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Comparative Efficacy of Biofungicides (NECO and ASTOUN) and Synthetic Fungicide against Black Pod Rot of Cocoa Caused by *Phytophthora palmivora* in Côte d'Ivoire

Fofana Balakissa [a*], Camara Brahima [a,b], Kassi Koffi Fernand J-M [a,b], Silué Nakpalo [c], Zouzou Michel [a] and Koné Daouda [a,b]

[a] University of Félix Houphouët-Boigny, UFR Biosciences, Plant Physiology and Pathology Teaching and Research Unit, 22 BP 582 Abidjan 22, Côte d'Ivoire.
[b] Wascal/African Center of Excellence on Climate Change, Biodiversity and Sustainable Agriculture (Wascal/CEA-CCBAD), 22 BP 463 Abidjan 08, Côte d'Ivoire.
[c] University of San-Pedro, UFR Agriculture, Allieutic Resources and Agro-Industries, Côte d'Ivoire.

Authors' contributions

This work was carried out in collaboration among all authors. Author FB worked on the research topic, designed the methodology, and drafted the manuscript. Authors CB and KKFJM produced the biofungicides NECO and ASTOUN and revised the manuscript for the final version. Author SN assisted in setting up the experimental setup and applied the biofungicides in the field. Authors ZM and KD supervised the research and reviewed the manuscript. All authors read and approved the final manuscript.

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**Corresponding author: E-mail: balakisfof@yahoo.fr;*

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ABSTRACT

Black pod rot, caused by the oomycete *Phytophthora* spp., is one of the most important and widespread diseases of cocoa worldwide. In Côte d'Ivoire, production losses are estimated at 25 % to 60 %. The use of synthetic fungicides, which are still dangerous for producers and the environment, remains the most widely practiced method against this pathogen. This study evaluates the efficacy of two plant-based biofungicides, NECO and ASTOUN, in comparison to Callomil super, a conventional fungicide, for managing black pod rot in Côte d'Ivoire. However, the field application rates of these two biofungicides need to be controlled. To this end, a complete randomized Fisher block design was set up with seven treatments (NECO at 5 and 10 ml/l; ASTOUN at 5 and 10 ml/l; Callomil super at 5 and 10 ml/l and controls) and three blocks. Each block was a replicate. The products were applied at 1.7 and 3.4 l/ha in a 332.5 l spray mixture once a month, followed by weekly evaluation of the parameters. A total of three applications were made during the trial. Healthy and diseased pods and cherries were counted to determine black rot rates. A significant difference was observed between treatments for all parameters (P=0.00). The lowest rate (3.00 %) was recorded with NECO at 5 ml/l and the highest rate (6.43 %) with Callomil super at 10 ml/l in 2018. Whereas in 2019, the lowest rate of black rot (4.99 %) was obtained with NECO at 10 ml/l and the highest rate (12.24 %) with ASTOUN at 10 ml/l. In contrast, untreated cocoa trees had a high rate of pod rot during 2018 (15.78 %) and 2019 (22.30 %). NECO at 5 and 10 ml/l, synthetic fungicide at 5 ml/l and ASTOUN at 10 ml/l were more effective in controlling infected pods. It should also be noted that climatic conditions influenced the incidence of black pod rot. The average black rot rate curves varied with the amount of rainfall recorded each week. Temperature and relative humidity varied very little. Rainfall in 2019 was higher (863 mm) than in 2018 (496 mm), as were black rot rates. The biofungicides NECO and ASTOUN have been registered for use against black pod rot in Côte d'Ivoire.

Keywords: Black pod rot; biological fungicide; Phytophthora palmivora; synthetic fungicides.

1. INTRODUCTION

Côte d'Ivoire has been the world's leading producer of cocoa beans since 1970, with 2.200 million tonnes in 2022 (Statista Research Department, 2022). Black pod rot caused by the oomycete *Phytophthora* spp. is one of the most important, widespread and damaging cryptogamic diseases in the world, with an estimated production loss of 30 % worldwide (Coulibaly *et al*., CNRA 2017; Decloquement *et al*., 2021) and 30 to 40 % according to the International Cocoa Organization (ICCO, 2009). In Côte d'Ivoire, production losses are estimated at between 20 % and 45 %, and can reach 60 % if no phytosanitary measures are observed by the grower (Coulibaly *et al*., CNRA 2017).

