield (T/ha), Mltub, TMtub, Nltub, Ntub and TNtub are strongly positively correlated with anthracnose severity. In contrast, Nstub and Mstub are negatively correlated with anthracnose severity on dimension 1. Figure 5(B) explains 96.26% of the information on the first two axes of the analysis.

- **Hybrid clone CNRAiga15/00028**

Figure 5(C) shows the correlation circle after principal component analysis of yield parameters and anthracnose severity on variety CNRAiga15/00028. The analysis shows that all yield parameters are positively correlated with anthracnose severity on dimension 1. Figure 5(C) explains 96.26% of the information on the first two axes of the analysis.

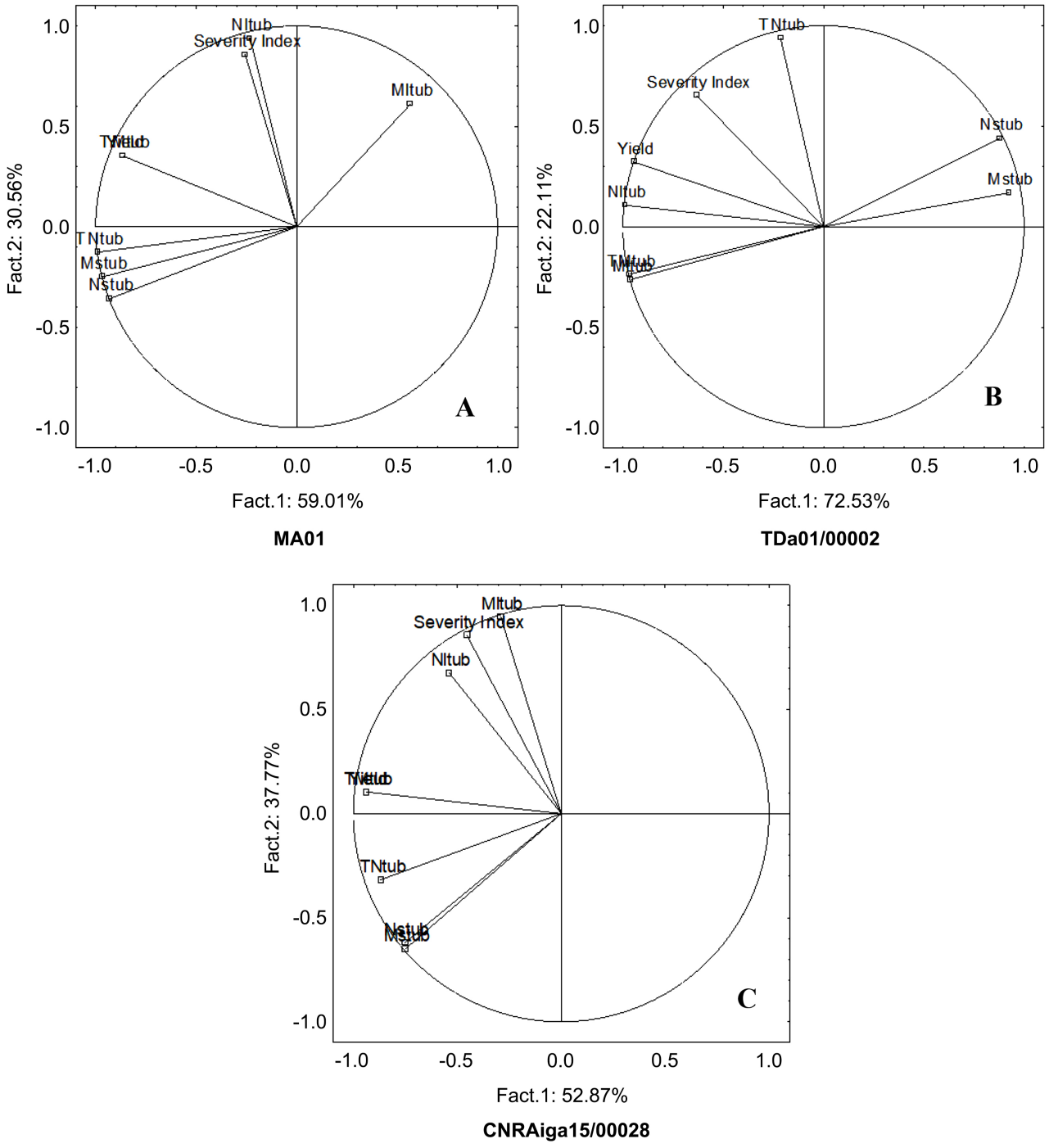

Figure 5. Correlation circle of yam local cultivar Ma01 and hybrid clones TDa01/00002 and CNRAiga15/00028.

