

Evaluation of the Insecticidal and Repellent Properties of Essential Oils Extracted from *Cymbopogon citratus* (DC.) Stapf, *Mentha piperita* L. Plants and Their Combination against *Glossina palpalis gambiensis* (Diptera: Glossinidae), a Vector of Trypanosomes in the Laboratory

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Abstract

Tsetse flies are primarily controlled using chemical insecticides, despite their negative environmental and health impacts. Developing safer and more sustainable alternatives is therefore essential. This study aims to evaluate the insecticidal and repellent activities of essential oils from *Cymbopogon citratus*, *Mentha piperita*, and their combination, against *Glossina palpalis gambiensis*. Insecticidal effects were assessed through tarsal contact on glass microfiber papers impregnated with the oils, while repellency was evaluated by exposing flies to essential-oil volatiles in flight tunnels. The results show that the *M. piperita* at 100% concentration produced the highest knock-down (48.4%) and mortality (61.3%) rates, with a significant difference compared with *C. citratus*

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($P < 0.01$). In repellency assays, *C. citratus* resulted in the lowest activation rate (46.5%), and all essential-oil treatments significantly reduced fly activation ($P = 0.01$). In arm-test assays, *C. citratus* recorded the lowest preference rate (21.7%) and differed significantly from *M. piperita* ($P = 0.04$; OR = 3.6) and from both controls ($P < 0.01$). Overall, the findings indicate that essential oils from *C. citratus* and *M. piperita* have promising potential and are environmentally safer alternatives to chemical insecticides for tsetse-fly control. Some studies must implement to know well the target chemicals compounds in oils.

Keywords

Insecticide, Repellent, Essential Oils, *Glossina palpalis gambiensis*

1. Introduction

Livestock farming constitutes a fundamental component of rural economies in sub-Saharan Africa, particularly in Burkina Faso, where it provides animal protein, draft power, manure, and substantial financial income for households. Beyond its contribution to livelihoods, the sector plays a key role in national food security and accounts for an estimated 10% - 20% of the country's gross domestic product [1]. Despite this strategic importance, livestock production systems remain highly vulnerable to numerous constraints, like as blood-feeding insects that act as vectors of major infectious diseases. Among these, tsetse flies, stable flies, and horseflies are of particular concern due to their direct harmful effects—such as blood loss, stress, and reduced productivity—and their major epidemiological role in transmitting viral, bacterial, and protozoan pathogens. African trypanosomiasis (AT), caused by protozoa of the genus *Trypanosoma*, is the most impactful vector-borne disease for livestock in the region. Transmitted exclusively by tsetse flies, AT affects both humans (human African trypanosomiasis, HAT) and animals (animal African trypanosomiasis, AAT) [2]. The disease remains endemic in 37 sub-Saharan countries, where nearly 60 million people and 50 million livestock are at risk [3].

Although notable progress has been made toward eliminating HAT [4] [5], AAT continues to exert a profound negative impact, causing approximately 3 million cattle deaths each year and generating economic losses estimated at over US \$4.75 billion [3] [6].

Current control strategies targeting tsetse flies—such as chemotherapy, the use of chemical insecticides on animals or resting sites, trapping devices, and the sterile insect technique—have contributed to reducing disease transmission in several areas. However, their widespread use is increasingly challenged by high operational costs, risks of insecticide resistance, environmental contamination, and concerns regarding food safety [7] [8]. These limitations underscore the urgent need to explore innovative, environmentally sustainable alternatives for vector control. Plant-derived substances, particularly essential oils, represent a promis-

ing avenue in this regard. Several studies have demonstrated their insecticidal, and repellent properties against mosquitoes, agricultural pests, and other hematophagous flies [9]-[13]. Yet, only limited research has investigated their effects on tsetse flies, with a few reports documenting activity of neem extracts on *Glossina fuscipes fuscipes* and *Glossina palpalis gambiensis* [14]. Our preliminary work conducted at CIRDES (not published), revealed the essential oils from *Cymbopogon citratus* and *Mentha piperita*, individually and in combination have insecticidal properties against *Glossina palpalis gambiensis*.

In this context, and within the framework of the COMBAT project, the present study aims to evaluate the insecticidal and repellent properties of essential oils from *Cymbopogon citratus* and *Mentha piperita*, individually and in combination, against *Glossina palpalis gambiensis*. By exploring plant-based alternatives, this study contributes to the search for sustainable, eco-friendly tools for integrated tsetse control strategies.

2. Methods

2.1. Study Site

The study was conducted at the International Center for Research and Development on Livestock in Subhumid Areas (CIRDES) located in the city of Bobo-Dioulasso (11°11'00" North and 4°17'00" South) in Burkina Faso. All flies were supplied by the CIRDES insectary. The CIRDES colony is maintained under controlled environmental conditions of 25°C ± 1°C and 75% ± 5% relative humidity.

