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Research Article

Assessment of the NECO's Effectiveness against Cassava Bacterial Blight Caused by *Xanthomonas axonopodis* pv. *manihotis* In Côte D'Ivoire

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Abstract

Background and Objective: Cassava (*Manihot esculenta* Crantz) is an important crop in intertropical areas. However, it is subject to many diseases, among them the bacterial blight caused by *Xanthomonas axonopodis* pv. *manihotis*. The objective of this study was to assess the behavior of two varieties of cassava, including a traditional one (Diarrassouba) and an improved one (Bocou 1) in the area of Ferkessédougou endemic to bacterial blight and on the other hand, to test the bactericidal activity of NECO, a biological product made of essential oil extracted from *Ocimum gratissimum*. **Materials and Methods:** Eight increasing concentrations of NECO in comparison with the reference Callicuivre were used to assess the *in vitro* antibacterial activity and the 5 mL L^{-1} NECO dose was used *in situ*. Data were analyzed with one way ANOVA and Statistica software. **Results:** The results show that NECO exhibits bacterial inhibitory activity with diameters ranging from 0.34-3.46 cm. *In situ*, the use of NECO at 5 mL L^{-1} dose significantly decreases bacterial blight in the range of 74.94-59.35%, depending on the crop season. **Conclusion:** NECO could, therefore, be used as an alternative to synthetic products in cassava bacterial blight control.

Key words: Cassava, *Xanthomonas axonopodis* pv. *manihotis*, antibacterial activity, NECO, severity

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Agriculture, implemented effectively, helps to reduce hunger and malnutrition in the rapidly expanding populations of Sub-Saharan African countries[1]. Thus, face the ever-increasing food demand, the Ivorian state has undertaken, over the past two decades, to promote food crops of great importance in order to achieve food self-sufficiency and improve farmers' incomes[2]. Cassava (*Manihot esculenta* Crantz) occupies an important place among these crops. This crop is the second most important food crop after yam. It is grown in all the agro-ecological zones of Côte d'Ivoire, with a high production around the big cities in the South, Center and West, particularly in Abidjan, Yamoussoukro, Bouaké, Man and Daloa.

However, cassava cultivation is undergoing some constraints that limit its productivity. Unlike the so-called cash crop sector, the cassava sector has remained traditional and informal. Indeed, the production, marketing and distribution are not organized. Moreover, climatic fluctuations also contribute to a decline in yields due to changes in rainfall patterns, with crop seasons beginning late and ending earlier[3]. To these constraints that limit cassava production, biological factors of parasitic nature are added. African cassava mosaic disease, mealybug, mites and bacterial blight caused by *Xanthomonas axonopodis* pv. *manihotis* (Xam)[4,5] are the most important ones[6]. Xam is a vascular bacterium and the symptoms include soaked and translucent angular spots on the leaves, surrounded or not by a chlorotic halo. The infection can spread to the entire plant leading to the death of infected plants. Infected planting material is the essential factor for its spread. The impact of this disease is the degradation of the organoleptic and nutritional quality of starch, the main constituent of the tuber.

Cassava bacterial blight (CBB) is responsible for significant yield losses ranging from 20-100%[7]. It was first reported in Latin America in 1912 and appeared in Uganda in 1937. It subsequently spread to a large number of African countries[8] where damage, estimated at more than 75%, have been reported in Ghana, Nigeria, Benin, Uganda,[9-11]. In Côte d'Ivoire, it was reported in 1979 in the North of the country on the basis of symptoms[12]. More recently, it has been described in Burkina Faso by Wonni *et al.*[13].

Given of the economic consequences that this disease might cause, anticipating appropriate strategies for control in crop areas will ensure sustained production of cassava, a guarantee of food security and improved incomes for producers.

This study aimed at assessing the *in vitro* and on-farm antibacterial effect of the essential oil of *Ocimum gratissimum*, formulated under the common and trade name of NECO, against cassava bacterial blight in order to develop an effective alternative control strategy for synthetic products.

