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Effect of *Cymbopogon citrates* (Poaceae) oil and citral on post-embryonic time of blowflies

Zeneida Teixeira Pinto[1,2]*, Félix Fernández-Sánchez[3], Arith Ramos Santos[4], Ana Claudia Fernandes Amaral[4], JoséLuiz Pinto Ferreira[4], Julio Cesar Escalona-Arranz[5] and Margareth Mariade Carvalho Queiroz[6]

[1]Laboratório de Educação em Ambiental e Saúde, Pavilhão Lauro Travassos, Instituto Oswaldo Cruz, Fiocruz, Av. Brazil 4365, 20045-900 Manguinhos, Rio de Janeiro, RJ, Brazil.
[2]Doctoral Program in Veterinary Sciences, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro (UFRRJ), Itaguaí, Brazil.
[3]Laboratoriode Aseguramiento de la Calidad, Moa, Holguín, Cuba.
[4]Laboratório de Plantas Medicinais e Derivados, Farmanguinhos, FIOCRUZ, Rua Sizenando Nabuco, 100, 21041-250 Manguinhos, Rio de Janeiro, RJ, Brazil.
[5]Departamento de Farmacia, Universidad de Oriente, Santiago de Cuba, Cuba.
[6]Laboratório de Entomologia Médica e Forense (LEMEF), Instituto Oswaldo Cruz, Fiocruz, Avenida Brazil, 4365, 20-045-900 Manguinhos, Rio de Janeiro, RJ, Brazil.

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***Cymbopogon citratus* oil is used in folk medicine asrepellent and insecticide against insects. This study evaluated the insecticidal activity of *C. citrates* oil extracted from Brazil and Cuba and its main component citral as insecticides against *Chrysomya megacephala*, *Chrysomya putoria* and *Lucilia cuprina*. Variables monitored were:duration of post embryonic development, larval weight (mg), sex ratio, mortality index and percentage of morphological deformities. The essential oils were dissolved in dimethylsulfoxide (DMSO) and tested at concentrations of 5, 10, 25, 50, 75 and 100%, andcitral was diluted in DMSO yielding a concentration of 17.5 µg/µL.Substances were applied (1µL on the newly-hatched larvae. Results showed that both oils and citral had toxic effects on post-embryonic development of all tested blowflies. While the mortality for *C. megacephala* reaches 80% of the flies treated with essential oil, and values under 50% for the LC_{50}; *C. putoria* has the highest ratio of deformities, once it becomes adult with values over 85%. The behavior for *C. citrates* essential oils and citral, changes from one variable to another, but in general sense, all are toxic to flies. Previous observations point out this essential oil as a potential alternative in those blowflies control.**

Key words: Arthropod, Calliphoridae, biopesticides, lemongrass, essential oil.

INTRODUCTION

The blowflies are vectors of a great number of pathogens with medical and/or veterinary importance(Greenberg, 1973; Barriga, 2002; Maldonado and Centeno, 2003). Their larvae also produce myiasis (Baumgartner, 1988; Guimarães and Papavero, 1999; Jiang, 2002; Sehgal et al., 2002).

Blowfly control largely relies on chemical insecticides. However, flies can develop resistance to those synthetic

Chemical substances (Shono and Scott, 2003; Levot and Sales, 2004). Chemical insecticides can affect men and others animals resulting in air and water pollution (Mendonça et al., 2011; Carriço et al., 2014), that is why natural insecticides emerge as a potential way of fly control. In this context, essential oilscan be an eco-friendly alternative form toprevent and control blowfly species.

Cymbopogon citratus (DC) Stapf (Poaceae) (Lemongrass) is a native plant of India and Sri Lanka (Zheng et al., 1993) distributed over several tropical countries, including Brazil and Cuba.It is internationally known as lemongrass. Some studies revealed itsinsecticidal properties against several agricultural and non-agricultural pests (Ishii et al., 2010; Andrade et al., 2013) as well as other pathogenic agents(Oliveiraetal., 2009; Kumar et al., 2011a,b). Previous phytochemical studies demonstrated that these propertiescan be attributed to some compounds identified in the essential oil; mainly citral, an isomeric mixture of neral and geranial (Khanikor and Bora,2011; Costa et al., 2013; Kumar et al., 2013).

