



Research Article

Effect of Fertilizer Application on Mealybug Infestation and Physiological Responses of Cocoa at the CNRA Station, Divo, Côte d'Ivoire

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Abstract

Applying fertilizer is one solution for improving cocoa production. However, while it improves cocoa productivity, it also promotes the development of pests and disease vectors. This study aimed to understand the effects of fertilizer on the proliferation of mealybugs known as vectors of cocoa swollen shoot virus. The experimental design was a Fisher block with four fertilizer formulations (T1 to T4) and an unfertilized control (T0), each replicated three times. Each elementary plot contained 45 cocoa trees from CNRA research station in Divo, Côte d'Ivoire. Mealybug infestation levels were evaluated every 15 days for one year, and three cocoa leaves were collected per treatment and replication in order to determine physiological and biochemical parameters. The data were analyzed using SAS 9.4 and XLSTAT 2025. The results revealed seven species of mealybugs in the treatments: *Formicococcus njalensis*, *Planococcus citri*, *Ferrisia virgata*, *Pseudococcus longispinus*, *Phenacoccus hargreavesi*, *Dysmicoccus brevipes*, and *Planococcus kenya*. Furthermore, analysis of variance revealed significant differences among the treatments regarding the content of chlorophyll A and B, total chlorophyll, carotenoids, protein, phenolic compounds, and proline. However, among the seven mealybug species identified, principal component analysis revealed a link between *Formicococcus njalensis*, *Ferrisia virgata*, and *Dysmicoccus brevipes*, as well as chlorophyll A and B, total chlorophyll, carotenoids, protein, phenolic compounds, and total sugars, in T0, T3, and T4. Meanwhile, the mealybug species *Pseudococcus longispinus* and *Phenacoccus hargreavesi* were linked to proline in treatment T2. Overall, the study showed that fertilizer has a small impact on mealybug and more on the physiological and biochemical parameters.

Keywords

Côte d'Ivoire, Mealybugs, Fertilizer, Physiological Parameters, Biochemical Parameters

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1. Introduction

Cocoa (*Theobroma cacao* L.) is a perennial plant belonging to the Malvaceae family [1]. In its natural state, the cocoa tree grows in the lower levels of the tropical rainforests in Central and South America [2]. The multipurpose beans were used as currency by the Mayan and Aztec peoples, who also used them in food preparations mixed with maize and chili peppers [3, 4].

This universally appreciated commodity forms the basis of many countries' economies, including Côte d'Ivoire. During the 2023–24 season, Côte d'Ivoire produced 1,674,000 tons of cocoa beans, representing 37.29% of the global supply [5]. This makes Côte d'Ivoire the world's leading cocoa producer. Cocoa cultivation directly supports the incomes of thousands of producers and accounts for around 20% of the country's gross domestic product (GDP). The cocoa sector contributes about one-third of Côte d'Ivoire's export earnings and employs nearly one-quarter of the population [6].

Despite these records, which are satisfactory at national and international records, the sustainability of cocoa farming is threatened by several constraints, including aging orchards, pests, diseases and climate change. Swollen shoot disease, which is spread by mealybugs, negatively impacts cocoa production in Côte d'Ivoire. It is considered as an important threat to cocoa cultivation in the country because it causes a rapid decline in production and the death of affected trees [7].

On the other hand, one solution for improving cocoa production is applying fertilizers [8]. However, the use of fertilizers would increase the cocoa productivity and promote the proliferation of certain pests and disease vectors, particularly mealybugs [9].

This study aims to determine and understand the effect of fertilizer application on the proliferation of mealybugs known as vectors of swollen shoot disease in cocoa plantations.

2. Materials and Methods

2.1. Study Site

The study was conducted at the National Agricultural Research Center (CNRA) research station in Divo, which is located 17 kilometers from the city. The station covers 3,520 hectares of land with dense secondary semi-deciduous forest vegetation [10]. The station receives an average of 1,200 mm of rainfall per year, which is distributed unevenly, and has an average temperature of 26°C. Atmospheric humidity reaches approximately 86% [11], and annual temperatures range from 19°C to 33°C.

2.2. Plant Material

The plant material consists of selected hybrids cocoa trees from the National Agronomic Research Center (CNRA). These cocoa trees were 3 to 5 years old.