MATERIAL AND METHODS

In vitro assessment of the effect of NECO on colonies of Xam

Bacterial strain: The bacterial strain used in this study was isolated from cassava leaves originating from the locality of Ferkéssedougou (Côte d'Ivoire) and showing the typical symptoms of bacterial blight. The strain has been referenced (Xam FeK_4) and preserved in the Laboratory of Plant Physiology of the Félix Houphouët-Boigny University, in Abidjan.

Preparation of culture medium and seeding: To this end, 1 mL of inoculum was prepared from a 48 h pure colony of Xam cultured on YPGA medium (Yeast, peptone, glucose and agar). The bacterial suspension obtained was then calibrated to the optical density (OD) of 0.02, corresponding to 10^8 bacteria mL^{-1}. A range of eight concentrations (v/v) of NECO and Callicuivre, of an arithmetic progression of reason 1, varying from 1 (C_1)-8 (C_8) μL L^{-1} were prepared in 15 mL tubes with a sterile (0.1%) water-agar (WA) mixture. Callicuivre, synthetic product, used as reference, is formulated to the concentration of 500 g kg^{-1} of copper oxychloride. The bacterial suspension was then mixed in a liquid YPGA medium (75%) at a rate of 15 μL of suspension for 4.5 mL of YPGA. Using a micropipette, 4.5 mL of the mixture were taken and uniformly poured on the surface of the solidified YPGA (100%) medium contained in Petri dishes. After solidification of the mixture, 1-3 deposits of 10 μL of each NECO concentration were carried out per Petri dish. A negative control, receiving only WA deposits, was provided. After diffusion of the deposits made, the cultures were incubated at 28°C. Three repetitions were performed per concentration.

After 96 h, the diameter of the inhibition zone was measured daily using a graduated scale. The average of the measurements of the three dishes for each concentration was calculated for 10 days of observations. As a principle, the inhibition was noted positive when it was greater than 1 mm[14]. The sterile distilled water was used as negative control and each trial was repeated 3 times.

On-farm trials

Location and description of the experiment site: The study was conducted in Northern Côte d'Ivoire, in the Tchologo

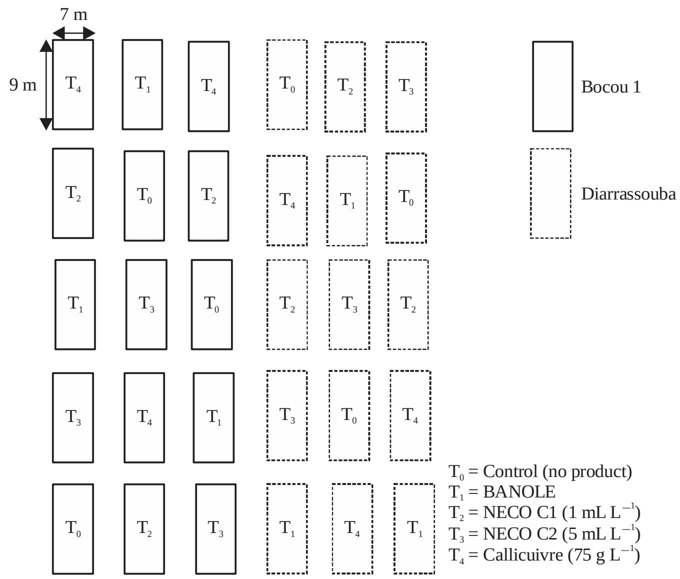

Fig. 1: Experimental design for on-farm assessment of the products

Region from 2015-2016. The test site is located at 10 km from Ferkessédougou city, administrative center of the region. Its position is 232 m altitude; 005°135.54'N and 06°479.42'W. This site belongs to the humid tropical savannah zone, with an annual rainfall ranging between 1150 and 1350 mm, belonging to agro-ecological zone VI of Côte d'Ivoire[15]. This zone is conducive to cassava cultivation, which is intensive, but often associated with maize. It has two seasons: A rainy season, extending from March-October and a dry one, from November-February. Between mid-November and February the Harmattan, a warm and dry wind of the Northern sector of Saharan origin, blows and influences the climate of the region[16].

