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Discriminative Study and Biological Control of *Lasiodiplodia theobromae* involved in the Foliar Desiccation of Cashew Tree Pricked by *Helopeltis* sp in Côte d'Ivoire

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Abstract

Context and Objective: A plantation control trial was carried out against the vector of foliar desiccation, *Helopeltis* sp in Côte d'Ivoire.

Material and Methods: A survey in cashew orchard was carried out in Béré, Poro, Hambol and Marahoué Regions. Buds showing attacks of *Helopeltis* sp were collected to isolate fungus. A biological control test was carried out *in situ* on *Helopeltis* sp in Dikodougou, Napié and Tortiya. The test used an essential oil, 30 mg/l *Ocimum gratissimum*, the synthetic pesticides based on 100 g/l Chlorantraniliprole + 200 g/l Thiamethoxam and 200 g/l Cypermethrin + 200 g/l Acetamiprid.

Results: All the dried cashew leaf buds procured fungal isolates of *Lasiodiplodia theobromae*. Chlorantraniliprole+Thiamethoxam and *Ocimum gratissimum* showed the best efficacy against *Helopeltis* sp at 300 ml/ha. Damage of the pest was reduced more than 10% for the *Ocimum gratissimum* compared to the untreated control. The economic gain generated by the biopesticide was more than 5000 F CFA.ha^{-1} compared to the untreated control.

Conclusion: These results suggest the adoption of the biopesicide *Ocimum gratissimum* and Chlorantraniliprole+Thiamethoxam at a dose of 300 ml/ha in the protection of cashew trees against the mosquito, *Helopeltis* sp, vector of cashew foliar desiccation due to *Lasiodiplodia theobromae*.

Keywords: Cashew, *Helopeltis* sp, *Lasiodiplodia theobromae*, biopesticide, Cote d'Ivoire

1. Introduction

In Côte d'Ivoire, cashew nuts (*Anacardium occidentale* L., 1753) are the most promising cash crop in its entire production area. In less than two decades, cashew nuts have become the leading agricultural export product for the entire northern half of the country. Cashew nuts are the third most important agricultural export product of Côte d'Ivoire after cocoa and rubber since 2010 (Djaha *et al*., 2010 ; Koné *et al*., 2015). This high production (711,236 tons) has made Côte d'Ivoire the leading producer and exporter of raw cashew nuts. Cashew contributes to increasing agricultural export income to 7% of agricultural and helps improve the income of many producers (Soro *et al*., 2019). Yields in Ivorian orchards remain very low (350 to 500 kg/ha) compared to international levels (1 to 1.5 t/ha) (Koné *et al*., 2015 ; Djaha *et al*., 2017). This low tree productivity is reportedly linked to the poor application of good agricultural practices, the use of unimproved planting material, poor production techniques and especially phytosanitary problems. Cashew trees are attacked by many biological pests such as pathogens and insect pests (Soro *et al*., 2015; Ouali *et al*., 2015). The mosquito, *Helopeltis anacardii* Miller, 1954 (Hemiptera: Miridae) have been identified among the major pests of cashew nuts in Côte d'Ivoire (website: smsillico.physioveg/).

Helopeltis anacardii is a biting insect sucking on the tender parts of the tree on young leaves or flowers and nuts. It is a vector insect for *Lasiodiplodia theobromae* (Pat.; Griffon & Maubl., 1909) that has a very damaging impact on the nut yield of the cashew tree. This study is an innovation in the management of this vector, *Helopeltis* sp on cashew trees in Côte d'Ivoire.

Lots of work has been done on the major insects of cashew but there is little information on the relative abundance, damage potential and management of cashew insect pests. Unfortunately, at the national level no data appear to exist on the direct loss of cashew yields caused by *Helopeltis* sp as a vector of *Lasiodiplodia theobromae*. In this context, the Council of Cotton and Cashew (CCA) established the National Cashew Research Program (PNRA) in 2016 in collaboration with FIRCA and PSAC, which involved all National Universities and the National Center of Agronomic Research (CNRA). This study is part of the overall objective of the Program to contribute to improving the productivity of cashew and specifically, it was to compare *in situ* the efficacy of the best biological pesticide and the synthetic pesticides on the vector *Helopeltis* sp of *Lasiodiplodia theobromae* on the cashew trees in Côte d'Ivoire.

