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Essential oils of *Cymbopogon citratus* (DC.) Stapf, *Cymbopogon nardus* L. and *Citrus sp*: Insecticidal activity on the Pink Bollworm *Pectinophora gossypiella* Saunders (Lepidoptera; Gelechiidae) and prospects for cotton pest management in Côte d'Ivoire

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ABSTRACT: In Côte d'Ivoire, the loss of cotton yield is mainly due to attacks caused by pest insects such as, the pink worm, *Pectinophora gossypiella* Saunders. For decades, the repeated use of chemical insecticides to control these pests has threatened the viability of the production system. Faced with the negative consequences linked to the use of synthetic chemicals, the search for alternative methods is essential. The objective of this study is therefore to evaluate the chemical properties and the insecticidal activity of essential oils extracted from *Cymbopogon citratus*, *Cymbopogon nardus* and *Citrus sp* on *P. gossypiella*. In the laboratory, nine concentrations (0.25; 0.50; 1; 2; 4; 8; 16; 32 and 64 %) for each of the three essential oils, with three replications, were tested by topical application method on adults of the pest using a micro-applicator. Results showed that the pest developed variable levels of sensitivity to those plant oils. The one extracted from *C. citratus* was the most toxic to *P. gossypiella*. The lethal concentrations (LC_{50} and LC_{90}) measured were 1.67 and 4.07 % respectively. Furthermore, the results of the gas chromatography coupled mass spectrometry (GS/SM) analysis indicated that the essential oils of the three aromatic plants evaluated were strongly composed of monoterpenes (91.57-100 %). *C. citratus* extract was the richest in oxygenated monoterpenes (73.71 %) followed by *C. nardus* extract (46.59 %). The essential oil of *C. citratus* can be used rationally as an alternative option to chemical in the current cotton pest control program in Côte d'Ivoire.

KEYWORDS: Cotton, Pectinophora gossypiella, essential oils, Cymbopogon citratus, Cymbopogon nardus, Citrus sp.

1 INTRODUCTION

In Côte d'Ivoire, cotton is the backbone of the rural economy in savannah areas and helps fight poverty thanks to the monetary income it provides [1]. It generates a contribution of 1.7 % to national GDP and 7 % as a share of exports since the early 2000 [2].

Although this crop has enabled Côte d'Ivoire and its people to make significant socio-economic progress, pests are a very serious constraint to cotton production [3]. Cotton is attacked by many species of lepidopteran insects at different stages of plant growth and the pink Bollworm, *Pectinophora gossypiella* (Saunders) is one of the most harmful [4] and widespread pest in the country [5]. Damage caused by insect pests is estimated at 61.9 % on the yield of cotton seed, 2.1 to 47.10 % on seed oil content and 10.70-59.20 % loss in normal capsule opening [6]. Thus, cotton protection method evolved since decades was based mainly on chemicals to fight cotton pests. The number of insecticide treatments is therefore 6 per season, including 2 or 3 during the vegetative phase and 3 or 4 in the fruiting phase of the plant [7]. With this phytosanitary strategy, cotton alone absorbs more than 60 % of the chemicals sold on the Ivorian market [8].

Indeed, the practice of chemical pest control is one of the successful factors that has facilitated the development of cotton sectors in West Africa [7]. However, it threatens the viability of production systems. The use of chemical pesticides in cotton cultivation, which requires several applications, has always posed potent danger for farmers and populations alike through environmental pollution, toxicity to mammals, and the resistance phenomena of harmful insects [9].