Against black pod rot, several control strategies have been carried out namely, genetic control (use of varieties resistant to *P. palmivora* not sustainable in general), cultural practices (sanitary harvests, pruning, etc.), chemical control (use of synthetic fungicides) and biological control with the use of natural antagonists and pesticidal plants (Mpika *et al*., 2009; Pohe *et al*., 2013; Gadji *et al*., 2015).

However, the use of synthetic chemicals remains the most common. For decades, chemical fungicides have proved their worth in controlling black pod rot in the field, enabling growers to increase their income. However, this method of control is expensive and encourages growers to acquire these products fraudulently on the market. Added to this is the fact that the use of synthetic fungicides in the field has harmful effects on the environment, biodiversity and human health, making the search for alternative methods all the more urgent (Kpatinvoh et al., 2017).

Indeed, pesticide residues can be found in foodstuffs and drinking water (Diallo, 2023). Pollution studies carried out on various rivers in Côte d'Ivoire have highlighted pesticide contamination (Coulibaly *et al*., 2012). Some molecules are found at levels higher than the standards defined by the WHO (Traoré *et al*., 2015). These waters, and groundwater in particular, therefore present risks for the health of the population and the environment.

Due to their characteristics and properties, the use of pesticidal plants combined with integrated pest management could be an effective alternative method. However, in Côte d'Ivoire, very few studies have been carried out with biological fungicides in cocoa production. The

biofungicides NECO and ASTOUN are registered and known to be effective against black spot of banana in Côte d'Ivoire (KASSI *et al*., 2014). It is therefore necessary to study their efficacy in the control of black rot of cocoa trees in order to reduce prodcution losses. For this reason, it is essential to master the application rates of these biofungicides, and a field trial is a prerequisite. This study evaluates the efficacy of two plant-based biofungicides, NECO and ASTOUN, in comparison to Callomil super, a conventional fungicide, for managing black pod rot in Côte d'Ivoire.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment took place in a small village in Abbé, 7 km from Azaguié at latitude 05° 39 N, longitude 04° 00 W and altitude 49 m. This region is located in the southeast of Côte d'Ivoire and belongs to the agro-ecological zone I defined by Halle and Bruzon in (2006). It is a humid forest zone with rainfall ranging from 1,400 to 2,500 mm and an average temperature of 29 °C.

2.2 Umbrothermal Diagram of the Area

Climatic factors play a very important role in the proliferation of brown rot in the field. For this reason, daily measurements of temperature, humidity and rainfall were taken during the experiment to study their influence on the proliferation of the disease. Relative humidity and temperature were recorded every 30 minutes using a thermohygrometer (Easy Loger USB brand), and these values were then converted to daily and weekly values. Rainfall was also recorded every day using a manual rain gauge. The average temperature was 24.71°C, the relative humidity was 91.50 and the total rainfall in 2018 was 2056 mm.

2.3 Biological Material

These are Mercédès varieties of hybrid pods selected by research and widely used by farmers. They consist mainly of the Amelonado and Criollo varieties, which are susceptible to black pod rot.

2.4 Pathogenic Fungal Material

Pathogenic fungal material consisted of pods naturally affected by black rot, identified from morphological and molecular analyses.

2.5 Biological Control Materials

These are plant extract-based formulations codified NECO and ASTOUN. The active ingredients in these formulations are Thymol, Eugenol, Gerenial, Neral and Myrcene. They are oily emulsions from the Unité de Recherche Industrielle (URI) at the Université Félix Houphouët-Boigny, Bingerville. The products were applied at rates of 1.7 and 3.4 l/ha in a 332.5 l spray mixture once a month, followed by a weekly evaluation of the parameters. Callomil super (600g/kg copper oxide + 60 g/kg Metalaxyl-M) was used as a synthetic fungicide registered for the control of cocoa pod black rot in Côte d'Ivoire.

