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Antifungal effect of the cumulative application of biostimulant and fertilizers on young cocoa fruits rot at Tafissou site, Centre-East of Côte d'Ivoire

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ABSTRACT

The use of biostimulants as an alternative way to chemical, often toxic, remains one of the best approaches to control cocoa black pod disease caused by *Phytophthora spp.* This study has been carried out to evaluate the effect of biostimulant's applications number and the cumulative action in case of applying previously fertilizers. The experimental design consisted of a Fischer block with six treatments (T01, T02, T1, T2, T3 and T4) and repeated three times. This design was replicated on two sites, one with previous fertilizer (DAE) and the other without previous fertilizer (DSE). Observations were made monthly on each test tree. The biostimulant Banzai was applied for 3 or 4 consecutive months depending on the treatment at each site. The data collected included the total number of cherelles produced and the number of rotten cherelles. From these, data were deduced the rates of healthy cherelles on which the Kruskal-Wallis test was applied to compare treatments between them and between sites. The results showed that four applications of the biostimulant with fertilizer provided better control than three applications without fertilizer. The results also showed that the majority of treatments at the DAE site were more effective than treatments at the DSE site. In conclusion, four applications Banzai coupled with fertilizer have achieved the best rates of healthy cherelles whatever the site. Regarding the cumulative effect of the previous fertilizer with Banzai, treatments of the site with the previous fertilizer were still better than the treatment site without fertilizer.

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Keywords: Cocoa, Antifungal effect, black pod disease, Banzai Biostimulant, Côte d'Ivoire.

INTRODUCTION

Cocoa tree is a perennial crop native from tropical rainforests of Central America (Janny et al., 2003). It belongs to the family of

Malvaceae, and the genus of *Theobroma* (Motamayor et al., 2002). Cocoa is a strategic product for Côte d'Ivoire economy as it accounts for 30% of export earnings and 15%

of the gross domestic product (CCC, 2015). Much of this production comes from small farms and is the unique source of income for some 5.5 million farming families (Koua, 2018). However, cocoa farming in Côte d'Ivoire is facing to several difficulties due to pests and diseases (N'guessan, 2016) including black pod disease and cocoa swollen shoot disease (Kouakou et al., 2011). Black pod disease is a fungal disease caused by *Phytophthora spp*, of which two species (*Phytophthora palmivora* and *Phytophthora megakarya*) are well known in Côte d'Ivoire cocoa growing areas (Akrofi, 2015; Coulibaly, 2015). These two fungal species cause rotting of cherelles and pods and cause significant yield losses (Kébé et al., 2009).

In Côte d'Ivoire, production losses due to this disease vary from 20 to 45%, or even 60% in some regions if no phytosanitary protection is provided (CNRA, 2017). In the view of this situation, each year a range of fungicides is used to control black pod in cocoa trees. These products, although often effective, are most of the time very expensive and poorly applied by producers (Mahob et al., 2014). This has as consequence in recrudescence of the disease and the impoverishment of the soil, hence the use of alternative products including biostimulants. Biostimulants are products that stimulate plant vitality (Oro et al., 2019). These biostimulants sometimes play the role of biocontrol and stimulators of natural defences depending on their composition (Faessel et al., 2014). According to some studies, the algae extracts contained in biostimulants give plants a strong resistance to cold and drought (Mooney and Van Staden, 1985). These algal extracts thus give plants increased resistance to fungal diseases, insect attacks and nematode infestation of roots (Wu et al., 1997). A biostimulant based on algal extracts is capable of controlling pathogens. Indeed, bacterial biostimulants enhance the defensive capacities of plants against diseases (Van Loon et al., 1998). The advantage of biostimulants is that they are less expensive, environmentally friendly and less hazardous to health (Faessel et al., 2016). The biostimulant Banzai is a product that improves the growth and yield of

cherelles and pods (Oro et al., 2019). It stimulates the vigour of cherelles and pods against diseases and external stresses (Callivoire, 2016). However, there is less information about the role of biostimulants in resistance to biotic stresses (Desfontaines et al., 2018). In recent years a few experiments have attempted to demonstrate this (Faessel et al., 2016; Yakhin, 2017). It is in this context that this study was being carried out to evaluate the antifungal effect of the biostimulant Banzai and fertilizer on the rot of cherelles. Specifics objectives are to:

- Evaluate the effect of the number of biostimulant Banzai applications according to the different doses on the control of cherelles rot.
- Evaluate the cumulative effect of the previous fertilizer and the different doses of Banzai on the control of cherelles rot.

