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Control of cassava mealybug *Phenacoccus manihoti* (Homoptera: Pseudococcidae) using NECO 50EC biopesticide in Grand Lahou (Côte d'Ivoire)

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Abstract

Cassava (*Manihot esculenta* Crantz) is a food crop with tuberous roots that plays an important role in feeding populations. The need to control cassava pests led to testing the effect of the biopesticide NECO 50 EC on *Phenacoccus manihoti* in a plot of the city of Grand-Lahou. The effectiveness of the biopesticide NECO 50 EC was tested on larvae and adults of P. *manihoti*, in comparison with a conventional insecticide K-OPTIMAL 35 EC. Dilutions gave 5 respective concentrations of NECO 50 EC: 8.33 g/l; 4.54g/l; 3.12g/l; 2.38 g/l and 1.92 g/l, and a concentration of 0.093 g/l for the control insecticide. Spraying of cassava plants infested with mealybugs was undertaken and observations were made 24 hours, 48 hours and 72 hours after treatment. On the larvae, the highest rate (92.07 ± 0%) is obtained at a concentration of 8.33 g/l for NECO and 98.51% for the control insecticide. In adults for NECO the highest rate (62.50 ± 0%) is obtained at the concentration of 8.33 g/l and 96.17% for the control insecticide. The biopesticide NECO 50 EC could be used as an alternative to the excessive use of synthetic insecticides to reduce the damage of the pest P. *manihoti*.

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Introduction

Cassava (*Manihot esculenta* Crantz) is a food crop with tuberous roots that plays an important role in feeding populations in the tropical and subtropical belts of the globe (Mabrouk and El-Sharkawy, 1993; Enete and Igbokwe, 2009). Originally from Central and South America, cassava was domesticated by pre-Columbian civilizations before being introduced to Africa in the 16th century, then to Asia in the 18th century (Silvestre and Arraudeau, 1983). World cassava production in 2017 was estimated at 292 million tonnes, of which 178 million tonnes came from Africa (FAOSTAT, 2017). Cassava is often grown by farmers, especially women, and most often on marginal land. For these farmers and their families, cassava is an important crop because it contributes to food security and generates substantial income (Enete, 2009; Howeler *et al.,* 2013).

In Côte d'Ivoire, cassava is the second food crop after yam (FAO, 2014). Several varieties of this plant are cultivated in Côte d'Ivoire, in particular the so-called traditional varieties (Bonoua, Kaman, etc.) with low yields and which are sensitive to diseases and insect pests and the improved varieties (Bocou 1, Bocou 2, TMS4 (2)1425, Yavo, etc.) with high yields and more resistant (N'zué *et al.*, 2013; Akpingny and Akoulou, 2017). A decline in tuber production is observed in Africa. This decrease could be partly explained by the presence and rapid spread of diseases (bacteriosis, viral infections) and pests (mites, mealybugs), recently introduced on the African continent (Herren, 1987), such as the cochineal floury cassava *Phenacoccus manihoti* (Matile-Ferrero, 1976). P. *manihoti* is an oligophagous insect that reproduces by thelytokous parthenogenesis and feeds on elaborate cassava sap (Calatayud and Le Ru, 1997). Cassava cultivation is exposed to considerable yield losses and reductions in both quantity and quality of planting material.

Several options for controlling this pest, including cultural and chemical methods as well as selection for host plant resistance, were explored. Cultivation methods, although quite effective, are limited in their use. Chemical control comes up against socio-economic constraints (Tata-Hangy, 1995). Unfortunately, the massive use of synthetic insecticides creates many problems: the resistance of insects, the resurgence of these to pesticide residues, environmental pollution, human poisoning, the elimination of pollinators and natural enemies of pests, destruction of wildlife and contamination of groundwater and rivers (Kadri *et al.*, 2013; Hénault-Ethier, 2015). Effective control without harming human health and the environment.