Plant material: It was composed of two varieties of cassava sensitive to bacterial blight, including a traditional variety "Diarrassouba" and an improved one "Bocou 1" popularized among producers by the National Center for Agronomic Research (CNRA). varieties both were selected on the basis of their sensitivity to bacterial blight[17] and their adoption in the region by most farmers.

Experimental design: The design adopted was a completely randomized Fisher block, with 6 replications that were spaced 2 m apart (Fig. 1). The main plot (Bloc) had a surface area of 8000 m^2. It was subdivided into 6 sub-blocks (repetitions) of 350 m^2.

The sub-blocks were spaced 2 m apart. Each sub-block was subdivided into five (elementary) plots of 300 m^2, which were spaced by an interval of 2 m. Each of them constituted a treatment. The elementary plot consisted of 7 rows of 9 plants arranged at 1×1 m, with 31 plants in the useful surface area. The useful surface excluded the plants from the rows of each elementary plot.

Each variety was assessed over three blocks (Fig. 1), with 5 treatments including two biological ones and two with the reference product. The treatments with the biological product NECO (T_2 and T_3) included two doses (NECO C_1 = 1 mL L^{-1} and NECO C_2 = 5 mL L^{-1}). These quantities were mixed in 1 L of mineral oil (Banole) in order to facilitate the spraying on the plants to be treated. As regards the treatment with the reference product Callicuivre (T_4), it was applied at the single concentration of 75 g L^{-1}. The effect of each of the treatments T_2, T_3 and T_4 was compared with a treatment (T_1) with mineral oil (Banole). Finally, all these treatments were compared with a control treatment (T_0) without application of NECO, Callicuive and Banole.

Treatment conditions and on-farm assessment: Application of the products took place during the rainy season, corresponding to the period when the disease is more explosive. In the case of this study, this period corresponded to the month of August, that is, 2 months after planting cassava cuttings. At that period, the incidence of the disease varies from 24.44-88.89% according to a previous study[17]. For the treatment, 5 L of product were prepared for each dose applied. Each dose of product was applied by spraying on both sides of the leaves as well as on the stems, at the rate of 30 plants/treatment, always starting with the lowest dose of the product to be tested (NECO C_1 and then NECO C_2). The amount supplied to each plant was 25 mL.

Each treatment was preceded by an assessment based on the IITA severity scoring scale IITA[18] ranging from 1-5. Healthy plants had level 1 severity and the sick ones had an index varying from 2-5, that is, from the least severe to the most severe. The severity index (I%) of the disease was subsequently calculated using the formula of Amini and Sidovich[19]:

$$I\ \% = \frac{\sum \text{Values} \times \text{Number of infected plants}}{\text{Highest value} \times \text{Total number of plants}} \times 100$$

Observations: The observations on the health status of both crop cycles of the trial were made monthly for 9 months and three applications were made respectively at the 2nd, 3rd and 4th month after planting.

As for the effectiveness of the assessed products, it was determined using the Henderson-Tilton Eq.[20]:

$$\text{Effectiveness (\%)} = 1 - \frac{Ta \times Cb}{Ca \times Tb} \times 100$$

Where:

Ta = Infection of the plot treated on the day of observation
Tb = Infection of the plot treated before application of the product
Ca = Infection of the control plot before trial commencement
Cb = Infection of the control plot on the day of observation

Statistical analysis: The data collected were saved with Excel 2007 spreadsheet and analyzed with Statistica software version 7.1. In order to assess the effect of the different doses of NECO and Callicuivre on Xam on-farm and in the laboratory a one-way analysis of variance ANOVA was used. The Newman-Keuls average comparison test at 5% threshold was performed in case of proven difference in the univariate analysis.

A repeated measuring analysis of variance was performed on the average inhibition zone diameters recorded daily (10 days) for each product.

Correlations were made between inhibition zone diameters and doses so as to demonstrate the possible link between these parameters. Regressions were also performed to determine the prediction line.

RESULTS

***In vitro* antibacterial activity of NECO:** The result, presented in Fig. 2, shows that NECO and Callicuivre have an inhibitory activity on the phytopathogenic Xam strain tested. The inhibition zones were clear and had distinct edges and variable diameter depending on the nature of the product, the concentration used and the time of application.