2.2. Species of *Glossina* Tested and Its Rearing

Glossina palpalis gambiensis (Vanderplanck, 1949) was the tsetse species used in this study. All flies were supplied by the CIRDES insectary where they were maintained under controlled environmental conditions of 25°C ± 1°C and 75% ± 5% relative humidity. For each experimental exposure, newly emerged teneral flies were selected. Flies are routinely fed using trays containing bovine blood covered with silicone membranes. A heating system maintains the plate temperature between 40°C and 42°C, ensuring a blood temperature of 36°C - 37°C beneath the membrane. The bovine blood used for tsetse feeding is collected at the Bobo-Dioulasso refrigerated slaughterhouse. After collection, the blood is defibrinated and subjected to both bacteriological and biological quality-control tests to confirm its suitability for fly consumption. It is subsequently irradiated using a Cesium-137 irradiator and stored under cold conditions until use.

2.3. Description and Dilution of Essential Oils Used

During this study, we used three essential oils derived from two locally available plant species: *Cymbopogon citratus* (Cc) essential oil, *Mentha piperita* (Mp) essential oil, and a combined formulation (Cc_Mp), consisting of 80% Cc and 20% Mp, as defined in previous investigations [15]. This combination aimed to improve the effectiveness of the oils' antioxidant effects while ensuring the pleasant

aroma of these aromatic plants [15]. *Cymbopogon citratus* DC. (lemongrass) belongs to the *Poaceae* family, which comprises approximately 660 genera and 9000 species [16]. It is a perennial, unbranched, lemon-scented grass that grows in dense clumps, characterized by light green, pubescent, strongly aromatic, tapering leaves united in a sheath along part of their length, with hyaline margins bearing numerous small, apically oriented teeth. Its subterranean system consists of a bulb or rhizome. The essential oil of *C. citratus* is primarily composed of Geranial and Neral [15].

Mentha piperita L. (peppermint) is a perennial, highly aromatic herbaceous plant belonging to the *Lamiaceae* family [17]. Mints have been used since antiquity and play a significant role in traditional therapy. As diffusible stimulants and sedatives, they are reputed to alleviate nervousness and related disorders. Their biological activity and characteristic scent derive largely from their essential oils [18]. The essential oil of *M. piperita* is mainly composed of Menthol, Menthone and Menthofuran [15].

All essential oils used in this study were produced and supplied by the Laboratory of Chemistry and Natural Substances at Nazi Boni University. Oils were extracted from dried leaves by hydrodistillation (steam distillation) using a Clevenger-type apparatus.

The oils were diluted according to the principle of conservation of mass in the solvent, using the equation $C_i \times V_i = C_f \times V_f$, where C_i represents the initial concentration of the pure essential oil or mixture, V_i the volume required to obtain the desired concentration, C_f the target concentration, and V_f the final volume of solution. Serial dilutions of each essential oil and the combined formulation were prepared in dimethyl sulfoxide (DMSO) to assess their insecticidal activity against tsetse flies. For each oil, three concentrations (100%, 50%, and 25%) were prepared through stepwise dilution. A volume of 200 μ l per oil and per concentration was prepared in glass vials for the insecticidal assays. After dilution under fume hoods, all solutions were stored at 4°C - 8°C until use.

2.4. Evaluation of the Insecticidal Properties of Essential Oils under Laboratory Conditions

The insecticidal properties of essential oils and their combinations were evaluated by tarsal contact of tsetse flies on impregnated glass microfiber paper. Before each exposure, the flies were lightly anesthetized in a freezer for five (05) minutes. Each fly was exposed for five seconds on the impregnated paper using soft tweezers; corresponding to the average contact time of tsetse flies on impregnated screens [19]. Pieces of glass microfiber paper with a surface area of 4.3 cm² were impregnated with 49 microliters of solution per concentration and per oil. Impregnation was carried out just a few minutes before exposure. At each repetition, the tsetse flies were exposed to the different essential oils one after the other. However, for each oil, exposure to the different concentrations was carried out simultaneously. The experiment was repeated three (03) times. Ten (10) males of tsetse were used per repetition and per concentration. One repetition involved exposing the tsetse

flies to the different essential oils and the different controls (positive and negative). A screen impregnated with Permethrin was used as the positive control and a piece of glass microfiber paper impregnated only with DMSO was the negative control. After exposure, the tsetse flies were placed in Roubaud cages by concentration and by essential oil and transported from the handling room to the storage room. The parameters measured were the number of flies knocked down at different times after exposure: 1 h, 2 h, 3 h, and 4 h; and mortality 24 h after exposure.

2.5. Evaluation of the Repellent Properties of Essential Oils under Laboratory Conditions

The experiment consisted of releasing tsetse flies into the release cage and observing their behavior in response to the treatments. Three (03) test treatments consisting of Cc vs Untreated Hand (Cc/UH); Mp/UH; and Cc_Mp/UH and two (02) control treatments (Empty/Empty and UH/UH) were the different combinations highlighted. The purpose of the controls was to demonstrate the reliability of the device by measuring the activation of flies in response to odors with Empty/Empty and untreated human hands in order to compare the effects of the treatments on fly activation. The treatments with essential oils consisted of soaking the palm of one hand with 50µl of essential oil and placing it on one of the collection boxes, while the other untreated hand (UH) was placed on the other box of the device. A repetition with each essential oil involved treating both hands one after the other in each test, which minimized the “arm” effect in attracting flies. The Vacuum/Vacuum control treatment was performed at the beginning and end of each repetition with the EOs.