2.6 Methods

2.6.1 Experimental plot

The experimental plot is a cocoa orchard with an average age of 11 years. The experiment took place in the village of Abbé, 11 km from Azaguié, at latitude 05° 39 N, longitude 04° 00 W and at an altitude of 49 m. It was divided into three blocks of 2640 m^2 each, for a total of 7920 m^2. Plants were separated by 2.5 m and cocoa lines by 3 m. The layout was a complete randomized Fisher block. A total of seven treatments were applied (T1C1, T1C2; T2C1, T2C2; T3C1, T3C2 and T0 control). Ten cocoa trees were treated per product and concentration. Each treatment was separated by intermediate and border trees so that one treated tree was surrounded by eight untreated cocoa trees. Each block was a repetition. Intermediate and border cocoa trees received no treatment or sanitary harvesting, so as to maintain inoculum in the field. The chemical fungicide Callomil super was used as a positive control.

The breakdown of treatments by block is as follows:

T1C1: NECO at a concentration of 5 ml/l;

- T1C2: NECO at 10 ml/l;
- T2C1: ASTOUN at 5 ml/l;
- T2C2: ASTOUN at 10 ml/l;
- T3C1: Callomil super at 5 ml/l;
- T3C2: Callomil super at 10 ml/l;
- T0: Untreated control.

2.6.2 Application of biofungicides and evaluation of parameters

The experiment was carried out in September, October, November and December 2018 and then in June, July, August and September 2019.

Healthy pods and cherries were marked with paint to monitor their development throughout the experiment. For each trial, the product was applied once a month, followed by four weekly parameter assessments. The first application is characterized by weeks S1, S2, S3 and S4, weeks S5, S6, S7 and S8 represent the second application and the third application corresponds to weeks S9, S10, S11 and S12. The biofungicides NECO, ASTOUN and the synthetic fungicide Callomil super were applied in two doses (5 and 10 ml/l) using a sprayer. In other words, a mixture of 1.7 and 3.4 l/ha of the biofungicides NECO and ASTOUN and the synthetic fungicide Callomil super in 332.5 l of water. One week after each application, healthy green and mature pods, pods affected by black rot, and healthy and infected cherries were counted weekly. These parameters allowed us to determine the rate of black pod rot and the rate of infected cherries for each treatment.

The rates of pod rot and infected cherries were determined using the formula described by Bérry and Cilas, (1994).

$$TPi = (\Sigma CABMAi / \Sigma CABSVi + \Sigma CABMAi + \Sigma CABMUi) X 100$$

where

TPi is the rate of pods affected by black pod rot in week i;
Σ CABMAi is the number of diseased pods in week i;
CABSVi is the number of healthy green pods in week i, and
CABMUi is the number of healthy mature pods in week i.

2.7 Statistical Analysis

All parameters measured in the field were subjected to statistical analysis using Statistica version 7.1 and Excel. Analysis of variance (ANOVA) was used to separate the means of the variables measured in the field. In case of a significant difference, the Newman-Keuls test was used at a 5 % threshold to separate the means.

3. RESULTS AND DISCUSSION

3.1 Effects of NECO, ASTOUN and CALLOMIL SUPER on Black Pod Rot Rates in 2018 and 2019

This experiment tested the efficacy of the biofungicides NECO and ASTOUN compared with the synthetic fungicide Callomil super during the 2 years of experimentation. A significant difference was observed between the products in 2018 and 2019 ($p = 0.000$), showing that cocoa trees treated with these biofungicides recorded a low rate of pod rot. The lowest rate (3.00%) was recorded with NECO at 5 ml/l and the highest rate (6.43%) with Callomil super at 10 ml/l in 2018. Whereas in 2019, the lowest rate of black rot (4.99%) was obtained with NECO at 10 ml/l and the highest rate (12.24%) with ASTOUN at 10 ml/l (Fig. 1). In contrast, untreated cocoa trees had a high rate of pod rot during 2018 (15.78%) and 2019 (22.30%). NECO at 5 and 10 ml/l, synthetic fungicide at 5 ml/l and ASTOUN at 10 ml/l were more effective in controlling infected pods. For NECO and Callomil super, the 5 ml/l dose was more effective in contrast to ASTOUN, which was more effective with the 10 ml/l dose in 2018. NECO at 10 ml/l and ASTOUN at 5 ml/l were more effective in 2019. NECO recorded the lowest rate of black rot. At equal dosage, NECO and ASTOUN biofungicides were better than the synthetic fungicide, and the rate of rotted pods was higher in 2019 than in 2018 for all treatments. NECO was more effective than ASTOUN and Callomil super in 2018 and 1019.