With regard to problems posed by the use of conventional insecticides, one of the alternative solutions is to control insects with natural substances. Thus, some recent work has highlighted the insecticidal or repellent power of certain plants, their extracts and allelochemical derivatives [10]. In many countries, several authors revealed biopesticide activities of aromatic plants such as *Cymbopogon citratus*, *Cymbopogon nardus* and *Citrus sp.* Indeed, in Cameroon, Tchoumbougnang *et al*. [11] stated that the essential oil of *C. citratus* would be very effective against *Anopheles gambiae* (an insect vector of malaria). Recently, in Burkina Faso, Ouédraogo *et al.* [12] confirmed the insecticidal activity of *C. nardus* on *Sitophilus zeamais* and *Rhyzopertha dominica*, the main pest insect of maize. As for the essential oil of lemon, in Algeria, Belguendouz *et al.* [13] proved its effectiveness on the population dynamics of *Parlatoria ziziphi* (Homoptera, Diaspididae), a formidable pest of citrus fruits.

In the present study, we propose to analyze on the basis of their biopesticide potential, chemical properties of essential oils extracted from three local aromatic plants *Cymbopogon citratus*, *Cymbopogon nardus* and *Citrus sp* and to evaluate their insecticidal activities on the cotton pink bollworm *P. gossypiella* in order to ascertain their rational use in the current cotton pest management.

2 Methods And Materials

2.1 Experimentation site

The experiment was carried out at the Laboratory of Agricultural Entomology of the cotton research program of the National Center for Agricultural Research of Bouaké (latitude 7°40' N and longitude 5°2' W).

2.2 Animal material

The last larval stages of *P. gossypiella* were obtained from green cotton capsules (*Gossypium hirsutum* L.) collected from a naturally infested experimental plots. The larvae were kept in the Laboratory, without any food, in plastic boxes (50 cm x 20 cm) containing cotton fiber. They were subjected to a photoperiod of 12 hours, a temperature of 26 ± 2 °C and a relative humidity of 70 ± 5 % [14]. After one week, the incubated larvae became chrysalis. This pupation lasted 13 days on average. Freshly emerged butterflies, that is, one-day old adults, were used for toxicological tests.

2.3 Essential oils

The study was conducted with three essential oils provided by the Laboratory of Biochemistry of Péléféro Gon Coulibaly University of Korhogo in the northern Côte d'Ivoire. They were extracted from the leaves of local aromatic plants belonging to the Poaceae family (*C. citratus*, *C. nardus*) and from lemon zest (*Citrus sp*) collected at the same area.

2.4 Extraction and analysis of essential oils

Extraction of essential oils was done on the dried leaves at room temperature (28 ± 2 °C) for 2 weeks and on fresh lemon zest. The oils were obtained by steam distillation using a stainless steel still (250L) for 3h.

The essential oils of were analyzed by selective gas chromatography (GC) coupled with selective mass spectrometry (GC / MS) in the Department of Chemical and Agri-Food Engineering (GCAA) of National Polytechnic Institute HOUPHOUËT-BOIGNY (INP-HB) at Yamoussoukro [15]. The analysis of the chemical constituents was carried out using an Agilent Technologies 6890 N Network GC System N.04.07 type chromatograph equipped with an HP-1 (phenyl-methyl polysiloxane) fused silica capillary column (25m x 200µm x 0.33µm). The oven was programmed at a temperature ranging from 50 to 150 ° C at a gradient of 5 °

C / min. The vector gas was helium with a flow rate of 0.8 ml / min. The temperatures of the injector (Agilent Technologies 7683) and the detector (Agilent 5973 Network) were respectively 250 and 280 ° C. A volume of 1 µl of essential oil sample diluted in diethyl ether was injected split mode.

The different volatile constituents were identified by their mass spectra and retention indices in comparison with those of the existing compounds in the database. The compounds were confirmed against standard reference data in the Nist 98.l and wiley275 database. The relative percentages of the different constituents were obtained by integrating their peaks on the gas chromatograph spectrum.

2.5 Toxicological tests

The Toxicological tests began with the preparation of essential oil concentrations over a range of concentrations that was determined based on several preliminary tests in the laboratory. Thus, for each of the three essential oils, the extract diluted 1/80th with acetone (80 % solution) was used to prepare the different concentrations tested: 0.25; 0.50; 1; 2; 4; 8; 16; 32; and 64 %.