2. Material and Methods

2.1. Experimental Sites

A sanitary survey in the cashew tree orchard was carried out in the Regions of Mankono, Korhogo, Katiola and Bouaflé. The control study was carried out in the Savannah District of Korhogo. Three parcels were selected from prospecting on the basis of criteria such as phytosanitary problems and accessibility of the plot to carry out the tests. The parcels are located in the localities of Dikodougou with a longitude of -005°35'34'', 09°06'49'' of latitude and a altitude of 420 m in the South of Korhogo; Napié 09°18'00'' latitude, -005°35'00'' longitude, altitude of 348 m in the Southeastern of Korhogo and Tortiya 08°46'00'' latitude, -005°41'00'' longitude, altitude of 323 m in the Department of Niakara. The experiment was performed according to a device consisting of 4 Fisher blocks completely randomized to 4 elementary repetitions. Each elementary repetition consists of 3 cashew trees arranged on one line and spaced between them 10 m is a basic plot of 300 m^2. A total of 12 cashew trees are selected for each treatment on a plot.

2.2. Material

The cashew trees use in this study were between 10 and 15 years. The essential oil of *Ocimum gratissimum* was used to control the pests in the orchards.

Helopeltis sp was used as the biological material in the fields. These pests were collected in the orchards of cashew in Côte d'Ivoire.

One pesticide and one biopesticide were tested compared to the control positive in the control of *Helopeltis* sp in this study (Table 1).

Table 1: Pesticides used

Pesticide role	Name of active substance	Dose	Chemical family
Positive control	200 g/l Cyperméthrine + 200 g/l Acétamipride	500 ml/ha	Pyrethroid + Neonicotinoid
Essay	100 g/l Chlorantraniliprole + 200 g/l Thiamethoxam	300 ml/ha	Diamides Anthraniliques + Neonicotinoid
Essay	*Ocimum gratissimum*	300 ml/ha	Essential oil

2.3. Methods

2.3.1. Prospecting Areas

Surveys were carried out throughout the cashew production zone in Côte d'Ivoire. About twenty localities were visited in order by Region to determine the level of *Helopeltis* sp attacks on cashew tree leaf buds. Observations were made on one hectare (100 trees) in each orchard. The orchards visited were at least 25 km apart and the trees observed had a minimum distance of 10 m between them in each orchard. Five buds showing *Helopeltis* sp attacks were collected from cashew trees in each orchard for the isolation of the fungus. Insects were collected by two methods. The first consisted in noting the damage caused to the flowers and the nuts on the tree. The second was to collect *Helopeltis* sp using mowing nets. The unkown insects were sent to the laboratory for identification (Koné *et al*., 2015).

2.3.2. Isolation and Identification of Pathogens

Cashew tree buds with desiccation were cut into 3 mm long explants. They were washed with water and put in 70% alcohol for 3 minutes. They were sanitized with 3% bleach for 3 to 5 minutes and rinsed three times with sterile distilled water. They were left to dry under the hood for about 2 minutes before being inoculated in Petri dishes containing potato dextrose agar (PDA) medium. The Petri dishes were then sealed with adhesive film and stored in the oven for 3-5 days.

The different fungal strains observed were purified in Petri dishes containing new PDA culture medium at the end of the 5-day. The identification of the fungi consisted of macroscopic and microscopic observation (Barnett and Hunter, 1998).

2.3.3. Application of Pesticides on Cashew Trees

Trees were marked with ribbons of different colors to differentiate the treatments between them. White (Control), yellow (Cyperméthrine+Acétamipride), blue (Chlorantraniliprole+Thiamethoxam) and red (*Ocimum gratissimum*) (Tonon *et al*., 2017). The application of the pesticides was carried out using a Cifareli brand atomizer with capacity of 15 liters. The treatments were repeated 3 times per month (November, December and January) in every orchard. One liter of formulation of pesticide was spread on each tree per treatment.

2.3.4. Rate of Insect Damage in Plantation

The pesticides efficacies were assessed through the rate of insect damage from cashew flowers or nuts yield (Sundararaju *et al*., 2004). The rate of insect damage was calculated from the ratio of the number of trees attack to the total number of trees for each treatment in the orchard.

The severity assessment was done on two opposite sides of the crown of each tree for *Helopeltis* sp. On each side, a quadrat of 1 m^2 was delineated at the foliage level of canopy of each plant. The number of healthy flowers or nuts and infested flowers or nuts was recorded and percent damage was calculated according Navik *et al*. (2015). The severity (S) of insect damage was assessed using a rating scale ranging from 0 to 4 as follows: 0 = no damage; 1 = 1-25% organs damage; 2 = 26-50% organs damage; 3 = 51-75% organs damage; 4 < 75% organs damage (infested flowers or nuts).

$$S = (ni^*\chi i) / N^*5$$

With S = Severity ; ni = number of trees with the same note χi ; N = Total number of trees.

