CHYSOMYA PUTORIA INFECTION IS INHIBITED BY OCIMUM BASILIUM LINN ESSENTIAL OIL

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ABSTRACT

The blowfly *Chrysomya putoria* was also capable of causing secondary myiasis due to its wide distribution in the Neo tropical region. The excessive use of synthetic insecticides has led to the development of pest resistance, resulting in diminished efficacy and detrimental consequences for the environment. Botanical insecticides give a different option to synthetic pesticides. Despite some reports of its insecticidal activity against some insects, no studies have been conducted on its efficacy against flies. A study was conducted to determine the effect of *Ocimum basilium* having essential oil on *Chrysomya putoria* at the post embryonic developmental stage. The Indian Laboratory established and maintained colonies of *Chrysomya putoria*. There are 6 concentrations of basil essential oil (5.14, 9.36, 21.65, 42.30, 62.90 and 81.30 mg/mL). Regular records of death rates and life cycle alterations were maintained. Basil essential oil contains β-caryophyllene, β-selinene, and eugenol. According to the experiments, there was a direct decrease in the viability of the fly at all concentrations tested, with the LC50 calculated at 8.50 mg/mL of oil. Also, at lower concentrations, the essential oil changed morphology of the abdomen, wings and pilinum. *Chrysomya putoria*, a disease vector blowfly, can be controlled with this essential oil.

KEYWORDS: Blowflies, holy basil, *chysomya putoria*, larvicidal effects, pathogens, essential oil
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INTRODUCTION

There has been a connection between humans and flies since ancient times. Like other blowflies of the Calliphoridae family, *Chrysomya putoria* was now widely distributed throughout the New World. Their feeding habits, like on excreta, urban garbage, decomposing meat, fresh food, and animal excrement, make blowflies of medical and veterinary importance worldwide.

A synanthropic fly, these flies could carry a wide variety of pathogens that may infect humans and domestic pets, including bacteria (*Escherichia coli, Proteus mirabilis, Citrobacter sp., Klebesiella sp., Morganella sp., Pseudomona sp., Salmonella sp.*), viruses (West Nile Virus), protozoa (*Entamoeba coli, Entamoeba histolytica, Giardia lamblia sp.*), and helminths (*Ascaris lumbricoides*, *Trichuris trichiura, Strongyloides stercoralis stercoralis stercoralis stercoralis stercoralis stercoralis stercoralis, Cryptosporidium, sp.*) and helminthes.

Humans and animals can get secondary myiasis from *Chrysomya putoria* larvae found in latrines. Chemical insecticides were being used to control insects in a manner that was causing untold damages to humans and animals, both in the short and long term. Agriculture, the economy, and public health have all been affected by insecticide resistance. Due to a progressive resistance of pests to synthetic insecticides resulting from excessive use of these chemicals, botanical insecticides offer an alternative to synthetic pesticides, reducing their effectiveness and producing negative environmental impacts.

There have been thousands of studies conducted over the last few decades in order to detect the presence of repellents and insecticides in plants. A secondary metabolite can be used to control insects, because it is a natural deterrent, toxic agent, or developmental disruptor. Multiple plant species have been found to be lethal and sub-lethal to various insects around the world, according to recent research.

There are two varieties of *Ocimum* basilium L. (Lamiaceae), holy basil and tulsi. Both of these are aromatic plants originating from the Indian continent, which are widely cultivated throughout much of tropical Southeast Asia. It is also locally cultivated in other tropical locations. Western countries lack information on cultural practices related to the plant, so it's less well known. Like aromatic herbs, it is cultivated in Cuba. In the towns and peasant dwellings, its seeds could be found spontaneously propagated by air.

This species has been used in treating coughs, dysentery, diarrhea, worms and kidney malfunctions, pneumonia, bronchitis, liver disease, genitourinary disorders, catarrhal fever, otalgia, lumbago, hiccough, ophthalmia, painful eye disease, gastric disorders, diabetes, skin diseases, poisoning, psychosomatic stress, arthritis, chronic fever, and insect stings. *O. basilium* essential oil has been found to be an insecticide in previous studies, however those studies were only conducted under laboratory conditions against mosquitoes (Anopheles stephensi Liston, Aedes aegypti Linnaeus and Culex quinquefasciatus), but no reports have been found concerning how well it works against flies. An evaluation was conducted of the insecticidal effects of *Ocimum basilium* essential oil on *Chrysomya putoria's* postembryonic development.