MATERIALS AND METHODS

Study Area

The study took place in the locality of Tafissou (Figure 1), located in the department of Djekanou (6°48'61.4 "N 5°11'110.6 "W) in central-eastern Côte d'Ivoire. The climate in this zone is classified into four seasons: a dry season which is between November and February, a large rainy season which starts in March and ends in July with rainfall of about 1,200 mm per year, a small dry season from August to September and a small rainy season from September to October. The climate is also characterized by a relative humidity of 80% (Yao, 2007).

Experimental design and treatments

The experimental design consists of a completely randomized Fischer block with six treatments (T01, T02, T1, T2, T3 and T4) repeated three times (Figure 3). Each treatment contains twenty (20) cocoa trees. T01 is the control without Banzai application but with fertilizer, T02 is the control without Banzai application and without fertilizer, T1 is a plot consisting of three applications of Banzai with fertilizer, T2 is four applications of Banzai without fertilizer, T3 is three applications of

Banzai without fertilizer and T4 is four applications of Banzai with fertilizer (Table 1).

In the study area, the experimental design was installed on two sites: one site with previous non-fertilizer application (DSE) and another site with previous fertilizer application (DAE). The Fertilizer Precedent Site (DAE) was a farm that had received fertilizer during the last three years prior to the trial set-up. The No Previous Fertilizer (DSE) system is a field that has not been fertilized in the last three years.

Trials experimentation on sites

Setting up the test consisted of a prospective survey that identified appropriate experimental sites and plots for study. The test plots were selected taking into account their cultural background and the age of the test plants. The systems set up on each of the experimental sites consisted of blocks of approximately 1 800 m² each, i.e. 300 m² for each elementary plot. The experimental blocks were delimited using a decameter and the boundaries were marked by posts. In each elementary plot, the selected trees were numbered from 1 to 20. The identification and marking of the test cocoa trees were followed by a sanitary harvest by removing all already rotten chérelles (Figure 2).

Application of Banzai biostimulant and SUPERCAO fertilizer

Banzai was applied to the basic plots or treatments (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 deduced from the initial dose (800 ml/ha), i.e. 144 ml of Banzai diluted in 16 L of water. SUPERCAO fertilizer was applied twice (July and August) during the study in the T01, T1 and T4 elementary plots. The fertilizer was applied at a dose of 150 g per tree within a radius of 30 cm around each test tree.

Data collection

Observations were made following each test tree up to 2.5 m above the ground. The data collection covered two parameters including the total number of cherelles produced and the total number of rotten cherelles. A cherelle is a cocoa young fruit which size is less than 6 cm. Each counted cherelle was tied with a string at the stalk to facilitate the counting of new cherelles produced by the test cocoa tree. The observation of the cherelles was carried out every month for nine months (June to March). From these data, the rate of healthy cherelles was deduced.

Statistical analysis of the data.

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

Equation 1:

$$HCR = \frac{TNCP - NRC}{TNCP}$$

HCR: Healthy cherelles Rate

NRC: Number of rotten cherelles

TNCP: Total number of cherelles produced.

Inferential analysis was used to compare the average rates of healthy cherelles per treatment. Thus, the boxplots were first performed to compare the distribution of the rate of healthy cherelles per treatment. Then, the Kruskal-Wallis statistical test was applied to evaluate the observed differences between the treatments and the control and between the treatments themselves. This test was also used to compare treatments within and between sites. Finally, a classification was performed to prioritize the treatments according to their effectiveness.