2.3. Experimental Design

The plot was established following a period of fallow. For this work, the experimental design on the plot was a Fisher block with five treatments repeated three times. The test involved comparing four different formulation doses with an untreated control (Table 1). Each plot contained 45 cocoa trees. The cocoa trees are spaced 3 meters apart between rows and 3 meters apart within the same row. Elementary plots are separate from 5 m to avoid contamination between treatments. The study was carried during the 2024-2025 cocoa campaign.

Table 1. Different formulations used in treatments.

Treatments	Formulation	Applications per year
T1	Urea-TSP-MOP-CaCO3-MgSO4-ZnSO4-H3BO3	Mars-avril (1) and August-September (1)
T2	Urea-MOP-CaCO3-MgSO4-ZnSO4-H3BO3	Mars-avril (1) and August-September (1)
T3	TSP-MOP-CaCO3-MgSO4-ZnSO4-H3BO3	Mars-avril (1) and August-September (1)
T4	Urea-TSP-MOP-CaCO3-MgSO4	Mars-avril (1) and August-September (1)
T0	Untreated control	Mars-avril (1) and August-September (1)

2.4. Counting Mealybugs

The method used to count mealybugs involved visually inspecting 25 cocoa trees in each block. From each cocoa tree,

organs up to a height of two meters high [12]. Mealybug colonies on the pods, twigs and chupons of the cocoa trees were counted every 15 days in the different blocks over the course of one year. One colony of mealybug consist in individuals located together on an organ of cocoa.

2.5. Determination of the Physiological and Biochemical Parameters of Cocoa Tree Leaves in Treatments

2.5.1. Collection of Cocoa Tree Leaves

The leaves were randomly selected from five plants per treatment and replication. Thus, fifteen cacao trees were used for each treatment. Three (3) leaves were randomly selected from each plant: one from the base, one from the middle and one from the top of three branches of the tree crown. A total of 45 leaves were used for each treatment, including 15 leaves per replication. All leaves from each replication were collected and had their veins removed. The leaf blades were cut finely and mixed to create three batches for each treatment. A known mass was taken from each batch for extraction.

2.5.2. Extraction and Measurement of Physiological and Biochemical Parameters from Cocoa Leaves

Extractions and measurements of physiological and biochemical parameters were performed using standard, well-established methods. Thus:

- 1) The extraction and measurement of chlorophyll pigments and carotenoids were performed using the method described by Lichtenthaler [13]. Various concentrations were calculated using Mackinney's formulas [14].
- 2) The extraction of phenolic compounds was carried out according to the method described by Kouakou [15]. The phenolic compounds were measured using the method described by Singh [16].
- 3) The total sugars contained in the cocoa leaves were extracted using the same method as the phenolic compounds. Total sugars were measured using the method described by Dubois *et al.* [17].
- 4) Protein extraction and measurement were carried out using the Bradford method [18].
- 5) Proline extraction and measurement were carried out using the Dreir & Goring method [19]. The proline concentration (C) was calculated using the following formula: $C \text{ (mM/g MF)} = DO/\epsilon \times L$; $\epsilon = 0.9986 \text{ mM.L}^{-1} \cdot \text{cm}^{-1}$, L: Length of spectrophotometer tube in centimeters.

2.6. Data analysis

All collected data were analyzed using analysis of variance (ANOVA) with the General Linear Model (GLM) procedure in SAS. Separation of means was performed using the Student-Newman-Keuls test at a 5% significance level. The average number of scale insects and the average quantity of biochemical variables per treatment were determined. Then, Principal Component Analysis (PCA) was performed using XLSTAT software to group the studied variables (mealybug species and biochemical parameters) and treatments.

3. Results

3.1. List and Distribution of Mealybug Species According to Treatment

A total of seven (07) species of mealybugs were recorded in the different treatments. These are: *Formicococcus njalensis*, *Planococcus citri*, *Ferrisia virgata*, *Pseudococcus longispinus*, *Phenacoccus hargreavesi*, *Dysmicoccus brevipes* and *Planococcus kenya*. The distribution of these species revealed that *Formicococcus njalensis*, *Planococcus citri*, *Ferrisia virgata*, *Pseudococcus longispinus* and *Dysmicoccus brevipes* were found in all treatments. *Phenacoccus hargreavesi* was observed in treatments T0, T2 and T3, and *Planococcus kenya* was observed in treatments T1, T2 and T3. All species of mealybugs were observed in treatments T2 and T3 (Table 2).

Table 2. Distribution of mealybug species according to treatment.