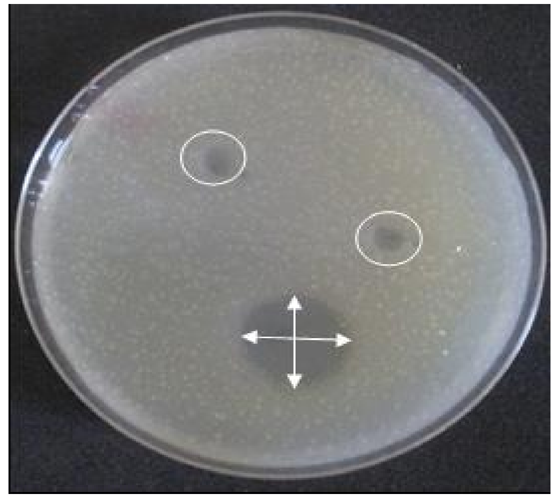

Fig. 2: Antibacterial activity of NECO reflected on Xam strains according to the method of Nguefack *et al.*[21]

Depending on the concentration of NECO: The analysis of these results, based on the comparison of average inhibition zone diameters, according to the LSD test (Least Significant Difference), at 95% confidence level, showed that there were significant differences both between NECO (C_1 and C_2) and Callicuivre and the concentrations of the same product. Thus, NECO having an average inhibition zone diameter ranging between 0.34 and 3.46 cm showed the greatest inhibitory activity on the Xam strain compared to Callicuivre, the reference product, which varied between 0 and 1.96 cm. The measured inhibition zone diameter of the products tested increased in the same direction as their concentrations. The inhibition graph (Fig. 3), depending on the assessed doses, reveals that the controls (C_0), doses C_1 and C_2 of Callicuivre had no inhibiting effect on the Xam bacterium. An inhibitory zone appeared as from concentration C_3 (3 μg L^{-1}). In contrast, all doses of NECO showed a reduction of bacterial growth. However, this inhibition (NECO) became greater as from C_3 (3 μL L^{-1}). A progressive inhibitory activity of Callicuivre was also found as from C_3, but its inhibition zone diameter remained well below that of the biological product.

Depending on the duration of incubation: Globally appreciate by Fig. 4 the inhibition zone diameters of the bactericides assessed over 10 days. The evolution of this regression curve, under the effect of NECO and Callicuivre, shows two and three distinct phases respectively, two of

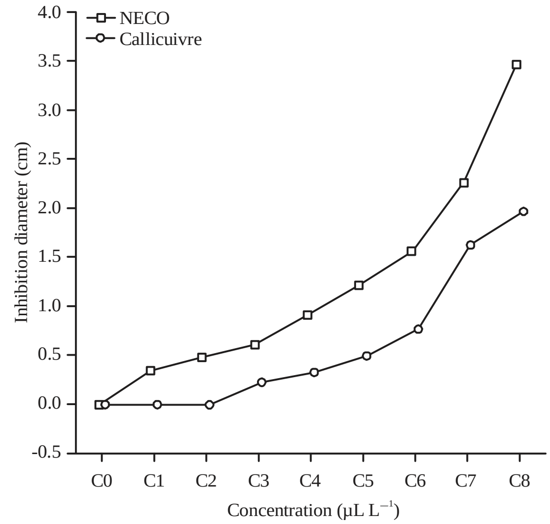

Fig. 3: Antibacterial activity of NECO reflected on Xam strains depending on the concentration of the assessed products