2.3.5. Measuring of Economic Impact of Pests in Orchard

At the production level, the assessment consisted of determining the daily mass of nuts falling under trees by treatment. The evaluation began with a collection of nuts under the 3 trees forming the treatment in each block. The harvested nuts were then sorted into 2 lots, a lot of good nuts and the second batch of bad nuts. The mass of the 2 batches was determined by weighing using an electronic weighing scale with the capacity of 50 kg. Finally, the economic impact was determined through the economic loss of performance (P) using equation (1) (Judenko, 1972).

$$P = W - RT$$

$$\text{With} \begin{cases} W = \frac{100 \text{ X } RT}{100 - L} \\ L = \frac{\alpha \text{ x p}}{100} \\ \alpha = \frac{(RTx - RT0) \text{ X } 100}{RTx} \end{cases}$$

P : Economic loss of performance ; **W :** Relative loss ; **RT :** Total return on the parcel ; **Rtx :** Total performance for treatment x ; **Rto :** Total tree yield in control ; **L :** Percentage of loss ; **α :** Coefficient related to nut loss ; **p :** Percentage of trees attacked in treatment.

2.3.6. Climate Data Recording

Precipitation, relative humidity and temperature were measured at each station using a TFA Digital Thermo-Hygrometer.

2.3.7. Data Analyse

The data collected were analyzed for variances (ANOVA) at the level of 5% using the Statistica 7.1 software. When the difference was significant, averages were classified as homogeneous by the Newman-Keuls test.

3. Results

3.1. List of Insects Inventoried on Cashew Orchard

The table 2 lists the insects collected and their prevalence levels on cashew trees during the survey.

Table 2: List of insects collected on cashew trees in the orchards

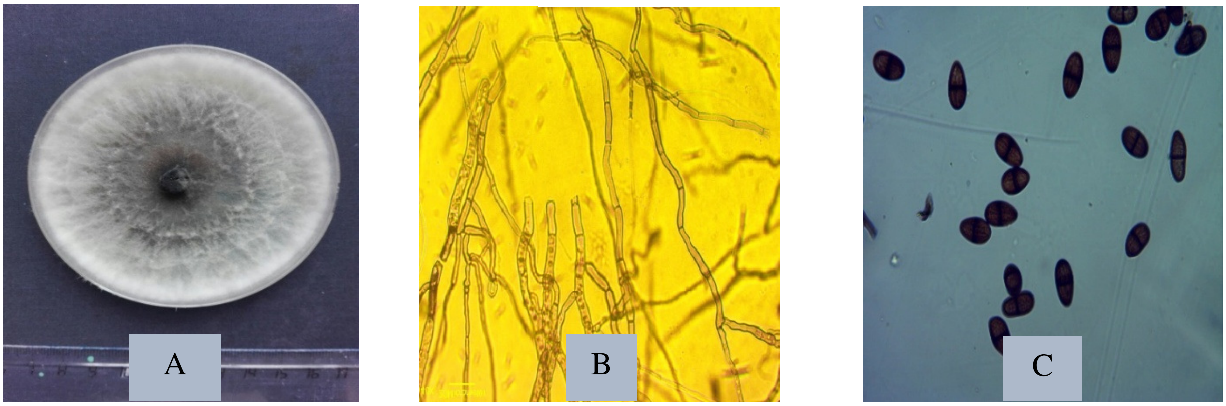
Scientific name	Order : Family	Prevalence level
Achaea lienardi (Boisduval)	Lepidoptera: Geometridae	+
Acrocercops syngramma (Meyrick)	Lepidoptera: Gracillaridae	+
Aleurocanthus woglumi (Ashby)	Homoptera: Aleyrodidae	+
Aleuroglyphus ovatus (Troupeau)	Acarina: Tyroglyphidae	+
Alphitobius diaperinus (Panzer)	Coleoptera: Tenebrionidae	+
Alphitobius laevigatus (Hbst.)	Coleoptera: Tenebrionidae	+
Anoplocnemis curvipes (Fabricus)	Hemiptera: Coridae	+
Apate terebrans (Pallas)	Coleoptera: Bostrychidae	++
Charaxes numenes (Hew)	Lepidoptera: Nymphalidae	+
Chrysomphalus ficus (Ashmead)	Homoptera: Diaspididea	+
Clania minuscula (Butler)	Lepidoptera: Psychidae	+
Coptops aedificator (Fabricius)	Coleoptera: Cerambycida	+
Diastocera trifasciata (Fabricius)	Coleoptera: Cerambycidae	++
Dysdercus spp.	Hemiptera: Pyrrhocoridae	+
Ferrisia virgata (Cockerell)	Homoptera: Coccidae	++
Helopeltis anacardii (MuLer)	Hemiptera: Miridae	+++
Helopeltis schoutedeni (Reuter)	Heteroptera: Miridae	+++
Mecocorynus loripes (Chevrolat)	Coleoptera: Curculionidae	+++
Othreis fullonia (Clerk)	Lepidoptera: Noctuidae	+
Palorus subdepressus (Woll)	Coleoptera: Tenebrionidae	+
Planococcus citri (Risso)	Homoptera: Pseudococcidae	++
Plocaedeus frenatus (Fahraeus)	Coleoptera: Cerambycidae	+
Plocaedeus spinicornis (Fabricus)	Coleoptera: Cerambycidae	+
Pseudotheraptus wayi (Brown)	Hemiptera: Coridea	+++
Scirtothripis dorsalis (Hood)	Thysanoptera: Thripidae	+
Selenothrips rubrocinctus (Giard)	Thysanoptera: Thripidae	++