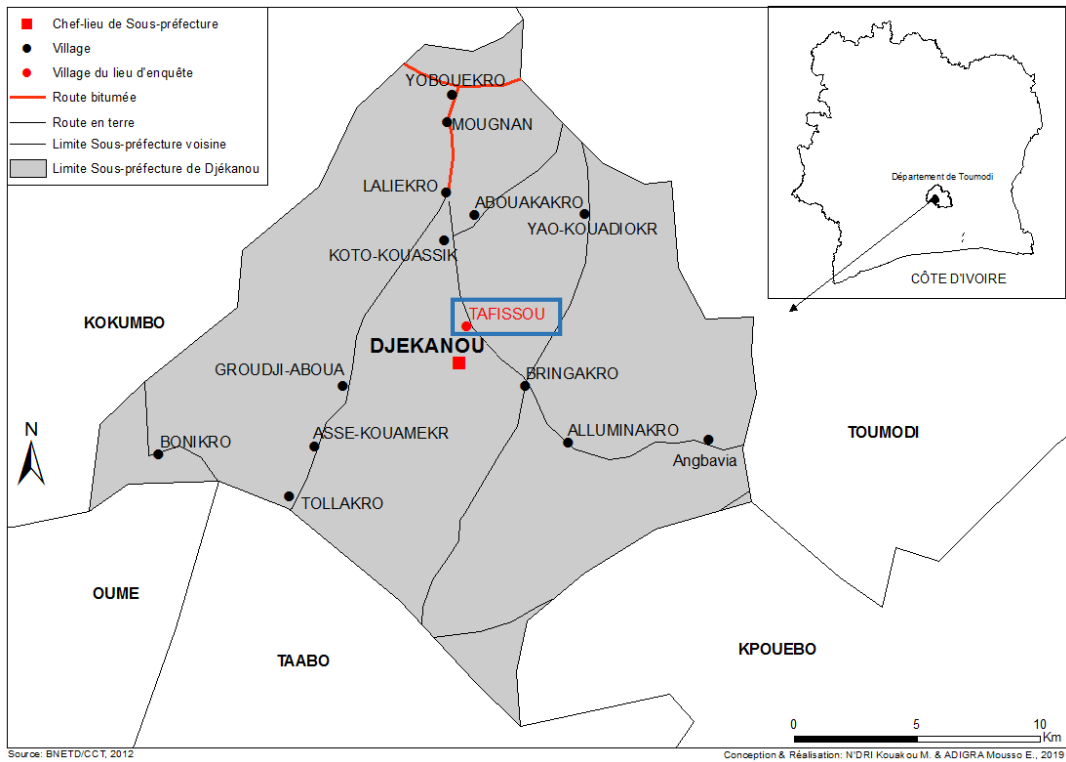


Figure 1 : Map of the study area showing the site of Tafissou.

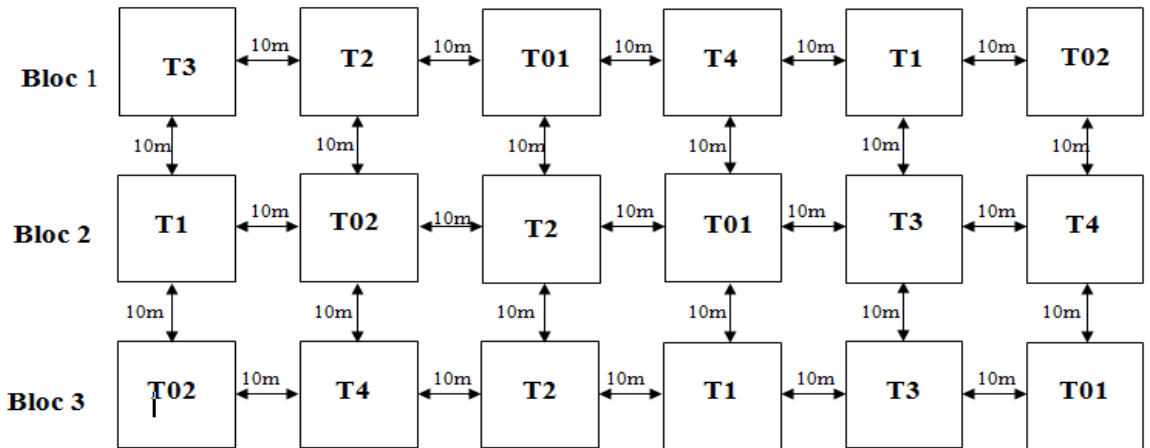


Figure 3: Diagram of the experimental set-up.

- T01:** Control without application of Banzai with fertilizer
- T02:** Control without application of Banzai without fertilizer
- T1:** 3 applications of Banzai with fertilizer
- T2:** 4 applications of Banzai without fertilizer
- T3:** 3 applications of Banzai without fertilizer
- T4:** 4 applications of Banzai with fertilizer

Table 1: Number of applications of SUPERCAO and Banzai.

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

Fertilizer: (+) one application, (-) no application

Banzai: (+) one application, (-) no application



Figure 2: Symptoms of cherelles rot and black pods in a peasant farm (Photo taken by Koffi Alain).

RESULTS

Effect of Banzai and the SUPERCAO fertilizer on the control of cherelles rot for the site with previous fertilizer (DAE)

The result of the data description showed overall that plots treated with Banzai had a better control rate than control plots with an average rate of healthy cherelles of 79% against 46% for T01 and 52% for T02 (Table 2).

The evolution of the healthy cherelles rate per treatment shows that the T4 treatment has the best healthy cherelles rate than the other treatments with 89% (Figure 4).

The boxplots

Rate of healthy cherelles around 89%, followed by group B composed of T3 and T2 with healthy representing the healthy cherelles rate as a function of treatment showed two trends. A first trend that groups the treated plots with the highest healthy cherelles rates around a minimum median of 70%, and a second trend relative to the control plots with median healthy cherelles rates of nearly 50% (Figure 5).