The use of biopesticides for crop protection as an alternative to synthetic insecticides would have many advantages. Their biodegradability with a low waiting period makes their products with low ecological impact (Isman, 1997). Several control trials using biopesticides have given good results on many insect pests (Tano *et al.*, 2012; Monfankye, 2014; Ossey *et al.*, 2018). The objective of this study is to evaluate the effectiveness of the biopesticide NECO 50 EC on the mealybug P. *manihoti.*

Material and methods

Study zone

The study was carried out on a plot located in the city of Grand-Lahou which is located in the region of the great bridges in the south of the country, on the edge of the Gulf of Guinea, at the embouchure of the Bandama River (Fig. 1). Its geographic coordinates are 5°25 North latitude and 4°55 West longitude. The climate of the Grand-Lahou region is of the equatorial type, characterized by a long dry season from December to March, a long rainy season from April to July, a short dry season from August to September (upwelling period) and a short rainy season from October to November (Konan *et al.*, 2013). The average annual rainfall of 1638 mm. Over the year, the average temperature in Grand-Lahou is 26.6°C.

Material

The plant material was cassava plants of the Yacé variety. The choice of this variety was justified by the fact that it was the most cultivated in the locality and the most adapted to the environment.

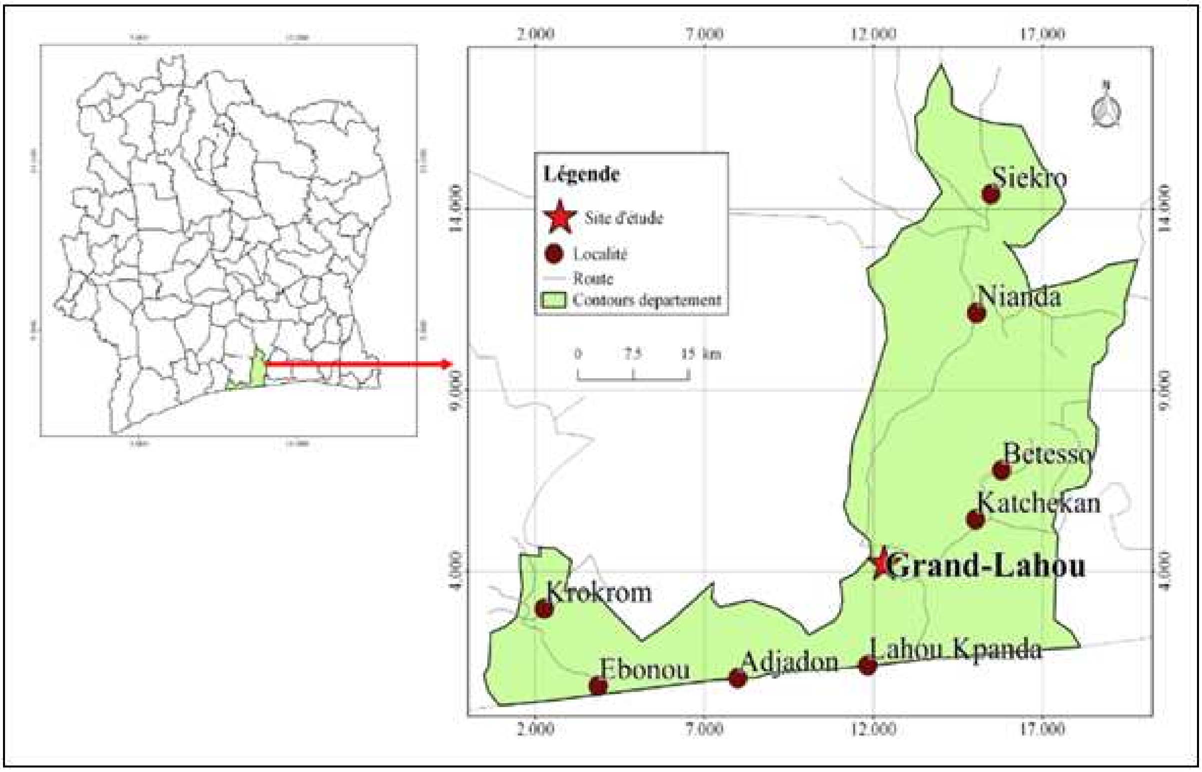


Fig. 1. Map of Côte d'Ivoire with the location of the study site in the Department of Grand-Lahou (INS, 2015).

