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Research Article
Rastrococcus invadens Control in Mango Orchards by Using NECO, ASTOUN and FERCA Plant Extracts-Based Biopesticides

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Abstract
Background and Objective: Fruit tree mealybug (*Rastrococcus invadens*), an emerging pest of the mango tree is rampant in the intensive mango production area in Côte d'Ivoire. The control methods in force, mainly mechanical and chemical have not, however, resulted in effective control of this pest responsible for increasingly significant damage. This study, conducted under natural infestation conditions, assesses the effectiveness of 3 biopesticides for *Rastrococcus invadens* control. **Materials and Methods:** Three formulations of *Ocimum gratissimum* (NECO), *Cymbopogon citratus* (ASTOUN) and *Eucalyptus citriodora* (FERCA) extract-based biopesticides were applied at a dose of 1.5 L ha^{-1}. Two successive biopesticide treatments, at 30 days interval (17/10/2019 and 18/11/2019) were carried out on the elementary plots, especially on the canopy. An infestation level assessment of the 5 mango trees in the center of each plot was carried out for 60 days. Data on the Total Number of Leaves (NTF), Number of Attacked Leaves (NFA), Number of Unattacked Leaves (NFNA) and Number of Fruit Tree Mealybug Colonies (NCC) were collected every ten days. **Results:** The number of unattacked leaves increased on plots treated with biopesticides. Thus, 192.33; 146.42 and 138 additional leaves were protected by NECO, ASTOUN and FERCA, respectively. Fruit tree mealybug colonies were also reduced on treated plots after two successive applications every 30 days. **Conclusion:** These results can be used as a basis for the development of an integrated management program for mango mealybug without resorting to synthetic pesticides.

Key words: *Mangifera indica*, mealybugs, *Rastrococcus invadens*, biopesticides, plant extracts

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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

Mango (*Mangifera indica* L.) is a very important fruit, cultivated in several tropical and subtropical regions and its distribution in world trade is expanding[1]. In 2018, the African continent provided about 15% of the total volume of mango yielded. Côte d'Ivoire, the leading African mango exporter and 3rd supplier to the European market, provided 42,000 t.

This yield is however subject to parasitic constraints and to pests. The diseases are of fungal and bacterial origin[2]. As for pests, the most important ones are fruit flies of the family Tephritidae[3] and the mango mealybug *Rastrococcus invadens*[4] (Hemiptera: Pseudococcidae). In Côte d'Ivoire, the presence of *Rastrococcus invadens* was reported in 1989 before invading in 1996 the northern region of the country (Korhogo), main producing area for exported mangoes[5]. *Rastrococcus invadens* very quickly became the main pest of mango tree vegetative organs with on average 53% yield losses[5,6].

In order to control it, physical and chemical control methods are the most widely used without, however, giving satisfactory results[5]. The physical or mechanical control which consists in pruning branches is rarely practiced by producers, as they consider it to be orchard-destructive[7]. As for chemical control, very few phytosanitary products are approved in Côte d'Ivoire specifically against mango mealybug. However, the few approved insecticides are not applied according to the manufacturer's recommendations and can be harmful to the environment, producers and consumers[8]. However, biological control offers solutions with the existence of natural enemies (predators and parasitoids) that naturally regulate mealybugs. These insects, useful for the mango tree and its fruit, however, remain sensitive to orchard treatments with chemicals[9]. Thus, for better efficiency, the biological control of the mango mealybug must be directed towards finding local solutions that are easily applicable and accessible for producers. It is in this context that this study was initiated to assess the sensitivity of the mango mealybug to 3 biopesticides (NECO, FERCA and ASTOUN) formulated respectively from the essential oils of *Ocimum gratissimum*, *Eucalyptus citriodora* and *Cymbopogon citratus*, in order to offer producers a sustainable alternative to the use of synthetic pesticides.