The result of the Kruskal-Wallis test applied on the healthy heart rate showed that there is a significant difference ($p=0.000<0.05$) between the treated and control plots on the one

hand and between the treated plots themselves on the other hand. This difference is confirmed by the classification of the treatments into five groups (A, B, C, D and E). Group A is represented by treatment T4 with the highest cherelles rates of 77% and 79% respectively. Group C is represented by treatment T1 with a healthy cherelles rate of 70% followed by controls T02 and T01 which belong respectively to group D and E with healthy cherelles rates of 52% and 46% respectively (Table 3). The existence of a significant difference between the treated plots and the control plots shows that the biostimulant Banzai was effective in controlling the cherelles rot. The fact that T2 (4 applications of Banzai without fertilizer) and T3 (3 applications of Banzai without fertilizer) belong to the same group shows that there is no difference between three applications and four applications of Banzai without fertilizer. In the case of fertilizer application during the experiment, four applications of Banzai give a better rate of healthy cherelles than three applications of Banzai. Therefore, the statistical difference observed in the T01 and T02 controls shows that the fertilizer applied (SUPERCAO) during the experiment did not have a positive effect on the control of cherelles rot. In fact, the control T02, which did not receive the SUPERCAO fertilizer, had a higher rate of healthy cherelles (52%) than the control T01 which received the fertilizer.

Effect of Banzai and SUPERCAO fertilizer on the control of rotting cherelles for the unpredecended fertilizer site (DSE)

The results of the data description showed in overall that the plots treated with Banzai had a better rate of healthy cherelles than the control plots with an average rate of healthy cherelles equivalent to 77% against 29% for T01 and 37% for T02 whatever the observation period (Table 4). Comparison of the treatments between them using the dynamics of the evolution of the healthy cherelles rate shows that treatment T4 (87%) had a higher rate of healthy cherelles than the other treatments T1, T2 and T3 which had on

average healthy cherelles rate of 74% (Figure 6).

Boxplots of healthy cherelles rate versus plots allowed the identification of three trends according to median dispositions. Thus, the controls T01 and T02 form a first trend with a median rate of healthy cherelles around 40%, the second trend contains T1 and T2 with a median rate of healthy cherelles around 75% and the third trend contains T3 and T4 with median rates of healthy cherelles close to 90% (Figure 7).

The Kruskal-Wallis test applied on the healthy cherelles rate showed a significant difference between the treated and the controlled plots. The classification of the treatments revealed that there are four groups (A, B, BC and C). Treatments T4 and T3 had the highest rate of healthy cherelles and form group A with average rates of 87% and 82% respectively. The lowest rate was observed in the T01 control represented by group C with an average rate of 30%. The intermediate average rates are represented by groups B and BC. Group B corresponded to treatments T2 and T1 with mean rates of 75% and 67% respectively. The BC group corresponded to the T02 control with a rate of 37% (Table 5). The statistical difference observed between the treated plots and the control showed that the Banzai biostimulant had a positive effect on the control of cherelles rot. The statistical difference observed between T4 (4 applications of Banzai with fertilizer) and T1 (3 applications of Banzai with fertilizer) showed that four applications of Banzai with fertilizer was more effective than the three applications with fertilizer. On the other hand, the significant difference observed between T2 (4 applications of Banzai without fertilizer) and T3 (3 applications of Banzai without fertilizer) shows that in the case of no fertilizer application, three applications of Banzai is better than four applications. In addition, the fact that the plots treated with fertilizer and the plots without fertilizer treatment belong to the same group shows that the SUPERCAO fertilizer applied during the experiment had no expected effect on the control of cherelles rot.

Cumulative effect of previous fertilizer and Banzai on the control of cherelles rot

Figure 8 shows the comparison of the rates of healthy cherelles at the two sites per treatment. Descriptive analysis shows that the site with previous fertilizer recorded the highest healthy cherelles rates with the exception of the T3 treatment.

The boxplots of healthy cherelles rates from the two sites allowed the treatments to be grouped into five trends based on medians. The first trend includes T01 and T02 of the DSE site with a lower healthy cherelles rate of approximately 40%, followed by the second trend which groups T01 and T02 of the DAE site with a healthy cherelles rate of approximately 50%. The third trend includes T1 treatment of both sites with a rate of about 75%, followed by the fourth trend with a rate of about 80% represented by T2 of the DAE site, T3 of the DAE site and T2 of the DSE site. Finally, the fifth trend is represented by T4 of the DAE group, T3 of the DAE site, and T4 of the DSE group with an average rate of healthy cherelles close to 90% (Figure 9). The Kruskal-Wallis test applied to the healthy cherelles rates of the two sites indicated that there was a

significant difference between the treated plots of the two sites. The classification resulting from this test showed eight distinct groups of treatments according to their effectiveness.