With the insects, the activity of *C. citratus* oil was demonstrated against *Aedes aegypti* (Linnaeus, 1762) larvae employing the dipping method (Cavalcanti et al., 2004). The oil was also toxic to the third instar of *Thyrinteina arnobia* (Stoll, 1782) (Lepidoptera: Geometridae) causing 100% mortality (Soares et al., 2011).

The bioinsecticidal activity of lemongrass oil against flies has also been tested before inhouseflies' larvae and pupae. Kumar and collaborators(2011, 2013) performed the contact toxicity assay (in a Petri dish and filter paper) showing a lethal concentration (LC_{50}) value of 0.41 µl/cm^2.Recently, our research group reported significant alterations in post-embryonic development of *Musca domestica* (Linnaeus), demonstrating its potential insecticidal activity (Pinto et al., 2015). Nevertheless, studies in whichanother flies' species are considered have not been developed as of today, with this in the focus of the study.To achieve it, essential oils originating from plantations that grow in geographic different conditions and three biological models (*Chrysomya megacephala, Chrysomya putoria* and *Luciliacuprina*) were considered.

MATERIALS AND METHODS

Essential oils

The Brazilian lemongrass was collectedat the Laboratory of Cultivation and Biomass Production of Farmaguinhos/Fiocruz-Jacarepaguá campus, Rio de Janeiro, Brazil (22°87'49"S, 43°24'53"W). A voucher specimen with the number RB3273021 wasdeposited at Rio de Janeiro Botanical Garden Herbarium (RB). The Cuban specimen was collectedin the district of Miraflores, municipality of Moa, Holguín, Cuba(20°38'21"N-75°01'44"W). A voucher specimen identified with the number 16443 wasdeposited at BSC Herbarium.Fresh leaves of *C. citratus* were extracted by hydrodistillation using a "Clevenger type apparatus", bottled in ambers flasks well wrapped and preserved at 4°C until accomplishing the analysis. Monoterpenecitral was purchased from Tedia®, Brazil.Oil extraction (Brazil/Cuba) followed previous methodology described in literature (Pinto et al., 2015).

Colonies of Diptera

Specimens were collected on campus of FundaçãoOswaldo Cruz, Rio de Janeiro, and were reared and maintained in Laboratório de EntomologiaMédica e Forense, InstitutoOswaldo Cruz, FIOCRUZ, following previous methodology described in literature(Queiroz and Milward-de-Azevedo, 1991). Flies were kept in cages, maintained in acclimatized chambers set at 27 ± 1°C and 70 ± 10% RH, 12:12 light/dark cycle,with water and sugar *ad libitum*. Decaying bovine ground beef was given for the maturation of the ovarioles and to stimulate oviposition. The second generation was reared following the same methodology and newly hatched larvae were used in the experiments.

Bioassay

For the preparation of the substances, the essential oils of *C. citratus* from Brazil and Cuba were dissolved in dimethylsulfoxide (DMSO-SIGMA, EUA) and tested in concentrations of 5% (25 µLoil + 475 µLDMSO); 10% (50 µL oil + 450 µLDMSO); 25% (125 µLoil+ 375 µLDMSO); 50% (250 µLoil + 250 µLDMSO); 75% (375 µLoil + 125 µL DMSO) and 100% (pure oil) to obtain the six different test concentration levels. The monoterpenecitral was diluted in DMSO yielding a concentration of17.5 µg/µL.