Treatments	<i>F. njalensis</i>	<i>P. citri</i>	<i>F. virgata</i>	<i>P. longispinus</i>	<i>D. brevipes</i>	<i>P. hargreavesi</i>	<i>P. kenya</i>
T0	X	X	X	X	X	X	-
T1	X	X	X	X	X	-	X
T2	X	X	X	X	X	X	X
T3	X	X	X	X	X	X	X
T4	X	X	X	X	X	-	-

3.2. Comparison of the Average Number of Mealybug Colonies According to Treatment

Analysis of variance revealed no significant differences in colony numbers among mealybug species across treatments (Table 3).

3.3. Comparison of the Average Quantity of Physiological and Biochemical Parameters According to Weeks

Analysis of variance from annual datas revealed significant differences ($p \leq 0.05$) in the levels of chlorophyll A (Chla), chlorophyll B (Chlb), total chlorophyll (Chlt), carotenoids (Carot), protein (Prot), phenolic compounds (Comph), and proline (Prol) among the treatments. However, there were no significant differences in total sugar content (Table 4).

3.4. Relationship Between Mealybugs, Biochemical Parameters and Treatments

A principal component analysis (PCA) of all the treatment data revealed that axes F1 and F2 explained 77.77% of the total variance. Treatments T0 (0.75 Cos^2), T1 (0.54 Cos^2), T3 (0.56 Cos^2) and T4 (0.79 Cos^2) were best represented on axis F1. Treatment T2, however, was the only one that was best represented on axis F2 with 0.95 Cos^2 (Table 5; Figure 1). The following variables were well represented on the F1 axis: Fnjalems (*Formicococcus njalensis*) with 0.48 Cos^2 , Fvirgata (*Ferrisia virgata*) with 0.54 Cos^2 , Phargre (*Phenacoccus hargreavesi*) with 0.96 Cos^2 , ChlaFF (Chlorophyll A) with 0.81 Cos^2 , ChlbFF (Chlorophyll B) with 0.95 Cos^2 , ChltFF (Total chlorophyll) with 0.91 Cos^2 , CarotFF (Carotenoids) with 0.96 Cos^2 , ProtFF (Proteins) with 0.81 Cos^2 , ComphFF (phenolic compounds) with 0.91 Cos^2 and SuctotFF (total sugars) with 0.90 Cos^2 . Meanwhile, the variables Plongis (*Pseudococcus longispinus*) with 0.57 Cos^2 , Dbrevipes (*Dysmicoccus brevipes*) with 0.60 Cos^2 , and ProlFF (Proline) with 0.64 Cos^2 were well represented on axis F2 (Table 6; Figure 1).

Table 3. Average number of mealybug colonies in each treatment.

Treatments	<i>F. njalensis</i>	<i>P. citri</i>	<i>F. virgata</i>	<i>P. longispinus</i>	<i>D. brevipes</i>	<i>P. hargreavesi</i>	<i>P. kenya</i>
T0	5.00±1.53a	02.67±0.88a	27.33±3.76a	2.00±0.52a	529.00±12.45a	1.00±0.01a	0.00±0.00a
T1	3.00±1.00a	02.33±0.45a	22.66±3.52a	1.00±0.00a	737.66±12.59a	0.00±0.00a	7.66±1.66a
T2	0.66±0.33a	04.00±1.52a	17.00±3.32a	1.00±0.01a	313.33±14.84a	0.66±0.00a	3.00±0.33a
T3	4.66±1.18a	14.00±2.02a	25.66±2.58a	1.66±0.66a	699.00±19.23a	1.00±0.01a	2.00±0.00a
T4	1.00±0.00a	05.00±1.88a	15.66±2.91a	2.00±0.02a	821.00±11.61a	0.00±0.00a	0.00±0.00a
Pr > F	0.23	0.19	0.73	0.95	0.26	0.72	0.61
Average	2.86	5.60	21.66	1.53	620.00	0.53	2.53
CV	34.46	10.96	32.06	14.08	45.21	22.70	25.90

* According to the Student-Newman-Keuls test, values in the same column that are followed by the same letter are not significantly different from each other at the 0.05 level of significance.

Table 4. Content of physiological and biochemical parameters in the treatments.

