

Screening of Two *Ocimum tenuiflorum* L. (Lamiaceae) Morphotypes for their Morphological Characters, Essential Oil Composition and Fruit Fly Attractant Ability

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Received December 14, 2014; Revised December 24, 2014; Accepted January 05, 2015

Abstract *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) is the destructive pest of over 300 cultivated and wild fruits all over the world. Present study describes the morphology, essential oil content and composition and bioassay on fruit fly attractant ability of essential oil of two *Ocimum tenuiflorum* morphotypes (MT₁ purple and MT₂ purple-green) grown in Sri Lanka. Four months old, *O. tenuiflorum* aerial parts were hydro-distilled for 4 h. GC-MS analysis of essential oils and bio-assay for *B. dorsalis* attractant ability was performed using previously established methodologies. The yield of the essential oils of *O. tenuiflorum* MT₁ and MT₂ were 1.51±0.02% and 1.45±0.01% (v/w), respectively. Eighteen compounds were identified, which encountered over 97% of the oil constituents. The main constituents found in the oil of MT₁ were methyl eugenol (72.50±1.03%) followed by β-caryophyllene (17.53±2.0%), germacrene D (1.55±0.10%), β-elemene (2.46±0.17%), while methyl eugenol (64.23±2.43%), β-caryophyllene (13.29±2.18%), β-elemene (6.94±1.41%), germacrene D (2.47±0.96%), were from extracted from MT₂. Bioassays conducted on essential oils of MT₁, MT₂ and purified Methyl Eugenol demonstrated that the *B. dorsalis* attractant ability of essential oil MT₁ (106±8.1), MT₂ 104±2 and commercial Methyl Eugenol (111±8.5) was not significantly different during the first week of the experiment. Results of our study open an avenue for use of essential oil of *Ocimum tenuiflorum* as potential natural para pheromone source for fruit fly control and monitoring in fruit industry in Sri Lanka.

Keywords: *Ocimum tenuiflorum*, Lamiaceae, fruit fly, methyl eugenol, parapheromone, essential oil

Cite This Article: R.M. Dharmadasa, D.A.S. Siriwardhane¹, K. Samarasinghe, S.H.C.S. Rangana¹, L. Nugaliyadda, Indika Gunawardane, and A.M.L. Aththanayake, "Screening of Two *Ocimum tenuiflorum* L. (Lamiaceae) Morphotypes for their Morphological Characters, Essential Oil Composition and Fruit Fly Attractant Ability." *World Journal of Agricultural Research*, vol. 3, no. 1 (2015): 1-4. doi: 10.12691/wjar-3-1-1.

1. Introduction

Bactrocera dorsalis (Hendel) (Diptera: Tephritidae) is the serious destructive pest of over 300 cultivated and wild fruits and vegetables including Annona (sugar apple), avocado, banana, citrus, coffee, guava, mango, papaya, passion fruit, peppers and tomato. This pest will apparently breed in all fleshy fruits [1]. The damage by oriental fruit fly is an impediment for establishment of commercial orchards of fruits than can be grown in Hawaii [2]. A number of methods to control these important pests of fruits, such as chemical control [3], cultural control [4], combinations of insecticides and plant products [5] and culture filtrates of fungi [6] have also been tried out. However, chemical control methods for fruit fly control were unsuccessful [7,8]. The substance

that contain single component (males lure) called parapheromone is only effective to capture male fruit fly. Meanwhile, pheromone traps with synthesized methyl eugenol have been used to eliminate the fruit fly damage by many people [9,10,11,12]. Moreover, this technique has been successfully practiced to monitor the fruit fly populations in India and Pakistan [13,14]. However, commercial synthesizing process of methyl eugenol is risky and expensive (US\$ 200/kg) and importation of required chemicals for the methylation process is difficult due to its high toxic nature. Therefore, development of an effective, plant based insect attractant, at an affordable price, is urgently needed.

Ocimum tenuiflorum L. (Lamiaceae) is one of the most famous, perennial herbs native to Africa, India and Asia, cultivated in temperate climate throughout the world with about 150 varieties. Interestingly essential oil of *Ocimum*

tenuiflorum contains 0.7% essential oils, of which 20-50% is methyl eugenol [15]. In the present study we identified chemical composition of essential oils of two morphotypes of *Ocimum tenuiflorum* and compared fruit fly attractant ability of two *O. sanctum* morphotypes.

2. Materials and Methods

2.1. Test Plants of *Ocimum Tenuiflorum*

Four-month-old plants of two morpho-types (MT) of *Ocimum tenuiflorum* grown under same environmental conditions at the Institutional premises (6.54°13900 N, 79.52°12492 E) were used for the extraction of essential oil. Prominent morphological characters of the above two morpho-types were recorded and herbarium specimens of the same (MT₁ as HTS 19 and MT₂ as HTS 20) were deposited at institutional herbarium.

