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Evaluation of the Biostimulant Banzaï's Effect and the Previous Fertilizer on the Control of Cocoa Black Cherries Disease in N'Gouamoinkro, in the Department of Toumodi, Côte d'Ivoire

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Abstract The overall objective of this study was to compare the effect of the biostimulant Banzaï and fertilizer on black cherries disease. More specifically, the aim was to evaluate the effect of the number of applications of the biostimulant Banzaï and to evaluate the cumulative effect of the previous fertilizer and Banzaï. The experiment was conducted in N'Gouamoinkro in the department of Toumodi. The design consists of a Fischer block with six treatments repeated three times and each treatment contained 20 test cocoa trees. This design was replicated on two sites, one with previous fertilizer (DAE) and the other without previous fertilizer (DSE). Banzaï was applied for three or four consecutive months depending on the treatment at each site. The SUPERCAO fertilizer was applied twice during the experiment. The data collected included the total number of cherries produced and the number of black cherries. The results obtained revealed that at both sites (DAE and DSE), the plots treated with Banzaï had better control the black cherries disease than the control plots. At DAE site, the control of black cherries disease was independent of the number of Banzaï biostimulant and fertilizer applications. At the DSE site, the three applications of Banzaï combined with the SUPERCAO fertilizer had a better control effect than the three applications of Banzaï without fertilizer. The cumulative effect of the previous fertilizer, and the Banzaï, did not have a positive impact on the control of black cherries disease.

Keywords: *cocoa tree, black cherries disease, Banzaï, biostimulant, Côte d'Ivoire*

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1. Introduction

The cocoa tree (*Theobroma cacao L*) is a perennial plant native to the Amazon rainforest. It belongs to the Malvaceae family and was first introduced to West Africa in the 20th century [10]. In terms of socio-economic importance, cocoa farming in Côte d'Ivoire provides about 40% of the world supply of cocoa beans [6], and contributes 15% of the Ivorian gross domestic product (GDP) with an annual production of 1 600 000 tons [15]. In spite of this socio-economic importance, cocoa farming is facing a significant decline in production due to several constraints [12]. These constraints include factors related to market prices and the lack of soil fertilization [8] and related to pests and diseases like black cherries diseases. This disease attacks both the cherries and the cocoa pods, which can cause significant damage [9]. In Côte d'Ivoire, the damage caused by this disease is estimated at 40-80% crop losses depending on the species [13] and on the most favourable areas [12]. Black pod or black cherries is a fungal disease caused by *Phytophthora sp* which is one of the most important bio-aggressors in terms of damage [9]. *Phytophthora sp* is represented by two major species in Côte d'Ivoire like *Phytophthora palmivora* and *Phytophthora megakarya* [11]. These two *Phytophthora* species cause pods and cherries to rotting (Figure 1),

thereby reducing cocoa production in Côte d'Ivoire [12]. The control of these pathogens has long been based on the use of fungicides [3]. The misuse of these chemicals therefore causes resistance in plant pathogenic fungi, thus leading to the recrudescence of the disease [3]. In addition, these chemicals have a negative impact on the environment, soil and human health. To counter this, it was therefore important to direct the fight against *Phytophthora sp* towards sustainable alternative solutions [14]. As part of these solutions, there are biostimulants that can provide often innovative solutions in the field of fertilization and crop protection [4]. Indeed, biostimulants include a wide range of products and substances capable of improving the functioning of the soil, the plant and the interactions between soil and plant [5]. However, the effectiveness of biostimulants in crop protection is still poorly known, because crop conditions (crop varieties, stage of plant development and environmental conditions) are poorly controlled [5]. In addition, the efficacy of biostimulants compared to the efficacy of chemical fertilizers is limited, because the effect of chemical fertilizers depends largely on growing conditions, rainfall and sunshine [1]. Unlike biostimulants, the effect of chemical fertilizers is retrospective and not immediate [5]. Biostimulants are composed of living microorganisms, natural substances or substances of natural origin, or mineral extracts that are capable of improving the productivity of cocoa farming [4]. Indeed, Banzaï is one of these biostimulants that acts on the cherries of cocoa tree by promoting flowering and limiting the fall of flowers. The biostimulant Banzaï has a great capacity to stimulate the vigor of pods against diseases and external stresses [2]. Since the using of Banzaï, very few tests on its efficacy against cocoa tree diseases have been carried out. This justifies this study whose main objective is to evaluate the effect of the biostimulant Banzaï and fertilizer on the control of cocoa black cherries disease. Specifically, this study was focused on:

- Evaluating the effect of the number of applications of the biostimulant Banzaï on the control of black cherries disease

- Evaluating the cumulative effect of the previous fertilizer and Banzaï on the control of black cherries disease.