MATERIALS AND METHODS

Material and study environment: The biopesticide effectiveness test was carried out in an orchard in the PORO region, which is naturally infested with mealybugs. The experimental site is located in the municipality of Waraniéné (Department of Korhogo) between 09°25'27.4"N and 005°40' 15.8"W, with a total surface area of 10 ha (Fig. 1). Mango trees of the 10-year-old Kent variety were planted at a density of 100 trees ha^{-1} (10×10 m in row).

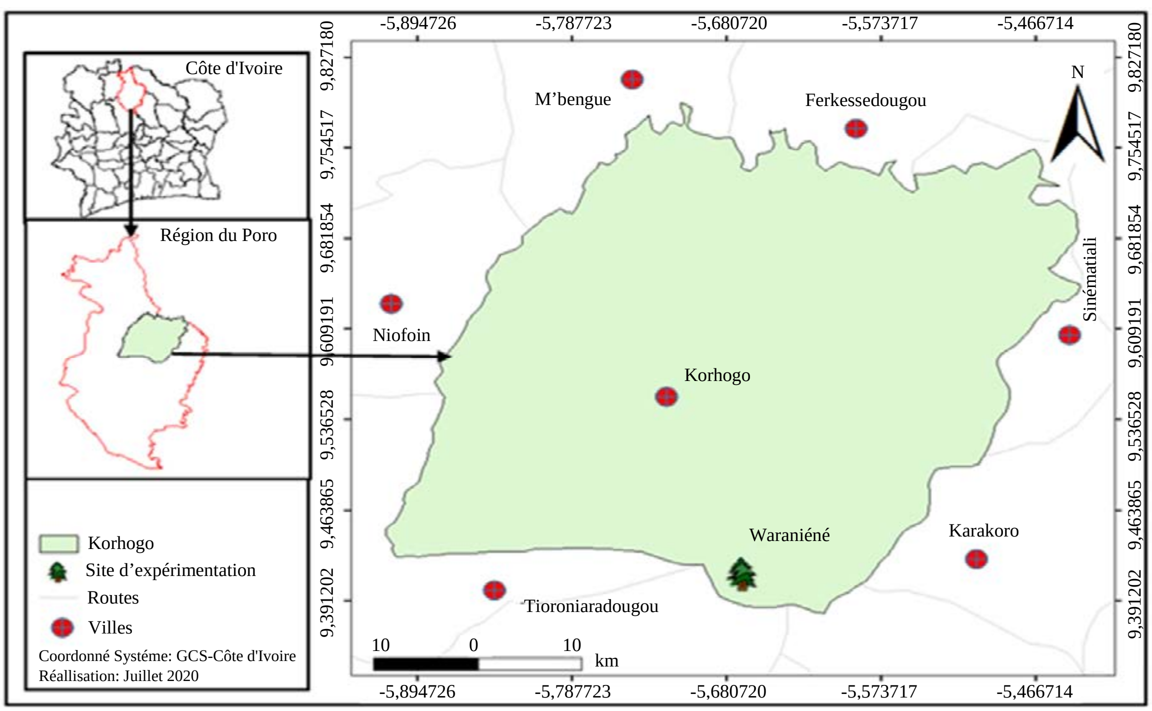

Fig. 1: Mapping of the study area

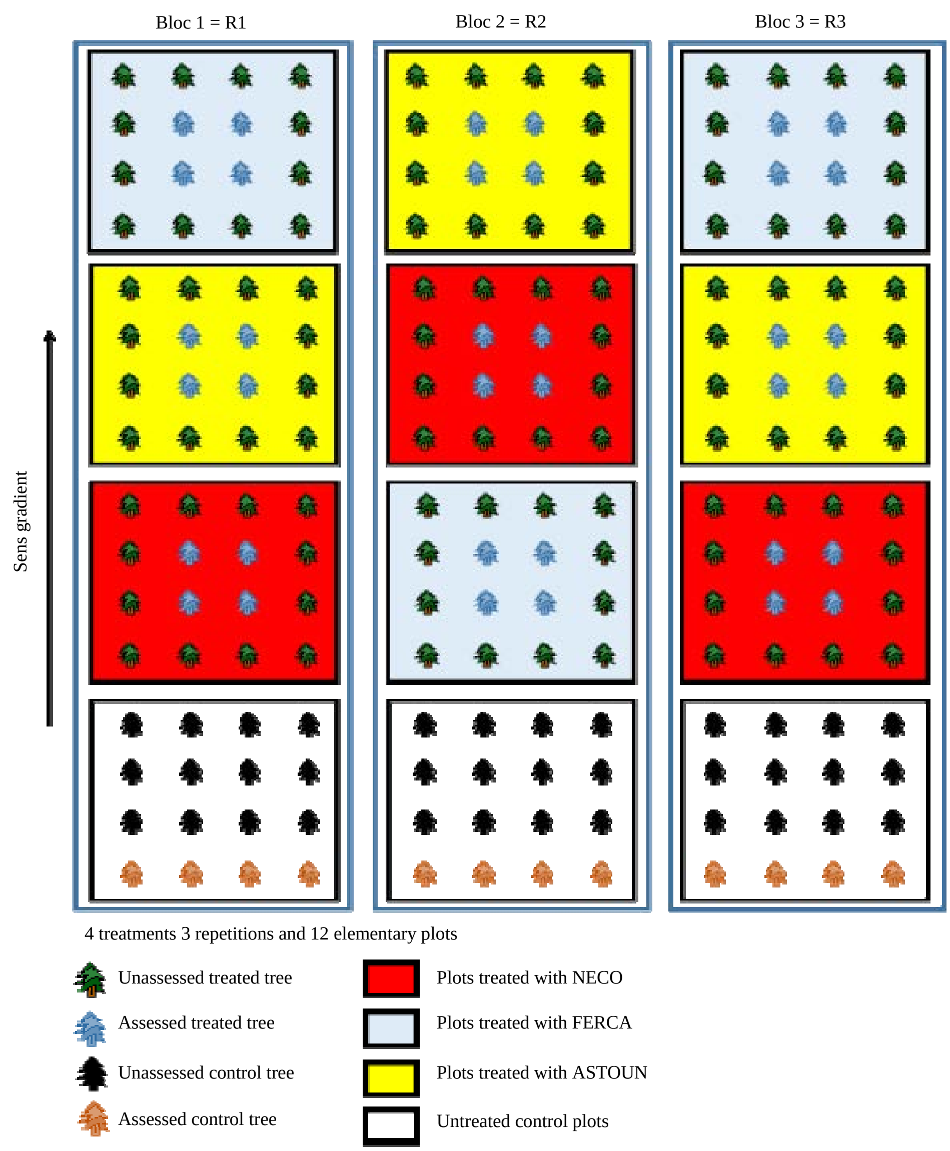

Fig. 2: Experimental design

Experimental design: The trials were carried out according to a 3-repetition Fisher block experimental design (Fig. 2). Three (3) biopesticide treatment modalities (NECO, FERCA and ASTOUN) were applied on plot units of 0.16 ha in comparison with the controls (without treatment). The trial was carried out over a surface area of 2 ha.

Biopesticide applications: A pre-treatment assessment at time (T_0) was made in order to assess the infestation level of each plot unit by *Rastrococcus invadens* colonies. The biopesticides, NECO, FERCA and ASTOUN were then applied at times T_1 and T_2 at a dose of 1.5 L ha^{-1} in 25 L aqueous slurry. These applications at times T_1 and T_2 were carried out on 17/10/2019 and 18/11/2019 on the elementary plots respectively using a backpack sprayer. This 15 L, adjustable-nozzle, carry-away sprayer was used to apply the biopesticide slurries on the canopy of target mango trees. Regular monitoring at 10-day intervals after applications was carried out for 2 months (October-November, 2019).