Thus, group A is represented by the T4 DAE treatment, T4 DSE treatment and T3 DSE treatment with rates of 90%, 87% and 82% respectively. Group AB is represented only by T2 DAE treatment with a rate of healthy cherelles close to 79%. The T3 DAE, T2 DSE, and T1 DAE treatments belong to group B with respective rates of 77%, 75% and 71%. The BC group is represented only by T1 DSE with a healthy cherelles rate of 67%. The T02 treatment of the DAE site belongs to group C and has a rate of 52% of healthy cherelles. T01 DAE treatment, T02 DSE treatment and T01 DSE treatment belong to groups DC, D, and E respectively with respective rates of 47%, 37% and 30% (Table 6).

Comparison of the treatments across sites shows that overall the DAE site treatments had a better rate of healthy cherelles than the DSE site treatments (Table 7). This shows that the previous fertilizer had a positive effect in controlling the cherelles rot.

Table 2: Global rate of healthy cherelles per treatment for the site with previous fertilizer.

Treatments	Number of produced cherelles	Number of rotten cherelles	Global rate of healthy cherelles
T01	1716	924	46,15%
T02	1615	772	52,20%
T1	4110	1203	70,73%
T2	2539	529	79,17%
T3	2775	632	77,23%
T4	3977	430	89,19%

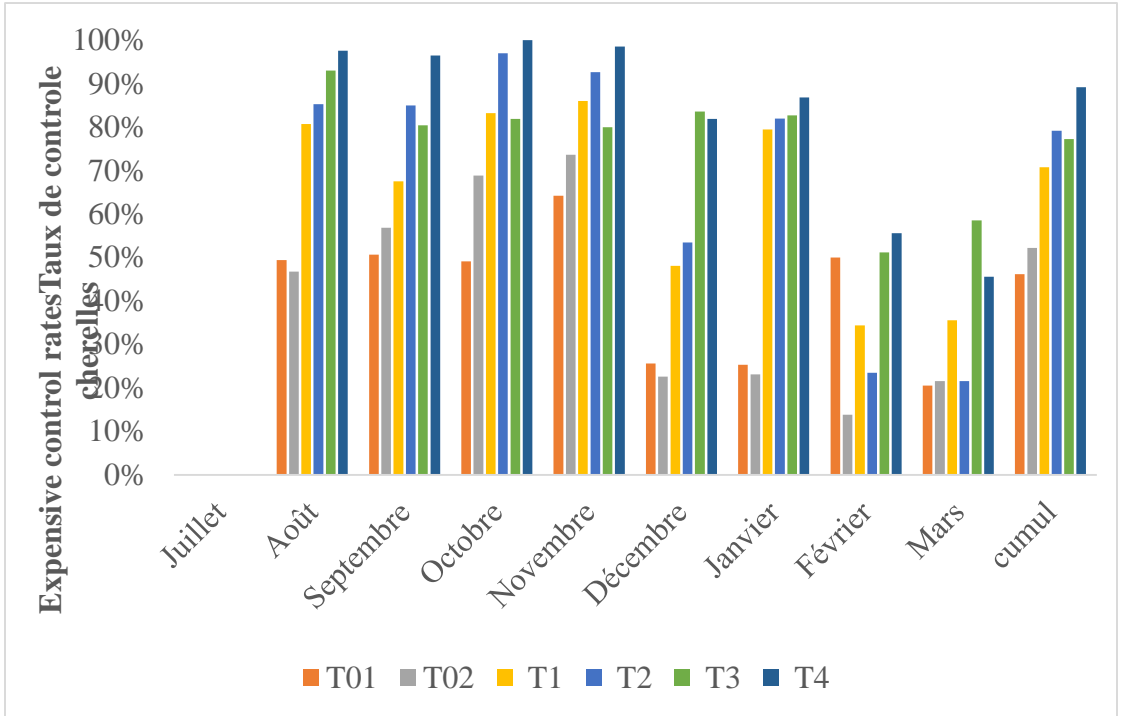
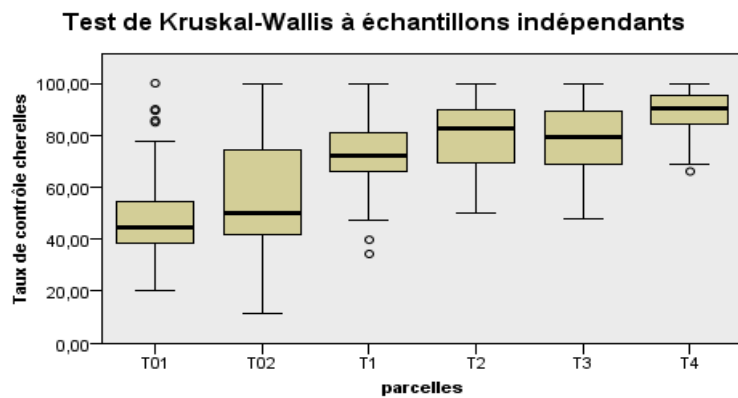


Figure 4 : Control rate of cherelles rot by observation and treatment for the site with previous fertilizer (DAE).