3.2 Effects of NECO, ASTOUN and Callomil Super on the Weekly Evolution of Black Pod Rot with Climatic Parameters

The weekly evolution of the average rate of pod rot was evaluated taking into account climatic parameters over the two years of experimentation (Fofana et al., 2025). The average black rot rate curves varied with the amount of rainfall recorded each week. Temperature and relative humidity varied very little. The average rate of pod rot was higher on untreated control cocoa trees than on treated ones in both years of experimentation. Rainfall in 2019 was higher (863 mm) than in 2018 (496 mm), as were black rot rates. During the first trial in 2018, rainfall (276 mm) was higher in October (S5, S6, S7 and S8) than in September (S1, S2, S3 and S4) and November (S9, S10, S11 and S12). The rate of black pod rot was higher in October on untreated control cocoa trees, whereas it was lower on those treated with the biofungicides NECO, ASTOUN and the synthetic fungicide Callomil super, despite the high rainfall during the month. Fungicides therefore considerably reduced the rate of rotten pods during this period (Figs.). A significant difference was observed between the average rot rate of

untreated cocoa trees and those treated with biofungicides and synthetic fungicide.

In the second year of experimentation (2019), rainfall amounts were highest in June (S1, S2, S3 and S4) with 381 mm of water, then in July (S5, S6, S7, S8) with 327 mm of water. In August, 155 mm of rain was recorded (Fig. 2). The rate of rotten pods was highest in June for untreated cocoa trees. On the other hand, those treated with the biofungicides NECO, ASTOUN and the synthetic fungicide Callomil super showed a low rate of black pod rot during June and July. During August, the rate of pod rot was low for all treatments, including the untreated controls.

This study examined the effectiveness of the biofungicides NECO and ASTOUN in comparison with the synthetic fungicide Callomil Super and an untreated control group over the course of two years. A substantial discrepancy was detected between cocoa trees administered biofungicides at a reduced rate of pod rot in 2018 and 2019. Conversely, untreated cocoa trees exhibited a high prevalence of pod rot. The application of NECO at concentrations of 5 and 10 milliliters per liter (mL/L), synthetic fungicide at 5 mL/L, and Astoun at 10 mL/L was found to be more efficacious in the management of infected pods. In the case of NECO and Callomil super, the 5 ml/L dosage proved to be more efficacious, while ASTOUN exhibited superior

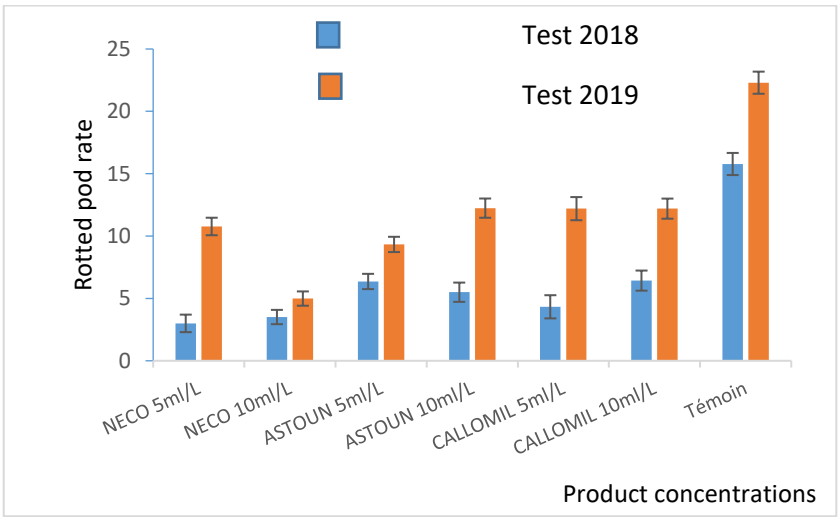

Fig. 1. Effect of fungicides on the average rate of rotted cocoa pods according to trial periods (2018 and 2019)