For the actual tests, butterflies of the same age (1 day after emergence) were distributed in groups of 10 individuals and three repetitions were carried out for each concentration, 3 boxes x 10 individuals. After this, the butterflies in the same group were put to sleep with carbon dioxide at a rate of 20 l / min for 10 seconds to facilitate their treatment by topical application. Each one of them received 1 µl of solution on the dorsal part opposite to his thorax using an Arnold Micro micro-applicator (Burkard, UK) [16]. For each extract, the application was made in ascending order of the concentrations prepared. The control was made with acetone solution. Each group of treated insects was immediately transferred to a 12 cm × 16 cm nest box in a cellophane bag. They were fed with a 10 % (v: v) honey solution as advocated by Wu *et al.* [17] during storage at 26 °C ± 2 °C and 70 % relative humidity for the observations. Experiments involved a total of 600 individuals (butterflies).

2.6 Determination of mortality rates

After topical application, mortality in the different groups was observed at 24 h, 48 h and 72 h. The immobile and visibly moribund insects, which can not fly normally, were considered dead. When dead or moribund insects were observed among the controls, mortality rates were corrected by Abbott's formula [18]: $\mathbf{Mc}\ (\%) = \frac{\mathbf{M_t - M_0}}{\mathbf{100 - M_0}} \times \mathbf{100}$

M_c: corrected mortality rate

M_0: mortality rate in the control group

M_t: Mortality rate in the treated group

2.7 Data analysis

An analysis of variance or ANOVA was done using the SPSS 22.0 software on the rate of insect-corrected mortality according to treatments (essential oils and concentrations). In case of significant difference between treatments, the comparison of averages was done by the Duncan test at the 5 % threshold.

Using the software WinDL 32.0 (CIRAD, Montpellier, version 1998), the lethal concentrations respectively causing the death of 50 % (LC_{50}) and 90 % (CL_{90}) of the populations tested, were determined for each of the essential oils, two days (48 h) after butterfly exposure. They have been deduced from the regression lines. For this, the corrected mortality percentages have been transformed into probits. Finney's method or probit analysis [19] based on regression of probabilities of mortality by logarithms was used for this purpose.

3 Results

3.1 Chemical compositions of the essential oils of *C. citratus*, *C. nardus* and *Citrus sp*

Chemical analysis data for samples of essential oils of *C. citratus*, *C. nardus* and *Citrus sp* are presented in Table 1. According to the results, the essential oil samples are composed of 4, 5 and 8 chemical elements respectively for *C. citratus*, *C. nardus* and *Citrus sp*. These elements represent 100 % of the components identified in each of the analysed samples. Relatively to the essential oil of *C. citratus* only composed of monoterpenes, the β-Myrcene with a content of 26.29 % was the only monoterpene hydrocarbon compound. The oxygenated monoterpenes (73.71 %) detected in this oil were α-Citral (31.89 %), Neral (39.33 %) and Sulcatone (2.49 %). For *C. nardus* essential oil, the hydrocarbon and oxygen monoterpenes determined in this sample were comparable (44.98 and 46.59 % respectively). The majority were β-Myrcene (11.19 %), Carene (29.09 %), Neral (20.23 %) and Linalool (23.38 %). To these compounds were added hydrocarbon sesquiterpenes (8.43 %) such as β-Elemène at a content of 1.77 % and 6.66 % δ-guaiene. As for lemon essential oil, strongly composed of hydrocarbon monoterpenes (89.63 %), it consisted mainly of Limonene (59.36 %), β-Pinene (19.42 %) and γ-Terpinolene (10.85 %). A low oxygenated monoterpenes content (10.37 %) characterized this oil with Terpene-4-ol and α-Terpinol as compounds at 3.63 % and 6.74 % respectively.