Treatments	Chla (ng/g) of Frle	Chlb (ng/g) of Frle	Chlt (ng/g) of Frle	Carot (ng/g) of Frle	Prot (µg/g) of Frle	Comph (µg/g) of Frle	Suctot (µg/g) of Frle	Prol (mM/g) of Frle
T0	1.56±0.00e	0.63±0.01d	2.19±0.02c	0.94±0.00d	111.97±0.89c	62.31±5.12c	73.22±5.94a	0.38±0.02c
T1	4.61±0.16a	2.50±0.13b	7.11±0.29a	2.72±0.09b	148.49±5.08b	151.07±0.53b	97.85±5.18a	0.46±0.01c
T2	2.37±0.04d	0.99±0.01c	3.36±0.02b	1.44±0.01c	121.95±0.07c	134.56±3.64b	95.45±1.76a	0.73±0.05a
T3	2.90±0.05c	0.77±0.05cd	3.68±0.00b	1.39±0.04c	120.85±0.44c	53.48±0.05c	81.64±4.66a	0.63±0.03b
T4	4.27±0.14b	2.96±0.07a	7.23±0.07a	3.13±0.16a	201.51±4.57a	200.61±7.10a	105.17±4.00a	0.49±0.00c

Treat-ments	Chla (ng/g) of Frle	Chlb (ng/g) of Frle	Chlt (ng/g) of of Frle	Carot (ng/g) of Frle	Prot (µg/g) of of Frle	Comph (µg/g) of Frle	Suctot (µg/g) of Frle	Prol (mM/g) of Frle
Pr > F	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.09	<.0001
Average	3.14	1.57	4.72	1.92	140.95	120.41	90.66	0.54
CV	5.54	7.61	4.89	7.62	3.79	8.30	14.98	9.66

* According to the Student-Newman-Keuls test, values in the same column that are followed by the same letter are not significantly different from each other at the 0.05 level of significance. Chla: Chlorophyll A Chlb: Chlorophyll B; Chlt: Total chlorophyll; Carot: Carotenoids; Prot: Protein; Comph: Phenolic compounds; Suctot: Total sugars; Prol: Proline; Frle: Fresh leave.

Table 5. Cos^2 of treatments.

Treatments	F1	F2	F3	F4
T0	0.755	0.100	0.005	0.141
T1	0.536	0.002	0.451	0.011
T2	0.024	0.946	0.026	0.004
T3	0.559	0.043	0.004	0.394
T4	0.790	0.058	0.152	0.000

Table 6. Cos^2 of variables.

Variables	F1	F2	F3	F4
Fnjalsens	0.483	0.374	0.139	0.004
Pcitri	0.103	0.027	0.012	0.858
Plongis	0.037	0.572	0.389	0.002
Fvirgata	0.540	0.242	0.217	0.000
Dbrevipes	0.287	0.600	0.013	0.101
Pkenya	0.108	0.092	0.799	0.002
Phargre	0.957	0.002	0.021	0.019
ChlaFF	0.814	0.033	0.091	0.062
ChlbFF	0.948	0.048	0.001	0.003
ChltFF	0.913	0.041	0.033	0.013
CarotFF	0.958	0.036	0.001	0.004
ProtFF	0.808	0.079	0.111	0.002
ComphFF	0.907	0.027	0.037	0.030
SuctotFF	0.900	0.082	0.010	0.008
ProlFF	0.003	0.643	0.030	0.324