2. Material and Methods

2.1. Location of the Study Area

The experiment took place at N'Gouamoinkro site (5°00′00″ North, 6°15′ 00″Ouest) in the department of Toumodi (Figure 2). This area is characterized by a humid tropical climate with an average rainfall of between 1 000 and 1 200 mm per year, and an average temperature of 30°C per year [16]. The zone of Toumodi is marked by two rainy seasons (March-June and October-November) and two dry seasons (November-February and July-September) [16]. Vegetation in the study area is characterized by open forests, ferrallitic and clay-humus or hydromorphic soils adapted to all types of food and industrial crops.

2.2. Experimental Device

The experimental device (Figure 3) consisted of a Fisher block with six treatments or elementary plots (T01, T02, T1, T2, T3 and T4) randomized and repeated three times on two sites. One site with no previous use of any fertilizers (DSE) and another site with previous any fertilizer use (DAE). The DAE site is a plantation that received fertilizer during the last three years before the trial was set up. The DSE site is a cocoa field that has not received any fertilizer in the last three years. However, during the experiment the SUPERCAO fertilizer was used. Each elementary plot contains twenty test cocoa trees. T01 is the control without application of Banzaï but with SUPERCAO, T02 is the control without application of Banzaï without SUPERCAO, T1 is a plot consisting of three applications of Banzaï with SUPERCAO, T2 is four applications of Banzaï without SUPERCAO, T3 is three applications of Banzaï without SUPERCAO and T4 is four applications of Banzaï with SUPERCAO (Table 1).

Figure 1. Symptoms of black cherries disease (Photo taken by Koffi Alain)

