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Assessment of *Ocimum basilicum* as Potentially Fruit Flies Attractant

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Abstract:

The Basil plant (*Ocimum basilicum* L) is an annual herb; belongs to family *Lamiaceae*. It is found to be an important source for Methyl Eugenol (ME), the fruit flies attractant. This study was carried out at Shendi area with main objective to assess the amount of ME extracted from *Ocimum basilicum* plant to evaluate its potentiality as fruit fly attractant. Plants of basil were grown till to maturity, then the differential harvest of leaves, flower and seeds were chemically investigated for volatilome. Five basil formulations (paste and powder of leaves, paste and powder of flowers, isolated ME from basil oil) were used for trapping potential of *Bactrocera* spp in mango orchards, as compared to the synthetic ME. The oil was extracted using Soxhlet apparatus, steam and water distillation, and analyzed using GCMS. The results revealed that highest extracted oil percentages was obtained from flowers, leaves, seed and stem were 5.75%, 3.03%, 0.02% and 0.21% respectively. Moreover, the basil traps catch was found to be lower compared with that of the control traps. Furthermore, when extracted ME from basil plant was compared with the standard synthetic, it gave lower catch fruit flies numbers, but the difference was not significant (>0.05). In conclusion, the basil raw derivates are confirmed not to be attractive for flies but the oil distillable from the leaves could be in force of its attractant ME contents and insecticide potency.

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Introduction

Ocimum basilicum L. (basil), which is known in Sudan as Rihan, belong to the plant family Lamiaceae. Basil is an annual, 20–60 cm long, white-purple flowering plant, which is originally native to India and other regions of Asia. Basil leaves containing essential oils of distinctive aroma, and can be used both fresh and dried to spice up various kinds of meals. In addition to that, basil has been traditionally employed as a medicinal herb in the treatment of headaches, coughs, diarrhea, constipation, warts, and/or kidney malfunction. Moreover, basil has a strong antioxidant effect that is beneficial for serum antioxidant levels, leading to improved fertility via the reduction of oxidative stress[1, 2]

Various basil species and cultivars provide essential oils with different compositions and aroma. There are usually considerable variations in the contents of the major components within this species. The type of cultivar, the agronomical practices and environmental conditions are all together affect the composition of sensory important compounds[3, 4].

The chemical characteristics and functional properties of essential oils from basil have been the subject of many studies. The components of essential oil mainly include phenyl propanoid derived compounds (methyl chavicol, chavicol, eugenol, methyl eugenol) and terpenoids (cineole, camphor, linalool citral)[5].

Tan and Nishida[6] reported that the biosynthesis of Methyl Eugenol (ME) evolved independently in many of the plant orders and families. Furthermore, its varies greatly within and between species as well as within and between the plant families. Current information on essential oils and/or their constituents from plants revealed that they have a broad spectrum of activity against insect and mite pests, plant pathogenic and other fungi, and nematodes, and consider as phototoxic.

All these information about basil as well as the importance of essential oil constituents, rank it as one of the important plants to be selected for our study. Moreover, few researches were reported to characterize the presence of ME in Sudanese *O. basilicum*, and to evaluate its efficiency as insect antifeedant/repellant or attractant[7]. Mangoes orchards in Sudan are threatened by the fruit flies. The damage has increased with the invasion of *Bactrocera spp*. The use of synthetic ME allows detection and monitoring of *Bactrocera* population. Thus, this study was conducted to achieve two objectives: i) the evaluation of the amount of ME available from different part of *O. basilicum* plant growing in Shendi; ii) to assess the potentiality of using different preparation and extracts from *O. basilicum* leaves and inflorescence, as well as the isolated Methyl Eugenol (IME) as attractant to males of *Bactrocera* spp.

Materials and Methods

Field Experiment Set-Up

Field observation were conducted in Shendi, area which is in the River Nile State-Sudan, where the average annual rainfall ranges from 14-60 mm to (60) mm, occurring mainly during the period from July to September. The climate of the experimental site is semi arid, relatively cool and dry in winter, with maximum and minimum temperature ranging from 34 - 36°C and 14 - 16°C, respectively and hot in summer with maximum and minimum temperature ranging from 40 - 46°C and 20 - 22°C respectively, and relative humidity ranges from 50 to 75 % in the rainy season and far below 50 % otherwise[8].