3.3.1. Classification of Four Hybrid Clones and the Local Variety of Yams According to Their Agronomic Performance

The yam cultivars were classified into four homogeneous groups by Ascending Hierarchical Classification (AHC) based on the similarity of their agronomic performance with respect to biostimulants and fungicides. The local variety MA01 and the hybrid clones TDa01/00012 and CNRAiga15/00020 form groups 1, 2 and 4, respectively. These three varieties have very different agronomic performances. On the other hand, group 3 hybrid clones CNRAiga15/00028 and TDa01/00002 have very similar agronomic performances (Figure 6).

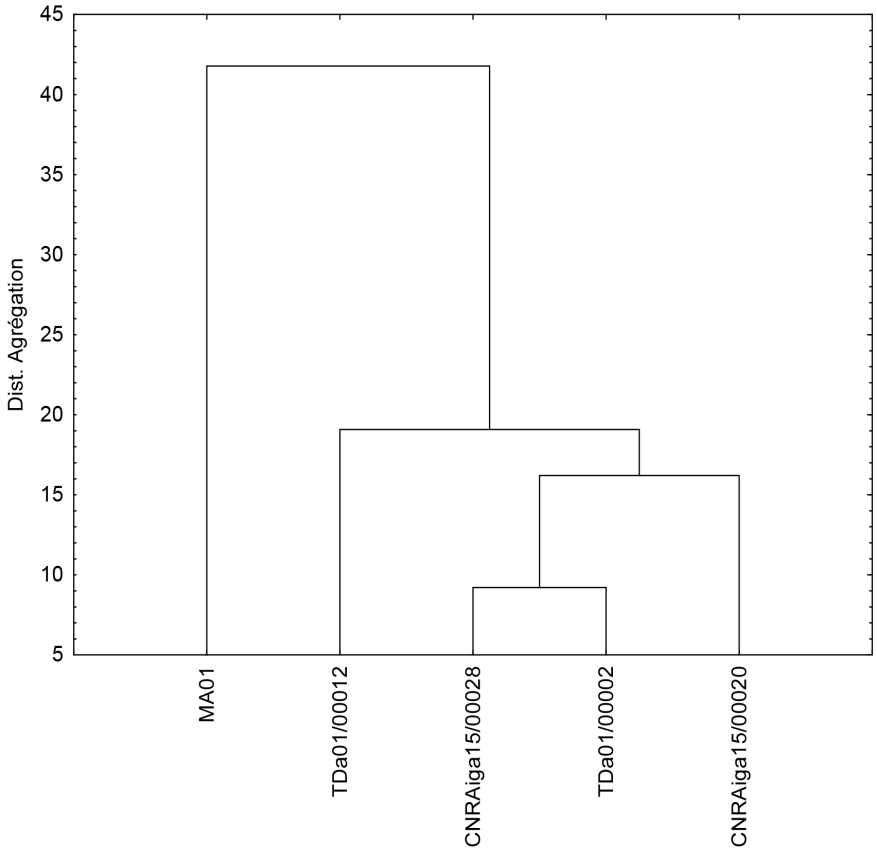

Figure 6. Hierarchical Classification of agronomic performance four hybrid clones and one local cultivar of yam.

3.3.2. Classification of Four Hybrid Clones and the Local Variety of Yams Based on Health Performance

The yam varieties were grouped into two homogeneous groups by Ascending Hierarchical Classification (AHC) based on the similarity of their severity with respect to biostimulants. Group 2 includes the yam hybrid clones CNRAiga15/00028, TDa01/00002, TDa01/00012 and CNRAiga15/00020. These varieties have very similar health performances, except for the control variety MA01, which alone forms group 1 (Figure 7).