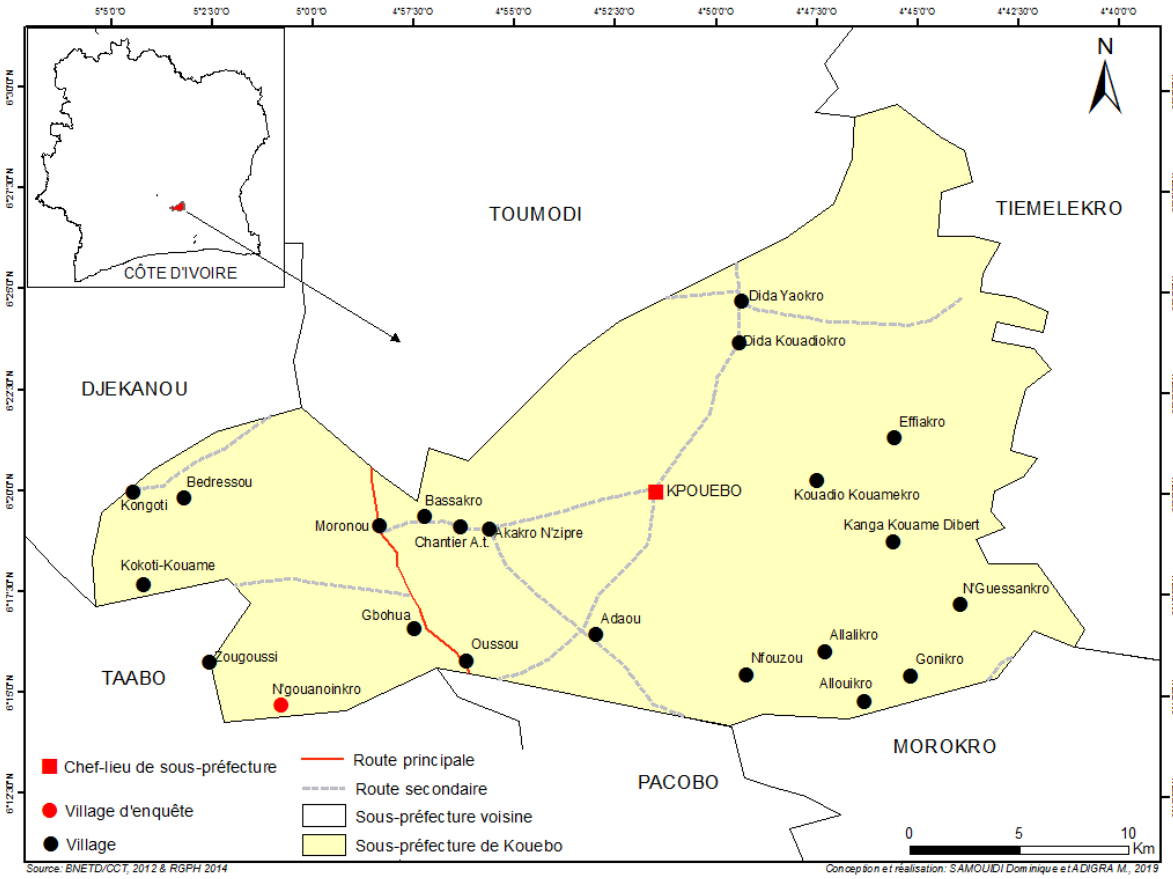
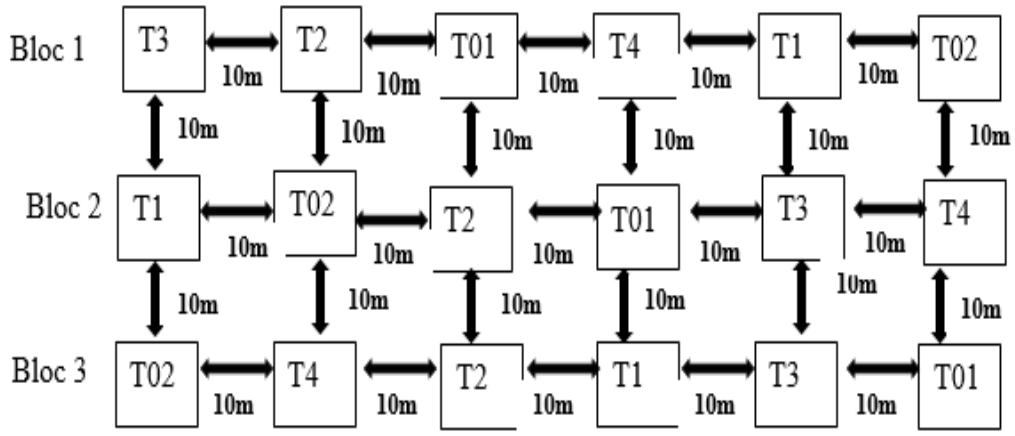


Figure 2. Map of the sub-prefecture of Kpouèbo showing N'Gouamoinkro, experimental site



T01: Control without application of Banzaï with SUPERCAO
T02: Control without application of Banzai without SUPERCAO
T1: 3 applications of Banzaï with SUPERCAO
T2: 4 applications of Banzaï without SUPERCAO
T3: 3 applications of Banzaï without SUPERCAO
T4: 4 applications of Banzaï with SUPERCAO

Figure 3. Experimental device for the study of the effect of the biostimulant Banzaï on the control of rot cherries

Table 1. Number of applications of SUPERCAO and Banzaï

Treatments	SUPERCAO	Banzaï
T01	+ +	-
T02	-	-
T1	+ +	+ + +
T2	-	+ + + +
T3	-	+ + +
T4	+ +	+ + + +

SUPERCAO Fertilizer: (+) one application, (-) no application
Banzaï: (+) one application, (-) no application.

2.3. Conduct of Trial Set

The implementation of the test consisted in identifying the experimental sites. In the system set up on each of the two sites, the blocks are about 1 800 m^2 in area each, i.e. 300 m^2 for each elementary plot. The overall system is about 12 800 m^2 in size. The experimental blocks were delimited using a decameter. In each elementary plot, the selected test trees are marked and numbered from 1 to 20. The identification and marking of the cocoa

trees were followed by a sanitary harvest which consists to remove the cherries with disease's symptoms. In addition, each tree selected is assigned a specific label coded according to the site (DAE/DSE), the number of the block, treatment or elementary plot, and the number of the tree.