METHODS AND MATERIALS

Materials derived from plants

During October 2017, *Ocimum basilium* leaves were collected in the city of Chennai, India. Vegetative growth was evident on the plants. Plants of this species were collected in an area with more than 25 plants. A random selection of plants was made and their taxonomy was determined. Using a previous voucher specimen archived at the same center's BSC Herbarium, the new specimen was verified with the previous specimen.

Oil extraction using essential oils

A traditional Clevenger apparatus was used for hydro distilling the essential oil from fresh *Ocimum basilium* leaves until exhaustion was reached. A Hewlett Packard model 5892 mass spectrometer coupled with a Gas Chromatography Mega 2 series was employed to identify the essential oil chemical composition. These conditions were similar to those used in a previous study.

As a carrier gas, helium was used in a DB-5 MS capillary column. At 65°C (120s), an incremental 4°C/min was applied to the programming temperature, followed by a 20°C/min increment to 120°C, and finally a 18°C/min increment to 155°C until 300°C. By comparing their mass spectra with the mass spectra from the NISTE mass spectrometry library and by using their Kovat's retention indexes, the compounds were characterized using electron impact ionization at -75 eV.

An overview of Diptera colonies

Collection of flies from the laboratory of India, provided the flies used in the study. Insects were handled by placing a black fabric sleeve over the opening of wooden cages with nylon screens on the sides. A cage with a volume of about 3.8 m3 (35 x 35 x 35 cm) was used to house the animals. A topical application of 2L/larva was applied on the larvae bodies using an automatic pipette to four groups of 55 newly-hatched larvae in Petri dishes.

As a solvent, dimethylsulfoxide (DMSO) was used to prepare 6 concentrations of the essential oil: 1 = 5.12 mg/mL, 2 = 9.36 mg/mL, 3 = 21.65 mg/mL, 4 = 42.30 mg/mL, 5 = 62.90 mg/mL, and 6 = 81.30 mg/mL. There were three control groups: DMSO control (just pure DMSO), classic control (without any substance added), and an oil control containing a natural insecticide. Based on previous laboratory experience with this substance on this biological model, *Cymbopogan citratus* essential oil at 84.4 mg/mL was used. A quadruplicate bioassay was conducted.

In a vessel containing 0.05 kg of bovine putrefied meat, the larvae were transferred after the essential oil was applied at different concentrations. A nylon web clamped with a rubber band was then placed around the 0.05 L vials and a 0.5mL vial containing vermiculite (Vermiculite Expanded Medium, expansion volume 0.2 m3) was added as the pupation substrate. Acclimatized chambers were used for the experiments, which kept temperatures at 28 ± 2°C, relative humidity at 69 ± 15%, and light cycle of 13:13 (light/dark).

It was recorded on a daily basis how many died and how their life cycle changed. Larvae abandon their diet when they reach maturity and are collected. A weight was determined for each larva, followed by transfer to glass tubes containing vermiculite and sealing with cotton plugs. A distance between the eyes was used to separate adults by gender after they emerged. Biological assays on the second generation were conducted in quadruplicate. During development (larval, pupal, newly-hatched larvae, and adult) corrected mortality and age were calculated. To accomplish this, the Abbot equation (equation 1) was used to calculate a corrected mortality parameter. Additionally, the sex ratio (equation 2) and the weight of mature larvae were taken into account.

Mortality corrected = (%) mortality in the treated group - Mortality in the control group) × 100 / 100 – Mortality rate in the control group (%) ----------------1

Gender Ratio = the Number of Female Flies That Emerged / (The Number of Female Flies That Emerged + the Number of Male Flies That Emerged) ---------2

Changes in morphology

A stereoscope was used to observe all insects in order to examine possible morphological changes to their heads, wings, and abdomens. An attached stereoscope was used to take pictures.

Analyses of statistics

ANOVA was used to determine the length of development periods and larval weights of this fly species. Means were compared using a Newman-Keuls test at a significance level of ≤ 0.06, and mortality was calculated using a Chi-square test. Abbot's formula was used to correct mortality. This objective was achieved by normalizing the data using the square root of the concentrations.

RESULTS

The main compounds in the compound were β-cayophyllene, β-seslinene, and eugenol (Table 1). Alternatively, the researchers found that two of the three main compounds were present (β-caryophyllene and eugenol) based on their previous investigations. Among the three main compounds, bicyclogermacrene (that accounted for 21.40% in the previous study) appeared in only 8.85% in this study. Across both samples, seven compounds comprise more than 55 percent (Table 1).