+: Low prevalence
++: Medium prevalence
+++: Hight prevalence

3.2. List of Fungi Isolated from Cashew Orchard

All fungi isolated from the dried buds showed a white, fluffy mycelium that blackened after one week of storage. Under light microscopy, the mycelium is partitioned with ovoid to ellipsoidal conidia, septate and without appendages. This is the mycopathogen *Lasiodiplodia theobromae* (Figure 1).

Figure 1: Macroscopic (A) and microscopic (B and C) aspects of *Lasiodiplodia theobromae* strain on PDA medium



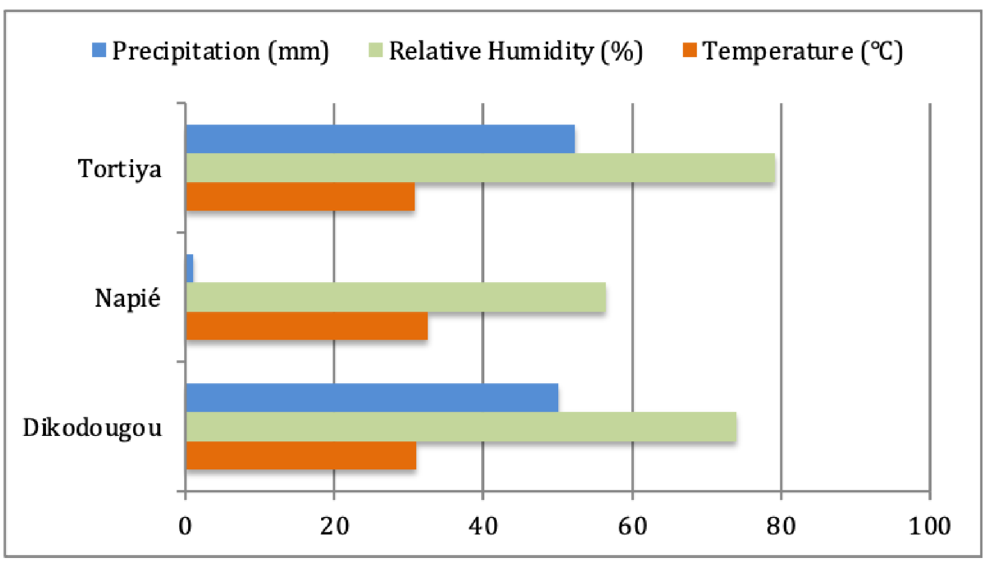
Scale: 40×20 Micrometer for microscopic view
A: Culture of *Lasiodiplodia theobromae* in Petri dish; B: Microscopic aspect of the mycelium Gx400; C: Microscopic aspect of the spores Gx400.

3.3. Prevalence of *Helopeltis* sp after Treatments

The prevalence of *Helopeltis anacardii* was found at level 3 in all three orchards before treatments. No presence of the insect was observed after the treatments. All pesticides showed significant efficacy against *H. anacardii.*

The temperature in Napié (32.6 °C) is slightly higher than in Dikodougou (31.03 °C) and Tortiya (30.86 °C). Napié's average rainfall is practically negligible (1.03 mm) and its relative humidity has remained very low (56.5%) (Figure 2).