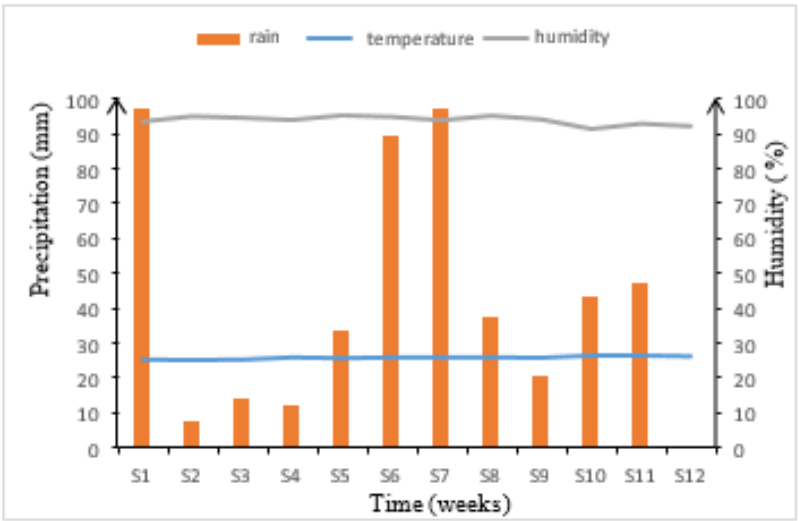

Fig. 2. Total weekly rainfall (Pmm), mean temperature (T°C), relative humidity (Hm) in Azaguié from September to November 2018

performance with the 10 ml/L dosage in Trial 1. In contrast to the findings observed in Trial 1, NECO at 10 ml/L and ASTOUN at 5 ml/L demonstrated enhanced efficacy in Trial 2. At equivalent dosage, the biofungicides NECO and ASTOUN demonstrated superiority over the synthetic fungicide. Furthermore, the rate of rotted pods was higher in 2019 than in 2018 for all treatments. The observed outcomes can be attributed to the sensitivity of *P. palmivora* to these fungicides. As demonstrated by Ngoh Dooh *et al*. (2015), *Thevetia peruviana* plant extracts have been shown to be efficacious in the mitigation of black pod rot and the reduction of *P. megakarya* inoculum pressure in Cameroon. Moreover, the positive effect of biofungicides on reducing the rate of black pod rot is likely attributable to the presence of chemical compounds contained in the formulation of these biofungicides. The medicinal, therapeutic, and biocidal properties of the plant are attributed to the presence of phenolic compounds, which are more concentrated in the leaves (Kpètèhoto *et al*., 2017; Soumanou & Adjou, 2016).

The experiments were conducted from June to September of 2019, which corresponded to the primary rainy season. In contrast, the experiments were conducted in September, October, November, and December of 2018, which corresponded to the shorter rainy season. A substantial body of research has demonstrated a correlation between climatic parameters and the progression of black rot, a condition caused by *Phytophthora* spp. Ngoh *et al*. (2015) have demonstrated that precipitation, elevated humidity levels, and low temperatures are conducive to the proliferation of black rot.

Coulibaly *et al*. (2017) demonstrated the field efficacy of synthetic fungicides, plant extracts, and antagonistic organisms, including Trichoderma spp.

The field efficacy of biofungicides (NECO and ASTOUN) and Callomil super was demonstrated on black pod and cocoa rot caused by *P. palmivora*. Biofungicides (NECO and ASTOUN) have been demonstrated to possess biocidal properties, including toxic, repellent, and anti-appetite effects, as reported by Boni *et al*. (2017). The fungicidal properties of these substances have been demonstrated by several authors. This phenomenon was previously observed in the studies by Kassi *et al*., (2021) and Kobenan *et al*., (2023), who demonstrated the inhibitory effect of NECO 50 EC and the *O. gratissimun* plant on black cercosporiosis of banana, mycelial growth, and the germination of spores of *Fusarium oxysporum* f. sp. *radicis lycopersici* and *Pythium sp*. The inhibitory effect of NECO 50 EC and the *O. gratissimun* plant was found to be comparable to that of synthetic fungicides.