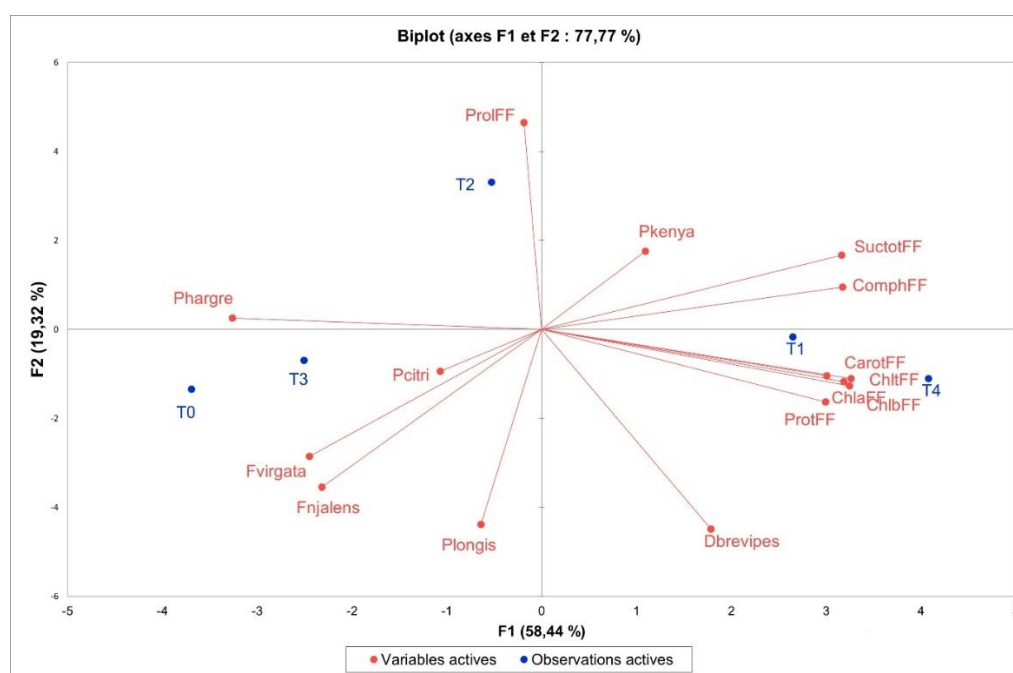


Figure 1. Principal component analysis between colonies of mealybugs species and physiological and biochemical parameters in the treatments.

ProlFF: Proline, ChlaFF: Chlorophyll A, ChlbFF: Chlorophyll B; ChltFF: Total chlorophyll; CarotFF: Carotenoids; ProtFF: Protein; ComphFF: Phenolic compounds; SuctotFF: Total sugars; Dbrevipes: *Dysmicoccus brevipes*; Fvirgata: *Ferrisia virgata*; Fnjalens: *Formicococcus njalensis*; Plongis: *Pseudococcus longispinus*; Phargre: *Phenacoccus hargreavesi*; Pcitri: *Planococcus citri*; Pkenya: *Planococcus kenya*.

4. Discussion

The objective of this study was to determine the impact of fertilizer application on the proliferation of mealybug species in cocoa plots. The results revealed seven species of mealybugs in the various treatment groups that comprised the trial. These species are: *Formicococcus njalensis*, *Planococcus citri*, *Ferrisia virgata*, *Pseudococcus longispinus*, *Phenacoccus hargreavesi*, *Dysmicoccus brevipes*, and *Planococcus kenya*. Previous studies as part of the mealybug inventory have revealed these species to be present in Ivorian cocoa orchards [20-12]. These species are also known as vectors of Cocoa swollen shoot virus [21]. Therefore, transmission tests were conducted as part of this work. Furthermore, *Formicococcus njalensis*, *Planococcus citri*, *Ferrisia virgata*, *Pseudococcus longispinus*, and *Dysmicoccus brevipes* were present in all treatments. Others authors [20] also found these species to be predominant in all study areas in their research on mealybugs that vector the swollen shoot virus in Abengourou, Bouaflé, and Divo counties. Apart from these species, *Phenacoccus hargreavesi* was observed in treatments T0, T2, and T3, and *Planococcus kenya* was observed in treatments T1, T2, and T3. Overall, treatments T2 and T3 harbored all species of mealybugs. With these treatments, which received fertilizer, more mealybug populations were observed than the control treatment. Other investigation of the impact of fertilizers on mealybug and mite populations in Moroccan

citrus trees revealed higher mealybug numbers on trees treated with potassium nitrate [22]. However, the results of the variance analysis showed no significant difference in the average number of colonies among the different treatments. The results also showed that there was no significant difference in total sugar content among the treatments. However, there was a significant difference in the content of chlorophyll A, chlorophyll B, total chlorophyll, carotenoids, proteins, phenolic compounds, and proline. Therefore, fertilizer application influences several of the cocoa tree's biochemical and physiological parameters, including its photosynthetic pigments (chlorophyll A and B, total chlorophyll, and carotenoids), proteins, phenolic compounds, and proline. Nevertheless, fertilizer application does not affect the average number of mealybug colonies or total sugar content. Thus, fertilization affects the plant's physiology and secondary metabolism more than insect abundance. Several studies have demonstrated the influence of biochemical parameters on cocoa trees. For example, applying multi-nutrient fertilizers stimulates the growth of young cocoa trees and increases their chlorophyll content [23]. Similarly, it was found that combining organic fertilization with cultural practices such as pruning improves cocoa tree vigor and increases leaf photosynthetic pigments [24]. Moreover, the total phenolic profile changes depending on the fertilization regimen [25]. Furthermore, fertilizers heavily impact total sugar accumulation depending on the element used for fertilization, the type of crop, and soil conditions [26]. Therefore, total sugar regulation in cocoa trees may be less