For each test, twenty-two-day-old male tsetse flies that had never fed on blood were released for twenty-five minutes (latency time + observation time). Knowing that tsetse flies are naturally attracted to light, a light was shone on the “arms” of the device at the collection boxes five (05) minutes after the flies were released in order to stimulate their natural activation, and the observation time for the behavior of the tsetse flies according to the treatments was twenty (20) minutes. The number of activated flies (*i.e.*, those that moved from the release cage to the glass tunnels) was recorded per arm in order to calculate the activation and attraction of *Glossina palpalis gambiensis* according to the three essential oil treatments. The number of flies knocked down according to the different treatments in the release cage was also noted in order to consider the number of potentially active flies in the evaluation of tsetse flies’ activation. Activation corresponds to the number of flies counted in the two collection arms relative to the total number of potentially active flies released into the cage. Attraction (or preference) for an essential oil is the ratio between the number of tsetse flies counted in the “test arm” (containing the treatment with that oil) and the total number of flies activated in both “arms”. To do this, one of the handler’s hands was impregnated with the oil to be tested and the other hand, which was not treated (UH), was considered the control.

As flies are diurnal insects, all tests were conducted during the day between 8 a.m. and 1 p.m., and the release cage was covered with a black cloth to create

darkness inside the release cage, which increased the flies' natural attraction to the light emitted from the collection boxes. For each essential oil, the tests were repeated three (03) times.

2.6. Ethical Consideration

The arm-test repellency assays were conducted using a single adult human volunteer who participated voluntarily after providing informed consent. According to institutional guidelines, formal ethical clearance was not required for this type of bioassay.

2.7. Statistical Analysis

All statistical analyses were performed using R.4.4.2 software [20]. The Shapiro test was used to test the normality of the data, and Pearson's chi-square test was applied at a 5% significance level. Statistical analyses of the effects of treatments on knock-downs (KD) were performed using the non-parametric Kaplan-Meier estimator. Multiple pairwise comparisons were made using the (glht) function of the (Multcomp) package when there was a significant effect of treatment or concentration on fallen tsetse flies or mortality.

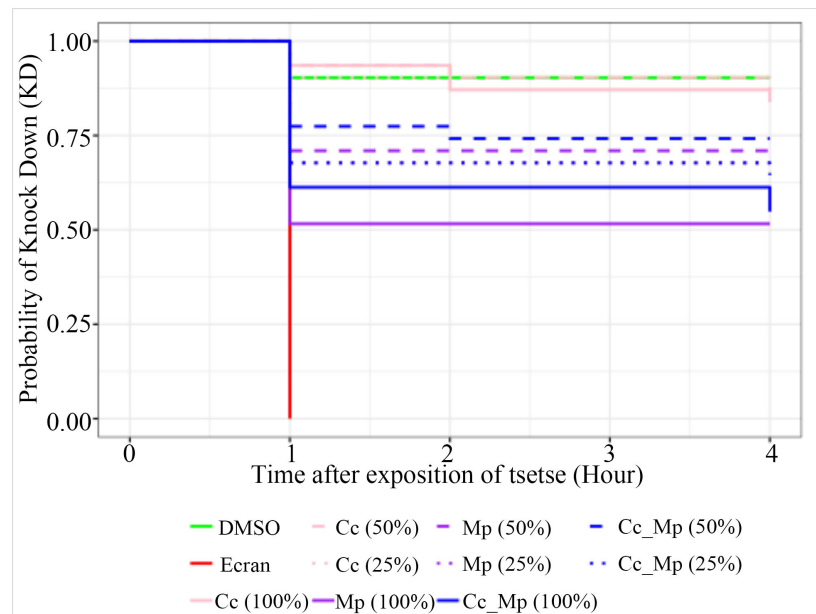
Statistical analyses of the effects of treatments on fly activation and attraction were performed using the generalized linear binomial model, followed by likelihood ratio tests (LRT) to evaluate the effects of different treatment combinations on tsetse fly activation. The analysis of tsetse fly attraction was performed by "combination" according to the treatment, and odds ratios were calculated to compare the rate of tsetse flies that were attracted to the "treated arm" compared to the "untreated arm" according to the different combinations tested.

3. Results

3.1. Knockdown Effects of Essential Oils and Their Combinations on Tsetse Flies

Results show that all essential oils tested exert knock-down (KD) effects on *Glossina palpalis gambiensis* (Figure 1). The highest knock-down rates were observed at the highest concentration (100% or 10,000 ppm). These rates were 16.1%, 48.4%, and 45.2% for *Cymbopogon citratus* (Cc), *Mentha piperita* (Mp), and their combination (Cc_Mp), respectively. The KD rates recorded in the controls were 9.6% and 100% for the negative control (DMSO-impregnated glass microfiber paper) and the positive control (impregnated screen), respectively. A low KD effect on tsetse flies exposed to essential oils compared to the positive control (impregnated screen) was observed ($P < 0.0001$). The KD probability curves show that the sensitivity rate of the essential oils tested on tsetse flies is very low, as the tsetse flies that fell in the oil treatments were spread out over time (1 h, 2 h, 3 hours, and 4 hours) of KD observation, compared to 100% of tsetse flies knocked down in the positive control group from the first hour of observation. The analyses showed an interaction between the knock-down effect and the treatment ($P < 0.001$). How-

ever, no significant interaction was observed between the knock-down effect and the concentration of essential oils, nor between the interaction of concentration and treatment ($P = 0.6$). A multiple comparison between the different essential oils tested showed a significant difference between the essential oil of *C. citratus* and that of *M. piperita* ($P < 0.01$). A significant difference was also observed between *C. citratus* essential oil and the combination essential oil (Cc_Mp) ($P = 0.01$). However, no significant difference was observed between *M. piperita* essential oil and the combination essential oil ($P = 0.99$).

