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Attractiveness of Essential Oil Extracted from *Melaleuca leucadendra* L. Leaves to the Mango Fly *Bactrocera dorsalis*

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Abstract: The oriental fruit fly, *Bactrocera dorsalis*, is one of the most significant pests in all mango-growing areas. In Senegal, its attacks have caused losses of around 30 to 60% of production, depending on the region. Thus, the strategy used to control fruit flies includes orchard sanitation, chemical control through insecticide spraying, mass trapping of males, etc. However, pesticides pose a risk to the health of both farmers and consumers. The objective of this study is to improve a technological package for the agroecological control of this fruit fly pest by using an essential oil extracted from *Melaleuca leucadendra* L. leaves. This essential oil, obtained by hydrodistillation, was analyzed using gas chromatography coupled with mass spectrometry (GC-MS). Its attractant effect on *B. dorsalis* at a dose of 2.5 ml was compared to that of a synthetic methyleugenol lozenge. Additionally, different doses—D1 (1.5 ml), D2 (2 ml), D3 (2.5 ml), and D4 (3 ml)—of this essential oil were tested for their attractiveness to fruit flies in Casamance and the Niayes region. The essential oil of *M. leucadendra* leaves consisted mainly of methyleugenol (>99.5%). At an application dose of 2.5 ml/trap, *M. leucadendra* essential oil exhibited greater attractiveness than synthetic methyleugenol, though without significant difference in Kafountine (174 vs. 160 flies/day/trap). However, a significant difference was observed in the Niayes area, where the number of flies caught was 183 compared to 150 flies/day/trap. The attractiveness of the essential oil at different doses showed a significant difference between the D1 (1.5 ml) dose and the D2 (2 ml), D3 (2.5 ml), and D4 (3 ml) doses. The D2, D3, and D4 doses had similar effects across all orchards in the two study areas. *M. leucadendra* essential oil could serve as an alternative to synthetic pesticides for controlling *B. dorsalis* in Senegal and other mango-producing countries.

Keywords: essential oil; *Melaleuca leucadendra*; *Bactrocera dorsalis*; mango; GC/MS

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1. Introduction

Fruit tree cultivation is a strategic sector of rural economies in Africa, providing essential income for farmers and contributing significantly to food security and the socio-economic development of rural areas. Among the major fruit crops, mango (*Mangifera indica* L.) stands out due to its economic value and nutritional benefits, holding a central position in agricultural exports from the continent [1]. However, the mango sector faces major phytosanitary challenges, particularly infestations by pest insects, with fruit flies being the most damaging, leading to significant yield losses and threatening the sustainability of this crop [2].

Phytophagous Diptera, particularly fruit flies, pose an increasing threat to fruit production, especially in West Africa. In addition to direct harvest losses, their presence also leads to strict trade restrictions imposed by importing countries, limiting the export of African mangoes [3]. *Bactrocera dorsalis* (Hendel), or the oriental fruit fly, is one of the most concerning species due to its ability to rapidly infest orchards, with its short reproductive cycle and strong dispersal capacity [4].

In Senegal, *B. dorsalis* has been reported on more than 58 fruit species, particularly in the Niayes region, a key horticultural area. This infestation is particularly problematic as the peak of the insect's proliferation coincides with the mango production period, exacerbating post-harvest losses. Since its detection in 2004, controlling this species has become critical for Senegalese producers, with yield losses ranging from 30 to 60% depending on the production area. This impacts both local and international marketing of mangoes and increases the need for quarantine measures and phytosanitary controls [5].

In response to this threat, several management strategies have been developed, including the collection of infested fruit [6–8], the use of insecticides and protein baits [9,10], the installation of attractive traps [11,12], the introduction of natural enemies [13,14], and the sterile insect technique [15]. However, the intensive use of pesticides raises concerns due to their harmful effects on human health and the environment [16,17], which has led to the search for alternative, eco-friendly solutions, particularly through the use of plant extracts.

Essential oils present a promising approach for managing *B. dorsalis* infestations. Some essential oils, particularly those derived from *Syzygium spiceum* [16], *Melaleuca bracteata* [17], and the *Ocimum genus* [17,18], rich in methyleugenol, have shown strong attractant properties for male *B. dorsalis*. In contrast, other oils, such as that of *Pogostemon cablin*, have repellent properties [19], while the essential oil of *Limnophila geoffrayi*, with high levels of *d*-Pulegone (27.1%) and perillaldehyde (19.1%), exhibits insecticidal effects against *B. dorsalis* [20]. In Senegal, the leaves of *Ocimum americanum*, used for several years to control *B. dorsalis*, contain 77% methyl eugenol in their essential oil, as revealed by our study [21].