The substances (essential oil of Brazil/Cuba and citral) were applied (1 µL) onto the larval bodies of *C. megacephala*, *C. putoria* and *L. cuprina* using micropipettes. Concentration oils (Brazil and Cuba) were appliedbyquadruplicating onfifty newly hatched larvae for each replicate. Citralwas used in groups of thirty specimens in the three bioassays. The control groups consisted in: untreated insects,and insectsthat were treated only with solvent (DMSO). After treatment, larvae were placed on putrefied bovine meat (50 mg). After reaching maturity (L3), the larvae spontaneously abandoned the meat and weremoved to recipients with vermiculite placed below the rearing containers. They were collected, individualized, weighed and then transferred to glass tested tubes containing vermiculite and sealed with cotton plugs, monitoring the duration of each phase. After emergence,*C. megacephala*, *C. putoria*and *L. cuprina* adults were separated by gender.Also, an exhaustive analysis (visual and with the stereoscope) with a view to identify the principal somatic deformities present in the biological models, was realized. The observations were made daily. Concentrations were selected on the basis of preliminary experiments conducted in the laboratory.

Statistics analysis

The results were analyzed by one way analysis of variance

*Corresponding author. E-mail: zeneida@ioc.fiocruz.br. Tel: (55) 21 2562 1061.

Table 1. Duration (days) of post embryonic development of *C. megacephala*, *C. putoria* and *L. prina* (Diptera:Calliphoridae), treated with essential oil of *C. citratus*(DC) Stapf from Brazil and Cuba.