2.4. Application of the Biostimulant Banzaï and the Fertilizer SUPERCAO

Banzaï biostimulant was applied to the elementary plots (T1, T2, T3 and T4) at both sites using a sprayer. It was applied for three or four consecutive months depending on the type of treatment. The different doses of each treatment were deducted from the initial dose (800ml/ha), i.e. 144 ml of Banzaï diluted in 16 L of water. SUPERCAO (NPK 0 - 23 -19 + 1 Ca + 5 Mg + 6.5 S) fertilizer was applied twice (July and August) during the study in the T01, T1 and T4 plots. The fertilizer was applied at a dose of 150 g per tree within a radius of 30 cm around each test tree.

2.5. Observations and Data Collection

Observations were made on each test tree within a height limit of 0 to 2.5 m above the ground. The data collection covered two parameters including the total number of cherries produced and the total number of black cherries. A cherry is an immature pod less than 6 cm in length. Each counted cherry was tied with a string at the stalk to facilitate the counting of new cherries produced by the test cocoa tree. The observation of the cherries was carried out every month for nine months. From these data, the rate of healthy cherries was deduced.

2.6. Statistical Analysis of the Data

The statistical analysis focused on the rate of healthy cherries, which represents the rate of disease control. The rate of healthy cherries is the proportion of healthy cherries in relation to produced cherries (Equation 1). The descriptive analysis consisted in representing the dynamics of the evolution of the healthy cherries rate per treatment and per observation period for the two sites using the Excel 2013 spreadsheet.

$$TCP = \frac{NCP - NTCP}{NCP} \quad (1)$$

TCP: Healthy cherries Rate
NCP: Number of produced cherries
NTCP: Number of black cherries.

Inferential analysis was applied on the rate of healthy cherries to compare the different treatments. Thus, the boxplots were first carried out to compare the distribution of the rate of healthy cherries per treatment. Then, the Kruskal-Wallis statistical test was applied to evaluate the differences observed between the treatments and the control and between the treatments themselves. This test was also used to compare treatments within and between sites.

3. Results

3.1. Effect of the Number of Applications of Banzaï and SUPERCAO Fertilizer on the Rate of Healthy Cherries for the Site with Previous Fertilizer (DAE)

Figure 4 shows the monthly change in the rate of control of rot of cherries per treatment. In general, the rates of healthy cherries were higher in the treated plots than in the control plots, with respective mean rates of 73% versus 58% (Table 2). In particular, treatments T1, T2 and T4 had higher rates of healthy cherries than the other treatments, while control T01 had the lowest rate of healthy cherries. The highest rates of healthy cherries were recorded in August, December and March.

The boxplots (Figure 5) show three evolutionary trends including the T01 control, the T02 control and all the treated plots with respective medians around 55%, 65% and 75%. At the level of statistical analysis, the Kruskal-Wallis test showed a significant difference ($p=0.00<0.05$) between the rates of healthy cherries in the treated plots and the control plots (T01 and T02). This test also showed that there was no significant difference between the rates of healthy cherries in the treated plots themselves. The classification resulting from the Kruskall-Wallis test made it possible to group the treatments into three (03) descending classes: A, B and C (Table 3). Class A, which includes treatments T1 (76%), T2 (76%), T3 (75%) and T4 (69%), has the highest rate of healthy cherries while class C represented by the control T01 (51%) has the lowest rate of healthy cherries (Table 3). Through these two classes A and C, was class B which contains only the T02 treatment with a rate of healthy cherries of 65%.