In this context, *Melaleuca leucadendra*, a plant species found in Senegal, is of particular interest due to its essential oil, which is rich in methyleugenol [22], a compound known for its effectiveness in controlling *B. dorsalis*. The aim of this study is to evaluate the effectiveness of the essential oil from *M. leucadendra*. in attracting and eliminating *male B. dorsalis*, with the ultimate goal of significantly reducing post-harvest losses and ensuring the production and marketing of Senegalese mangoes.

2. Materials and Methods

2.1. Plant Materials

Samples of *M. leucadendra* fresh leaves were collected from Fatick-Senegal (14°20’24.99” N, 16°23’1.284'O): The leaves of a sample were taken on the same tree. The plant material was identified by the technicians from the department of botanical of the Fundamental Institute of Black Africa (IFAN) of University Cheikh Anta Diop of Dakar.

2.2. Extraction of Essential Oil

Plant material was air-dried for 14 days at room temperature. Samples were hydrodistilled (5h) using a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia [23]. The yields of essential oils (w/w, calculated on dry weight basis) were given in the Table 1.

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Table 1. Chemical composition of the leaf essential oils from *M. leucadendra.*

N[a]	Compounds	lRIa[b]	RIa[c]	RIp[d]	%
1	Methyleugenol	1369	1367	2009	**99.5**
2	Trans-methylisoeugenol	1463	1461	2175	0.1
3	Germacrene D	1491	1490	1695	0.3
4	Bicyclogermacrene	1494	1493	1712	0.1
	Phenylpropanoids				99.6
	Hydrocarbon sesquiterpenes				0.4
	Total identified (%)				100
	Yields (w/w vs dry material)				

a Order of elution is given on apolar column (Rtx-1). b Retention indices of literature on the apolar column (lRIa). c Retention indices on the apolar Rtx-1 column (RIa). d Retention indices on the polar Rtx-Wax column (RIp).

2.3. GC and GC/MS Analysis

The chromatographic analyses were carried out using a *Perkin-Elmer Autosystem XL GC* apparatus (*Walthon*, MA, USA) equipped with dual flame ionisation detection (FID) system and fused-silica capillary columns, namely, Rtx-1 (polydimethylsiloxane) and Rtx-wax (poly-ethyleneglycol) (60 m × 0.22 mm i.d; film thickness 0.25 μm). The oven temperature was programmed from 60 to 230°C at 2°C/min and then held isothermally at 230°C for 35 min: hydrogen was employed as carrier gas (1 mL/min). The injector and detector temperatures were maintained at 280°C, and samples were injected (0.2 μL of pure oil) in the split mode (1:50). Retention indices (RI) of compounds were determined relative to the retention times of a series of n-alkanes (C5–C30) by linear interpolation using the Van den Dool and Kratz (1963) equation with the aid of software from *Perkin-Elmer* (Total Chrom navigator). The relative percentages of the oil constituents were calculated from the GC peak areas, without application of correction factors.

Samples were also analysed with a *Perkin-Elmer Turbo mass* detector (quadrupole) coupled to a *Perkin-ElmerAutosystem XL*, equipped with fused-silica capillary columns Rtx-1 and Rtx-Wax. The oven temperature was programmed from 60 to 230°C at 2°C/min and then held isothermally at 230°C (35 min): hydrogen was employed as carrier gas (1 mL/min). The following chromatographic conditions were employed: injection volume, 0.2 μL of pure oil; injector temperature, 280°C; split, 1:80; ion source temperature, 150°C; ionisation energy, 70 eV; MS (EI) acquired over the mass range, 35–350 Da; scan rate, 1 s.

Identification of the components was based on: (a) comparison of their GC retention indices (RI) on non-polar and polar columns, determined from the retention times of a series of n-alkanes with linear interpolation, with those of authentic compounds or literature data; (b) on computer matching with commercial mass spectral libraries [24–26] and comparison of spectra with those of our personal library; and (c) comparison of RI and MS spectral data of authentic compounds or literature data.