Figure 2: Climatic parameters recorded on the cashew tree experimental plots

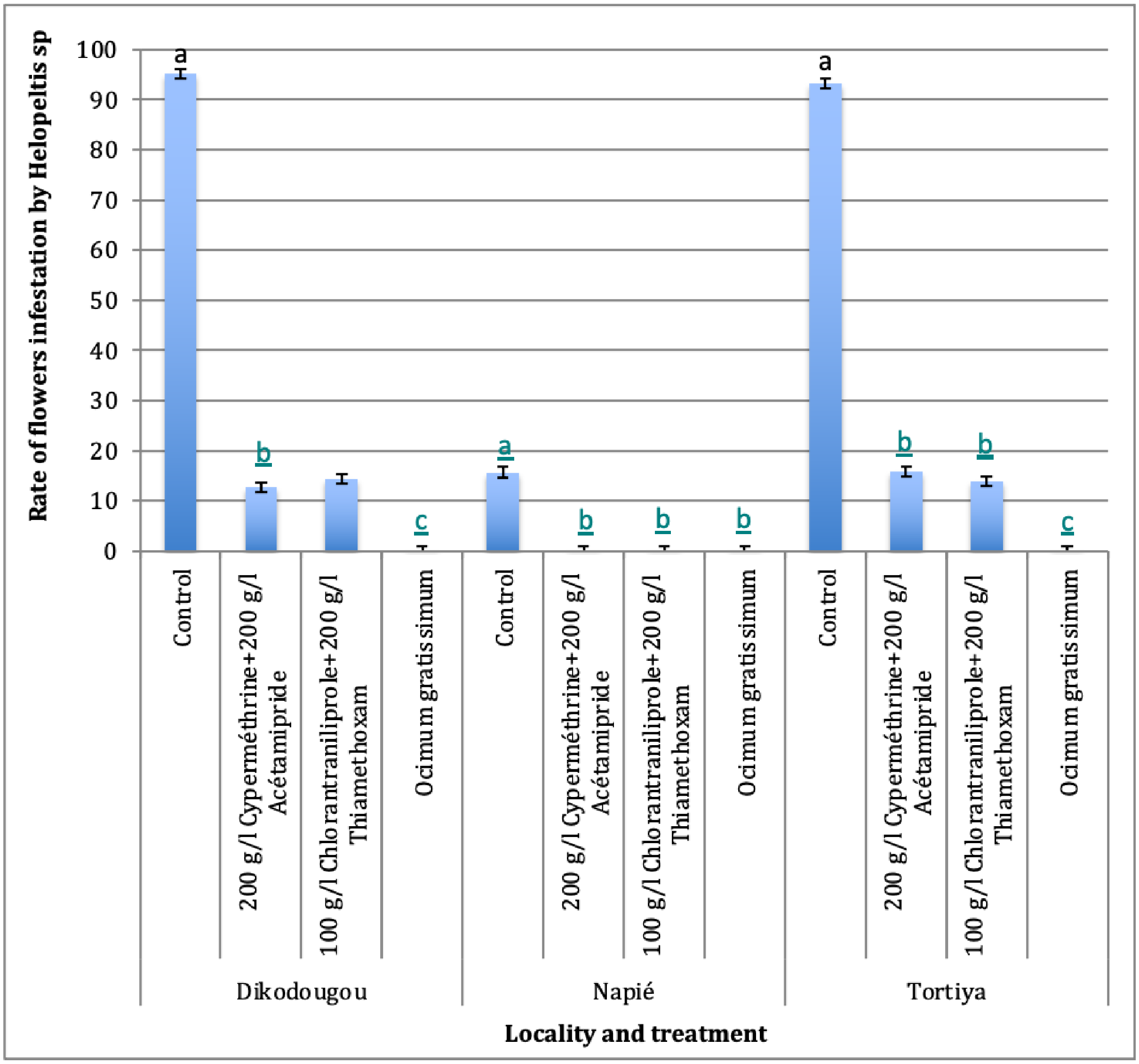


3.4. Rate of Infestation of Flowers by *Helopeltis* sp

Figure 3 shows the infestation of flowers by *Helopeltis* sp in cashew orchards. Attacks vary by location and treatment. All controls showed a significant difference from the trials in all three orchards. The Napié orchard had the lowest infestations. The treatments 100 g/l Chlorantraniliprole + 200 g/l

Thiamethoxam, 200 g/l Cypermethrin + 200 g/l Acetamiprid and *Ocimum gratissimum* reduced infestations to over 86%, 87% and 95% respectively.