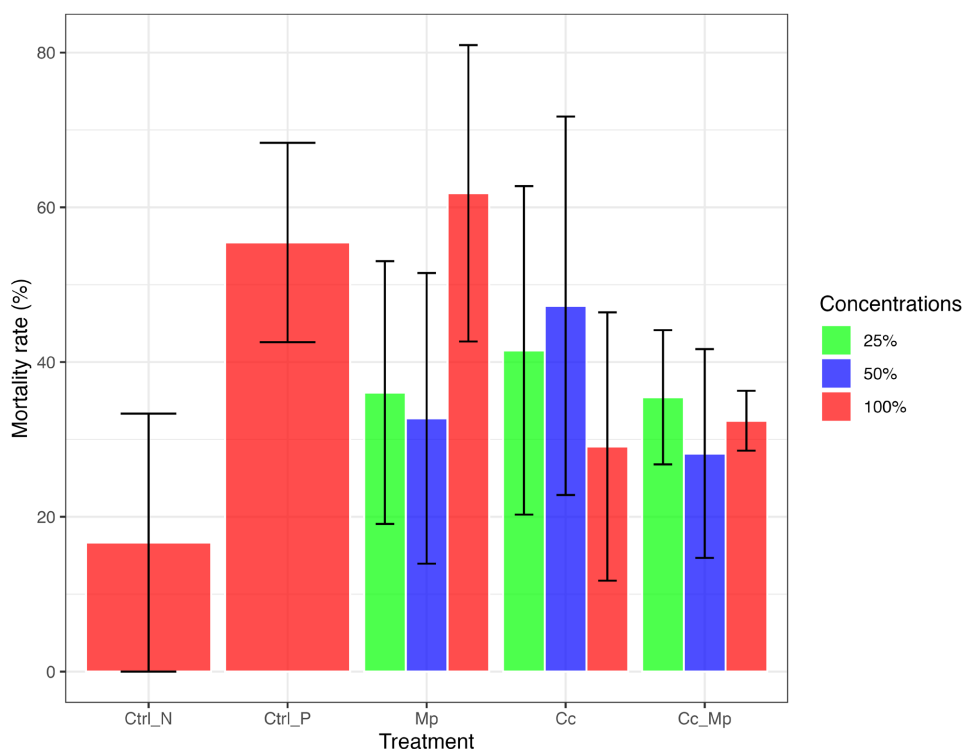


DMSO: Dimethyl Sulfoxide, Ecran: Impregnated screen, Cc: *Cymbopogon citratus*, Mp: *Mentha piperita*, Cc_Mp: Combination of *Cymbopogon citratus* and *Mentha piperita*.

Figure 1. Probability of knock-down in *glossina* according to essential oils and concentrations.

3.2. Effects of Essential Oils on Tsetse Fly Mortality

The insecticide test results for essential oils (Figure 2) show that all the essential oils tested cause mortality in tsetse flies. With the essential oils tested (for all concentrations combined), mortality rates for *G. palpalis gambiensis* ranged from 29.0% to 61.3%. The highest mortality rate recorded on *G. palpalis gambiensis* was obtained with *M. piperita* essential oil at a concentration of 100%. Mortality rates of 16.1% and 54.8% were recorded for the negative control and positive control, respectively. Significance tests at the 5% threshold revealed that there was a highly significant effect of all treatments on fly mortality ($P < 0.001$) while there was no significant effect between concentrations ($P = 0.65$) or interaction between concentrations and treatments ($P = 0.16$) on the different mortality rates. A multiple comparison between essential oils showed that there was no significant difference between the different essential oils tested on the mortality rates of *Glossina palpalis gambiensis*, with $P(\text{Cc_Mp/Cc}) = 0.82$, $P(\text{Mp/Cc}) = 0.99$, and $P(\text{Mp/Cc_Mp}) = 0.55$, respectively.



Ctrl_N: negative control, Ctrl_p: positive control, Cc: *Cymbopogon citratus* essential oil, Mp: *Mentha piperita* essential oil, Cc_Mp: Combination of *Cymbopogon citratus* and *Mentha piperita* essential oil.