2.2. Extraction of Essential Oil

Ten plants each of *Ocimum tenuiflorum* MT1 and MT2 were harvested at flowering and the leaves were hydro-distilled in a Clevenger-type apparatus for 5h. The extracted volatile oils were dried over anhydrous sodium sulphate and stored in sealed vials at 4°C until analysis. The yield of the oils was calculated based on dry weight of plant materials.

2.3. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis was carried out on a Hewlett-Packard 6890 Gas Chromatograph fitted with a fused silica HP-5MS capillary column (30 m × 0.25 mm; film thickness 0.25µm). The oven temperature was programmed from 60 - 280°C at 4°C/min. Helium was used as carrier gas at a flow rate of 2 mL/min. The gas chromatograph was coupled to a Hewlett-Packard 6890 mass selective detector. The MS operating parameters were ionization voltage, 70 eV; and ion source temperature, 200°C.

2.4. Bioassay of Essential Oil

Bioassays were conducted in home-gardens, where *B. dorsalis* infestation was predominant in mango, and other fruit crops (guava, avocado, and banana) Pheromone traps used to catch the fruit flies were prepared from round-shaped hollow plastic containers (20 cm length x 10 cm diameter). The lid on each side of the container had a pipe fixed in its center to allow the flies to enter into the trap. The inner diameter of each pipe was 1 cm and it was protruded into the plastic container for a length of 5 cm. In each trap 0.1 mL of essential oil was injected into a cotton wick that was suspended in the center of the trap with a wire and the same amount of methyl eugenol was used as a control. The traps were placed at a spacing of 50 m each in the home garden and they were fixed at a height of 1.5 m above ground. Experiment was conducted from January to August 2013. Attracted fruit flies for each trap containing ME, essential oil of MT₁ and MT₂ were identified with the help of an Entomologist for the correct species. The data on number of fruit flies attracted to each treatment was counted and tabulated.

2.5. Statistical Analysis

The data were analyzed using the analysis of variance (ANOVA) and the means were compared by using the Tukey's Multiple Range Tests (P<0.05) of SPSS statistical package 11.5 version.

3. Results and Discussion

Present study compares the morphological, essential oil content, essential oil composition, fruit fly attractant ability of two methyl eugenol rich *Ocimum tenuiflorum* morphotypes (MT₁ purple and MT₂ purple-green) grown in Sri Lanka. Since both morphotypes were grown in same soil and climatic conditions, as well as same aged plants, the observed results reflected the true morphological, phytochemical and insect attractant ability of both morphotypes.

3.1 Morphological Variations of Two Morpho-types of *Ocimum Tenuiflorum*

Out of the 35 morphological characters recorded, 11 were identified as distinguishable features of MT₁ (Purple) and MT₂ (Purple-green) (Table 1). Leaf and stem colour, leaf margin, leaf apex and leaf width can be used to differentiate the two morphotypes.

Table 1. Morphological characters of the two *Ocimum tenuiflorum* morphotypes used for the extraction of essential oils

Character	MT1 (Purple)	MT2 (Purple Green)
Plant habit	Erect, much branched	Intermediate
Plant height	30-75 cm	40-60 cm
Leaf margin	Serrate	Undulate
Leaf shape	Elliptic-Oblong	Subulate
Leaf apex	Acute	Cuspidate
Colour of the dorsal surface	Dark Purple	Purplish Green
Colour of the ventral surface	Purplish Green	Green
Leaf length	3.5-4 cm	3.1-3.5 cm
Leaf width	2.5-3 cm	1.8-2.2 cm
Length of the petiole	1.3-2.5cm	1.0-2.0 cm
Internodal space	2.5-4.0 cm	2.0-2.5 cm

3.2. Essential Oils of Two Morphotypes of *Ocimum Tenuiflorum*

Chemical composition of essential oil mainly depend on soil and climatic conditions of the location, growing season and maturity stages [16]. However, plants subjected to present study were cultivated at the same location under same soil and climatic conditions and harvested at the same age. Therefore, variability of essential oil composition could be considered as variation between two morphotypes. The yield of the essential oils obtained from aerial parts of *Ocimum tenuiflorum* MT1 and MT₂ were 1.51±0.02% (v/w) and 1.45±0.01% (v/w), respectively.

The GC-MS analysis of the oils identified more than 97% of the oil constituents (Table 2). As demonstrated in Table 2, the main constituents present in the oil of both *Ocimum tenuiflorum* morphotypes was methyl eugenol (MT₁, (72.50±1.03%) and MT₂, 64.23±2.43) followed by, β-caryophyllene (17.53%), β-elemene (2.46%).

Correspondingly, oil of *Ocimum tenuiflorum* morphotype -2 (MT₂) contained methyl eugenol (64.23%), β -caryophyllene (13.29%), β -elemene (6.94%) as major components.