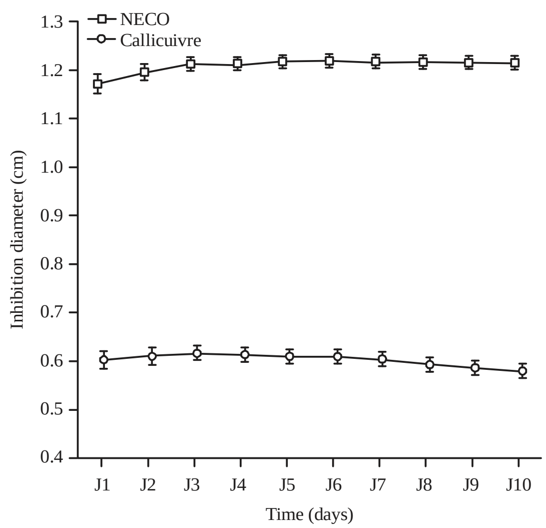

Fig. 4: Antibacterial activity of NECO reflected on Xam depending on the time of assessment

which are similar. From the 1st-3rd day, it shows a positive gentle slope, inferring an inhibition zone diameter of 1.17-1.22 cm for NECO and 0.6-0.62 cm for Callicuivre. In the second phase, a dwell was observed for both products, with an average diameter of 1.22 and 0.62 cm, respectively for NECO and Callicuivre. This dwell phenomenon observed on the 3rd day remained constant over the last 7 days of the experiment only with NECO. In contrast, there was a slowdown in Callicuivre, as from the seventh day, regressing towards its initial pace (0.58 cm).

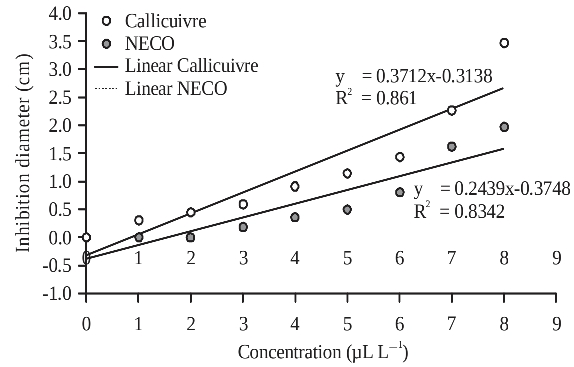

Fig. 5: Prediction line shows that NECO concentrations have similar effects as Callicuivre concentrations on inhibition diameter

The coefficients of determination of NECO and Callicuivre of 0.86 and 0.83, respectively indicate that in 87 and 83% cases the variation in inhibition zone diameter can be explained by the dose applied. Projection of C_7 and C_8 concentration of the reference product on NECO's prediction line shows that NECO concentrations C_4 (4 µL L^{-1}) and C_5 (5 µL L^{-1}) have similar effects as Callicuivre concentrations C_7 (7 µL L^{-1}) and C_8 (8 µL L^{-1}) (Fig. 5).

Assessment of bacterial blight on the two sensitive varieties of cassava: After two cycles of 9 months of study at Ferkessédougou on the behavior of two varieties of cassava sensitive to bacterial blight, it was found that the disease affected the variety "Diarrassouba" as well as "Boucou 1" but with a different level of sensitivity and at different periods. The disease was characterized by fluctuations from one season to another, with greater amplitudes in "Diarrassouba". The angular, translucent spots followed by droplets of exudates on the underside of the leaf blade (Fig. 6a) were seen in July, that is, 1 month after the cuttings were planted. High levels of disease progression were observed in the first 2 months when cumulative precipitation levels reached those of the months of August-November. The corresponding average severities were 40.5 and 48.11%, with an incidence of 64.72 and 79.44%, respectively on "Bocou 1" and "Diarrassouba". During this period, the level or grade 5 of severity was noted. The variety "Diarrassouba" would be particularly affected. On the latter, attacks occurred on the stems in addition to the leaves and twigs (Fig. 6b). This resulted in a mortality of 26.54% of the plants (Fig. 6c) as opposed to 3.17% for "Bocou 1".

These variations were gradually less important from December until the end of assessments in March.