The seeds of *Ocimum basilicum* L. (basil) were collected from Elmisktab area (North Shendi), and sown in a heavy clay soil in January 2014 in a 100 m^2 divided into 8 plots at Shendi University farm. Irrigation and cultural practices were done as recommended. Some plots were harvested as stems and leaves, some as flowers and the rest as seeds in different time.

Plant Preparations

Essential oils were extracted from different parts of basil plant. Samples containing 150, 200, 100, and 50g of leaves, flower, stem, and seeds respectively were dried and powdered. The samples were placed in the Soxhlet extraction setup to extract essential oils with n-hexane for 5h. The obtained mixture of oil hexane was distilled with separating funnel to collect volatile constituents, and the volume of the obtained essential oil was calculated as percentage volume per dry weight.

Essential Oils Analysis

The chromatography/mass spectrometry (GC/MS) was used for analyzing the leave and flower, and n-hexane extracts was performed by a Shimadzu (LC

2010) used with flame ionization detector and HP-capillary column (30 × 0.25) the mobile phase was set at 1.83 ml /min, the sample injection oven temperature (60 C^{o}) and run fully at a range of 75-600 m/z and the composition rate was reported at related percentage at total peak area[9].

Further analyses were performed on the flowers extract by the High Performance Liquid Chromatography (HPLC) using a Shimadzu LC–210 ATH high performance liquid chromatography c18 (250× 4.6mm). The mobile phase composition was 70:30 acetonitrile water the flow rate of the mobile phase was adjusted to 1 ml/min and the analysis was done at room temperature. The sample was dissolved in water and injected into the column. The detection was performed in UV detector at 250 nm.

Purification and Identification of Methyl Eugenol from *O. basilicum* inflorescence extract was carried out by HPLC, and the identification of the purified Methyl Eugenol was carried out by comparing the retention times of HPLC chromatographic peaks, obtained from *O. basilicum* inflorescence extract, with those of authentic compound, Methyl Eugenol, run under identical conditions.

Bioassay Experiments

This study was conducted in Shendi area, during the period of May-August /2015 (mango fruiting time), in 5 neighboring mango orchards to evaluate the potentiality of the raw plant parts as attractant for fruit flies. Each orchard was provided with 5 traps baited with powder leaves, paste leaves, powder flowers, paste flowers and ME (control), replicated 3 times.

To assess the efficiency of the isolated ME (IME) extracted from the basil *vs.* the synthetic one (ME), traps baited with the two compounds were distributed throughout the 5 orchards and replicated 3 times. Throughout the study period May to August, trap catch was collected every 10 days; specimens counting were done in the laboratory.

In details, Lynnfield traps were locally made from a clear cylindrical plastic container (10 cm diameter and 10 cm height), with four holes (3 cm diameter each), evenly spaced around the upper half of trap, each trap baited with 2 ml of IME or ME and 0.5ml of Malathion in a cotton wick (4 cm long and 1 cm diameter); powder and paste of the row plant traps, 5 g of each was placed in the trap, Malathion 57% EC was used.

Statistical Analysis

Statistical package of social science (SPSS) was used as computerized program for the data analysis. Particularly the statistical inferences.

Results

Essential Oil Composition

Table 1, shows the oil content of flower, leaf, stem, and seed parts. The highest value for the oil content was obtained from flower (5.75%), followed by leaf part (3.03 %) then stem part (0.21%) and the lowest was the seeds part (0.022).

Table 2 showed the chemical composition of essential oil of the *O. basilicum* leaves sorted according to their order of elution in the column. Their abundance percentage varies from major compounds: [1,6- Octadien-3-ol,3,7-dimethyl] (45.04%), [4-Hexen-1-ol,5-methyl-2-(1-Methyl)] (32.25%), to minor compounds:[Beta-phellandrene] (0.04%), [Benzene,1,2-dimethoxy-4-(2propenyl)] (0.1%). The Eugenol, which is the precursor of ME (Benzene, 1,2-dimethoxy-4-(2-propenyl), is one component of the analyzed oil and was eluted at retention time 22.9, M/Z 164 and its abundance percentage was considered to be 1.38%. Noteworthy that the high abundance percentage of Eugenol (1.38 %) and low abundance percentage of ME (0.1%) in leaf part revealed that the process of Methylation in progress.

As far as the essential oil constituents of *O. basilicum* inflorescence is concerned**, t**he GC/MS analysis of the essential oil of the inflorescence revealed that the oil contains a mixture of thirty-two compounds (Table 3).