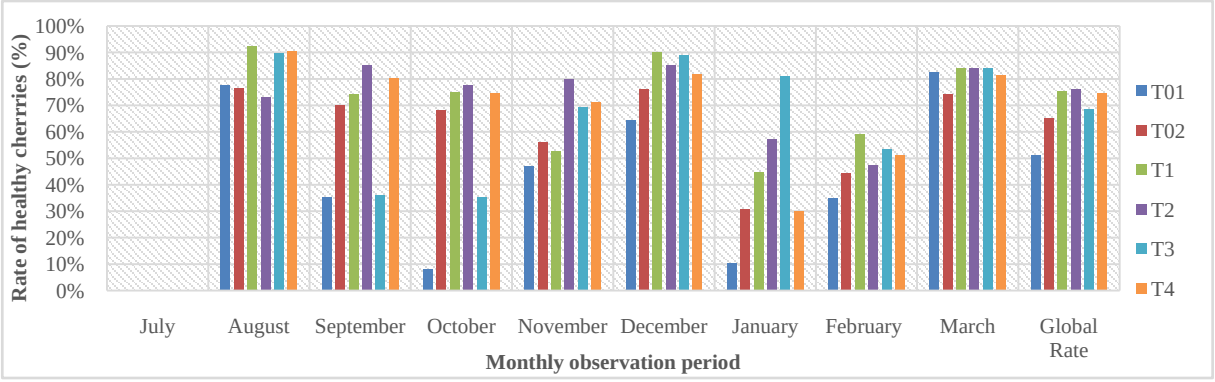
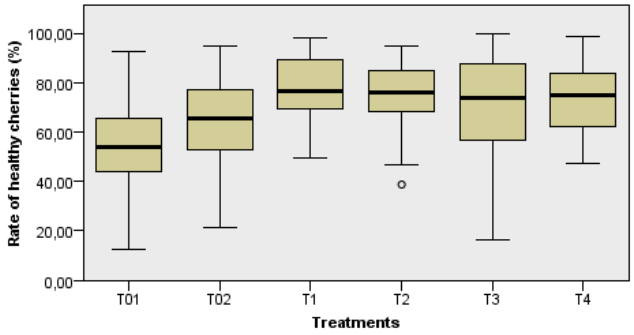


Figure 4. Evolution of the rates of healthy cherries for the site with previous fertilizer (DAE)

Table 2. Rate of healthy cherries for the site with previous fertilizer (DAE)

Treatments	Number of produced cherries	Number of black cherries	Number of healthy cherries	Rate of healthy cherries
T01	4706	2288	2418	51%
T02	6431	2245	4186	65%
T1	8346	2039	6117	76%
T2	8125	1932	5927	76%
T3	7645	2384	5261	69%
T4	7011	1780	5231	75%
Total	42264	12668	29140	70%

The significant difference observed between the treated and control plots showed that the biostimulant Banzaï had a stimulating effect on the control of black cherries. The lack of significant difference between treated plots itself indicates that the three applications of Banzaï had the same effect as the four applications on the control of black cherries. The significant difference between the controls in favor of T02, which had not received fertilizer, also shows that the SUPERCAO fertilizer applied during the experiment had no effect on the control of black cherries disease.



N Total	360
Statistique de test	68,638
Degrés de liberté	5
Sig. asymptotique (test bilatéral)	,000

Figure 5. Boxplots representing the rates of healthy cherries per treatment (DAE)

Table 3. Classification of treatments according to the rate of healthy cherries for the site with previous fertilizer (DAE)

Treatments	Rate of control	Groups
T1	76%	**A**
T2	76%	**A**
T4	75%	**A**
T3	69%	**A**
T02	65%	**B**
T01	51%	**C**

3.2. Effect of the Number of Applications of Banzaï and SUPERCAO Fertilizer on the Rate of Healthy Cherries for the Unprecedented Fertilizer Site (DSE)

Figure 6 shows the monthly change in the rate of healthy cherries per treatment for the unprecedented fertilizer site. Healthy cherries rates are highest in August, November, December and March. In general, during the nine months of observation, the rates of healthy cherries in the treated plots are higher than the rates of healthy cherries in the control plots except for the T3 treatment. In particular, the T2 treatment recorded the highest rate of healthy cherries with 86%, followed by T1 with 76%, T4 with 72%, T02 with 72% and T3 with 66% (Table 4). The difference between the rates of healthy cherries of the treated plots and the rates of healthy cherries of the control plots remains small. However, the control T01 recorded the lowest rate of healthy cherries with 64%.