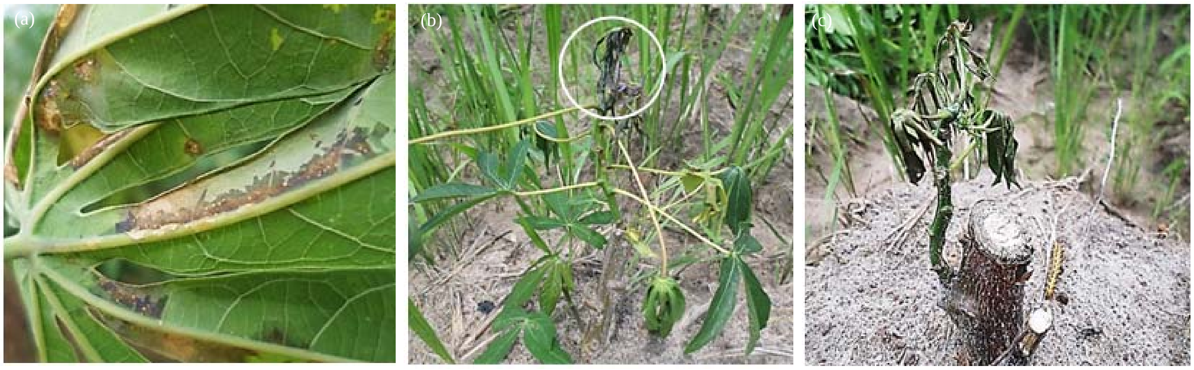

Fig. 6(a-c): Evolution of bacterial blight on cassava varieties "Diarrassouba" and "Bocou1", (a) Angular spot on the lower leaf blade (Stages 2 and 3), (b): Extensive leaf burns, wilting, defoliation and necrosis of extremities (die-back) (stage 4) and (c) Complete defoliation and cauline necrosis; Stunting and necrosis of lateral twigs with vegetative stoppage (stage 5)
The bars on the strips indicate the standard errors on the mean (Mean±SEM)

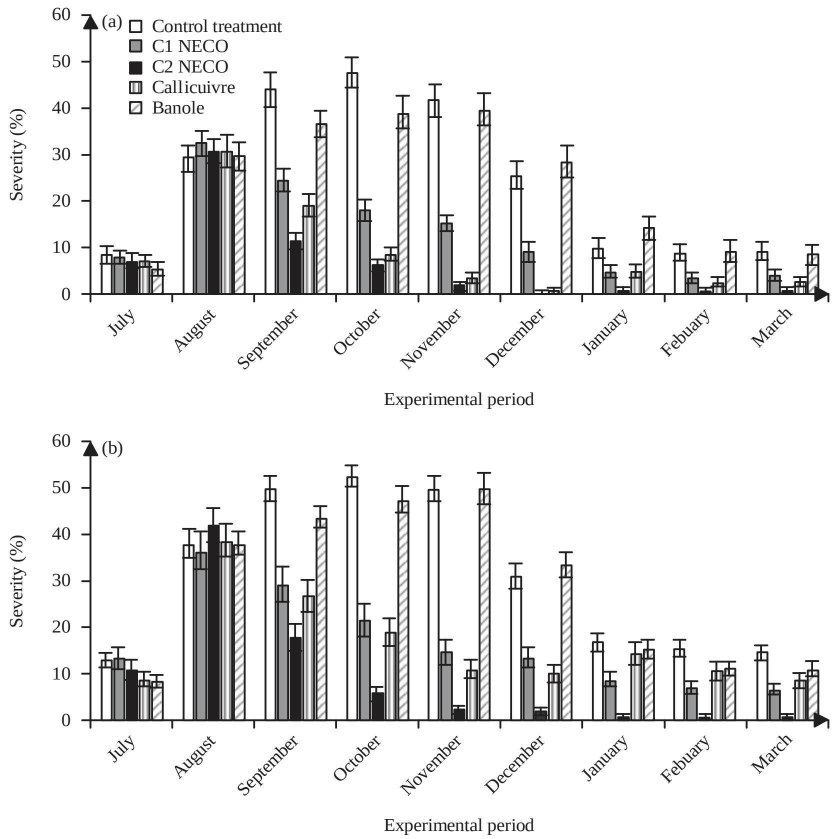

Fig. 7(a-b): Effect of treatments on cassava varieties "Diarrassouba" and "Bocou1", (a) Treatment on variety "Bocou 1" and (b) Treatment on variety "Diarrassouba"