Table 1. Chemical composition of essential oils of C. citratus, C. nardus and Citrus sp

Compounds	Mass percentages (%)		
	C. citratus	*C. nardus*	*Citrus sp*
Monoterpenes	**100**	**91,57**	**100**
Hydrocarbon monoterpenes	**26,29**	**44,98**	**89,63**
β-Pinene	-	-	**19,42**
γ-Terpinolene	-	-	**10,85**
β-Myrcene	**26,29**	**11,19**	-
Carene	-	**29,09**	-
d-Limonene	-	4,70	**59,36**
Oxygenated monoterpenes	**73,71**	**46,59**	**10,37**
α-Citral	**31,89**	-	-
Neral	**39,33**	**20,23**	-
α-Citronellal	-	2,98	-
Linalool	-	**23,38**	-
Sulcatone	2,49	-	-
Terpene-4-ol	-	-	3,63
α-Terpinol	-	-	6,74
Sesquiterpenes	-	**8,43**	-
Hydrocarbon sesquiterpenes	-	**8,43**	-
β-Elemene	-	1,77	-
δ-guaiene	-	6,66	-
Total	**100**	**100**	**100**

3.2 VARIATION OF THE MORTALITY RATE OF *P. GOSSYPIELLA* AS A FUNCTION OF THE CONCENTRATION OF ESSENTIAL OILS 24, 48 AND 72 H

The results of the mortality rates of *P. gossypiella* as a function of the concentration of the essential oils throughout the test (24 h, 48 h and 72 h) are presented in Figs. 1. In general, the results indicate that the mortality of insect has been in direct relationship with the concentration of the three essential oils. Thus, 24 hours after the test, the essential oil of *Citrus sp* used at concentrations ranging from 0.25 to 16 % allowed mortality between 0 and 46.66 % of the population of tested individuals. On the other hand, there are only two concentrations 32 and 64 % which caused mortalities of more than 50 % (80 % and 100 % respectively). However, with the essential oils of *C. citratus* and *C. nardus* more than 50 % mortality of the tested pest was obtained with five concentrations ranging from 4 to 64 %. Mortality of 100 % of the insect was obtained from 16 % (Fig. 1a). At 48 h, lemon oil (*Citrus sp*) had a very insignificant insecticidal activity with a slight increase in insect mortality ranging from 80 to 83 % at only 32 % concentration. Apart from this concentration no else allowed to obtain more than 50 % of the mortality of the tested individuals (Fig. 1b). An evolution of the insecticidal effect of the essential oil of *C. citratus* was observed from 0.5 to 4 % concentration. With that extracted from *C. nardus*, this increase in the mortality of the individuals tested was obtained with the concentrations of only 0.5 and 4 %. However, all the others had no insecticidal effect at this date of observation (48 h).

At 72 hours of exposure of insects to essential oils, an increase in the mortality of the individuals tested was general with the three plants evaluated (Fig. 1c). Thus, Citrus essential oil, at concentrations ranging from 2 to 32 %, has led to a growth in the mortality of the pest, even if this increase is weakly marked. Nevertheless, during the days of insect exposure to Citrus essential oil, no mortality was observed at concentrations of 0.25 %; 0.50 %; and 1 %. The first mortalities of *P. gossypiella* were observed from the concentration of 2 % of this essential oil. A totally different finding was made with the essential oils of *C. citratus* and *C. nardus*. In fact, the essential oil of *C. citratus* continued to have an insecticidal effect on the insect tested even 72 hours after the test. This allowed to obtain 100 % of the deaths from the 8 % concentration. Similarly, with *C. nardus* extract, mortality increased from the lowest concentration (0.25 %) to the 4 % concentration. The mortality of all insects tested was obtained at a concentration of 16 % during the three days of exposure of *P. gossypiella*.