N Total	360
Statistique de test	156,150
Degrés de liberté	5
Sig. asymptotique (test bilatéral)	,000

Figure 5 : Moustache box showing median of treatments as a function of the control rate of cherelles rot.

Tableau 3 : Classification showing the average rate of control of cherelles rot by treatment.

Treatments	Average rate	Groups
T4	89,19%	A
T2	79,17%	B
T3	77,23%	B
T1	70,73%	C
T02	52,20%	D
T01	46,15%	E

Tableau 4 : Global rate of healthy cherelles per treatment for the unprecedented fertilizer site.

Treatments	Number of produced cherelles	Cumulation of rotten cherelles	Global control rate of cherelles
T01	710	498	29,86%
T02	701	441	37,09%
T1	1429	474	66,83%
T2	937	234	75,03%
T3	1000	183	81,70%
T4	1035	131	87,34%

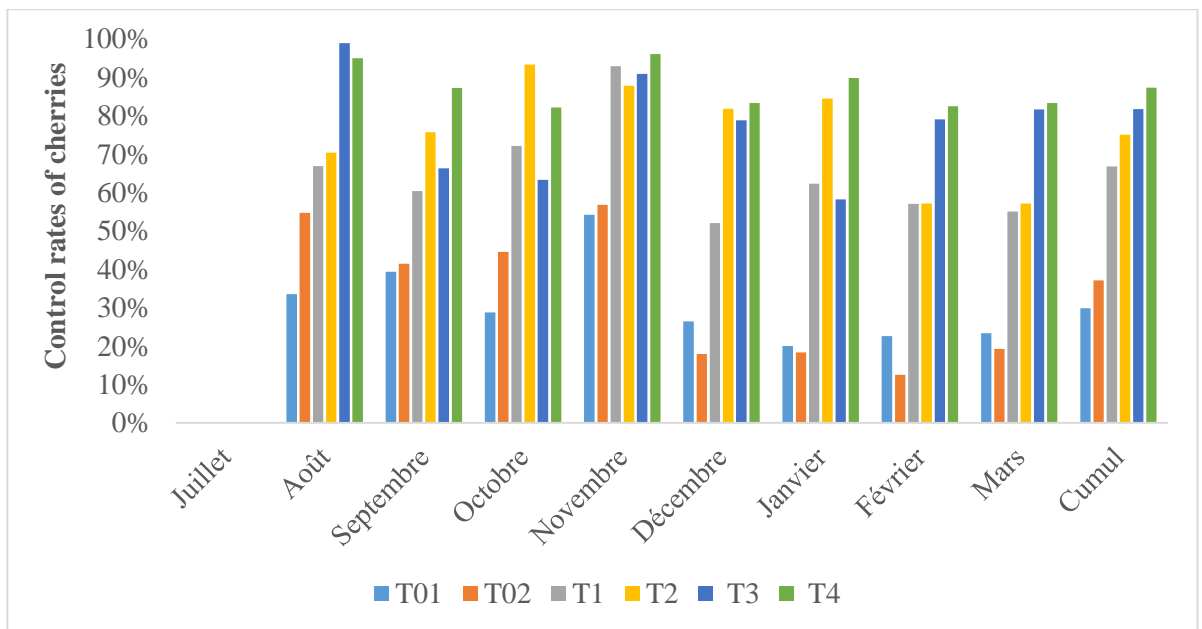
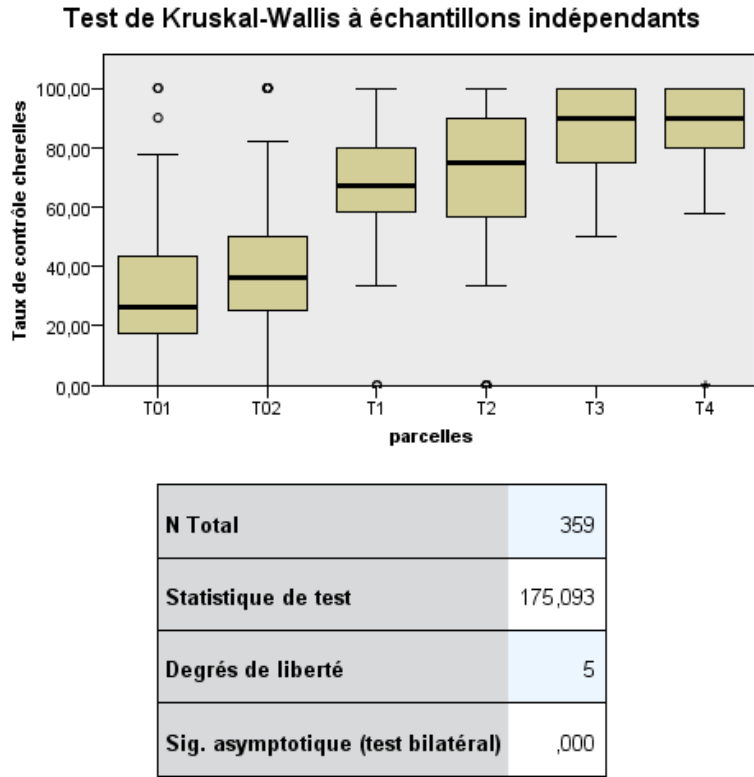


Figure 6 : Control rate of cherelles rot by observation and by treatment for the No Previous Fertilizer Site (DSE).