***In situ* effectiveness of NECO:** Doses 1 and 5 mL L^{-1} and 75 g L^{-1} of NECO and Callicuivre respectively, effective *in vitro*, were used on-farm. The supply of products, by the spraying of leaves and stems, contributed to a significant decrease in parasitic pressure. By comparing the severity of bacterial blight on the treated plants with those of the controls (Fig. 7a and b), it was noted that the symptomatic response differed, on the one hand, from the nature of the product and from the dose used and, on the other hand, from the variety. In general, estimates of the effectiveness of the three phytosanitary treatments (NECO, Callicuivre and Banole) varied from 74.94-48.11% (Fig. 8).

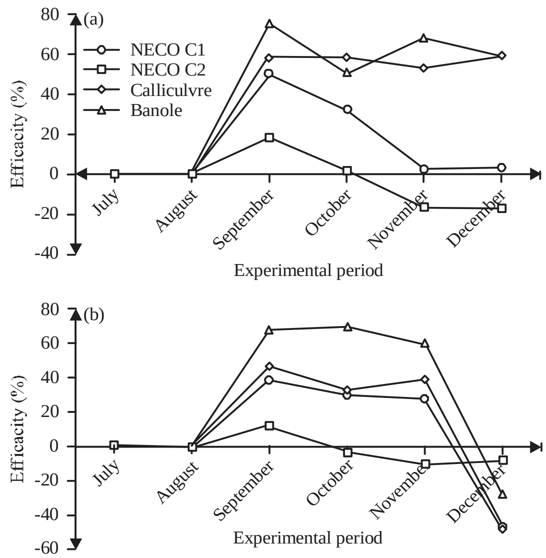

Fig. 8(a-b): Effectiveness of products according to the formula of Henderson and Tilton[21] on, (a) varieties "Bocou 1" and (b) "Diarrassouba"'

As for the nature of the products, NECO had a high potential against the disease, followed by Callicuivre. Banole showed the lowest response of effectiveness. As for the dose, the plots treated with NECO, treatments T_3 (5 μL L^{-1}) caused the highest rates of disease reduction on both varieties. In fact, 30 days after the first application, the severity of the disease decreased sharply in September with an effectiveness of 74.94 and 67.68%, respectively on "Bocou 1" (Fig. 8a) and "Diarrassouba" (Fig. 8b). Figure 8a and b shows that at the same time period, treatment T_2 at 1 mL L^{-1} of NECO and T_4 at 75 g L^{-1} of Callicuivre had a respective efficiency of 49.9 and 58.56% on "Bocou 1" and 38.94 and 47.17% on "Diarrassouba".

The last two treatments of October and November were also satisfactory overall over the two varieties tested, with the exception of the Banole-based treatment which remained without bactericidal effect. The assessment of the two varieties also shows an effectiveness around 60% of treatments T_3 (5 mL L^{-1}) NECO and T_4 at 75 g L^{-1} of Callicuivre, whereas that of NECO T_2 at 1mL L^{-1} was about 30%.

The extension of the assessment until December showed that the phytosanitary response is a function of the time with which a gradual decrease in effectiveness is observed, especially on "Diarrassouba" (Fig. 8b).

Until one month after the three treatments, we did not notice any sign of toxicity neither on the plants treated with the synthetic product nor with NECO.

DISCUSSION

This study investigated the influence of antibacterial treatment on two sensitive varieties of cassava ("Boucou1" and "Diarrassouba")[17] from a product of biological origin; NECO in comparison with a synthetic product; Callicuivre. *In vitro* inhibition tests were performed on the pathogen (Xam) followed by on-farm trials in Ferkessedougou, a bacterial blight endemic zone.