The boxplots show the graphical representation of the rates of healthy cherries by treatments (Figure 7). They allow three trends to be distinguished. One trend includes the T2 treatment with the highest rate of healthy cherries around 85%, a second trend including the T1 treatment with a median rate of 75%, and finally the third trend which includes the T3, T4 and control treatments with a median rate around 70%. The result of the Kruskal-Wallis test showed a significant difference ($p=0.00<0.05$) between plots. Indeed, treated plots T1 and T2 had significantly higher rates of healthy cherries than the rates of healthy cherries in plots T3, T4, T01 and T02. The classification of the treatments allowed them to be grouped into three (03) descending classes A, B and C (Table 5). Treatment T2 is class A, which has the highest rate of healthy cherries at 86%. The T1 treatment is class B with a 76% rate of healthy cherries and class C which includes treatments T4 (72%), T3 (66%) and controls T01 (64%) and T02 (72%).

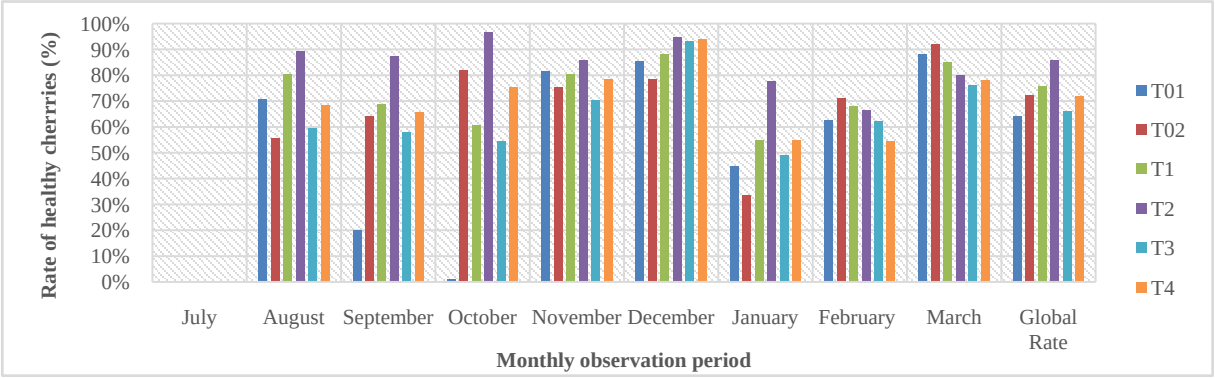
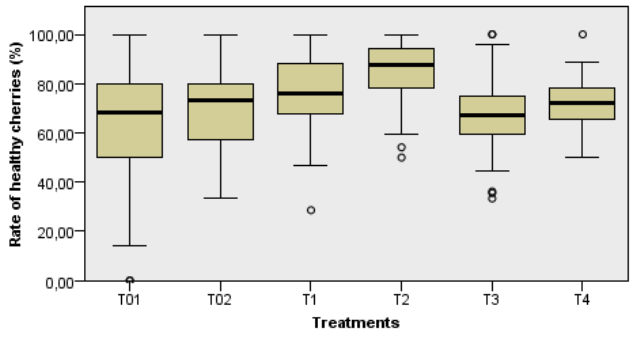


Figure 6. Evolution of the rates of healthy cherries for the site with previous fertilizer (DSE)



N Total	360
Statistique de test	60,621
Degrés de liberté	5
Sig. asymptotique (test bilatéral)	,000

Figure 7. Boxplots representing healthy rates for the site with previous fertilizer (DSE)

Table 4. Rate of healthy cherries for the site with previous fertilizer (DSE)

Treatments	Number of produced cherries	Number of black cherries	Number of healthy cherries	Rate of healthy cherries
T01	2201	796	1405	64%
T02	2162	604	1558	72%
T1	3112	762	2350	76%
T2	2854	411	2443	86%
T3	3057	1037	2020	66%
T4	3638	1030	2608	72%
Total	17024	4640	12384	73%

Table 5. Classification of treatments according to the rates of healthy cherries for the site with previous fertilizer (DSE)

Treatments	Global rate	Groups
T2	86%	**A**
T1	76%	**B**
T4	72%	**C**
T02	72%	**C**
T3	66%	**C**
T01	64%	**C**

In the case of the effect of Banzaï and fertilizer on the control of cherries, three applications of Banzaï with fertilizer optimize the control of cherries unlike three applications without fertilizer. On the other hand, when four applications of Banzaï are applied, the combination with fertilizer no longer has its effect.