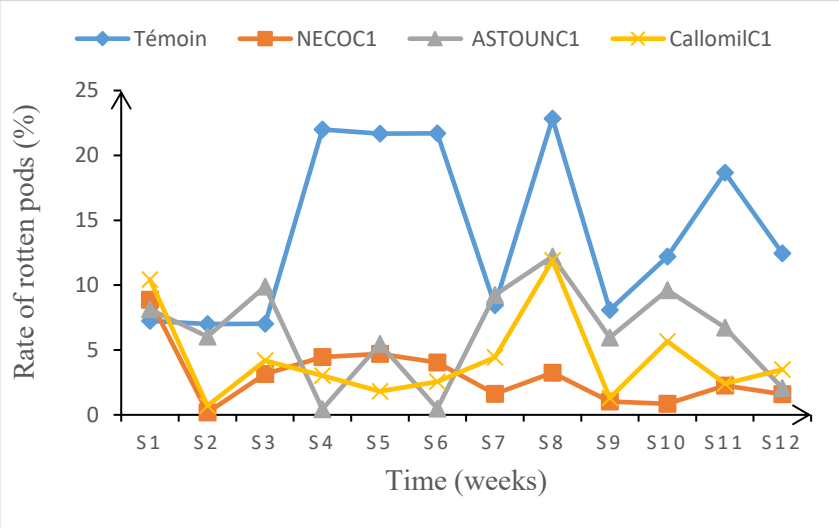

Fig. 3. Weekly variation in the average rate of rotten pods caused by *P. palmivora* after treatment with 5 ml/L of NECO, ASTOUN and Callomil super in 2018

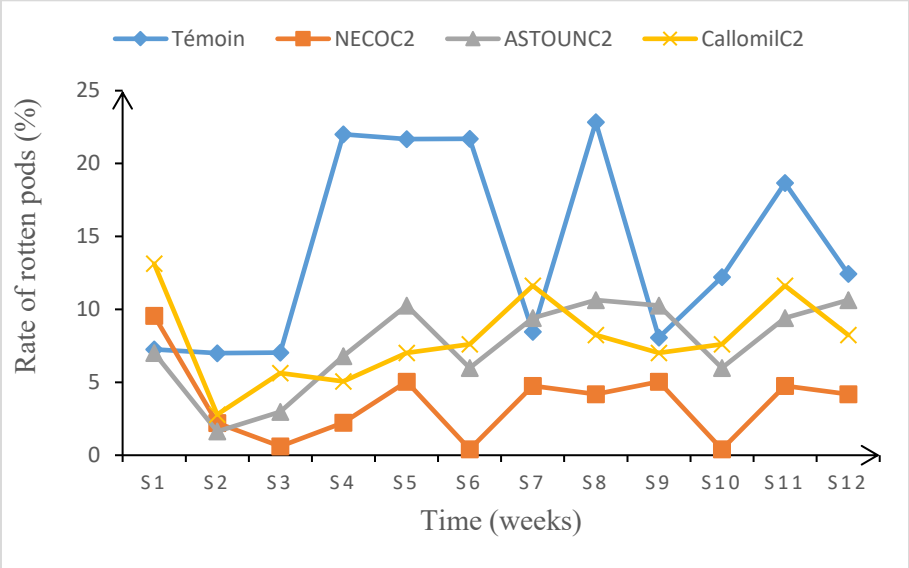

Fig. 4. Weekly variation in the average rate of rotten pods caused by *P.palmivora* after treatment with 10 ml/l of NECO, ASTOUN and Callomil super in 2018