Assessment of leaf infestation level and number of mealybug colonies: The assessment of the survival of mealybug colonies concerned the 4 trees located in the center of each elementary plot on each block and for each treatment. All the trees in each elementary plot were treated. Thus, on each assessed tree, 12 small branches were selected. They were distributed over the four cardinal points at the rate of 3 small branches selected in each direction and marked with fabrics of different colors to make their identification and the assessment easier. The selection of small branches was made at random. On each small branch, the following variables were noted using an assessment sheet:

- Total number of leaves on the small branch (NTF)
- Number of leaves attacked by mealy bugs on the small branch (NFA)
- Number of unattacked leaves on the small branch (NFNA)
- Number of mealybug colonies on the small branch (NCC)

Statistical analysis: The data collected was processed with an Excel 2013 spreadsheet and analyzed with STATISTICA version 7.1 software. The average rates of leaves attacked by mealybugs were determined and subjected to Factorial ANOVA [2 factors ([products$\times$time] leaves attacked)]. The comparison of means test (Fisher LSD: Least Significant Difference) at 5% probability threshold was combined with the previous analysis in order to group together products whose effects were similar at a given time. The $p<0.05$ of each test were kept so as to determine the biopesticides and effective or not, compared to the control.

RESULTS

Effect of treatments on leaf infestation level: The assessment of the initial infestation level before applying the biopesticides showed a significant difference at the level of plot units (Table 1). The infestation level of the control plots was statistically identical to the ones that had to receive FERCA and significantly lower than those of NECO and ASTOUN. This infestation of the plots by *Rastrococcus invadens* was increasing on the control plots and decreasing on the plots treated with biopesticides during the 30 days of assessment. Indeed, the number of leaves infested by mealybugs increased from 247.5-293.7 on the control plots. During the same period, on the treated plots, a decrease from 397.6-346.3, 373.5-330.25 and 255.83-227.33 infested leaves was found on plots treated with NECO, ASTOUN and FERCA, respectively (Table 1). The sanitizing effect of the treatments became noticeable and highly significant from the second application of the biopesticides. At the end of the first assessment phase (T_1+30) following the first application of the biopesticides, the average number of leaves infested by *Rastrococcus invadens* was 293.7 for the control plots and 346.3, 330.25 and 227.33 for plots treated with NECO, ASTOUN and FERCA, respectively. At the end of the second assessment phase (T_2+30) a significant reduction in leaves bearing *Rastrococcus invadens* colonies were observed on the treated plots. Thus, a decrease from 346.3-205.33, from 330.25-227.08 and from 227.33-117.83 infested leaves was observed on plots treated with NECO, ASTOUN and FERCA, respectively (Table 1). On the control plots, an increase in the number of infested leaves from 293.7-367.16 was rather observed.

The number of leaves unattacked (NFNA) by mealybugs was also taken into account in the assessment of the pesticidal effect of the applied bioproducts (Table 2). These allowed the protection of additional leaves which grew from 179-371.33, from 154.33-300.75 and from 120.41-258.41 on plots treated with NECO, ASTOUN and FERCA, respectively. On the control plots, a significant reduction in healthy leaves from 137.58-17.91 was rather observed (Table 2).

Effect of treatments on the number of mealybug colonies: Biopesticide treatments (NECO, ASTOUN and FERCA) induced a reduction in mealybug colonies at the end of both assessment phases (Fig. 3). Depending on their effects on the survival of *Rastrococcus invadens* colonies, two different development phases were observed on the plot units treated with biopesticides (Fig. 3). The average number of mealybug colonies for the plot units treated with the biopesticides was 523 colonies/434 leaves assessed. At the same time, the control plots have an average of 390 mealybug colonies/385 assessed leaves. Twenty days after treatment, at [T_1+20j], The results showed a slight increase in scale insect colonies for the mango trees treated with ASTOUN

Table 1: Average number ($\pm$standard error) of leaves attacked (NFA) by mealybugs