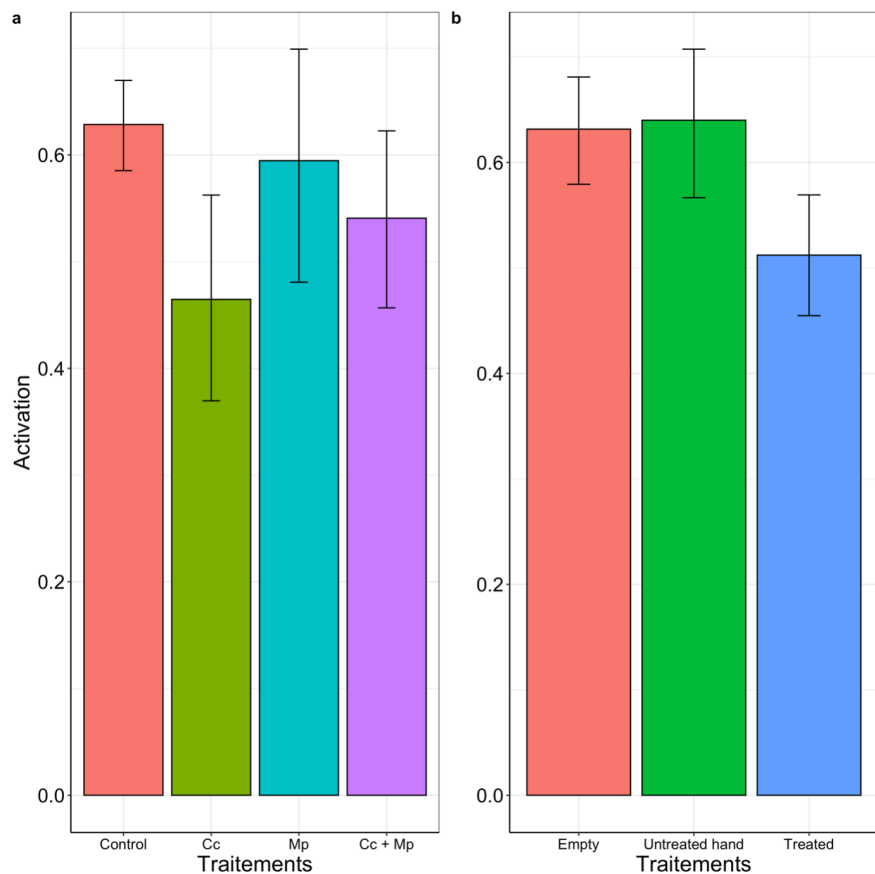
Figure 2. Effect of different concentration of essential oils on the mortality of tsetse.

3.3. Repellent Properties of Essential Oils and Their Combinations on Tsetse Flies

The activation of tsetse flies in the experimental device was evaluated according to treatments with essential oils (Cc; Cc_Mp; and Mp) and controls (Empty = Ctrl1; and Untreated hand = UH = Ctrl2). The results on activation (**Figure 3(a)** and **Figure 3(b)**; and **Table 1** and **Table 2**) show that tsetse flies were activated in the device regardless of the type of combinations tested. With all combinations, activation rates ranged from 46.4% to 63.9% (**Table 2**). The presence of essential oils in the combinations resulted in lower activation rates compared to the controls (**Figure 3(b)**). The presence of *C. citratus* EO resulted in a decrease in tsetse fly activation to below 50% ($\bar{X} = 46.46\%$). Fly activation was higher in the vacuum ($\bar{X} = 63.15\%$) and untreated hands ($\bar{X} = 63.99\%$) compared to the essential oils tested (**Table 2**).

A multiple comparison showed that there was a significant difference between all the test treatments combined on the activation of *Glossina palpalis gambiensis* compared to the different control treatments (Treated/Ctrl1: $P < 0.01$; Treated/ Ctrl2: $P = 0.02$). However, there was no significant difference between the two controls ($P = 0.98$) or between the essential oils tested on the activation of *glossina* in the experimental device, with $P > 0.05$ respectively (**Table 1**). Furthermore, *Cymbopogon citratus* essential oil showed a highly significant effect on activation

compared to the different controls combined ($P = 0.01$), but there was no significant difference in the repellent effects of *M. piperita* essential oil and the combination essential oil on fly activation compared to all controls (Table 1).



Panel a. Comparison of the activation rate of all control against different treatments with essential oils; **Panel b.** Comparison of the activation rate of all treatment with essential oils against different control arm. Ctrl_N: negative control, Ctrl_p: positive control, Cc: *Cymbopogon citratus* essential oil, Mp: *Mentha piperita* essential oil, Cc_Mp: Combination of *Cymbopogon citratus* and *Mentha piperita* essential oil.

Figure 3. Comparison of activation rate of *G. palpalis gambiensis* between the treatments.

Preference or attraction is the ratio of the number of tsetse flies attracted to the treated arm to the total number of tsetse flies attracted to both arms of the experimental device. The preference of tsetse flies in choosing between the arms (“test arm” and “control arm”) was assessed based on combinations of each essential oil with an untreated control (Cc/UH; Cc_Mp/UH; Mp/UH). Fly releases were carried out using only control treatments (untreated or empty hand) to compare the effectiveness of the two arms of the olfactometry device. The preference results (Figure 4 and Table 2) showed that with all the essential oils tested, preference rates ranged from 21.7% to 52.6% (Table 2). In fact, all essential oils were less preferred except for *M. piperita* EO (Table 2). The lowest attraction rate (Rate = 21.7%) was obtained with *Cymbopogon citratus* EO.