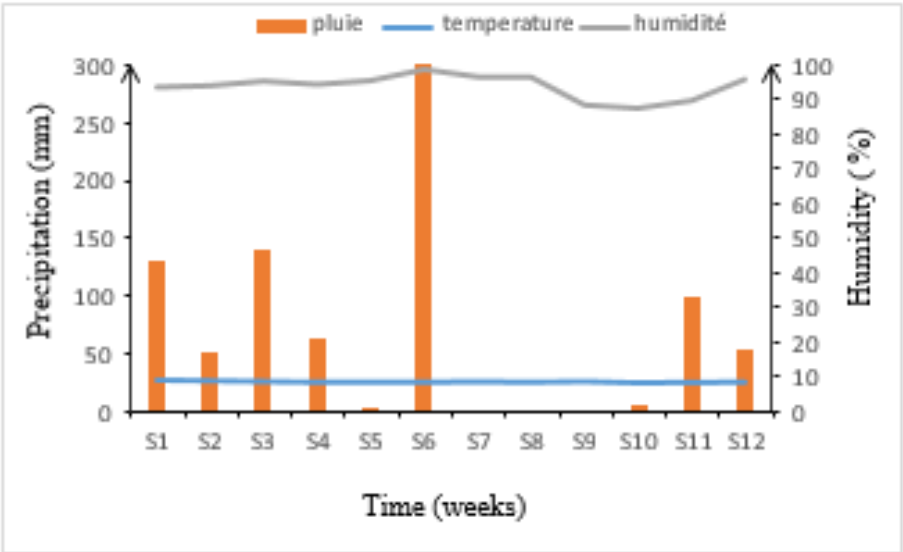

Fig. 5. Total weekly rainfall (P mm), mean temperature (T °C), relative humidity (Hm) at Azaguié from June to August 2019

According to N'guessan *et al*. (2009), these pesticidal plants, which are comprised of phytochemicals including phenol, have been shown to possess both anti-inflammatory and antimicrobial properties. Phenol has been demonstrated to impart a biocidal effect on tropical basil, thereby enhancing its capacity for defense against bioaggressors (Attou, 2011). In fact, according to Ogayo *et al*. (2015), extracts from *O. gratissimum* have been shown to regulate the population of *Tetranychus urticae* Koch by impeding oviposition and resulting in 90 % mortality among this pest. Furthermore, cocoa trees treated with biofungicides (NECO and ASTOUN) exhibited a reduced susceptibility to the disease caused by *P. palmivora* in comparison with the reference fungicide Callomil super at doses of 5 and 10 ml/l. Consequently, the biofungicides exhibited superior efficacy in comparison to the synthetic fungicide when administered at equivalent rates. The findings of this study substantiate the fungicidal and

fungistatic properties of these biofungicides. This outcome can be attributed to the fact that the products were applied once a month, whereas synthetic fungicides are typically applied once every 21 days, as prescribed by the manufacturer. This observation provides a rationale for the observed lower efficacy of these chemical fungicides over an extended evaluation period.

The incidence of black rot was minimal during the initial weeks following application. The rates exhibited an oscillatory pattern, increasing and then decreasing over the course of several weeks for all three applications. The observed fluctuations in black rot rates over the 12-week period are believed to be attributable to two primary factors. Firstly, the presence of volatile compounds in the biopesticides, which undergo evaporation over time, may contribute to the observed variations. Secondly, the seasonal variation in rainfall could play a significant role in the dynamics of black rot rates.

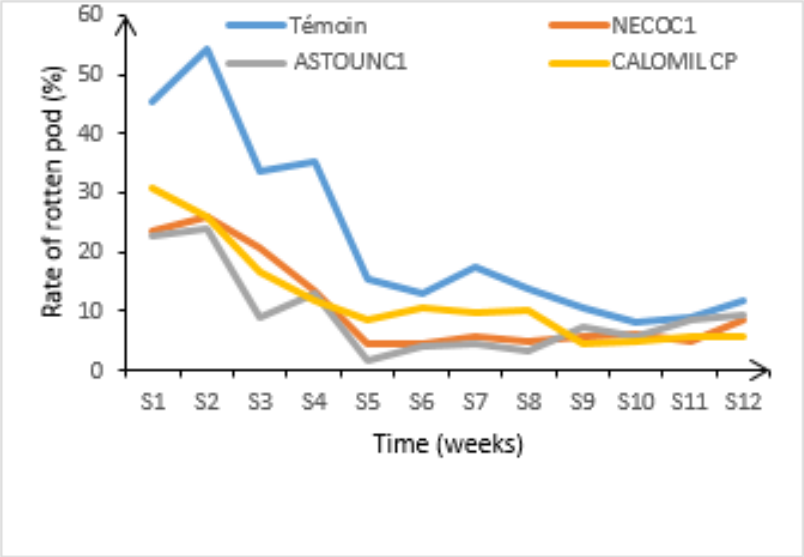

Fig. 6. Weekly variation in the average rate of rotten pods caused by *P. palmivora* after treatment with 5 ml/l of NECO, ASTOUN and Callomil super in 2019