It appeared during the tests carried out *in vitro* that the NECO extracted from *Ocimum gratissimum* showed an inhibitory activity by the induction of inhibition zones on the germs of *Xanthomonas axonopodis* pv. *manihotis* (Xam) strains in a dose-response relationship. These results corroborate those obtained by Nguefack *et al.*[21], Bupesh *et al.*[22], Paret *et al.*[23], Camara[24] and Lucas *et al.*[25] which attest that essential oils possess antibacterial and antifungal properties. The average diameters obtained varied from 0.34 and 3.46 cm. By performing daily measurements of the zone of inhibition on each NECO modified Petri dish, no regression (decrease in borders) of inhibited surface could be observed depending on application time, unlike the reference product (Callicuivre) where a slowdown was observed from the seventh to the tenth day of observation. This suggests that the zone inhibited by the NECO active principle would still have the same antibacterial property 10 days later. The two products tested on Xam, in the same proportions, in terms of concentrations, reveal a resistance of the bacterial strain at the doses of 1 and 2 μL L^{-1} of Callicuivre. In addition, by comparing the effect of each dose, Callicuivre's antibacterial activity was significantly lower than that of NECO. This result suggests the existence of at least one active principle with a very high antibacterial property which would inhibit the growth of Xam. The study carried out in Côte d'Ivoire by Oussou[26], Kanko[27] and Kassi *et al.*[28] shows that the essential oil of *Ocimum gratissimum*, active substance of NECO, might be predominantly composed of thymol (46.1%.) and ɣ-terpinene, (17.6%.). Previous studies conducted by Traboulsi *et al.*[29] and Nguefack *et al.*[30] showed the importance of thymol and ɣ-terpinene in the strong antiparasitic activity of the essential oil of *Ocimum gratissimum*.

The monitoring of bacterial blight development was carried out following a visual diagnosis based on the observation of the characteristic symptoms on the leaves and stems of cassava plants. The period of onset and evolution of the disease varies according to the agro-ecological zones[17], the crop seasons and the varieties cultivated. The treatment trials carried out the two varieties tested were based on previous results that showed a greater presence of diseases[17]. These attacks gradually evolved through angular spots on the leaves, leading to wilting and sometimes vegetative stoppage. The period of high parasitic pressure occurred during the rainy season between August and November. These results confirm those obtained in Togo by Boher and Agbobli[31], indicating that the rainy season facilitates the multiplication of the parasite in the host tissues with visible symptoms. This indicates that under strong inoculum pressure facilitated by the environment, even some resistant varieties developed in IITA control strategies become sensitive to the bacterium.

During this experiment, the reduction in the disease severity index varied according to the product and the associated dose, with an optimum effect observed by treatment T_3 at 5 mL L^{-1} of NECO followed by treatment T_4 of the reference product, Callicuivre at 75 g L^{-1} and treatment T_2 of NECO at 1 mL L^{-1}. The comparison of control treatments with mineral oil (banole) treatments showed a significant effect of the latter on the severity of the disease compared to control treatments alone.

Moreover, the works of Kassi *et al.*[28] on banana black leaf streak showed that a better reduction in the growth of the ascomycete fungus *Mycosphaerella fijiensis* is obtained only at 20 mL L^{-1} of NECO. These results suggest that the phytosanitary treatment of cassava Xam would be more sensitive than that of banana tree fungi.

CONCLUSION

The phytosanitary situation of cassava (*Manihot esculenta* Crantz) caused by *Xanthomonas axonopodis* pv. *manihotis* (Xam) is therefore a real problem for which a treatment trial based on an essential oil extract of *O. gratissimum* (NECO) was undertaken in a prevalence zone in Ferkessédougou (Côte d'Ivoire). An improvement was observed after three applications of this biological product (NECO) at a dose of 5 mL L^{-1} for the rainy season (critical) with an average effectiveness of 65% against 48.16% for Callicuivre, the reference product. The results obtained are interesting for the future in sustainable agriculture. However, despite the success, further studies are needed to understand and improve the treatment conditions of NECO. Other varieties of cassava and different agro-ecological sites will have to be taken into account.

SIGNIFICANCE STATEMENTS

This study revealed a new strategy for bacterial blight control by using NECO a biopesticide formulated from natural plant substances and easily applicable in farmers field. It thus proves to be a promising alternative to the use of synthetic products which are harmful to the environment, the applicator and the health of the consumer. NECO could be advised in the integrated control programs for cassava bacterial blight in Côte d'Ivoire.

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