A total of 6concentrations were tested and their effects on fly life cycles were evaluated. The larval period of *C. putoria* significantly decreased when the essential oil *O. basilium* was applied to all concentrations compared to the control group and DMSO control, but not significantly in comparison to the positive control *C. citratus* essential oil (Table 2). When compared with the control group or the control with DMSO, all essential oil concentrations treated insects had a significantly shorter pupal period, except for the dosage of 21.70 mg/mL.

When analyzing the newly hatched larvae to adult period, however, these time reductions were statistically different from those for the positive control (Table 2). Also, basil essential oil had a direct effect on larval weight. All concentrations (all above 50 mg) of larvae treated with *O. basilium* essential oil were lighter than larvae treated with pure control (37.0±11.18 mg) and DMSO control (35.8±6.30 mg), with highly significant differences ($p < 0.002$). No statistical behavior was observed with the positive control.

Table: 1 An analysis of the chemical composition of *Ocimum basilium* essential oil.

CHEMICAL COMPOUND	PERCENTAGE OF AREA	EXPERIMENTAL RETENTION INDEX	INDICATOR OF RETENTION (REPORTED)
Elemene (delta)	3.95	1437	1438
Eugenol	13.45	1476	1478
Eugenol(methyl)	4.82	1498	1494
Caryophyllene	20.39	1535	1534
Elemene(gamma)	3.48	1539	1540
Farnesene (beta)	2.88	1562	1565
Humulene(	4.7	**1566**	**1565**
Alloaromadendrene	2.75	1569	1568
Gurjunene	8.28	1586	1586
Selinene	17.25	1594	1597
Bicyclogermacrene	8.92	**1600**	1600
Bisabolene	6.9	1608	1610
Spathulenol	4.43	1689	1688
Caryophyllene oxide	2.07	1692	1693
Eudesmol	2.27	1624	1622
Unidentified compound	2.42	1745	00

Hinesol	2.0	1750	1750
Eudesm-7	3.80	1755	1757
Cadin-4-en-10-ol	3.92	1769	1767
Phytol	2.20	2207	2206

Using holly basil essential oil resulted in the larvae gaining more weight as well as reducing the larval period (Table 2). At all concentrations tested, holly basil oil caused mortality. In larvae, essential oil at a concentration of 5.15 mg/mL was most effective, while at 62.90 mg/mL it was least effective. Five of the six concentrations tested, as well as the positive control, showed higher pupal mortality than larval and newly hatched larvae to adult mortality.

Only one group received essential oil of 81.30mg/mL. Sexual ratios were not statistically different among all control groups, including the positive group, according to the chi square test. Consequently, this natural extract does not affect this biological variable in any way. *O.basilium*, besides affecting the postembryonic cycle, also altered the morphology of adult animals receiving 5.14 mg/mL or 9.30 mg/mL treatments.

Table: 2 *Ocimum basilium* essential oil effects on *Chrysomya putoria* post-embryonic development.

THE TREATMENTS	PERIOD OF LARVAL DEVELOPMENT (DAYS)	PERIOD OF PUPALIZATION (DAYS)	LARVAE HATCHING TO ADULTHOOD (DAYS)
Control	4.17 ± 0.40	6.17 ± 0.46	9.32 ± 0.70
Dimethylsulfoxide as a control	4.80 ± 0.55	6.13 ± 0.43	9.87 ± 0.55
Pure Cymbopogon citratus oil	3.00 ± 0.30	5.52 ± 0.34	8.51 ± 0.69
5.12	4.04 ± 0.20d	5.71 ± 0.46	8.73 ± 0.49
9.26	4.01 ± 0.02d	5.58 ± 0.60	8.60 ± 0.60
21.64	4.01 ± 0.04d	5.95 ± 0.23	8.95 ± 0.25
42.26	4.01 ± 0.05	5.74 ± 0.65	8.75 ± 0.75
62.90	4.01 ± 0.02	5.72 ± 0.74	8.69 ± 0.75
81.30	4.40 ± 0.50b	5.69 ± 0.54	9.20 ± 0.85

DISCUSSION

The essential oil of *O. basilium* exhibits both similarities and differences as shown in Table 1. Considering that the main compounds remain almost undeviated in both studies, they may be considered important chemical patterns of the Cuban *Ocimum basilium* essential oil. Several Indian studies have also found that β-caryophyllene and eugenol are the most important components.