In the absence of Banzaï, SUPERCAO fertilizer has no effect on the control of black cherries disease.

3.3. Cumulative Effect of Banzaï and the Previous Crop on the Control of Black Cherries Disease

Figure 8 and Table 6 represent the comparison of the rates of healthy cherries from the two sites (DAE / DSE) by treatment over the observation period. In general, the rates of healthy cherries at DSE site remain higher than those observed for DAE site regardless of treatment except for treatments T3 and T4. However, the rates of healthy cherries in the control plots remain lower than those in the treated plots at both DAE and DSE sites.

Figure 9 shows the boxplots of healthy cherries rates by treatment at both sites. Three trends emerge from it. The first is the trend consisting of the T2 DSE treatment which has the highest median rate of healthy cherries, followed by the trend which contains all DAE treated plots, the T1, T3 and T4 DSE plots and DSE controls with a median rate close to 75%. Finally, the trend containing DAE treatments T01 and T02 with the lowest median rate of healthy cherries close to 65%. In addition, the Kruskal-Wallis test result shows a significant difference ($p=0.00<0.05$) between the rates of healthy cherries of the treatments at the two sites. The classification of the treatments according to their efficacy made it possible to group the treatments into five descending classes (A, B, BC, C and D) (Table 7). The T2 treatment of DSE corresponds to class A, while the T4, T1, T2 of DAE and T1 of DSE are class B treatments. The BC class, which includes T4, T02 of DSE and T3 of DAE, remains an intermediate class between class B and class C. Class C includes T3, T01 of DSE and T02 of DAE. The last class is class D, which corresponds to T01 OF DAE.

The significant difference observed in favor of the T2 DSE site shows that the previous fertilizer did not have a decisive effect on the control of DAE, even though some treatments of DAE site had relatively higher rates of healthy cherries than some treatments of DSE site.

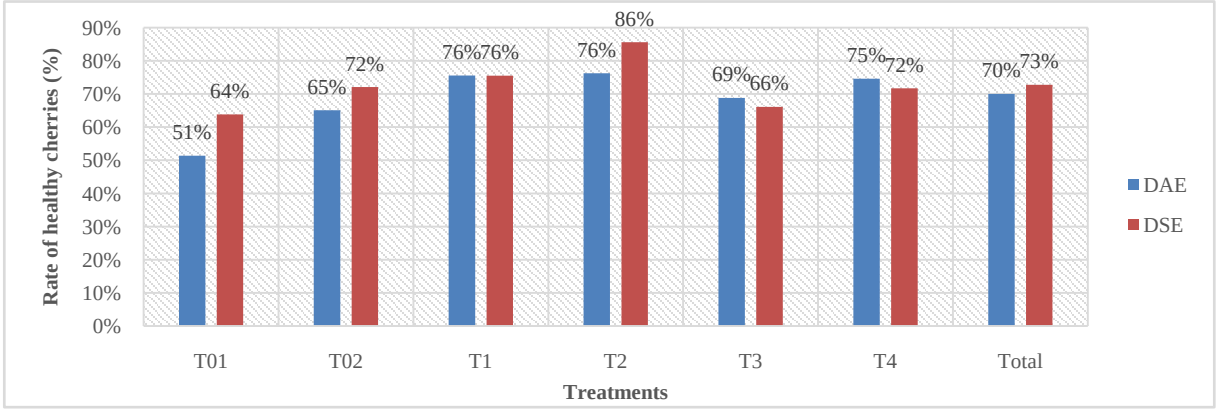
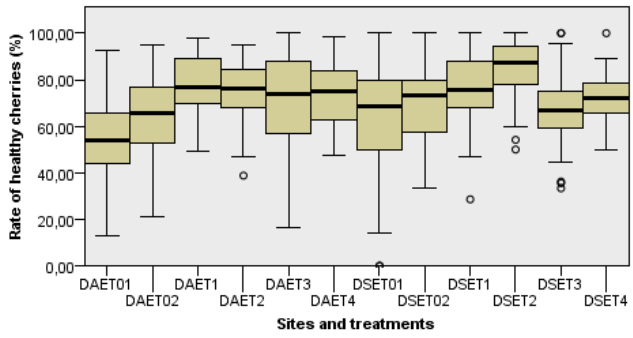