The animal material was the larvae and adults of the mealybug measuring between 1 to 5mm. These mealybugs are characterized by the absence of wings, a pink color, an oval shape, and very short body filaments. They are covered with a white powdery wax evoking a mealy appearance (Braima *et al.*, 2000). The technical equipment consisted of equipment for protection, treatment and preparation of extracts.

The protective equipment consisted of a pair of gloves, a nose mask to avoid contact with insecticides and inhalation of products.

The two treatment products were NECO 50 EC (Fig. 2A) and K-OPTIMAL 35 EC (Fig. 2B). K-OPTIMAL 35 EC has two active ingredients: Lambda-cyhalothrin (25 g/l) and Acetamiprid (20 g/l). It is a broad-spectrum systemic chemical insecticide commonly used to control insect pests of vegetable crops. It served as a reference chemical insecticide (the control). NECO 50 EC is a biopesticide based on *Ocimum gratissimum* essential oil. A hand sprayer (a jet at 0.25 ml/s wetting a leaf area of 650 cm² at a distance of 30 cm from the leaf) was used for the treatments. Distilled water, pipettes and beakers were used for the preparation of the different concentrations.

Methods

Experimental dispositif

This study was carried out on an experimental plot with an area of 435 m² (29 m × 15 m). This plot is divided into 3 blocks 2.5 m apart. Each block comprised 03 elementary plots 7 m long and 4 m wide. Two consecutive elementary plots are separated by 1 m. The arrangement of the cassava cuttings is made in hols equidistant from 0.5 m (Fig. 3). A cassava cutting was put in each hole. Each elementary plot is made up of 30 plants (Fig. 4). There are 270 plants in total on the entire experimental plot.

Evaluation of the of NECO 50 EC

Two insecticides were used: K-OPTIMAL 35 EC and NECO 50 EC.

Determination of concentrations: The reference chemical insecticide used is K-OPTIMAL 35 EC, whose active ingredients are Lambda-cyhalothrin 25 g/l and Acetamiprid 20 g/l. The recommended dose for the treatment of plants is 40 ml of the product diluted in 15 liters of water, i.e., 4 ml of the product in 1.5 l of water. This corresponded to a concentration of 0.093 g/l. NECO 50 EC is a biopesticide based on *Ocimum gratissimum* essential oil. The dilution of NECO 50 EC (1ml) in distilled water (5ml; 10ml; 15 ml; 20 ml and 25 ml), made it possible to have 5 respective concentrations: 8.33 g/l; 4.54g/l; 3.12g/l; 2.38 g/l and 1.92 g/l.

Spraying larvae and adults of *P. manihoti* : Per the elementary plot, 21 cassava plants infested by adults and larvae of *P. manihoti* were chosen and marked. The larvae and adults of *P. manihoti* (30 to 40 individuals) present on the leaves and stems of the plants were counted using a hand magnifying glass. Mealybugs were treated with insecticides with different concentrations. Three repetitions were made per concentration and per insecticide. The dead insects were counted 24; 48 and 72 hours after treatment.

For each concentration, the mortality rates were calculated and corrected by Abbott's formula (1925) :

$$M = \frac{\text{number of dead insects}}{\text{total number of insects}} \times 100$$

$$MC = \frac{mo - mt}{100 - mt} \times 100$$

M: mortality rate; Mc: corrected mortality rate; Mo: mortality rate observed in the trial; Mt: mortality rate observed in control.

The lethal concentration 50 or LC50 is that which causes the death of 50% of a population of treated insects after 24 hours. It was determined for the NECO by the method of Finney (1971).

Data analysis

Data were processed using Statistica software, version 7.1. Analysis of variances (ANOVA) and the Student-Newman-Keuls (SNK) test at the 5% threshold were used to analyze and compare the average adult mortality rates. The results of the dose-response test are subjected to a probit analysis according to the method of Finney (1971) using the XLSTAT software version 2015 for the calculation of the LC_{50}.

Results

Effect of insecticides on P. manihoti mortality

On the larvae: The mortality rates varied from 14.10 to 94.91%, from 16.66 to 95.77 and from 17.44 to 98.51%, respectively 24, 48 and 72 hours after the treatments (Table 1).