Table 1. Comparison between the treatments on the activation of *Glossina palpalis gambi-ensis*.

Treatment	z. ratio	P value	OR
Cc/Ctrl	-3.01	0.01	0.51
Mp/Ctrl	-0.156	0.94	0.86
Mp/Cc	1.168	0.33	1.68
Cc_Mp/Ctrl	-1.84	0.25	0.69
Cc_Mp/Cc	1.14	0.65	1.35
Cc_Mp/Mp	-0.74	0.87	0.8
Ctrl2/Ctrl1	0.18	0.98	1.03
Treated/Ctrl1	-3.01	0.01	0.61
Treated/Ctrl2	-2.68	0.02	0.59

SE = standard error; OR = Odds ratios; Cc: Essential oil from *Cymbopogon citratus*; Mp: Essential oil from *Mentha piperita*; Cc_Mp: Combination of *Cymbopogon citratus* and *Mentha piperita* essential oil; Ctrl1: Control with empty box; Ctrl2: Control with untreated hand.

Table 2. Average activation and attractivity rate of *glossina* according to the treatments.

Treatment	Average rate of activation (%)	Average rate of attractivity (%)
Cc	46.46	21.70
Cc_Mp	54.07	34.25
Ctrl	62.8	NA
Ctrl1	63.1	50.2
Ctrl2	63.9	52.6
Treated	51.2	-
Mp	59.4	50.0
Tested arm	NA	47.7
Control arm	NA	34.2

Cc: Essential oil from *Cymbopogon citratus*; Mp: Essential oil from *Mentha piperita*; Cc_Mp: Combination of *Cymbopogon citratus* and *Mentha piperita* essential oil; Ctrl1: Control with empty box; Ctrl2: Control with untreated hand.

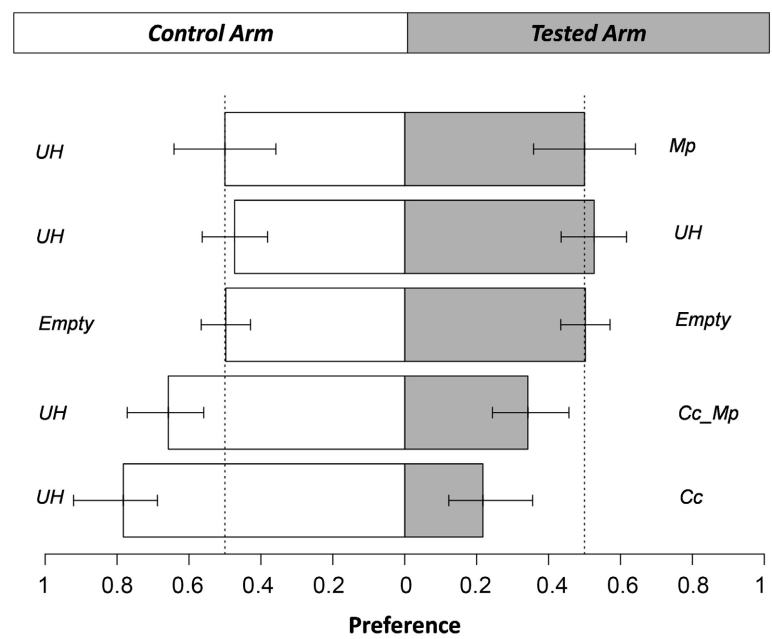
Multiple comparisons showed differences between the oils themselves and between the oils and controls on preference (**Table 3**). There was a significant difference between *Cymbopogon citratus* EO and the controls in terms of tsetse fly preference, and *C. citratus* essential oil was slightly less preferred than the two controls (with Ctrl1/Cc: $P < 0.01$; OR = 3.6; Ctrl2/Cc: $P < 0.01$; OR = 4.0). Furthermore, we noted a significant difference in the attractiveness of tsetse flies between *C. citratus* and *M. piperita* essential oils ($P = 0.04$; OR = 3.6). However, there is no significant difference between Cc and Cc_Mp ($P = 0.59$; OR = 1.8) or between Mp and Cc_Mp ($P = 0.44$; OR = 1.9). The analysis showed a significant difference between Cc_Mp and Ctrl1 ($P < 0.01$; OR = 1.9) but not with Ctrl2 ($P =$

0.1; OR = 2.1). Furthermore, there was no significant difference between *Mentha piperita* essential oil and the two controls ($P > 0.05$).

Table 3. Comparison between different treatments in the preference of de *G. palpalis gambiensis*.

Treatment	z. ratio	Pvalue	OR
Cc_Mp/Cc	1.44	0.59	1.8
Ctrl1/Cc	3.35	<0.01	3.6
Ctrl1/Cc_Mp	2.33	0.01	1.9
Ctrl2/Cc	3.43	<0.01	4.0
Ctrl2/Cc_Mp	2.44	0.10	2.1
Ctrl2/Ctrl1	0.41	0.99	1.1
Mp/Cc	2.73	0.04	3.6
Mp/Cc_Mp	1.67	0.44	1.9
Mp/Ctrl1	-0.02	0.99	0.9
Mp/Ctrl2	-0.30	0.99	0.8
Control arm/Test arm	-2.15	0.03	0.5

SE = Standard Error; OR = Odds ratios; Cc: Essential oil from *Cymbopogon citratus*; Mp: Essential oil from *Mentha piperita*; Cc_Mp: Combination of *Cymbopogon citratus* and *Mentha piperita* essential oil; Ctrl1: Control with empty box; Ctrl2: Control with untreated hand.