2.4. Attractiveness of Essential Oil

2.4.1. Attractiveness of Essential Oil (EO) Compared to Synthetic Methyleugenol (ME)

The study of the comparison between essential oil and synthetic methyleugenol was conducted in Kabar, a village located in Kafountine, in three orchards (KV1, KV2, and KV3), each at least 1 hectare in size. These are traditional orchards, mainly consisting of Kent and Keitt mangoes, with a few citruses occasionally present. The distance between the trees was not consistent. The fallen mangoes were not picked, and the grass cover was dense, with no irrigation. The orchards were not fenced against livestock. The presence of Biofeed feed traps was noted in the third orchard. In the Niayes area, the survey was conducted in Seugheul. The site consisted of three grafted mango orchards, where the Kent and Keitt varieties were dominant. A large number of weeds were present, and sanitation was not applied in the orchards. The irrigation system used was drip irrigation in the SV2 orchard and sprinkler irrigation in the SV1 and SV3 orchards. The experimental design

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implemented was in randomized dispersed blocks, with an "Attractant factor" consisting of EO and ME treatments, each with three replicates. In each block, three methyl eugenol (ME) traps and three *Melaleuca leucadendra* essential oil traps were placed, with a dose of 2.5 mL in each. Each trap was provided with a DDVP wafer to kill the captured insects. Tephritraps were used with synthetic methyl eugenol as the reference control. The required amount of essential oil was soaked in hydrophilic cotton. The attractants were renewed after one month of exposure in the traps. The distance between traps was 100 meters to limit interactions between attractants.

2.4.2. Evaluation of the Attractiveness of Different Doses of Essential Oil

The evaluation of the effect of essential oil dose on Tephritidae catches was carried out in Kafountine and Notto in the Niayes. In Kafountine, three orchards (MV1, YV2, and SV3) of 10 hectares, consisting mainly of Kent and Keitt grafted mango trees, were used. The mangoes were not harvested and had not been subjected to phytosanitary treatments.

The effect of different doses of essential oil was studied in these orchards. The Notto site consisted of three orchards, two of which were contiguous (NV2 and NV3). These orchards mainly consisted of grafted mango trees over 4 years old. Phytosanitary treatments had not been applied in these orchards. The fallen mangoes were collected and incinerated in all the orchards.

Thirty-six traps were placed at this site, with 12 traps per orchard. The traps were hung on tree branches at a height of 1.5 m to 1.8 m. To prevent any predatory activity, especially that of red ants, the branches supporting the traps were coated with solid grease on both sides of the attachment points. Four doses of *M. leucadendra* essential oil were tested: 1.5 mL, 2 mL, 2.5 mL, and 3 mL per trap to determine the optimal dose. Each dose was repeated 3 times in all three orchards. A distance of 100 m was maintained between traps to avoid interactions between doses. Trap surveys were conducted weekly in the morning at a specific time for 16 weeks. The captured flies were placed in bags with the trap number and the name of the orchard and transported to the laboratory for counting and species identification. Tephritrap traps were used with essential oil soaked in cotton wool, accompanied by DDVP insecticide wafers.

2.5. Mesurements

The parameter measured was the number of flies caught per trap. This parameter was expressed as the number of fruit flies per day per trap (FTD). It was calculated using the following formula:

$FTD=F/(T\times D)$

F is the total number of flies; T is the number of traps; D is the number of days of exposure

2.6. Data Processing

The data were subjected to the Shapiro-Wilk normality test to verify their normal distribution. A comparison of capture averages was performed using the analysis of variance (ANOVA) test with R software version 3.6.0. Bonferroni comparison tests were then conducted after ANOVA when the effect of the factors tested was significant at a 95% confidence interval and the 5% significance level.

3. Results and Discussion

3.1. Chemical Composition of Essential Oils

This essential oil yield, calculated with relative to the mass of dry plant material, was 2.75%. Analysis of the leaf essential oil by GC/FID and GC/MS allowed the identification of 4 compounds accounting 100% of the total composition (Table 1). Methyleugenol was the main constituent of the essential oil. The richness in methyleugenol of leaf essential oils from *M. leucadendra* has also been reported from Australia (99.0%) [27], Brazil (96.6%) [28] and Senegal (98.4–99.5%)[22].

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3.2. *Attractiveness of Essential Oil*

3.2.1. Effect of the Attractant on the Number of Fly Captures

The number of flies caught (FTD) as a function of the two types of attractants was evaluated in both study areas (Figure 1). In Kafountine, the number of *B. dorsalis* captured (FTD) with *M. leucadendra* essential oil at a dose of 2.5 ml was 173.75 flies/day/trap. These fly captures were higher than those recorded with synthetic methyleugenol, which reached 160.29 flies/day/trap. This indicates a statistically non-significant difference between catches with essential oil and methyleugenol ($p > 0.05$). However, in Niayes, the number of flies caught per trap per day with *Melaleuca leucadendra* essential oil was 182.62 flies, while the trap with synthetic methyleugenol caught 149.65 flies/day/trap. A statistically significant difference was noted between catches with EO and ME ($p \leq 0.046$).