Table 1. Mortality rate (%) of P. *manihoti* larvae after treatments.

Control periods after treatment	NECO g/l					K-Optimal (0,093 g/I)	Témoin
	1,92	2,38	3,12	4,54	8,33		
24 hours	14,10 ± $2{,}72^{i}$	17,95 ± $2{,}72^{hi}$	27,20 ± $1{,}86^{f}$	65,71 ± $4{,}04^{d}$	88,10 ± $3{,}37^{c}$	94,91 ± $1{,}36^{b}$	1,59± $1{,}57^{j}$
48 hours	16,66 ± 0^{hi}	18,72 ± $2{,}72^{h}$	32,46 ± $1{,}86^{e}$	67,62 ± $2{,}03^{d}$	92,07 ± 0^{b}	95,77 ± $1{,}10^{ab}$	2,55 ± $0{,}20^{j}$
72 hours	17,44± $2{,}72^{hi}$	22,22 ± $2{,}35^{g}$	35,09 ± $1{,}86^{e}$	67,62 ± $2{,}03^{d}$	92,07 ± 0^{b}	98,51 ± $1{,}58^{a}$	2,92 ± $0{,}54^{j}$

Newman-Keuls test at the 5% threshold F= 1227.70; ddl= 20; p<0.001.

Average of three replicates (n=40). Means followed by the same letters are not significantly different.

The application of K-OPTIMAL 35 EC at a concentration of 0.093 g/l gave a mortality rate of 94.91% twenty-four hours (24 hours) after treatment. Seventy-two hours (72h) after treatment, the rate increased by 3.50% to reach 98.51%. Applications of NECO 50 EC, at concentrations of 1.92; 2.38; 3.12; 4.54 and 8.33 g/l, gave respective mortality rates of 14.10; 17.95; 27.20; 65.71 and 88.10% 24 hours after the treatments. Mortality rates increased by 2 to 4% forty-eight hours (48h) after treatment and by 2 to 4% seventy-two hours (72h) after treatment. The highest rate, obtained at a concentration of 8.33 g/l, was 92.07 ± 0%. Statistical analysis showed significant differences between mortality rates (F=% F= 1227.70; ddl= 20; p < 0.001). On the adults: The mortality rates varied from 7.50 to 90.33%, from 10.83 to 93.17 and from 28.33 to 96.17%, respectively 24, 48 and 72 hours after the treatments (Table 2).

Table 2. Mortality rate (%) of P. *manihoti* adults after treatments.

Control periods after treatment	NECO g/l					K-Optimal (0,093 g/I)	Témoin
	1,92	2,38	3,12	4,54	8,33		
24 hours	7,50 ± 2,50^{i}	15,83 ± 3,82^{g}	22,50 ± 2,50^{d}	51,67 ± 1,44^{d}	62,50 ± 5^{c}	90,33 ± 1,44^{a}	2,83 ± 1,44^{j}
48 hours	10,83 ± 2,89^{h}	24,17 ± 2,89^{f}	26,67 ± 1,44^{f}	58,33 ± 1,44^{c}	76,67 ± 6,29^{b}	93,17 ± 1,44^{a}	3,67 ± 1,44^{j}
72 hours	28,33 ± 1,44^{f}	30,83 ± 2,89^{e}	34,17 ± 1,44^{e}	63,33 ± 2,89^{c}	85 ± 6,61^{a}	96,17 ± 1,44^{a}	3,67 ± 1,44^{j}

Newman-Keuls test at the 5% threshold F= 1467.34; ddl= 20; p<0.001.

Average of three replicates (n=40). Means followed by the same letters are not significantly different.

The application of K-OPTIMAL 35 EC at a concentration of 0.093 g/l gave a mortality rate of 90.33% twenty-four hours (24 hours) after treatment. Seventy-two hours after treatment, the rate increased by 5.80% to reach 96.17%. Applications of NECO 50 EC, at concentrations of 1.92; 2.38; 3.12; 4.54 and 8.33 g/l, gave respective mortality rates of 7.50; 15.83; 22.50; 51.67 and 62.50% 24 hours after the treatments. Mortality rates increased by 2 to 4% forty-eight hours (48h) after treatment and by 2 to 4% seventy-two hours (72h) after treatment.