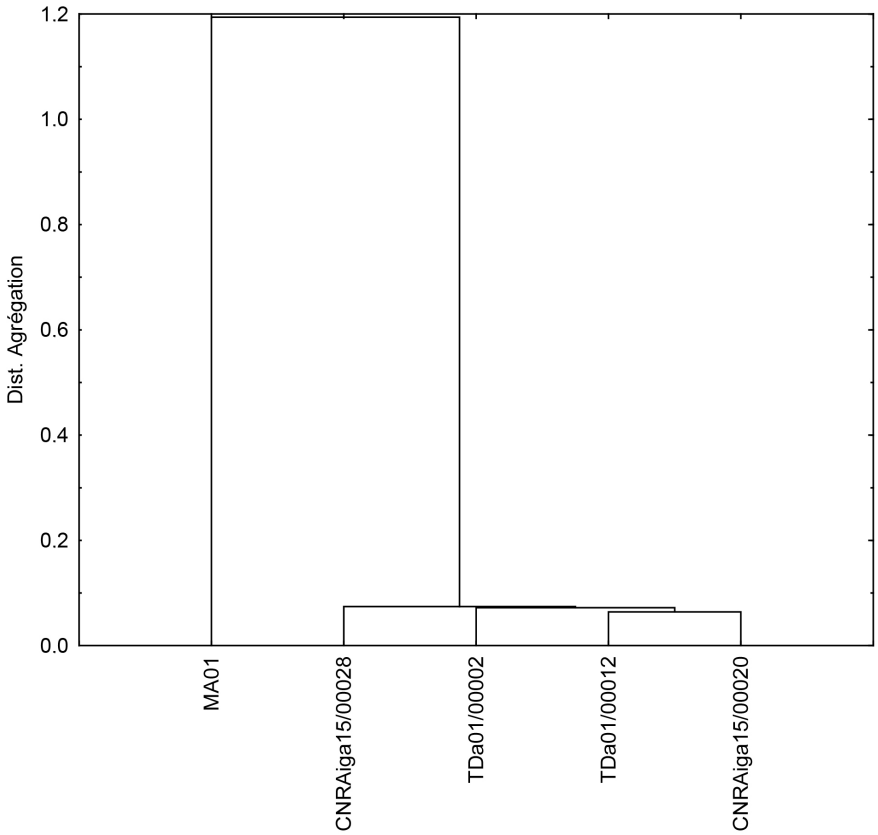

Figure 7. Hierarchical Classification of health performance of four hybrid clones and one local cultivar of yam.

4. Discussion

The symptoms observed on the different yam plants after planting could be justified by the presence of yam anthracnose. Similar symptoms were described by Degras in 1986 to indicate the presence of this disease [17]. In this study, the yams were less affected by anthracnose two months after planting. The severity of anthracnose increased until it reached its threshold in the eighth month after planting. In fact, the first symptoms of the disease were observed two months after planting, during the observations that preceded the first application of treatments. However, symptoms were observed throughout the plot starting six months after the second treatment application. Heavy rainfall was recorded in Bouaké, the center part of the country during the trial. This high level of severity can be explained by the heavy rainfall, high temperatures and humidity, which are favorable conditions for the development of the pathogenic fungus *Colletotrichum gloeosporioides*. Achar *et al.*, in 2013 also showed that one of the first and most important factors in the development of anthracnose is rain, especially heavy rainfall [18]. According to the authors, *C. gloesporioides* spores are dispersed on the stems and leaves of Dioscorea spp., by splashing during the rainy season. This allows water to penetrate the leaf stomata and carry the spores, resulting in maximum disease expression on the leaves of the clones in this case. The optimum temperature for strains of the

fungus is between 25˚C and 30˚C, as described by Bussière et Rivel in 2015 [19].

The increase in the severity of anthracnose may also be related to the use of *C. gloesporioides* contaminated plant material. Anthracnose spreads through infected tubers, and *D. alata* cultivars are more susceptible to this disease than other species, as shown by Ayisah *et al.*, in 2019 [20]. All the cultivars in this trial behaved differently in the face of infection and development of the pathogenic fungus (anthracnose). Some yams germinated late, while others did not germinate at all. This could also explain the variability in severity between varieties and treatments. The results obtained showed that the treatments had no significant effect on the hybrid clones CNRAiga15/00020 and CNRAiga15/00028. However, they did have a significant impact on the TDa01/00012 and TDa01/00002 hybrid clones, as well as on the local variety MA01.