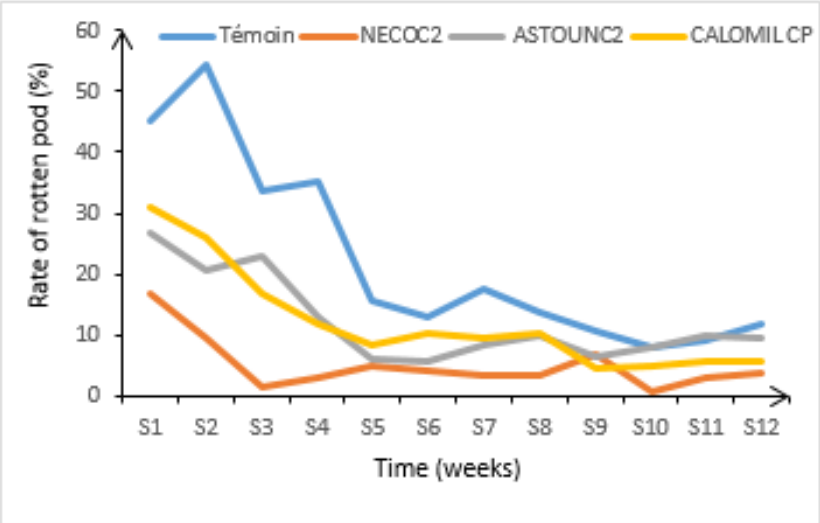

Fig. 7. Weekly variation in the average rate of rotten pods caused by *P. palmivora* after treatment with 10 ml/l of NECO, ASTOUN and Callomil super in 2019

The weekly progression of the black pod rot rate was assessed, incorporating climatic parameters from the two-year experimental period. The black rot rate curves exhibited variability in accordance with the precipitation data recorded during each week of observation. Conversely, temperature and relative humidity exhibited minimal variability. Pod rot rates were higher on untreated control cocoa trees than on treated cocoa trees in both years of experimentation. The precipitation levels in 2019 exhibited a marked increase compared to the previous year, with a recorded volume of 863 millimeters, significantly surpassing the 2018 precipitation levels of 496 millimeters. A similar trend was observed in the incidence of pod rot, which also showed a higher occurrence rate in 2019 compared to the previous year. In effect Leandro-Muñoz *et al*. (2017) demonstrated that temperature and water levels exerted a significant influence on the processes of germination and sporulation in Moniliophthora roreri (Cif.). The study demonstrated that pod rates exhibited a higher frequency during weeks characterized by elevated rainfall levels on untreated cocoa trees in comparison to those treated with biofungicides. Furthermore, a decline in decay rates was observed during weeks with low rainfall. The present study provides substantial evidence for the impact of climatic factors on the proliferation of black rot, a condition caused by the fungus *Phytophthora palmivora*. This finding contributes to our understanding of the role of environmental conditions in the spread of plant diseases, offering insights that could inform future research and management strategies (Ngoh Dooh *et al*., 2015).

4. CONCLUSION

The field experiments conducted in this study provided substantial evidence that biofungicides NECO and ASTOUN are more effective than the synthetic fungicide Callomil super. This conclusion was reached after a two-year experimental period. The biofungicide produced by NECO demonstrated a higher degree of efficacy, with concentrations of 5 and 10 milliliters per liter. However, during the primary rainy season, a dosage of 10 milliliters per liter would be optimal for the effective management of black pod rot, with two application periods per year. The initial phase occurred in May, June, and July, while the subsequent phase transpired in September, October, and November. Furthermore, the 5 ml/L biofungicide ASTOUN could be utilized in conjunction with NECO to avert the potential development of resistance in *Phytophthora* sp. to NECO. This study offers a glimmer of hope for the gradual replacement of chemical fungicides with the registered biofungicides NECO and ASTOUN in Ivorian cocoa production. Nevertheless, chemical analysis of cocoa beans to quantify residues of biofungicides applied during treatments would be essential for sustainable industrial production.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technology such as large language models (ChatGPT, COPILOT, etc.) and text-to-image generators were used in the writing or revision of this manuscript.re used in the writing or revision of this manuscript.

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COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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