UH: Untreated hand; Mp: hand treated with *Mentha piperita* essential oil; Cc_Mp: hand treated with combined essential oil from *Mentha piperita* and *Cymbopogon citratus*.

Figure 4. Preference of glossina according to the arm of test.

4. Discussion

Exploring the insecticidal properties of essential oils extracted from plants against tsetse flies may offer alternatives to chemical insecticides in controlling tsetse flies that transmit trypanosomiasis. The present study evaluated the insecticidal effect of essential oils from *Cymbopogon citratus*, *Mentha piperita*, and their combination on *Glossina palpalis gambiensis* in the laboratory. All essential oils tested showed an insecticidal effect on *G. palpalis gambiensis*. This insecticidal property, demonstrated by knock-down effects and mortality of treated flies, varied depending on the essential oil used and concentration. These results confirm several previous studies on the insecticidal effects of essential oils. Indeed, Joseph *et al.* [21] showed the insecticidal effect of essential oils from five (05) aromatic plants, including *C. citratus*, against the bean weevil (*Acanthoescides obtectus* Say) in the Republic of the Congo. Tial *et al.* [22] showed that essential oils from *Cymbopogon citratus* DC and *Ocimum canum* Sims are effective against *Cylas puncticollis* Boheman, a sweet potato weevil in Côte d'Ivoire. Balboné *et al.* [12] [23] demonstrated the adulticidal properties of essential oils from plants and their combinations on populations of *Anopheles gambiae* and *Aedes aegypti* in Burkina Faso. The essential oils of *Cymbopogon citratus* have been shown to have antiplasmodial and larvicidal activities against *Plasmodium falciparum* and mature larval stages of *Anopheles funestus* in Cameroon [10]. *Mentha piperita* essential oil had showed toxic by inhalation to adults of *Rhyzopertha dominica* [24] and has insecticidal effects on the development of two mosquito species (*Culiseta longiareolata* and *Culex pipiens*) in Algeria [25]. However, the essential oils in our study showed relatively weak insecticidal properties by tarsal contact on glass microfiber paper impregnated at all concentrations tested. The weak insecticidal effect of these tested essential oils may be linked to the genus, species, and stage of development of the insect used in the study, on the one hand, or to the fact that the active ingredients contained in the essential oils may have been absorbed by the glass microfiber papers. The low knock-down rates obtained with all the essential oils tested are attributable to the low toxicity of the essential oils evaluated on *Glossina palpalis gambiensis*. It can be explained also by the very low penetration rate of the active ingredients in these oils through the tarsi of the tsetse flies. These results are consistent with those obtained by Makoundou *et al.* [26] who showed that a natural insecticide extracted from neem (*Azadirachta indica* A. Juss) had no insecticidal effect on *Glossina fuscipes fuscipes* through forced tarsal contact with impregnated blue fabric and that the same formulation had a low insecticidal effect when applied topically to *Glossina fuscipes fuscipes*. In contrast to the low mortality rates, we obtained with the use of microfiber paper impregnated with essential oils, high mortality rates were recorded on *G. palpalis gambiensis* using neem oil [14]. The difference between our results may be due to the treatment used by these authors, which was pure neem oil, compared to diluted essential oils in our case. Previous studies have shown that essential oils from certain plants and their combinations cause significant mortality in *Anopheles gambiae* larvae and

adults [27]-[29]. Unlike *Glossina palpalis gambiensis*, which is a dipteran of the genus *Glossina* used in the present study, these authors used larvae and adults of *An. gambiae*, which is also a dipteran but of the genus *Anopheles*. Additionally, the short time of the exposure of flies to the essential oils, can limit their efficacy on the mortality.

The search for essential oils with repellent properties extracted from plants against tsetse flies may be an alternative to chemical insecticides in protecting livestock against bites from tsetse flies, which are vectors of AAT. In our study, we evaluated the repellent effects of *Cymbopogon citratus*, *Mentha piperita*, and their combination on *Glossina palpalis gambiensis* in the laboratory. The insect repellent properties demonstrated through the activation and attraction rates of tsetse flies tested in the experimental device varied depending on the essential oils used. Among all the essential oils tested, *Cymbopogon citratus* and the oil derived from the combination of *C. citratus* and *M. piperita* (Cc_Mp) showed interesting repellent properties on *G. palpalis gambiensis*. However, *Mentha piperita* essential oil did not have a repellent effect on tsetse flies. Our results confirm the repellent effects of essential oils on insects demonstrated by several authors. Previous studies [30] showed repellent effects of several essential oils on many pest species, among these, ticks [30], mosquitoes [9] [13] [31], bruch beetle [21] and houseflies [9]. These repellent effects were recorded with essential oils from *C. citratus*, *Lippia multiflora* L., *Cymbopogon citratus* Steud., *Chenopodium ambrosioides* L., *Ocimum gratissimum* L., *Zingiber officinale* Rosc. *Clausena anisata*. The aforementioned authors evaluated the repellent properties of essential oils on insects other than *G. palpalis gambiensis*, which was used in our case. After coating the flanks of cows and goats with several solutions of *Azadirachta indica* essential oil solutions, a very large proportion of tsetse flies exposed to the treated animals refused to feed after 72 hours [14]. Based on the high non-feeding rate, these authors concluded that *A. indica* essential oil repelled tsetse flies. The difference between our study and that of these authors is that we evaluated the repellent effects of our tested EOs in flight tunnels in an olfactometry room, whereas they applied neem oil directly to feeding hosts.