3.3 TOXICITY OF THE ESSENTIAL OILS OF *C. CITRATUS*, *C. NARDUS* AND *CITRUS SP* ON *P. GOSSYPIELLA* AT TWO DAYS (48 H) OF EXPOSURE

The lethal concentrations causing respectively 50 % and 90 % death of the populations tested (LC_{50} and LC_{90}), were determined for each of the essential oils. According to the results presented in Table 2, the oil extracted from *C. citratus* and *C. nardus* was much more toxic than that extracted from lemon zest (*Citrus sp*). Indeed, the lethal concentration values determined for the two plants of the genus Cymbopogon are respectively: LC_{50} = 1.67 ± 0.08 % and LC_{90} = 4.57 ± 0.07 % for *C. citratus* and: LC_{50} = 1.71 ± 0.15 % and LC_{90} = 7.12 ± 0.10 % for *C. nardus*. The lethal concentration values 50 of these two plants are approximately equal and extremely low, 7 to 11 times, in comparison with those obtained with lemon essential oil (LC_{50} = 12.80 ± 0.10 % and LC_{90}= 54.68 ± 0.10 %). These results imply that the insecticidal activity of *C. citratus* essential oil is very close to that of *C. nardus*.

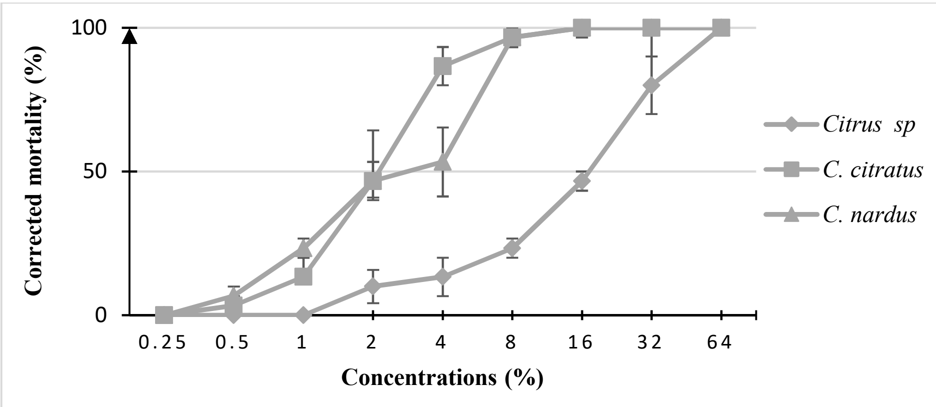

Fig. 1a. Insecticidal effect of the essential oils of C. citratus, C. nardus and Citrus sp on adult P. gossypiella 24 h after topical application

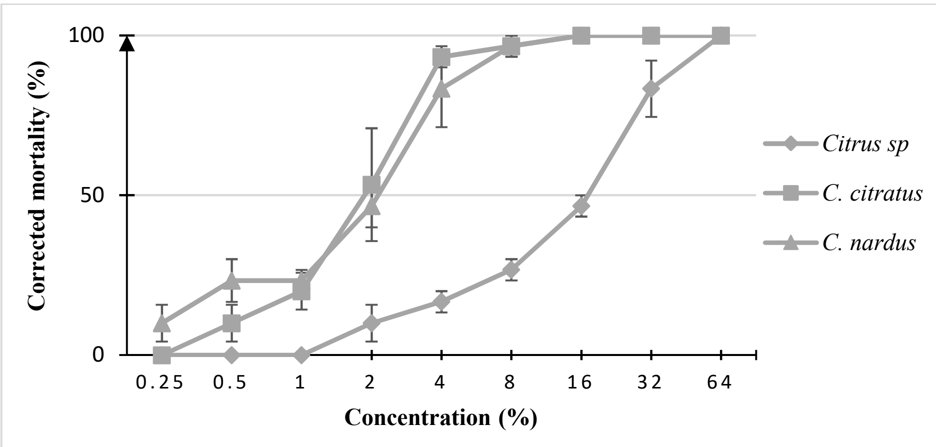

Fig. 1b. Insecticidal effect of the essential oils of C. citratus, C. nardus and Citrus sp on adult P. gossypiella 48 h after topical application