	Before treatment	1st application (T_1)			2nd application (T_2)		
Products	T_0	T_1+10j	T_1+20j	T_1+30j	T_2+10j	T_2+20j	T_2+30j
Control	247.50$\pm$41.17^{abc}	263.00$\pm$41.17^{abcd}	272.20$\pm$41.17^{abcd}	293.70$\pm$41.17^{abcde}	326.33$\pm$35.94^{ef}	350.83$\pm$35.94^{f}	367.16$\pm$35.94^{f}
NECO	397.60$\pm$41.17^{e}	362.40$\pm$41.17^{cde}	350.90$\pm$41.17^{cde}	346.30$\pm$41.17^{cde}	274.30$\pm$35.94^{bcef}	217.20$\pm$35.94^{abcd}	205.33$\pm$35.94^{abcd}
ASTOUN	373.50$\pm$41.17^{de}	342.08$\pm$41.17^{bcde}	331.91$\pm$41.17^{bcde}	330.25$\pm$41.17^{abcde}	292.83$\pm$35.94^{cef}	234.75$\pm$35.94^{abce}	227.08$\pm$35.94^{abce}
FERCA	255.83$\pm$41.17^{abc}	216.58$\pm$41.17^{b}	216.41$\pm$41.17^{b}	227.33$\pm$41.17^{ab}	190.75$\pm$35.94^{abd}	152.25$\pm$35.94^{ad}	117.83$\pm$35.94^{d}
F-test	3.76^{S}	2.76^{NS}	2.10^{NS}	1.62^{NS}	2.18^{NS}	5.55^{HS}	9.54^{HS}
Probability	0.017	0.053	0.11	0.19	0.10	0.0025	0.000057

NS: Not significant at 5% threshold, S: Significant at 5% threshold, HS: Highly significant at 5% threshold. Values with different letters on the same line are significantly different at p<0.05 (Fisher's LSD test)

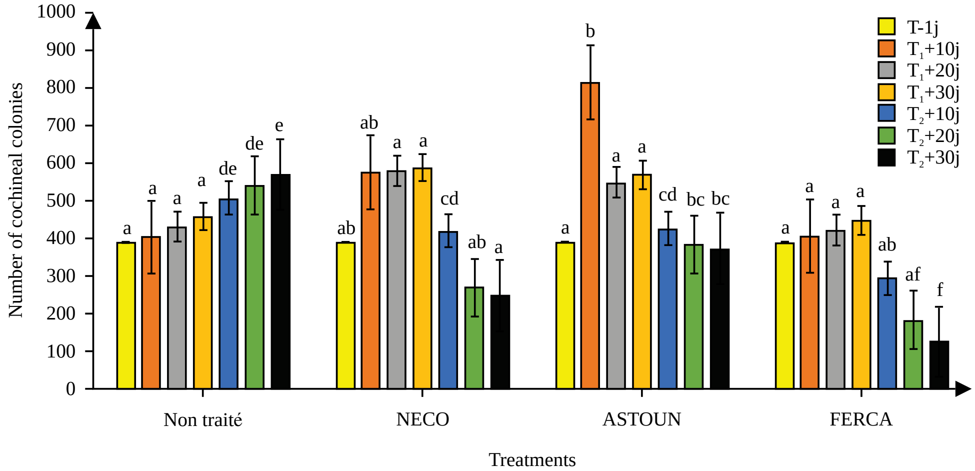

Fig. 3: Variation in the number of mealybug colonies depending on the treatments and the duration of exposure on the Waraniéné site

Table 2: Average number (±standard error) of leaves unattacked (NFNA) by mealybugs