Nevertheless, the number of flies caught with *M. leucadendra* essential oil was greater than that with synthetic methyleugenol. Studies conducted by [29] showed larger captures of *B. dorsalis* (226 flies) at a dose of 0.2 mL with *Ocimum sanctum* essential oil, which contained 82.29% methyleugenol. Chemical analysis revealed that 40% of the essential oil of *O. sanctum* was composed of methyleugenol, based on an experiment conducted in the mango orchard from December 2016 to May 2017 in Kapur, India. The attractive effect of all parts of the *O. sanctum* plant was tested on *B. dorsalis*. The results showed that the maximum number of standard fruit flies was attracted by basil leaf extract, with an average of 12.67 flies, followed by stems (8.67 flies), inflorescences (10.67 flies), roots (10.33 flies), and synthetic methyleugenol, which attracted an average of 127 fruit flies [30].

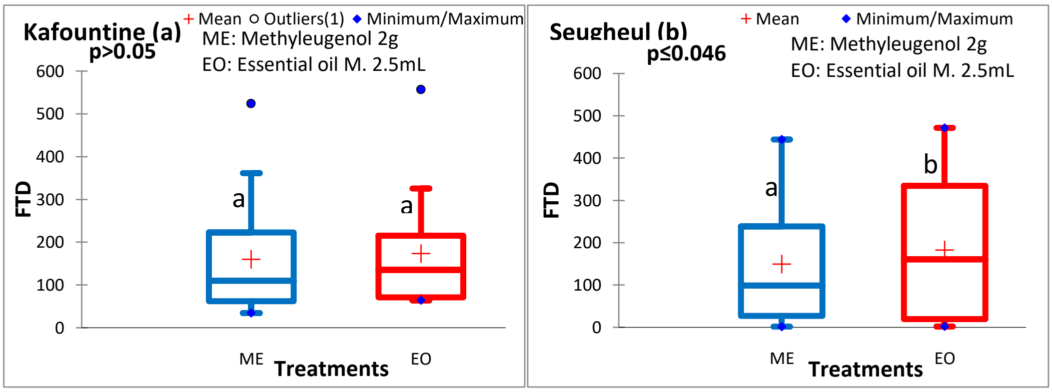

Figure 1. Effect of attractant on the number of flies caught in Kafountine (a) and Seugheul (b).

3.2.2. Attractive Power of Essential Oil

The results in Figure 2 show the attractiveness of essential oil and methyleugenol in orchards. Fly captures were 154.51, 169.62 and 156.75 flies/day/trap in orchards KO1, KO2, and KO3 with methyleugenol, and 176.76, 189.01 and 155.49 flies/day/trap in the same orchards KO1, KO2, and KO3, respectively, with essential oil in Kafountine. These data show a statistically non-significant interaction between the attractant and the orchards. The effect of the attractant on FTD varied between orchards. Moreover, in Seugheul, the attractive power of the essential oil was 178.16, 189 and 180.72 flies/day/trap in orchards SO1, SO2, and SO3, respectively, compared to 162.25, 149.91 and 136.8 flies/day/trap in the same orchards SO1, SO2 and SO3 with methyleugenol. The analysis conducted on these data shows a non-significant interaction between the attractant and the orchards. A similar study was conducted in Sri Lanka. The species *Ocimum tenuiflorum* (MT1 purple and MT2 purple-green) grown in Sri Lanka has a methyleugenol content of 72.50% for MT1 and 64.23% for MT2. Bioassays performed on MT1, MT2 essential oils, and synthetic metyleugenol demonstrated that the attractiveness of *B. dorsalis* from MT1 essential oil (106 ± 8.1), MT2 essential oil (104 ± 2), and commercial methyleugenol (111 ± 8.5) was not significantly different. [31].