Table 3 presents the GC\MS composition of essential oil of extracted from the *O. basilicum* inflorescence growing in Shendi. They are listed according to their order of elution in the column, and their abundance percentage varies from major compounds: [Methyl-cis-isoeugenol] (20.23%), [L-Linaloon] (19.38%), to minor compounds: [Bicyclo 2.2.1] heptan-2-ol, 1, 3, 3trimethyl] (0.02%), [1, 3,7- Octatriene, 3,7-dimethyl] (0.01%). Moreover, the analysis also recorded that p-Eugenol, was prevalent in the essential oil, and was eluted at retention time

Table 1. Percentages of oils in different parts of *Ocimum basilicum* plant growing in Shendi area –Sudan (2014)

Oil's Percentage (%)	Flowers	Seeds	Stems	Leaves
	5.75	0.02	0.21	3.03

Table 2. Chemical composition of essential oil of *Ocimum basilicum* leaves according GC\MS analyses.

Peak No.	Compound	M /Z	Retention time	Abundance %
1	Alpha-phellandrene	193.00	7.094	0.21
2	Bicycle [3.1.1] heptane, 6, 6-dimethyl	193.00	7.177	0.37
3	Beta-Myrcene	169.00	7.588	0.59
4	D-limonene	168.00	8.945	0.51
5	Beta- phellandrene	193.00	9.251	0.04
6	Eucalyptol	143.00	9.756	3.82
7	1, 6- Octadien -3- ol, 3, 7-dimethyl	171.00	12.337	45.04
8	Bicycle [2.2.1] heptan-2- one , 1, 3, 3	181.00	13.106	9.16
9	3- Cyclohexane -1-methanol, alpha	159.00	16.242	0.71
10	2,6-Octadien-1-ol,3,7- dimethyl	169.00	17.151	0.78
11	2, 4-Decadienal, (E,E)	181.00	17.618	0.41
12	4-Hexen-1-ol, 5-methyl-2- (1-methyl)	169.00	18.106	32.25
13	Cyclopropanmethanol, 2-methyle-2	169.00	18.407	0.83
14	2, 6-Octadien, 3,7-dimethyl-,(E)	169.00	19.403	1.71
15	1H-3a,7-Methanoazulene, 2, 3, 4, 7, 8	119.00	21.035	0.53
16	Eugenol	164.00	22.951	1.38
17	Naphthalene, 1, 2, 3, 4, 4a, 5, 6, 8a-octa	161.00	24.354	0.26
18	Benzene, 1, 2-dimethoxy-4-(2propenyl)	178.00	24.636	0.1
19	Bicycle [4.4.0] dec-1-ene,2-isoprop	161.00	28.623	1.19

Table 3. Chemical composition of essential oil of *O. basilicum* inflorescence according GC\MS analyses.

Peak No.	Compound	Retention time	Abundance %
1	1,3,7-Octatriene, 3,7-dimethyl-	13.033	0.01
2	Cis- beta, Ocimene	13.167	0.53
3	1,3,6-Octatriene, 3,7-dimethyl-	13.251	4.28
4	trans-beta-OCIMENE	13.520	0.05
5	1,3,6-Octatriene,3,7-dimethyl-, (E)-(CAS)Beta OCIMENE	13.858	1.21
6	Heptan-2-one, 1,3,3-trimethyl-,	14.681	0.01
7	1,6-Octadien-3-ol, 3,7-dimethyl-,	15.173	1.81
8	LINALOON	15.362	0.06
9	1,1′-Dimethyl-2′-propenyl Benzoylformate	15.433	0.01
10	L-LINALOON	15.657	19.38
11	D-Fenchyl alcohol	16.044	1.75
12	Bicyclo[2.2.1]heptan-2-ol, 1,3,3trimethyl	16.294	0.02
13	1,3,3-trimethyl-Bicyclo[2.21]-trimethyl	16.630	0.01
14	CIS-EPOXY-OCIMENE	16.925	0.86
15	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl	17.012	1.15
16	(+)-2-Bornanone	17.606	0.02
17	NORBORNAN-2-ONE	17.695	0.47
18	ALPHA TERPINEOL	18.041	0.06
19	3-Cyclohexene-1-methanol, alpha, 4-trimethyl-,	18.451	1.10
20	BETA FENCHYL ALCOHOL	18.649	0.24
21	p-menthan- len-8-ol	18.978	0.02
22	GERANIOL	20.080	0.08
23	Trans-GERANIOL	20.379	3.89
24	2,6-Octadiene-1-ol,3,7-dimethyl (z)	20.840	0.61
25	Phenol, 2-methoxy-3-(2-propenyl)	23.109	0.22
26	Phenol, 3-ally-2-methoxy	23.349	2.59
27	p-Eugenol	23.866	2.52
28	Methyl-cis-isoeugenol ME	24.113	20.23
29	Benzen1-2dimethoxy-4(2propenyl)	24.308	3.57
30	Methyl-trans-isoeugenol	24.414	0.07
31	Trans-methyl-isoeugenol	24.603	0.04
32	Anisyl-Aston-Methoxyphenyl	24.914	0.05