Figure 3: Rate of infestation of flowers by *Helopeltis* sp

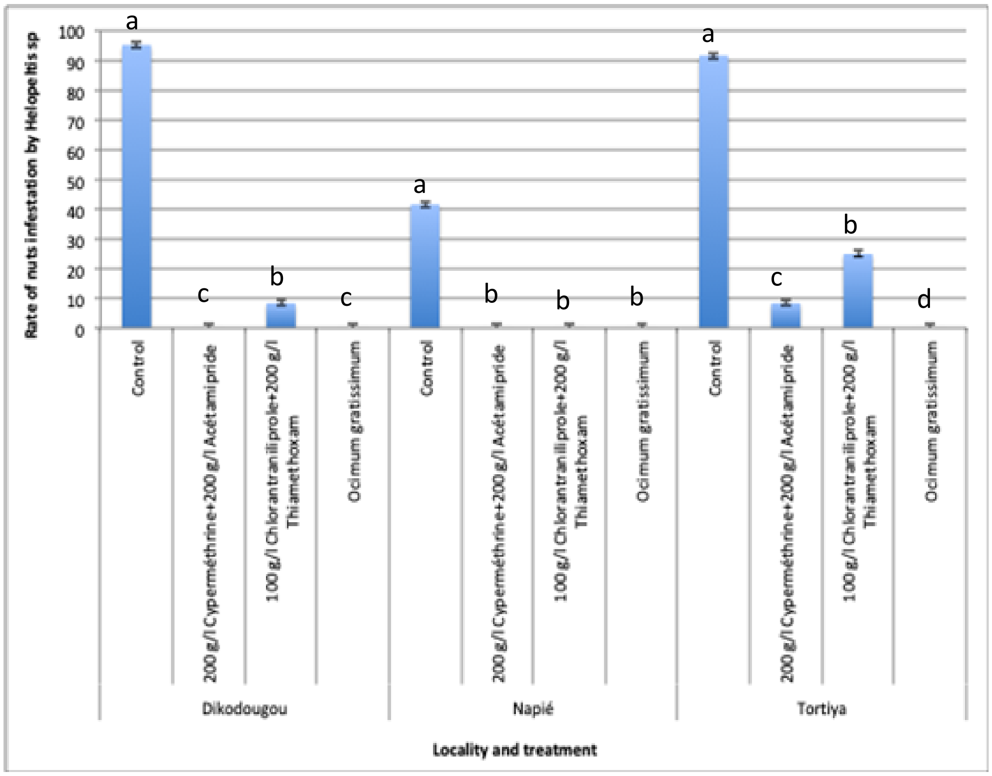


The diagrams with the same letters in the same Locality are not significantly different according to the Newman-Keuls test at the level of 0.05.

3.5. Rate of Infestation of Young Nuts by *Helopeltis* sp

Infestations of *Helopeltis* sp on young cashew nuts differ from one locality to another. Attacks are higher in controls in all three orchards. Napié locality showed zero attacks, less than Dikodougou (8.33% and 0%) and Tortiya (25% and 0%) with the tested pesticide (100 g/l Chlorantraniliprole + 200 g/l Thiamethoxam) and biopesticide (*Ocimum gratissimum*) respectively. In the absence of treatment, 95.25%, 91.66% and 41.66% of young nuts were pricked in Dikodougou, Tortiya and Napié respectively. The attack rate was reduced to 100% with the biopesticide in the three orchards (Figure 4).