Our results clearly demonstrated the presence of more than 60% of methyl eugenol in both morphotypes and it was significantly higher in MT₁ ($p > 0.05$). Moreover, all 13 constituents identified were common to both morphotypes tested. This is quite acceptable as both morphotypes belonged to the same species. Further, all the constituents we have identified have been reported by previous studies and methyl eugenol has been identified as major constituent in *Ocimum* species grown in India, and Thailand [17]. Moreover, varietal methyl eugenol content, of *Ocimum tenuiflorum* grown in Thailand and Indian conditions exhibited the higher content of Methyl eugenol [18]. Therefore, the findings on essential oil content and composition in the present study are in agreement with previous studies conducted elsewhere.

Table 2. Composition of the essential oils of *Ocimum tenuiflorum* MT₁ (purple) and MT₂ (purple green)

Compound	Compound percentage (%)	
	MT ₁	MT ₂
α -pinene	0.16±0.02	0.77±0.11
Camphene	0.13±0.01	0.74±0.03
Sabinene	0.04±0.01	0.11±0.01
β -pinene	0.08±0.01	0.43±0.01
1-Limonene	0.04±0.01	0.2±0.00
γ -Terpinene	0.04±0.01	0.03±0.00
α -Terpinolene	0.04±0.01	0.03±0.00
Camphor	0.04±0.01	0.14±0.01
Eugenol	0.17±0.02	<0.03±0.00
β -elemene	2.46±0.17	6.94±1.41
β -Caryophyllin	17.53±2.00	13.29±2.18
Methyl eugenol	72.50±1.03	64.23±2.43
α -humulene	1.0±0.0	1.08±0.02
Germacrene D	1.55±0.10	5.58±1.21
β -cubebene	2.55±1.46	2.47±0.96
γ -Cadinene	0.2±0.0	<0.03±0.01
γ -murolene	0.04±0.10	0.16±0.01
α -cadinene	0.71±0.10	0.87±0.01

3.3. Bioassay of Essential Oil on Fruit Fly

Methyl eugenol (4-allyl-1, 2-dimethoxybenzene-carboxylate, ME), is the most effective naturally occurring male-fruit fly attractant and hence widely used in population estimation, and as detection, monitoring, and control of male fruit flies [18,19,20]. The highest methyl eugenol sensitivity of fruit fly species was observed for *B. dorsalis*, followed by *B. papayae* and *B. carambolae* [20].

Once methyl eugenol rich essential oils are identified, they should be bio assayed against fruit fly species in order to ensure the effectiveness of the essential oils. Therefore, in the present study we have bioassayed essential oils of two morphotypes with a positive control (Commercial methyl eugenol) as described in section 2.4. The number of fruit flies attracted to essential oil from MT₁ and MT₂ were found to be similar to those attracted to Methyl Eugenol from 1st to 4th week of field exposure (Table 3). The fruit flies attracted to traps went down by 40% in 4 weeks after exposure in all treatments tested. Therefore, the tested morphotypes of *Ocimum tenuiflorum* can be considered as sources for commercial extraction of para-pheromone. These results are in agreement with Llopis et al., who reported the attraction of *Bactrocera oleae* and *Ceratitis capitata*, to different emission levels

of pheromone and parapheromone [21]. The present study indicated that 0.1 mL of the extracted essential oil per trap would provide similar results which are observed in same amount of commercial methyl eugenol. Results of the fruit fly attraction is in agreement with Ravikumar (year), who investigated the fruit fly trapping systems by using methyl eugenol and protein food baits in guava and mango orchards [22].

Table 3. Total number of fruit flies attracted to essential oils of *Ocimum tenuiflorum* MT₁ and MT₂ and positive control (n=5); (Mean values to the nearest whole number)

Time	Number of fruit flies (mean ± SE) attracted to		
	Essential oil of MT ₁	Essential oil of MT ₂	Positive control (ME)
1st week	106±8.1 ^a	104±2 ^a	111±8.5 ^a
2nd week	93±4.1 ^{ab}	84±4.5 ^b	99±6.2 ^a
3rd week	76±3.0 ^a	65±5.2 ^{ab}	53±9.2 ^b
4th week	48±6.0 ^a	38±2.0 ^{ab}	37±3.0 ^b

Means followed by the same letter are significantly different at P=0.05. ME – Methyl Eugenol

4. Conclusion

Results clearly demonstrated two morphotypes of *O. tenuiflorum* with high methyl eugenol content for the first time in Sri Lanka. The essential oil extracted from two morphotypes of *O. tenuiflorum*, exhibited similar fruit fly attraction ability as purified methyl eugenol. Therefore, these two morphotypes could be used as initial materials for the large scale cultivation of *O. tenuiflorum* for commercial extraction of oils and to develop an essential oil based parapheromone in order to control the fruit fly menace in Sri Lanka as well as globally.

Conflict of Interest

Authors declare that there is no conflict of interest.

Acknowledgement

Authors wish to acknowledge National Research Council for financial support (PPP/12/26)

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