A different composition of minor compounds, on the other hand, was in line with the suggestion that essential oil aroma is influenced by genetics, vegetative development, agro climatological factors and chemical compounds during essential oil synthesis. Despite its insecticidal properties, essential oils often contain high concentrations of eugenol and β-caryophyllene. Many insect species, including *Musca domestica* L. and *Drosophila melanogaster*, were known to be resistant to eugenol, as well as sesquiterpenes.

Blowflies' larval period is recognized internationally as the most important part of their life cycle. When larvae are in this stage, they are eating as much food as possible in a short period of time. Moreover, larvae remain on the diet for shorter periods of time if nutrients and weight are sufficient to support pupation. Nevertheless, when all experimental and control groups were conducted under the same conditions, we were able to assume that the results obtained were caused by the essential oil of holly basil, the same as the positive control.

On the same blowfly species, aqueous crude extracts of *Pouteria sapota* (Jacq.) (Sapotaceae) exhibited larvicidal effects, resulting in a longer larval period. Fly larvae are one of the most vulnerable stages in their development cycle, making this finding of great importance. Predation is more likely to occur during this period. The pupal period is when holometabolous insects, such as blowflies, undergo their most important hormonal changes.

Physiological processes such as those influenced by hormones, the endocrine system, and the neuroendocrine system might be altered by compounds extracted from plants and tested to control insects, Cabral suggested. These physiological processes could be affected by holy basil essential oil in some way. A similar neurotoxic mechanism of action to that produced by carbamates and organophosphates has been proposed by Kostyukovsky for the use of essential oils or their purified constituents. Other research has shown that essential oil terpenes inhibit both the electric eels' acetyl cholinesterase, and the domestic fly and cockroach heads' cholinesterase.

An evaluation of the substance efficacy as an insecticide is most effective based on the newly hatched larvae or the entire developmental period, owing to the fact that it avoids abnormalities seen between larval and pupal phases. A higher concentration does not affect this variable as much as a lower one, which is unusual. This fact may be explained by considering the type of substance applied. Under lab conditions, pure essential oils are more susceptible to evaporating than dissolutions in DMSO under these circumstances.

The larvae treated with lower concentrations could be in contact with the tested substance for a longer period of time, whereas the larvae treated with higher concentrations were susceptible to evaporation. DMSO use could also be a factor. The permeability of membranes has been reported to be increased by DMSO, according to some authors.

Gurtovenko and Anwar reported that DMSO caused the membrane to thin and expand, increasing the fluidity of phospholipid bilayers. Notman and his colleague reported that DMSO induced pores in the membrane. Hydrophobic cores are thought to explain why DMSO is effective at permeating hydrophobic solutes, especially those that are hydrophobic. In spite of this, and regardless of any theoretical explanation, the essential oil was effective against the development of insects at all concentrations.

According to Carriço, the larval period of the blowfly species was reduced by crude extracts of *P.sapota* as well. The same effects were observed by Carvalho on *Chrysomya albiceps* and *C.putoria*, when cocaine was tested on their developmental rate. This particular behavior in the present study could also be explained by some effect on the endocrine system in flies. Compared to the lower concentrations, the corrected mortality in group 3 was 75%. The values obtained for the 3 and 2 groups exceeded those achieved for the positive control, demonstrating a good activity. Moreover, groups with higher essential oil concentrations showed fewer efficacies than those with lower concentrations. A possible DMSO effect as well as evaporation may help explain this particular behavior. LC50.0 was computed ignoring mortality values of the two most concentrated experimental groups (4, 5), resulting in a value of 8.50 mg/mL.

Out of 95 flies that emerge as adults, 14.5% (13 flies) are morphologically altered; but seven of 68 (11.55%) are handicapped as well. Interestingly, this behavior appears only when these oils are applied at minor concentrations, indicating how toxic these oils could be to this fly's postembryonic cycle since when; if they are not capable of killing, they produce some psychological effects.

An abdominal contraction, a non-retracted ptilinum, and twisted hemolymph were the most common morphological abnormalities. As far as the control group was concerned, all insects were normal. Flying ability was directly affected, as was the ability to find food and reproduce as a result of the morphological alterations. Thus, basil essential oils were found to be an effective alternative to spraying for blowflies.

CONCLUSIONS

Based on the results from this study, *Ocimum basilium* essential oil is a promising option for controlling *Chrysomya putoria* and, in some cases, performs better than its positive control *Cymbopogon* citrates. Besides the first report of its effect on post-embryonic blowflies , the current research also demonstrated that minimal proportions of this essential oil can have a good effect on this medically and sanitarily important fly, with a concentration of 21.70 mg/mL and an estimated LC50 of 8.50 mg/mL achieving 75% mortality.

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