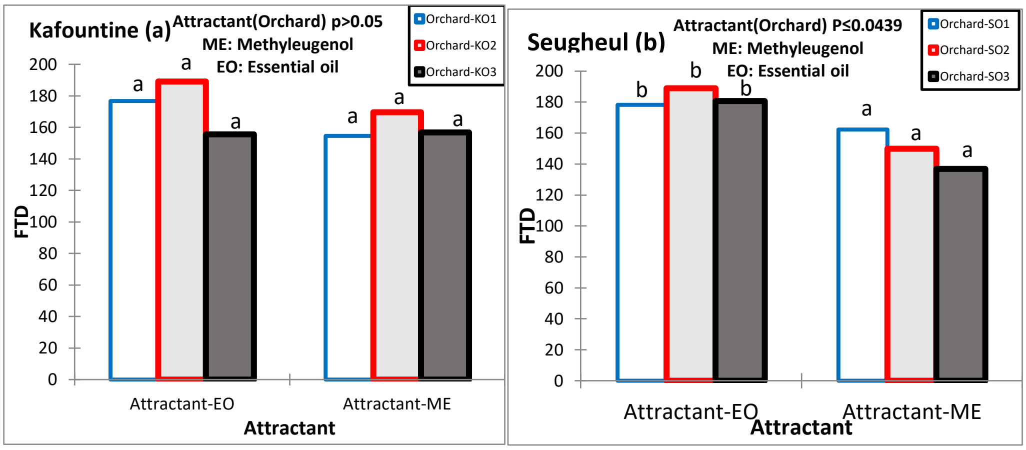

Figure 2. Attractant power in Kafountine (a) and Seugheul (b) orchards.

3.2.3. Abundance of Fruit Fly Populations in Orchards

Population dynamics of *B. dorsalis* in the Kafountine and Seugheul orchards showed that the highest peak catches were obtained from July to August, and the lowest from September to October. From the first to the fourth week, *M. leucadendra* essential oil caught 556 to 131 flies in Kafountine and 417 to 217 flies in Seugheul. For synthetic methyleugenol, it was 361 to 224 flies, with an increase in catch during the third week, from 141 to 524 flies in Kafountine and from 168 to 395 flies in Seugheul. From the fifth to the tenth week, catches increased after product renewal in the fourth week, with a decrease noted in the tenth week. For essential oil, catches ranged from 273 to 67 flies in the orchards of Kafountine and from 370 to 3 flies in Seugheul. For synthetic methyleugenol, it was 215 to 63 flies in Kafountine and 255 to 5 flies in Seugheul. From the eleventh to the sixteenth week, an increase in catches was noted in the eleventh week after product renewal in the tenth week, followed by a gradual decrease in fly catches until the sixteenth week. The number of flies caught by *M. leucadendra* essential oil and synthetic methyleugenol was higher in July-August and lower in October and November. This finding is consistent with those of [32–34] which showed peaks and *B. dorsalis* damage recorded in July and August. The high proliferation of fruit flies can be explained by the maturity of many mango varieties and the emergence of generations of pupae that evolved on the young fruits that fell under the crowns of mango trees during this period [35]. *B. dorsalis* is very abundant during the rainy season, which coincides with the mango ripening period in areas where it has been [36–38]. Added to this is the intensity of the rain and the ripening of other mango varieties. Vayssieres et al., (2008 ; 2009)[39,40] reported that *B. dorsalis* is a species that grows best in conditions of high humidity. According to Ouedraogo (2007) [41], humidity affects the abundance of Tephritidae populations through the reduction of fecundity of females during dry periods and by the high mortality of newly emerged adults in dry conditions. Humidity influences fluctuations in fruit fly populations during host plant fruiting periods [42]. The synchronization of fruiting time and abiotic factors (rainfall, temperature, and humidity) is essential for fruit fly population dynamics [43]. This would explain the higher abundance of flies in Kafountine, with 336,730 individuals captured, compared to 334,948 individuals in Seugheul, because it rains more in the south than in the Niayes area.

The duration of effectiveness of essential oils is four weeks in mango orchards. This result corroborates that of [44] which showed an efficacy of four weeks in mango orchards in Djibélor. In contrast, Kardinan et al. (1980) [45] showed a two-week efficacy with *M. bracteata* in star fruit and guava orchards. This difference in the duration of effectiveness can be explained by the amount of methyl eugenol in the essential oil, which is 76% in *M. bracteata* and 99.5% in *M. leucadendra*. Indeed, [45] showed that the efficacy duration of the product is longer if the percentage of methyl eugenol is greater. Also, the amount of methyl eugenol is decreased by environmental factors (temperature, humidity, and wind), which cause it to evaporate over time [46].