23.866, and its abundance percentage was considered to be 2.52 %. Noteworthy that ME is one of the oil constituents obtained from the inflorescence extract, and its abundance percentage was 3.57 %.

HPLC analysis was used to verify the ME extracted from basil oil, and compared with a standard, these confirmed the presence of ME (Figure 1).

The retention time of ME in *O. basilicum* inflorescence given away at retention time 2.671 min (Figure 2).

Bioassay Experiment

The catch per trap obtained from leaves in form of powder and paste were 7.52 and 11.14 insect\trap\month respectively (Table 4, P ≤ 0.01).

Slightly better results were given using the powder and paste from flowers (12.23 and 14.89, respectively, However, the control trap with synthetic ME performed an average of 473 monthly catch, what means that the raw preparations from the leaves were about fifty times less attractive for the insects, and the flowers preparations about thirty-five lower, Better results came out using the oil extracted from the leaves (150.78) and the flowers (177.56) (Table 5) what means that the oil preparations resulted about only three times less attractive than control (P 0.001)

The result of trapping of fruit flies obtained from a pair wise between synthetic ME and the purified one from *O. basilicum* were 473.0 and 448.9 respectively (Table 6) what means a non-significant 5% reduction.

Discussion

The result showed that the leaves and flowers produced the highest percentage (3.031% and 5.75%) respectively, while lower percentage was obtained from the seed part (0.022%). This obtained result is coincide with the fact that the plant part harvested depends upon project use. When basil is grown for its dried leaves, it is harvested just prior to the appearance of flowers. For essential oil, it is harvested during full bloom[10].

Percentages of oils in different parts of *Ocimum basilicum* leaves and flowers of plants material (150g), the volume of the obtained essential oil was calculated as a percentage volume per weight (%v/w), the percentage of leaves 0.35% and flowers 0.31%,[10] the different results it may by cause by age of plant and geographical source. Noteworthy that the high abundance percentage of Eugenol (1.38 %) and low abundance percentage of ME (0.1%) in leaf part revealed that the process of methylation in progress.

GCMS analyses for basil oils produced 19 compounds from leaves and 32 compounds from flowers[10]. GCM analysis showed the chemical components of extract leaves, the chemical composition of essential oil Cineol, Cymene, Methanol, Linalool, Caravone, Camphor, Caranol, Geranial, Estragol, Geraniol, Thugo, Cy. Citral, Eugenol, M. cinnamate, ME, Bergamotene, Bisabolene, Isoledene. The different results may be attributed to the differences of methods used fresh leaves *vs.* dry ones. ME was detected in all basil oils analyzed by GCMS.

Azhari et *al.*[11] reported that in *Clarkia breweri*, ME derived from Eugenol by a specific Methylation involving an S-adenosyl Methionine dependent O-methyl transferase. The strict correlation between ME and

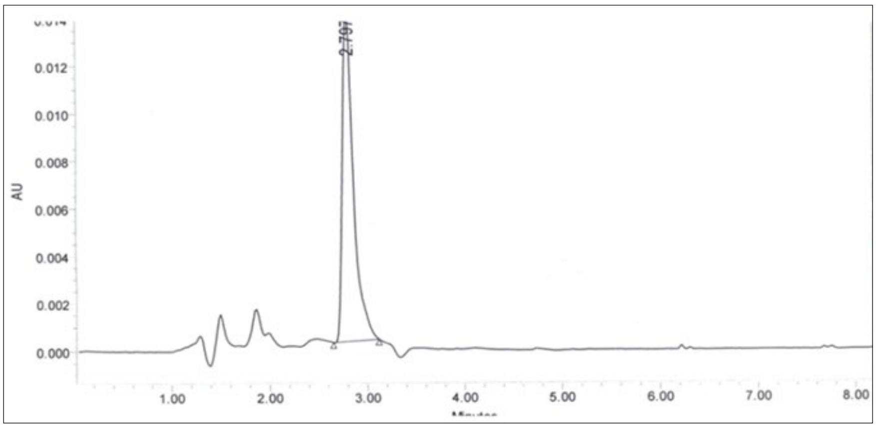

Figure 1. HPLC chromatogram of methyl eugenol (standard)