	Before treatment	1st application (T_1)			2nd application (T_2)		
Products	T_0	T_1+10j	T_1+20j	T_1+30j	T_2+10j	T_2+20j	T_2+30j
Control	137.58 ± 22.14^{abcd}	122.08 ± 22.14^{abc}	112.83 ± 22.14^{ab}	91.33 ± 22.14^{a}	58.75 ± 23.97^{d}	34.25 ± 23.97^{d}	17.91 ± 23.97^{d}
NECO	179.00 ± 22.14^{cdef}	214.25 ± 22.14^{ef}	225.75 ± 22.14^{f}	230.33 ± 22.14^{f}	302.33 ± 23.97^{bc}	359.41 ± 23.97^{cg}	371.33 ± 23.97^{g}
ASTOUN	154.33 ± 22.14^{bcde}	185.75 ± 22.14^{def}	195.91 ± 22.14^{def}	197.58 ± 22.14^{def}	235.00 ± 23.97^{aef}	293.08 ± 23.97^{abc}	300.75 ± 23.97^{abc}
FERCA	120.41 ± 22.14^{abc}	159.66 ± 22.14^{bcde}	159.83 ± 22.14^{bcde}	148.91 ± 22.14^{abcd}	185.50 ± 23.97^{e}	224.00 ± 23.97^{ef}	258.41 ± 23.97^{abf}
F-test	1.32^{NS}	3.11^{S}	4.62^{HS}	7.57^{HS}	20.18^{HS}	35.08^{HS}	36.79^{HS}
Probability	0.27	0.035	0.006	0.0003	0.00000	0.00000	0.000000

NS: Not significant at 5% threshold, S: Significant at 5% threshold, HS: Highly significant at 5% threshold. Values with different letters on the same line are significantly different at $p<0.05$ (Fisher's LSD test)

with an average of 548 colonies/434 leaves evaluated at [T_1+20j] and 569 colonies/434 leaves evaluated at [T_1+30j]. For the plot units treated with FERCA this average is 420 colonies/434 leaves evaluated at [T_1+20j] and 447 colonies/434 leaves evaluated at [T_1+30j]. On the other hand, the plot units treated with NECO recorded a non-significant increase of mealybug colonies with an average of 557 colonies/434 leaves evaluated.

However, after the second application, the statistical values of the effect of the products on the number of mealybug colonies showed a highly significant difference for all treatments at [T_2+30j]. Thus, an increasing decrease in the number of mealybug colonies was observed for mangoes treated with the biopesticides ranging from 247 mealybug colonies/434 leaves assessed for the plot units treated with Neco, those treated with Astoun were 372 mealybug colonies/434 leaves assessed and those treated with Ferca recorded an average of 125 mealybug colonies/434 leaves assessed. Compared to the (untreated) controls an increase in the number of 568 mealybug colonies/434 leaves assessed at [T_2+30j].

DISCUSSION

The biopesticides NECO, ASTOUN and FERCA had positive effects on leaf sanitation in the treated plots. These results might result from both a toxic effect on mealybugs and protection of new leaves generated by treated mango trees. Some studies have already looked into the use of botanical extracts as an alternative to synthetic insecticides in *Rastrococcus invadens* control, which is the case of the work of Gahukar[10] which demonstrated the effectiveness of aqueous extracts from different parts of neem (leaves, ripe and unripe fruits, bark) against the cotton mealybug.