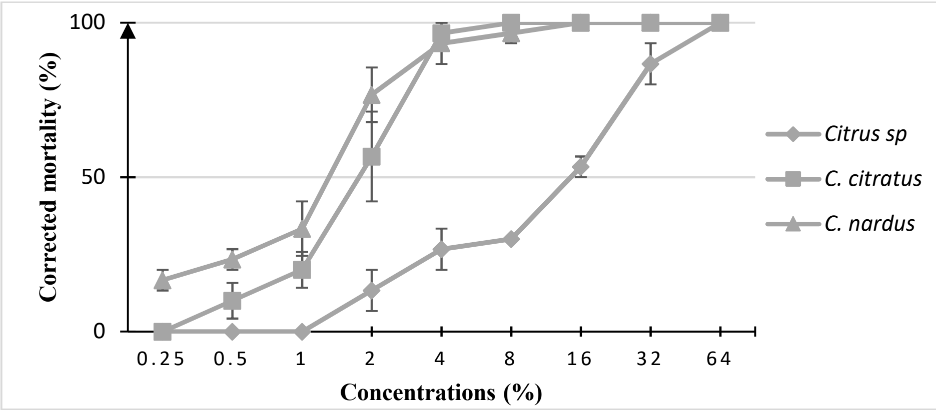

Fig. 1c. Insecticidal effect of the essential oils of C. citratus, C. nardus and Citrus sp on adult P. gossypiella 72 h after topical application

Table 2. Lethal concentrations (LC_{50} and LC_{90} in %) of the essential oils of C. citratus, C. nardus and Citrus sp 48 h after application to adults of P. gossypiella

Local plants used	N	λ^2	DF	LC_{50} ± SD (%)	LC_{90} ± SD (%)
C. citratus	300	3,05	3	1,67 ± 0,08	4,57 ± 0,07
C. nardus	300	4,88	3	1,71 ± 0,15	7,12 ± 0,10
Citrus sp	300	4,86	4	12,80 ± 0,10	54,68 ± 0,10

***N**: number of adults tested, **λ^2**: Chi 2, DF: degree of freedom, **LC_{50}**: lethal concentration at 50 %, **CL_{90}**: lethal concentration at 90 %, **SD**: standard deviation*

4 DISCUSSION

Chemical analysis of the essential oils of the three aromatic plants revealed that the extract of *C. citratus* was the richest in oxygenated monoterpenes with predominance of the compounds such as α-Citral (or geranial) and Neral. This same observation has been made by several authors in many countries [20], [11], [21], [22]. In Côte d'Ivoire, Kouamé *et al.* [23] worked on chemotypes that were characterized by a high percentage of Citral and Neral. A single hydrocarbon molecule, Myrcene, at a percentage of 26.29 % was detected in this essential oil. Our results corroborate those of Chisowa *et al.* [24] in Zambia. Indeed, the chemotype used by these authors was characterized by the presence of this molecule as the sole terpene hydrocarbon compound. With regard to the essential oil extracted from *C. nardus*, the chromatographic analysis of this extract demonstrated that it consisted mainly of Neral (20.23 %) and Linalool (23.38 %) with a low content in Citronellal (2.98 %). This chemotype differs from that studied in 2009 in Togo by Koba *et al.* [20]. In fact, the chemical analysis of the essential oil extracted from their chemotype showed a strong composition of Citronellal (35.5 %) and Geraniol (27.9 %) with a low fraction in Neral (0.4 %). However, the work of Wei *et al.* [25] revealed a small proportion of citronellol (4.8 %) in the essential oil of their chemotype. Recently in Burkina Faso, the essential oil extracted from *C. nardus* and analyzed during the work of Ouédraogo *et al.* [12] was rich in Citronellal (30.58 %) and Geraniol (23.93 %) with zero Linalool content (0 %). Relatively to the hydrocarbon terpene element composition of the essential oil of *C. nardus*, our results are in opposition to those obtained by Koba *et al.* [20]. Indeed, the essential oil of our chemotype was made up of more than 44 % of hydrocarbon monoterpenes against 1.9 % of the chemotype of the previous authors. The extract of lemon zests (*Citrus sp*), which is very little composed of oxygen-containing terpene molecules (10.37 %), was, however, very rich in hydrocarbon monoterpenes (89.63 %) with Limonene (59.36 %) and Pinene (19.42 %) strongly in the majority. Our results are therefore in line with those obtained by Boukabouche and Boudjefdjouf [26]. These authors showed that lemon zests contained high levels of Limonene and Pinene but were relatively low compared to our data. In Algeria, Ammad *et al.* [27] obtained an essential oil extracted from lemon zests rich in Limonene (61,68 %) with a considerable proportion of Neral (21,66 %). The lemon extract studied in this study contained only a small proportion of oxygenates with 10.37 % but higher than those obtained in India by Sharma and Abhishek [28].