Figure 4: Rate of infestation of young nuts by *Helopeltis* sp

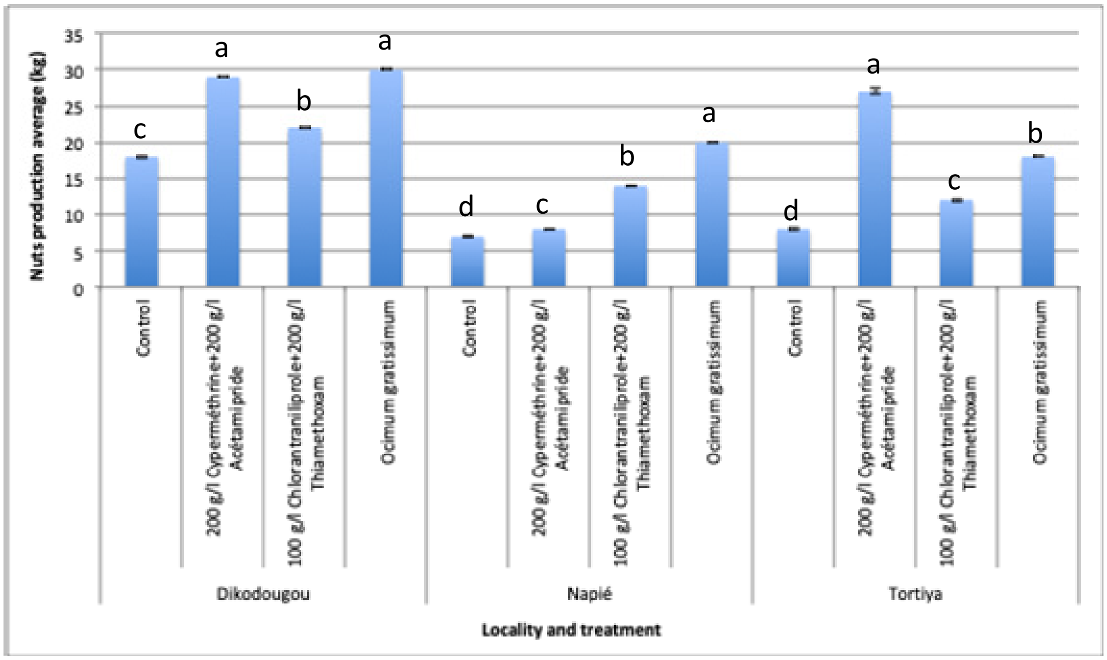


The diagrams with the same letters in the same Locality are not significantly different according to the Newman-Keuls test at the level of 0.05.

3.6. Cashew Yield by Treatment

Figure 5 shows the production of cashew trees treated with pesticides. Cashew tree production varies by location and treatment. All treatments showed a significant difference from control. The treatments were able to increase cashew nut production by more than 27.27% in Dikodougou, 28.57% in Napié and 29.23% in Tortiya. The Dikodougou orchard produced more nuts with the treatments (831.08 kg.ha-1) than the other two orchards in Napié and Tortiya (415.50 kg.ha-1 ; 549.33 kg.ha-1). The positive pesticide (200 g/l Cypermethrin + 200 g/l Acetamiprid) showed a significant difference in cashew nut production in Tortiya (77.14%), while the biopesticide (*Ocimum gratissimum*) was more effective in Dikodougou (62.50%) and Napié (74.07%).

Figure 5: Cashew nut yield of the orchards by treatment



The diagrams with the same letters in the same Locality are not significantly different according to the Newman-Keuls test at the level of 0.05.

3.7. Economic Loss Related to Nut Loss According Treatments

The table 3 presents the cashew nuts production per treated tree. The mass of good quality nuts remains between 2.89 and 13.48 Kg/ha with a maximum obtained with the tested pesticide (100 g/l Chlorantraniliprole + 200 g/l Thiamethoxam) treatment at Dikodougou. The mass of poor-quality nuts is between 0.15 and 1.64 Kg/ha at Napié and Tortiya. Statistical analysis shows that the effect of treatments is significant at the 5 thresholds on this production. Locality*treatment produces a significant effect to 5% in good quality nuts.

Gains on the calculated economic loss, loss varies from treatment to treatment depending on the locality. The highest gain 10355 F CFA/ha and 6710 F CFA/ha is obtained with essay treatments (100 g/l Chlorantraniliprole + 200 g/l Thiamethoxam) and the biopesticide (*Ocimum gratissimum*) respectively in the localities of Tortiya and Napié.

Table 3: Economic loss related to nut loss according treatments

Locality	Treatment	Mass of good quality nuts (kg.ha-1)	Mass of poor quality nuts (kg.ha-1)	Mass of total nuts lost (kg.ha-1)	Economic loss of total return (500 F CFA.kg-1)
Dikodougou	Control	1,52 ± 0,06f	0,25 ± 0,25d	-	-
	100 g/l Chlorantraniliprole + 200 g/l Thiamethoxam	13,48 ± 0,53b	0,0 ± 0,0e	11	5500
	200 g/l Cypermethrine + 200 g/l Acetamipride	7,47 ± 0,29d	0,79 ± 0,79c	4,79	2395
	Ocimum gratissimum	8,34 ± 0,33d	0,31 ± 0,31d	12,31	6155
Napié	Control	2,68 ± 0,10e	0,20 ± 0,20d	-	-
	100 g/l Chlorantraniliprole + 200 g/l Thiamethoxam	2,89 ± 0,11e	0,15 ± 0,15d	1,15	575
	200 g/l Cypermethrine + 200 g/l Acetamipride	3,23 ± 0,12e	0,0 ± 0,0e	7	3500
	Ocimum gratissimum	17,6 ± 0,70a	0,42± 0,42d	13,42	6710