The repellent properties of *C. citratus* essential oil against *Glossina palpalis gambiensis* are consistent with the results obtained by [9] who found that *C. citratus* essential oil repelled *Stomoxys calcitrans*. Since the tsetse fly, in our study, were exposed to the vapor phase (odors) of the essential oils tested, it is possible that their action of these EOs occurs via the olfactory receptors of these insects. Using electroantennography (EAG), Baldachinno *et al.* [9] demonstrated that *C. citratus* essential oil was an active substance on the olfactory receptors of the antennae of *Stomoxys calcitrans*. The repellent effect of *C. citratus* EO observed against tsetse flies in our study could therefore be explained by a potential action on the olfactory receptors in the antennae of *G. palpalis gambiensis*, justifying the low preference rate in the “test arm” with *C. citratus*. The low repellent properties observed with *M. piperita* essential oil could be due to the high number of tsetse

flies that were knocked down in the release cage of the experimental device when the flies were exposed to the smell of *Mentha piperita*. This could be explained by a toxic effect of inhaling *M. piperita* essential oil on *Glossina palpalis gambiensis*. These results are similar to those previously reported by other authors [13] [24]. The intermediate repellent effects observed with the combination of *C. citratus* and *M. piperita* essential oils can be attributable to the action of *C. citratus*. The repellent effects of combinations of certain essential oils on mosquitoes is already reported [13].

Overall, all essential oils and their combination used in our study showed varying insecticidal and repellent properties against *Glossina palpalis gambiensis*. *Mentha piperita* essential oil had higher insecticidal effects against *G. palpalis gambiensis* than *C. citratus* essential oil. *C. citratus* EO showed interesting repellent effects against *G. palpalis gambiensis*. The essential oil from the combination of *C. citratus* (80%) and *M. piperita* (20%) was found to have intermediate insecticidal and repellent effects against tsetse flies. Contrary to the results of some previous studies [14] [22] [23] which showed very interesting insecticidal properties of certain essential oils and plant extracts against several insect species at low concentrations, the essential oils we tested showed relatively weak insecticidal effects despite the high concentrations used. This suggests that the combination of *C. citratus* and *M. piperita* essential oils could not be used as alternative bio-insecticides to chemical insecticides in large-scale tsetse fly control. However, *M. piperita* essential oil could be used by farmers around farms by impregnating fabrics due to its knock-down effects and mortality rates comparable to those of the screen impregnated with chemical insecticide used in our study as a positive control. However, the significant repellent effects of *C. citratus* essential oil on *Glossina palpalis gambiensis* suggest that *Cymbopogon citratus* essential oil could be used as a natural repellent against tsetse flies, replacing chemical repellents by applying it to the skin of livestock or spraying it on livestock resting areas, as mentioned by some authors in their studies in Mali [14]. One limitation of this study is the absence of chemical characterization of the oils used. Variations in their chemical composition may partly explain the low efficacy observed against tsetse flies. Further studies incorporating detailed chemical analyses are therefore required to better understand the biological effects of these oils and to assess their potential role in trypanosomiasis control.

5. Conclusions

This current study aimed to evaluate the insecticidal and repellent properties of essential oils from *Cymbopogon citratus*, *Mentha piperita*, and their combination against *Glossina palpalis gambiensis*. The results obtained showed that the two essential oils and their combination tested have different insecticidal and repellent properties against tsetse flies. The essential oils evaluated have relatively low insecticidal activity against tsetse flies. *Mentha piperita* essential oil showed a stronger insecticidal effect than *Cymbopogon citratus* essential oil and can be used

as a bioinsecticide against tsetse flies at livestock farms level. *C. citratus* essential oil has repellent properties against *G. palpalis gambiensis* and could be applied to livestock as a natural repellent against tsetse fly bites while grazing in infested areas. Intermediate insecticidal and repellent properties were obtained with the combination of the essential oil from the two plant species.

In short, the essential oils tested in our study could be used either as bio-repellents or bioinsecticides in vector control against animal trypanosomiasis. Combining the essential oils would enhance their action against insects.

In light of these laboratory results, we suggest that further studies be conducted to: 1) evaluate the insecticidal and repellent properties of these essential oils when applied to animals in a semi-controlled environment; 2) evaluate the persistence of essential oils after application to animals; 3) determine the toxicity of these essential oils to livestock; and 4) evaluate the cost-effectiveness of essential oils in vector control against tsetse flies.

Authors' Contributions

SHP, PA, MS, ES conceived the design of the study. SHP, NJK, and AMB did the laboratory works. All authors contributed to writing the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

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