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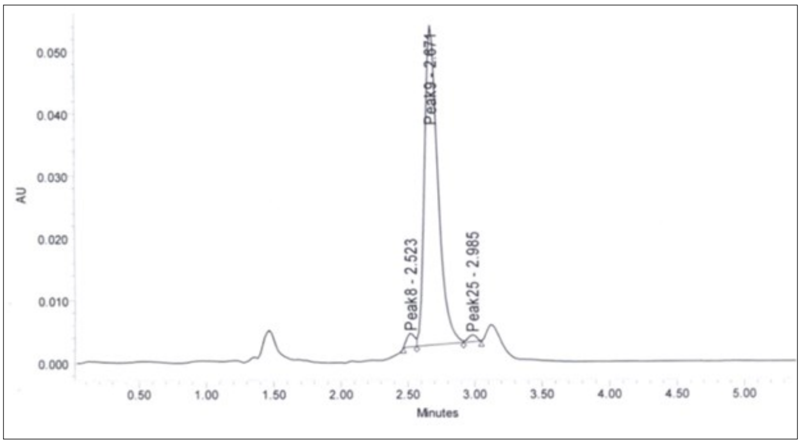

Figure 2. HPLC chromatogram of methyl eugenol of *O. basilicum* inflorescence n-hexane extract

Table 4. Monthly traps collections of fruit flies using basil plant parts (2014).

Trap content	Insect\Trap\Month Mean±SE
Powder leaves	7.52 ± 2.25[a]
Paste leaves	11.14 ± 1.57[a]
Powder flowers	12.23 ± 5.03[a]
Paste flowers	14.89 ± 1.01a
ME(control)	473 ± 5.39[b]

[a,b] At significant level ≤0.001

Table 5. Monthly traps collections of fruit flies using oil of leaves and flowers (2015).

Trap content	Insect\Trap\Month M±SE
Oil of leaves	150.78 ± 2.65[a]
Oil of flowers	177. 56 ± 3.92[a]
ME (control)	473 ± 5.39[b]

[a,b]At significant level≤0.05

Table 6. Monthly traps collections of fruit flies using basil plant parts- Shendi –Sudan (2015)

Traps content	Insect\Trap\Month M±SE
Extracted Isolated ME	448.9±9.28[a]
Synthetic ME	473.0±5.72[a]

[a]At significant level≤0.05

Eugenol found in basil could be explained by a similar pathway. The activity of the invoked enzyme decreases when plants grow. In fact, leaf age was considered to be a factor that influences the composition of essential oil in sweet basil[12]; Eugenol and ME predominated (28-77%) and Eugenol levels were higher in younger leaves (53%), while ME levels predominated in older leaves (68%). The amount of ME in an essential oil extracted from a given type of plant differs according to variety, plant maturity at the time of harvesting, the method of harvesting, storage condition, and the method of extraction[13].

Assessment of the efficiency of the isolated ME (IME) extracted from basil *vs.* the pure synthetic one (ME), in attraction of fruit flies revealed that the lower catch (-5%) obtained from the extracted ME may attributed to its degree of purity.

The raw basil plant parts leaves and flowers (powders and paste) when were used inside the insect traps, gave very poor attraction of fruit fly, that may be accounted by the presence of some repellent compounds or from low levels of ME. Our results partially agree with N'Dépo *et al.*[7] that observed ME to 416 catch of males / week a value fourfold than the present of 473 / month with synthetic or basil extracted ME; but here we cannot confirm the reported high values as 150 catches / week by the leaf basil powder. Our best result obtained from the basil leaf oil was 150 catches / month. According Ling, *et al.*[14] the basil oil contains three major active constituents (trans-anethole, estragole, and linalool) that are active insecticide. Thus, our results can suggest extended possibilities for a potential application to "attract and kill" the fruit flies.

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