1. La statistique de test est réglée pour les ex aequo.

Figure 7: Boxplots showing median of treatments as a function of the control rate of cherelles for the unprecedented fertilizer design (DSE).

Table 5 : Showing the average row of the control rate of cherelles rot per treatment for the unprecedented fertilizer device (DSE).

Traitements	Taux Moyens	Groupes
T4	87,34%	A
T3	81,70%	A
T2	75,03%	B
T1	66,83%	B
T02	37,09%	BC
T01	29,86%	C

Table 6 : Classification of average rate healthy cherelles by Site (DAE and DSE) and by treatment.

Sites and plots	Average rate	Groups
DAET4	89,19%	A
DSET4	87,34%	A
DSET3	81,70%	A
DAET2	79,17%	AB
DAET3	77,23%	B
DSET2	75,03%	B
DAET1	70,73%	B
DSET1	66,83%	BC
DAET02	52,20%	C
DAET01	46,5%	DC
DSET02	37,09%	D
DSET01	29,86%	E

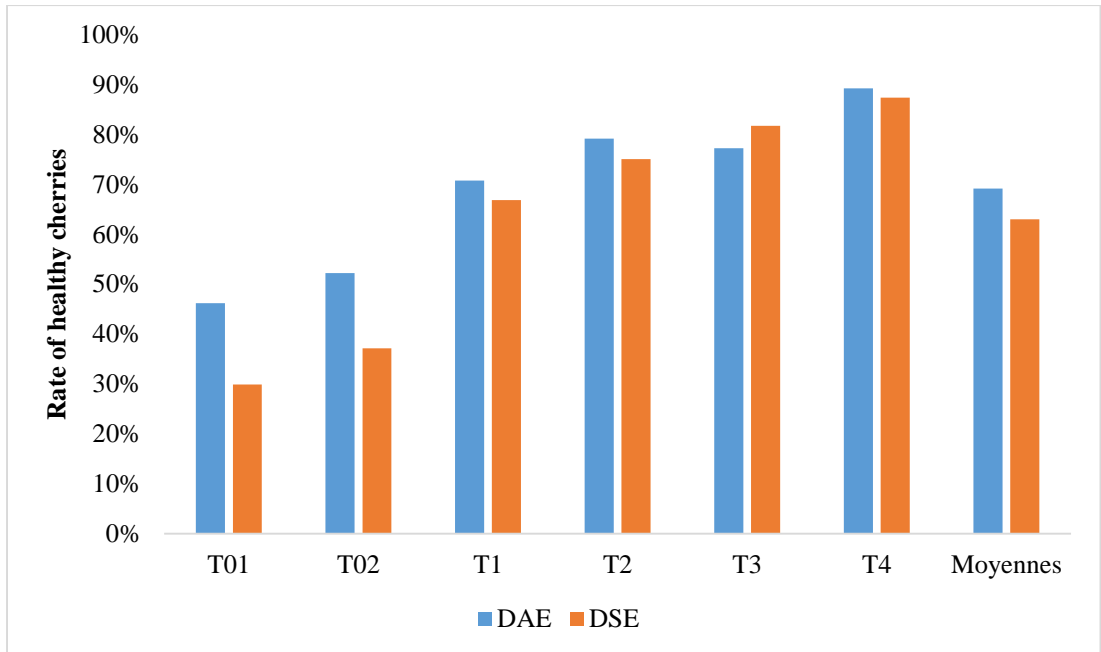


Figure 8 : Histogram of the rate of healthy cherelles by treatment and by site.