The essential oil of the three aromatic plants each demonstrated an insecticidal potential against *P. gossypiella* during the 72 hours of the toxicological tests. The mortality of the pest insects was significantly dependent on the concentration of the extracts of these plants. Our results confirm the biological activity of extracts of these plants highlighted by many authors [29], [30], [21], [31], [26]. Indeed, chemical analysis has revealed that these essential oils were strongly composed of monoterpene (hydrocarbon and oxygenated). This chemical composition would explain their insecticidal potential against this pest of cotton as Konstanpoulou *et al.* [32]. In fact, these authors had indicated that the biological activity of essential oils were closely related to their levels of monoterpenes.

The essential oil of *C. citratus* has had remarkable insecticidal efficiency on the pest. The toxicity of *C. nardus* extract was substantially similar to that of *C. citratus*. The insecticidal activity of these two plants belonging to the Poaceae family was much higher than that of *Citrus sp* essential oil, as according to lethal concentration data (LC_{50} and LC_{90}). The chemical composition of these oils could provide a clear answer to this observation. Thus, when one refers to the data of the chemical analyses of these plants, one can notice that the insecticidal effectiveness of the essential oils was made according to their compositions in oxygenated monoterpenes. The essential oil extracted from *C. citratus*, which was the most toxic for the pest, contained a high content of oxygenated monoterpenes (73.71 %), followed by *C. nardus* extract at 46.59 % and finally *Citrus sp* extract, the least effective, had a low content of 10.37 %. Indeed, several authors who have studied the biological power of monoterpenoids in essential oils have revealed that oxygenated terpenoids have a higher potential ratio than non-oxygenated terpenoids [10], [33]. The best effectiveness of the essential oil of *C. citratus* could also be explained by the presence of oxygenated terpenoids such as α-Citral (31.89 %) and Neral (39.33 %). In 2005, Tchoumbougnang *et al.* [11] reported that Citral was the most active ingredient in the essential oil of *C. citratus*. The insecticidal efficiency of the essential oil of *C. nardus*, which was found to be close to that of *C. citratus* with respect to *P. gossypiella*, it corroborates the results of Hernandez *et al.* [34]. Indeed, these authors, by their work relating to the evaluation of the essential oils of three plants of the genus Cymbopogon (*C. citratus*, *C. nardus* and *C. martinii*) on adults of *Oryzaephilus surinamensis* (L.) and *Sitophilus zeamais* Motsch, found that the toxicity of these essential oils were all comparable to those pests tested. Moreover, the very low toxicity of the *Citrus sp* extract for *P. gossypiella* butterflies, despite its strong hydrocarbon monoterpene composition with Limonene dominance (59.36 %), is due to its low content of oxygenated compound (10.37 %). Limonene, the main compound of lemon zest oil, is therefore not very toxic to this pest.

5 CONCLUSION

The present study revealed that the essential oils extracted from the leaves of *C. citratus*, *C. nardus* and lemon fruit zests (*Citrus sp*) have biological activities on *P. gossypiella*. The essential oil of *C. citratus* was the most toxic on the insect. The toxicity of *C. nardus* extract was very similar to that of *C. citratus*. The insecticidal efficacy of lemon oil was low for the pest. The essential oil of *C. citratus* or even that of *C. nardus* could be used as a basic substance for the development of new botanical insecticides in cotton cultivation. Field tests could better confirm this.

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