Fig. 2. Treatment and protection equipment (A: NECO 50 EC, B: K-OPTIMAL 35 EC).

The highest rate, obtained at a concentration of 8.33 g/l, was 62.50 ± 0%. Statistical analysis showed significant differences between mortality rates (F=% F= 1647.34; ddl= 20; p < 0.001). Lethal concentrations (LC50) were 3.46 g/l for larvae and 4.43 g/l for adults.

Discussion

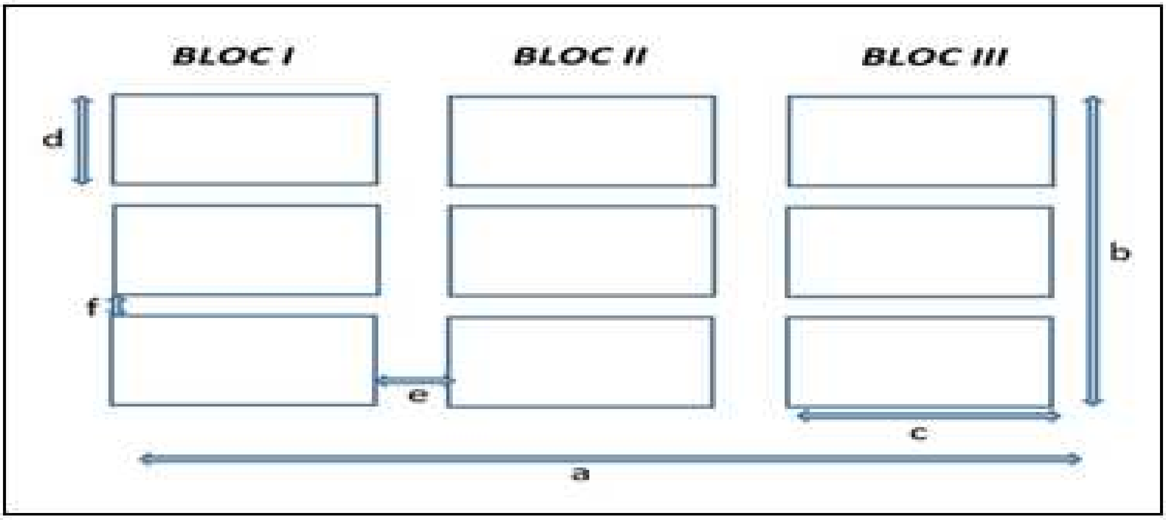
The results of the present study showed that compared to the chemical insecticide, the different concentrations of NECO 50 EC induced mortality rates in the larvae and adults of *P. manihoti* in the treated plots. Indeed, the highest mortality rates of P. *manihoti* larvae and adults with NECO 50 EC are obtained after 72 hours at a concentration of 8.33 g/l.

As for the chemical K-OPTIMAL 35 EC, the mortality rate is 94.91% after 24 hours and 98.51% after 72 hours at a concentration of 0.093 g/l.

The synthetic chemical (which has the active ingredient Lambda-cyhalothrin and Acetamiprid) was shown to be significantly more effective in reducing the cochineal population.

This would be due to the difference in insecticidal power and the mode of action of the different formulations on the one part and to the difference in the persistence of the different products on mealybugs.

In general, synthetic chemical products have higher insecticidal power and remain active for a relatively long time after application compared to biological products (Tounou *et al.*, 2018), which are characterized by biodegradability due to the sun.



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ig. 3. Diagram of the experimental dispositifa: Length of the plot: 29 m; b: plot width: 15 m; c: length of the sub-plot: 8m; d: width of the sub-plot: 4 m; e: distance between two blocks: 2.5m; f: distance between two consecutive subplots of the same block.

The results obtained with the NECO 50 EC treatment indicate that at a concentration of 8.33 g/l, mortality rates on P. *manihoti* larvae are greater than 70% after 24 hours of exposure. This observation is in agreement with that of Begon *et al.* (1990), who reported that a substance with an insecticidal effect is effective only when it induces a mortality rate of at least 70% on pests.