Test de Kruskal-Wallis à échantillons indépendants

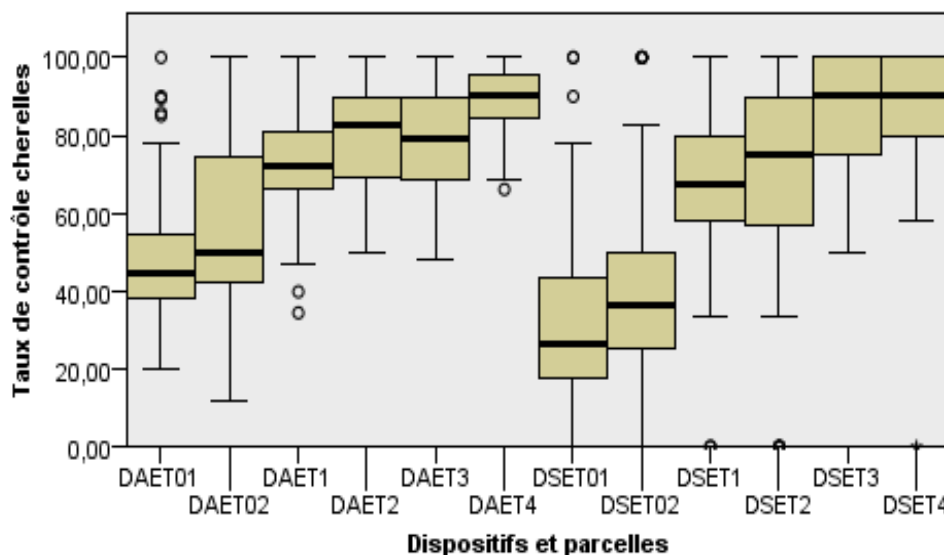


Figure 9 : Boxplots of healthy cherelles rates by site (DAE and DSE) and by treatment.

Table 7 : Comparison of the two sites (DAE and DSE) according to the rate of healthy cherelles per treatment.

Device and plots	T01	T02	T1	T2	T3	T4	Averages
DAE	46,15%	52,20%	70,73%	79,17%	77,23%	89,19%	69,11%
DSE	29,86%	37,09%	66,83%	75,03%	81,70%	87,34%	62,98%

DISCUSSION

At the previous fertilizer site (DAE) and at the site without previous fertilizer (DSE), statistical results showed that the biostimulant Banzai was effective in the control of cherelles rot with reference to the control plots. Indeed, the control plots T01 and T02, which had not received Banzai biostimulant application, were subjected to high parasitic pressure, particularly due to fungal pathogen (Muller, 1974). This is elucidated by the high rates of healthy cherelles in the treated plots and the low rates of healthy cherelles in the control plots.

The statistical test also showed that four applications of Banzai coupled with fertilizer were more effective than the three applications with or without fertilizer at any site (DAE and DSE). This result is in line with the recommendations indicated in the Banzai biostimulant data sheet (Callivoire, 2016).

On both sites, fertilizer applied alone during the experiment had no effect in limiting the decay of cherelles. In the case of the site with previous fertilizer (DAE), the control plot that did not receive fertilizer had a higher rate of healthy cherelles than the control plot that received fertilizer. For the No Previous Fertilizer Site, the unfertilized control plot had

a statistically identical rate of healthy cherelles as the fertilized control plot. Indeed, fertilizer stimulates the production of cherelles only, unlike biostimulant, which is capable of stimulating both the production of cherelles and the natural defense systems against pathogens (Oro et al., 2020). This is in line with the studies conducted by Fardeau and Jonis (2004), which state that the effect of the biostimulant can create a cell barrier that promotes resistance to the penetration of pathogens into the plant. These results are also in line with those of Lakhdar (2018), who showed that biostimulants trigger defence processes at the plant level through the elicitors they contain.

Concerning the cumulative effect of the biostimulant Banzai and the previous fertilizer, the results obtained show that the treatments of the site with previous fertilizer (DAE) mostly had higher rates of healthy cherelles than the treatments of the site with previous without fertilizer. This result is in line with the results of the report of the Coffee-Cocoa Council (2015) which showed that the effect of the fertilizer is visible provided that it is applied at least twice during two successive years.

Conclusion

At the end of this study, it appears that the biostimulant Banzai was effective both on the site with previous fertilizer and on the site without previous fertilizer in the control of the cherelles rot in reference to the control plots. Specifically, at the site with previous fertilizer (DAE), the T4 treatment (4 applications of Banzai with fertilizer) had the best rate of healthy cherelles. At the site without previous fertilizer (DSE), the T3 (3 applications of Banzai without fertilizer) and T4 (4 applications of Banzai with fertilizer) treatments better controlled the cherelles rot. In general, the four applications of Banzai coupled with fertilizer allowed to obtain the best rates of healthy cherelles whatever the site. Concerning the cumulative effect of the previous fertilizer with Banzai, the treatments of the site with previous fertilizer were always better than the treatments of the site without previous fertilizer.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

FZO managed the literature research, designed the study, wrote the protocol, participated in data collection and performed the statistical analysis, and wrote article. HDL managed the analyses of study, and wrote article. SS designed the study and approved final protocol. KMN participated in data collection, data analysis and wrote the first draft of the manuscript. HAD was the principal investigator.

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