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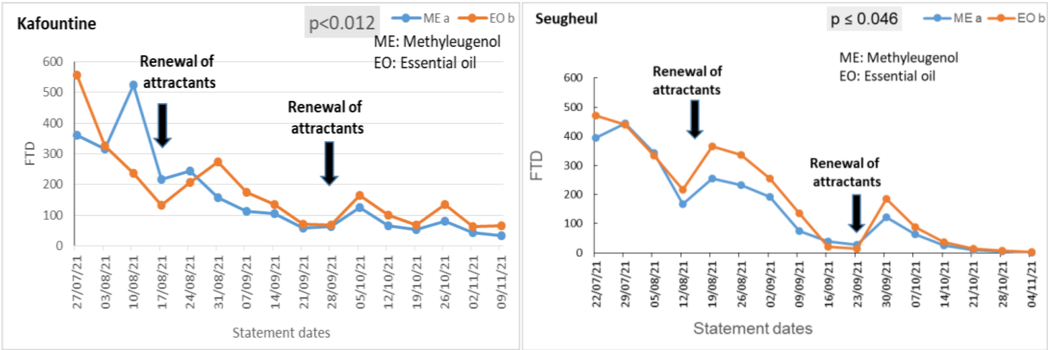

Figure 3. Population dynamics captured in the Kafountine and Seugheul orchards.

3.2.4. Effect of Different Doses of Essential Oil in Orchards

At Kafountine the number of flies caught was high with D4 (174 flies/trap/day), followed by D2 (151 flies/trap/day), then D3 (148 flies/trap/day) and finally D1 (135 flies/trap/day) (Figure 4). The highest catches were obtained with the D4 dose (174) and the lowest with the D1 dose (135 flies/day/trap) (Figure 4). At Seugheul, the catches obtained with Dose 1 (159.72) were lower than those with doses D2 (182.89 flies/day/trap), D3 (176.75 flies/trap/day), and D4 (200.26 flies/trap/day), with a significant difference between dose D1 and the other doses (D2 and D4) at $p<0.05$. However, there was no significant difference between doses D1 and D3 ($p>0.05$). In the set of catches with the different doses of *M. leucadendra*, the number of catches increased with the doses. This result is consistent with that of [47] *],* where *B. dorsalis* catches varied with the amount of methyl eugenol in the mixture. The higher the concentration of *Melaleuca lecadendra* oil used, the more fruit flies are trapped, and the greater the amount of methyleugenol that evaporates.

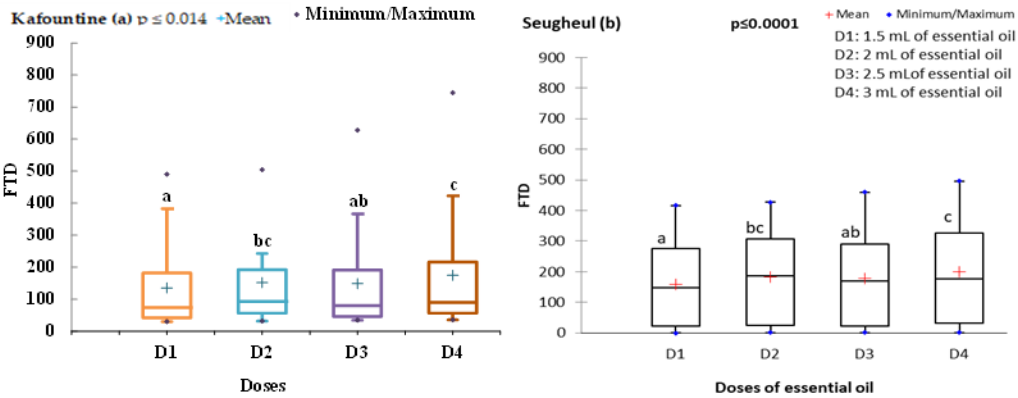

Figure 4. Effect of different doses of essential oil in orchards.

4. Conclusions

This study demonstrated that the essential oil of *M. leucadendra*, rich in methyl eugenol, is an effective alternative to synthetic attractants for trapping *B. dorsalis*. Trials conducted in the orchards of Kafountine and Seugheul confirmed its strong attractiveness, comparable to or even superior to synthetic methyl eugenol. The application of 3 ml per trap proved to be optimal. This agroecological approach reduces the use of chemical pesticides, thereby preserving health and the environment. Its integration into a sustainable fruit fly control strategy is promising. Further studies are needed to assess its large-scale effectiveness and cost-effectiveness for producers.

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