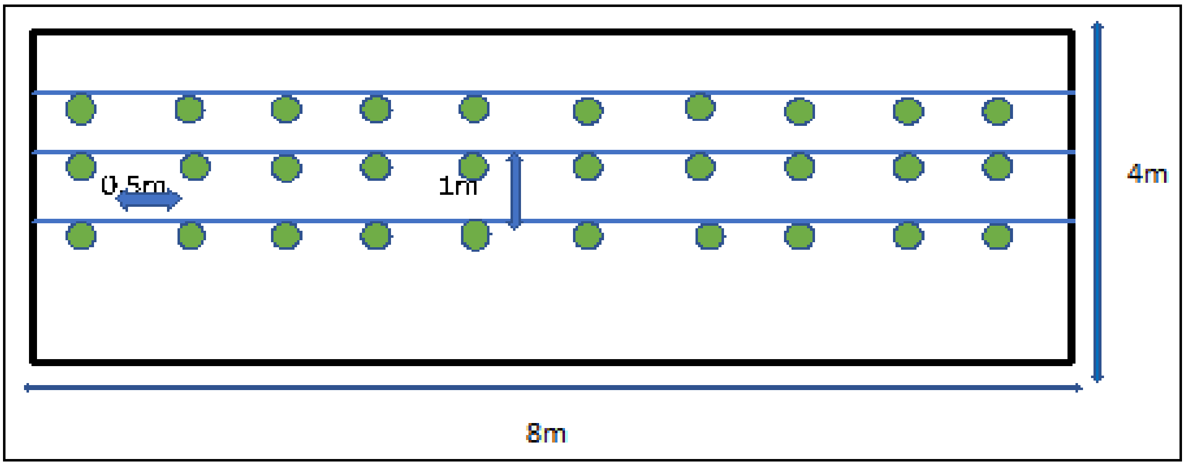

Fig. 4. Dispositif of seedlings on a block.

The insecticidal effect of the essential oil of *Ocimum gratissimum*, the main component of NECO 50 EC, was mentioned by Kouninki *et al.* (2005), Ouedraogo *et al.* (2016), Johnson *et al.* (2018) and Tano *et al.* (2019) which showed its effectiveness in controlling adults of Coleoptera *Sitophilus zeamais*, *Rhyzopertha dominica* and *Podagrica decolorata* in Côte d'Ivoire. Kouninki *et al.* (2005) showed that this oil, in contact with adults of S. *zeamais*, caused mortality rates reaching 100% depending on the doses and the duration of exposure. Indeed, the insecticidal activity of NECO 50 EC would be due to the action of terpene oxygenated compounds such as thymol which is the main compound of the essential oil of O. *gratissimum* from which NECO was formulated (Gueye *et al.*, 2011; Kassi *et al.*, 2014). Hymol, which is a recognized toxic compound, would act directly on the cuticle of insects and mites, especially those with soft bodies, causing its degradation (Cloyd and Chiasson, 2007). Thymol would also interfere with the activity of the synapses, which would prevent respiration by suffocation and lead to the death of the insect (Priestley *et al*., 2003; Gonzalez *et al*., 2013). Johnson *et al.* (2006) noted that these compounds played a repellent role at low concentrations and lethal at high concentrations in the control of Coleoptera *Callosobruchus maculatus* in foodstuffs stored in Côte d'Ivoire.

Conclusion

Evaluation of the efficacy of the biopesticide NECO 50 EC gave high mortality rates. These levels were between 62.50 and 88.10% at a concentration of 8.33 g/l, 24 hours after treatment. The NECO 50 EC induced a mortality rate greater than 92% seventy-two hours (72 h) after treatment.

The biopesticide NECO 50 EC can therefore be used as an alternative to the excessive use of synthetic insecticides to reduce the damage of the pest *P. manihoti* and increase cassava production in Côte d'Ivoire. This study leaves broader lines of research. Nevertheless, work must continue in order to study the bioecology of the *P. manihoti* population in order to carry out effective control.

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