Regarding biopesticide NECO, its application resulted in a reduction in the number of infected leaves from 346.3-205.33 and that of *Rastrococcus invadens* colonies from 523-247 colonies out of 434 assessed leaves. The work of Kassi *et al.*[11] demonstrated the sanitizing and protective effect of NECO on banana leaves, thus confirming the results obtained during our work on mango leaves. The sanitizing effect of this biopesticide was also demonstrated during the work of Fofana *et al.*[12] against cocoa pod brown rot. Its insecticidal effect on adults of *Diastocera trifasciata*, chopper of cashew tree branches was proven during the work of Akéssé *et al.*[13]. After NECO application, the latter obtained mortality rates higher than 70% with concentrations. They also demonstrated that 72 hrs after the treatments, biopesticide NECO induced a mortality rate of $94.67\pm2.32\%$. These results also showed that it is as effective as the reference insecticide (Acetamiprid 32 g L^{-1}+Lambda cyhalothrin 30 g L^{-1}) against adults of *Diastocera trifasciata*. Indeed, *Ocimum gratissimum* essential oil, main component of biopesticide NECO has already been the subject of numerous studies and its antiparasitic effect demonstrated[14,15]. The insecticidal properties of essential oils of species of the genus Ocimum in the fight against attacks by insects of various orders have already been reported by several authors, both in Côte d'Ivoire and elsewhere[16-19]. Indeed, the work carried out by Seri-Kouassi *et al.*[20] highlighted the insecticidal activity and the significant reduction in egg laying of the beetle *Callosobruchus maculatus* of *Ocimum gratissimum* and *Melaleuca quinquenervia* oils by fumigation at different volumes. Moreover, other authors have also observed the toxicity of this oil by contact, as with NECO. Indeed, Ogayo *et al.*[21] have shown the toxic effect by contact of an *Ocimum gratissimum* extracts on two-spotted spider Mites (*Tetranychus urticae*) for Improved yield and quality of french beans.. The effect of NECO on the survival and reduction of *Rastrococcus invadens* colonies might be due to the insecticidal action of Thymol and γ Terpinene which constitute the active molecules of *Ocimum gratissimum* essential oil[11,17,22,23]. Thymol is an insecticide that might interfere with synapse activity, which in turn might inhibit respiration and lead to the death of the insect[24]. Likewise, Ouédraogo *et al.*[25] have shown that the insecticidal activity of *Ocimum gratissimum* observed during their work seems to be a combined effect of several constituents with regard to its chemical composition.

Spraying biopesticide ASTOUN at micro-plot level resulted in a reduction in the number of infested leaves from 330.25-227.08 and that of *Rastrococcus invadens* colonies from 523-372 colonies out of 434 leaves assessed. Its antiparasitic effect is confirmed by the work of Fofana *et al.*[12] against *Phytophtora palmivora*, responsible for cocoa pod brown rot. *Cymbopogon citratus* essential oil, main component of biopesticide ASTOUN, has already been the subject of numerous studies. Previous studies had shown that *Cymbopogon citratus* essential oil has a significant insecticidal activity against certain insects, in particular *Anopheles gambiae* Giles larvae[26], adult insects of *Pectinophora gossypiella* Saunders[17], aphids *Aphis gossypii*[27]. This insecticidal activity is might be induced by geranial and neral[28]. Chemical analysis of essential oils extracted from *Cymbopogon citratus* leaves collected in various regions of Côte d'Ivoire revealed a high content of geranial, myrcene and neral[29].

Biopesticide FERCA is the one that caused the highest reduction in *Rastrococcus invadens* colonies. This great ability to reduce the survival of *Rastrococcus invadens* might be linked to its larvicidal effect and its composition of active elements. Indeed, this biopesticide is mainly composed of *Eucalyptus citriodora* essential oil. The possibility of using this essential oil as a biopesticide is demonstrated in the work of Idoko[30] and its demonstrated toxic effect on larvae[31].

Depending on their effects on the survival of *Rastrococcus invadens* colonies, two different evolution phases were observed after application of the biopesticides. A first phase from T_1-1j to T_1+30d characterized by statistically identical colony numbers, the second phase from T_1+30j to T_2+30j marked by a significant reduction in colony numbers. Similar results were obtained after application of Cyren 480 EC, Pyrical 480 EC and Pyriforce 480 EC in mango orchards in northern Côte d'Ivoire[4].

CONCLUSION

The results obtained with the 3 biopesticides on *Rastrococcus invadens* control are satisfactory. NECO, ASTOUN and FERCA provided protection for the newly generated leaves and also reduced the survival of *Rastrococcus invadens* colonies. However, a significant reduction in mealybug colonies requires 2 successive applications at 30 days interval. Thus, these formulations may well fit into an integrated management program for mango mealybug.

SIGNIFICANCE STATEMENT

This study has led to the discovery of ecological and responsible management of the mango mealybugthat can benefit mango growers through biological control. This study will help researchers to discover critical areas of aromatherapy in agriculture that many researchers have not been able to explore. In this way, a new theory on pest management can be obtained.

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