sensitive to variations in fertilization than other biochemical parameters. Regarding mealybugs, authors [27] reported that applying complete inorganic fertilizers to palm trees infested with *Aspidiotus rigidus* increased plant growth and reduced insect population density. This was in contrast to the use of organic fertilizers, which did not produce the same results. Therefore, the effects of fertilization on mealybugs depend on the type of fertilizer used. Furthermore, principal component analysis (PCA) of all the treatment data revealed that axes F1 and F2 accounted for 77.77% of the total variance. Treatments T0, T3, and T4, as well as the variables *Formicococcus njalensis*, *Ferrisia virgata*, *Phenacoccus hargreavesi*, chlorophyll A, chlorophyll B, total chlorophyll, carotenoids, proteins, phenolic compounds, and total sugars, were well represented on axis F1. Conversely, treatment T2, along with the variables *Pseudococcus longispinus*, *Dysmicoccus brevipes*, and Proline, exhibited a notable presence on the F2 axis. These results demonstrate that the F1 and F2 axes, which account for most of the total variability in the data, are sufficient to validate the principal component analysis (PCA) and accurately interpret the findings. The results also show that the mealybug species *Formicococcus njalensis*, *Ferrisia virgata*, and *Phenacoccus hargreavesi* are linked to chlorophylls A and B, total chlorophyll, carotenoids, proteins, phenolic compounds, and total sugars in treatments T0, T3, and T4. Conversely, the mealybug species *Pseudococcus longispinus* and *Dysmicoccus brevipes* are linked to proline in treatment T2. This variability shows that mealybug dynamics depend on the host's specific nutritional and physiological conditions [28]. However, proline accumulation is often interpreted as an adaptive response by the plant in the face of biotic or abiotic stress [29]. Therefore, the cocoa trees in treatment T2 may have experienced stress, either nutritional or related to interactions with these mealybug species, resulting in increased proline levels. Therefore, the abundance of certain mealybug species may hinder the proper growth of cocoa trees. This is evident in the higher levels of chlorophyll A found in all fertilizer-treated samples except T2. Treatment T2 included all the mealybug species inventoried in this study.

5. Conclusion

To conclude, this study examined the impact of fertilizer application on the distribution of mealybug species carrying swollen shoot disease. Overall, fertilization did not significantly impact the number of mealybug colonies. However, it did influence the physiological and biochemical parameters of cocoa trees. The novelty of these results suggests that using fertilizer should not impact the proliferation of mealybug which may spread the cocoa swollen shoot disease. However, this study does not permit a definitive assessment of the results as it was limited to one location. Therefore, the study should be carried out in different regions of Côte d'Ivoire where cocoa

is produced. Additionally, plots that are both untreated and treated should be selected, with the untreated and treated plots more than 100 meters apart.

Abbreviations

ANOVA	Analysis of Variance
Carot	Carotenoids
Chla	Chlorophyll A
Chlb	Chlorophyll B
Chlt	Total Chlorophyll
CNRA	National Agricultural Research Center
Comph	Phenolic Compounds
Dbrevipes	<i>Dysmicoccus Brevipes</i>
Fnjalems	<i>Formicococcus Njalensis</i>
Frle	Fresh Leave.
Fvirgata	<i>Ferrisia Virgata</i>
GDP	Gross Domestic Product
GLM	General Linear Model
PCA	Principal Component Analysis
Pcitra	<i>Planococcus Citri</i>
Phargre	<i>Phenacoccus Hargreavesi</i>
Pkenya	<i>Planococcus Kenyae</i>
Plongis	<i>Pseudococcus Longispinus</i>
Prol	Proline
Prot	Protein
Suctot	Total Sugars
TSP	Triple Superphosphate

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Author Contributions

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Stanislas Kouakou Koffi: Data curation, Methodology, Writing – original draft

Diallo Karim Aboudou: Formal Analysis, Writing – original draft

Alain Acka Jacques Kotaix: Investigation, Resources

Leonard Rusinamhodzi: Funding acquisition

Richard Asare: Funding acquisition

Kouassi Allou: Conceptualization, Project administration, Supervision

Conflicts of Interest

The authors declare no conflicts of interest.

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