However, the highest average severities were only observed in treatment T1 (control) for all varieties. These results could be explained by the fact that the susceptibility factors of all varieties are related to the great genetic diversity that varies from one clone to another. A difference in the virulence of the *C. gloeosporioides* strains in Bouaké and their ability to adapt could also explain the behaviour of these yams. In 2017, Yao *et al.*, showed that the virulence of the fungus, once present, is influenced not only by the microclimate of the area, but also by the preference of the plant as a preferred host [21]. In the hybrid clones CNRAiga15/00020 and CNRAiga15/00028, there was no significant difference between the different treatments. This could be explained by the fact that these clones were less sensitive to the treatments than the other varieties, since they have resistance and/or tolerance to the disease. Once the disease is detected, the plant activates a second line of defense based on the recognition of the pathogen's molecular patterns by protein receptors, triggering a series of signals that lead to the activation of defense mechanisms [22]. The different treatments applied to the hybrid clones TDa01/00012 and TDa01/00002 and to the local variety MA01 showed a significant difference. These varieties therefore needed help with disease resistance. In 2012, some phytopathologists claimed that natural defense stimulators, which are biostimulants, confer systemic and long-lasting resistance to plants. Indeed, the senescence of clones treated in the experimental field was delayed until the harvest [23].

Regarding the yield components, the treatments applied showed no significant difference in the clones studied, except for Number of small tubers (Nstub), Mass of large tubers (Mltub) and Total mass of tubers (TMtub) in TDa01/00002 and Mass of small tuber (Mstub) in CNRAiga15/00028. The yield components were not affected by the bio stimulants used. These results could be explained by the fact that the doses applied were insufficient, allowing poor uptake of mineral elements by the different varieties to increase yield parameters. These results contradict those of Agassoum *et al.* 2022, where the growth variables can be explained by a good uptake of mineral elements [24].

Fresh tuber yields varied according to the different treatments applied. They were generally high for treatments T1 (control), T3 (Mancozèb) and T4 (OCIBIO

5%), However, they were no significant difference between the treatments applied to the different varieties, except for TDa01/00002. These results could be explained by the late application of the stimulants and pesticides used, As the plant had already reached maturity, it did not have the time to absorb the molecules of the different products as it should have done to make use of all the soil resources necessary for its growth and to improve the quality and quantity of the tubers. These results contradict those of Yakhin *et al.* in 2017, who demonstrated that bio stimulants improve plant productivity because of the emergent properties caused by the complex of components [25]. The low yield of the MA01 variety could be explained by the quality of the seed used. The tubers used as seeds were stored for a long time, which contributed to their physiological and biological deterioration.

As a result, the tubers germinate in the boxes in which they are stored, using the assimilates stored in their organs. According to Cornet in 2015, this process activates metabolism and improves the tuber, reducing the autonomy of the future seedling when it is sown [5]. Yield does not depend on the bio stimulants used, but on other factors such as the environment or the heterozygosity of the varieties.

5. Conclusion

The purpose of this study was to show the effect of two bio stimulants and a synthetic fungicide on anthracnose susceptibility and yield of four hybrid clones and one local variety of yam (*Dioscorea alata*) in central Côte d'Ivoire. This study showed that anthracnose was very severe in the varieties studied. The results obtained led us to conclude that not all the objectives of our study had been achieved, as the treatments applied had no effect on the yield of certain varieties. However, all varieties were susceptible to anthracnose infection to varying degrees. Although, the severity was high in local cultivar MA01 and hybrid clone TDa01/00002, it was mild in the other varieties. All varieties had different yields at harvest after the trial. The best yields were attributed to the hybrid clones TDa01/00012 and CNRAiga15/00020. The bio-stimulants and pesticides used had different effects on the different varieties in our study. The classification in descending order of the effect of the treatments on the morphological parameters of the varieties is as follows Treatment T1 (Control) > Treatment T2 (Biofungicide) > Treatment T3 (Mancozèb) > Treatment T4 (OCIBIO 5%). Yam anthracnose is a major threat to food security in Côte d'Ivoire.

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Conflicts of Interest

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

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