Figure 8. Evolution in the rates of control of cherries by treatment and by device

Table 6. Comparison of the healthy cherries rates by treatment and by site

Treatments	T01	T02	T1	T2	T3	T4	Global rate
Control rate of DAE	51%	65%	76%	76%	69%	75%	70%
Control rate of DSE	64%	72%	76%	86%	66%	72%	73%



N Total	720
Statistique de test	131,713
Degrés de liberté	11
Sig. asymptotique (test bilatéral)	,000

Figure 9. Boxplots comparing median treatments of DAE and DSE devices as a function of the rate of control cherries rot

Table 7. Classification of treatments by rate of control per tree

Treatments	Global rate	Groups
DSE T2	86%	**A**
DAE T1	76%	**B**
DSE T1	76%	**B**
DAE T2	76%	**B**
DAE T4	75%	**B**
DSE T4	72%	**BC**
DSE T02	72%	**BC**
DAE T3	69%	**BC**
DSE T3	66%	**C**
DAE T02	65%	**C**
DSE T01	64%	**C**
DAE T01	51%	**D**

4. Discussion

4.1. Effect of the Biostimulant Banzaï on the Control of Black Cherries Disease

The results of the statistical test showed that treated plots had better control of black cherries disease than control plots on the previous fertilizer site (DAE). Unlike DAE site, the test also showed that at DSE site, only the T1 and T2 treatments had better control. In addition, these results also show that the three applications of Banzaï with SUPERCAO–fertilizer had as much effect as the four applications for a site without previous fertilizer. For a site with previous fertilizer, there is no difference between the three and four applications of the Banzaï biostimulant. This shows that the Banzaï had a positive impact on the control of black cherries disease. These results are in agreement with those of Callivoire [2], who demonstrated that Banzaï biostimulant, applied four months in succession, limited the decline of cherries due to diseases and external stresses [7].

The slight difference in the effect of the Banzaï biostimulant on the control of black cherries disease for a site without previous fertilizer unlike DAE site where this difference is more marked shows that the previous crop could play a decisive role in the antifungal action of this product. This assertion is in line with the statement made by the authors [5] who stated that the efficacy of biostimulants depends on several factors, which are still little known or poorly controlled, such as the previous crop, crop varieties, the stage of plant development and environmental conditions [5].

4.2. Cumulative Effect of Fertilizer and Banzaï on the Control of Cherries Rot

Regarding the cumulative effect of SUPERCAO fertilizer in Banzaï applications on the control of black cherries disease, the statistical test showed that the fertilizer used during the experiment had little effect both on the site with previous fertilizer (DAE) and on the site without previous fertilizer (DSE). The low antifungal impact of the Banzaï biostimulant cumulated with the SUPERCAO fertilizer is due to the retrospective effect of the fertilizer [1]. Indeed, the fertilizer must take some time to be assimilated by cocoa trees. Furthermore, the effect of fertilizer depends largely on the growing conditions, rainfall and sunshine.

5. Conclusion

At the end of this study, it appears that the biostimulant Banzaï was effective both on the site with previous fertilizer (DAE) and on the site without previous fertilizer (DSE) on the control of the black cherry disease in reference to the control plots. Specifically, at the previous fertilizer site (DAE), the control of the black cherries is independent of the number of applications of Banzaï and the fertilizer application. At the unprecedented fertilizer site (DSE), the three applications of Banzaï combined with SUPERCAO fertilizer during the experiment were better than three applications of Banzaï without fertilizer. However, from four applications of Banzaï, the effect of the fertilizer was not significant. Moreover, SUPERCAO fertilizer alone did not have a positive impact on the control of black cherries disease. The cumulative effect of the previous fertilizer, and Banzaï, did not have a positive impact on the control of black cherries disease.

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