Locality	Treatment	Mass of good quality nuts (kg.ha-1)	Mass of poor quality nuts (kg.ha-1)	Mass of total nuts lost (kg.ha-1)	Economic loss of total return (500 F CFA.kg-1)
Tortiya	Control	8,66 ± 0,34d	0,90 ± 0,09c	-	-
	100 g/l Chlorantraniliprole + 200 g/l Thiamethoxam	12,56 ± 0,50b	1,71 ± 0,71a	20,71	10355
	200 g/l Cypermethrine + 200 g/l Acetamipride	10,61 ± 0,42c	1,64 ± 0,64a	5,64	2820
	Ocimum gratissimum	10,27 ± 0,41c	1,16 ± 0,16b	11,16	5580
	P value (Treatments x localities)	0,01013	0,056871		
	P value (Treatments)	0,03459	0,06564		

4. Discussion

Approximately 20 fungal isolates of *Lasiodiplodia theobromae* were obtained from this prospecting on dried cashew tree leaf buds. The isolates are all similar in appearance, mycelial growth and spore shape and are mostly biseptate. *Lasiodiplodia theobromae* is a fungal species of the family Botryosphaeriaceae (Barnett and Hunter, 1998).

The effectiveness of insecticides against mosquito and borer on cashew nuts was tested during flowering and cashew production. The prevalence of insects varied greatly between localities. No attacks of borer were found in Napié, unlike Dikodougou and Tortiya. This difference in the severity of the insect would be linked to its adaptability in the different localities. The microclimate which is more humid in the orchards of Dikodougou and Tortiya would have an impact on the presence of the insect as reported by some authors (Yéo *et al*., 2019).

The prevalence of *Helopeltis anacardii* was also found to be lower in the locality of Napié compared to Dikodougou and Tortiya. The insect was observed at an attack rate of 20% of flower buds and young nuts of cashew trees in Napié compared to more than 90% in the other two localities. This low presence of *Helopeltis* sp is thought to be due to the temperature and level of sunlight in the Napié locality (Agboton *et al.,* 2014). *Helopeltis* sp does not tolerate open areas and also sun exposure. Vegetation cover, humidity level and low humidity would therefore be the primary causes of its strong presence in the localities of Dikodougou and Tortiya as Sundararaju (2004) showed in his work.

The control of borer and mosquito activities in cashew field was confirmed with essay pesticide (Chlorantraniliprole+Thiamethoxam), and the biopesticide (*O. gratissimum*).

The severity of *Helopeltis* sp on flowers and young nuts of treated trees, remains below 80% in the three localities. Sundararaju (2004) obtained the same rate of reduction. Chlorantraniliprole+Thiamethoxam and *Ocimum gratissimum* treatments carried out in the field to control the insects have been effective on nuts production and their quality. The quantity of nuts produced varies from the localities. The reduction of the insect's severity by treatments gains on the economic loss between 5000 to 10000 F CFA/ha.

Conclusion

This sanitary survey study of the cashew tree orchard in the regions of Béré, Poro, Hambol and Marahoué revealed a direct correlation between the drying of cashew tree leaf buds pricked by *Helopeltis* sp and *Lasiodiplodia theobromae*. The pathogen is associated with all dried buds showing cashew tree mosquito bites. *Helopeltis* sp appears as a vector of cashew tree foliar desiccation due to *Lasiodiplodia theobromae*. This is the first time that this mycopathogen has been identified as being involved in cashew tree leaf bud drying following cashew tree mosquito attacks in Côte d'Ivoire.

This study assessed the pesticides activity *in situ* of *Ocimum gratissimum* essential oil compared to the synthetic pesticides based on Chlorantraniliprole+Thiamethoxam. *Helopeltis* *anacardii* reduce the production of quality nuts, which has an impact on producer's income. The level of attacks due to *Helopeltis* sp decreased sharply after treatment. The trial determined the best insecticide treatments on cashew trees. Also, this study showed that synthetic pesticides based on Chlorantraniliprole+Thiamethoxam and essential oil of *Ocimum gratissimum* used in field reduce the damages of the pests on the flowers and the cashew nuts. The two pesticides increase cashew nut production. The essential oil of *Ocimum gratissimum* was effective as a biological control of *Helopeltis* sp as a vector of *Lasiodiplodia theobromae* in cashew orchard.

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