Species	Treatments	Larval stage (days)		Pupal stage (days)		Newly-hatched larvae to adult (days)	
		Brazil X ± DP#	Cuba X ± DP#	Brazil X ± DP#	Cuba X ± DP#	Brazil X ± DP#	Cuba X ± DP#
C. megacephala	C	4.42 ± 0.50^{a}	4.42 ± 0.50^{a}	5.23 ± 0.42^{a}	5.23 ± 0.42^{a}	9.63 ± 0.60^{a,b}	9.63 ± 0.60^{a}
	DMSO	4.38 ± 0.49^{a}	4.38 ± 0.49^{a}	5.18 ±.0.44^{a}	5.18 ± 0.44^{a}	9.54 ± 0.61^{a}	9.54 ± 0.61^{a}
	5 (%)	4.79 ± 0.41^{a,b}	4.83 ± 0.38^{a}	6.02 ± 0.15^{b}	6.06 ± 0.25^{b}	10.79 ± 0.40^{b,c}	10.87 ± 0.48^{b,c}
	10	4.66 ± 0.77^{a,b}	4.68 ± 0.77^{a}	6.03 ± 0.32^{b}	6.05 ± 0.36^{b}	10.66 ± 0.87^{a,b,c}	10.68 ± 0.87^{a,b,c}
	25	4.76 ± 0.56^{a,b}	4.79 ± 0.55^{a}	6.18 ± 0.58^{b}	6.23 ± 0.63^{b}	10.90 ± 0.88^{c}	10.99 ± 0.84^{b,c}
	50	5.57 ± 0.50^{b}	4.64 ± 0.48^{a}	5.74 ± 0.76^{a,b}	5.82 ± 0.76^{a,b}	9.60 ± 0.76^{a,b}	9.78 ± 0.84^{a,b}
	75	4.67 ± 0.26^{a,b}	4.77 ± 0.26^{a}	5.72 ± 0.35^{a,b}	5.78 ± 0.34^{a,b}	9.77 ± 0.37^{a,b,c}	9.88 ± 0.38^{a,b,c}
	100	4.68 ± 0.81^{a,b}	4.72 ± 0.78^{a}	6.07 ± 0.26^{b}	6.13 ± 0.34^{b}	10.62 ± 0.86^{a,b,c}	10.64 ± 0.72^{a,b,c}
C. putoria	C	3.30 ± 0.46^{a}	3.30 ± 0.46^{a}	4.33 ± 0.47^{a}	4.33 ± 0.47^{a}	7.55 ± 0.76^{a}	7.55 ± 0.76^{a}
	DMSO	3.26 ± 0.44^{a}	3.26 ± 0.44^{a}	4.30 ± 0.46^{a}	4.30 ± 0.46^{a}	7.51 ± 0.61^{a}	7.51 ± 0.61^{a}
	5 (%)	2.42 ± 0.50^{b}	2.47 ± 0.50^{b}	4.64 ± 0.48^{a}	4.65 ± 0.48^{a}	7.06 ± 0.23^{a}	7.10 ± 0.31^{a}
	10	2.60 ± 0.49^{a,b}	2.61 ± 0.49^{a,b}	4.49 ± 0.50^{a}	4.52 ± 0.50^{a}	7.03 ± 0.76^{a}	7.08 ± 0.70^{a}
	25	2.99 ± 0.23^{a,b}	3.02 ± 0.15^{a,b}	4.21 ± 0.41^{a}	4,24 ± 0.43^{a}	7.20 ± 0.46^{a}	7.26 ± 0.44^{a}
	50	2.87 ± 0.67^{a,b}	2.88 ± 0.40^{a,b}	4.49 ± 0.64^{a}	4.53 ± 0.65^{a}	7.37 ± 0.86^{a}	7.39 ± 0.76^{a}
	75	2.54 ± 0.50^{a,b}	2.53 ± 0.50^{a,b}	4.85 ± 0.35^{a}	4.87 ± 0.33^{a}	7.40 ± 0.62^{a}	7.40 ± 0.61^{a}
	100	2.96 ± 0.53^{a,b}	2.97 ± 0.58^{a,b}	4.45 ± 0.50^{a}	4.48 ± 0.50^{a}	7.37 ± 0.79^{a}	7.40 ± 0.80^{a}
L. prina	C	3.24 ± 0.43^{a}	3.24 ± 0.43^{a,b}	4.72 ± 0.58^{a}	4.72 ± 0.58^{a}	7.96 ± 0.67^{a}	7.96 ± 0.67^{a}
	DMSO	3.17 ± 0.38^{a}	3.17 ± 0.38^{a}	4.67 ± 0.60^{a}	4.67± 0.60^{a}	7.81 ± 0.51^{a}	7.81 ± 0.51^{a}
	5 (%)	4.22 ± 0.41^{b}	4.25 ± 0.44^{c}	8.60 ± 0.78^{b}	8.69 ± 0.85^{b,c}	12.84 ± 1.01^{b}	12.86 ± 1.50^{b}
	10	4.05 ± 0.21^{b}	4.03 ± 0.25^{b,c}	10.05 ± 0.86^{c}	10.11±0.84^{c}	14.11 ± 0.85^{b}	14.20 ± 0.90^{b}
	25	4.07 ± 0.30^{b}	4.13 ± 0.39^{c}	9.75 ± 0.99^{b,c}	9.90 ± 0.88^{c}	13.86 ± 1.02^{b}	14.11 ± 1.01^{b}
	50	4.11 ± 0.41^{b}	4.14 ± 0.46^{c}	9.74 ± 0.92^{b,c}	9.79 ± 0.90^{b,c}	13.88 ± 1.11^{b}	13.97 ± 1.13^{b}
	75	4.11 ± 0.63^{b}	4.18 ± 0.63^{c}	8.42 ± 0.88^{b}	8.42 ± 0.93^{b}	13.57 ± 1.10^{b}	13.59 ± 1.16^{b}
	100	4.17 ± 0.53^{b}	4.21 ± 0.58^{c}	9.78 ± 0.80^{b,c}	9.67 ± 0.93^{b,c}	14.10 ± 1.15^{b}	14.03 ± 1.20^{b}

#Values within a column followed by the same letter is not significantly difference at the 5% level according to Tukey's LSD. DMSO = dimetilsufoxide, C = control.

(ANOVA) (P <0.0001). The linear regression for the LC_{50} and the mean values were compared by the Tukey-Kramer (LSD) test at the 0.05 (%) significance level (Zar, 1999) computed with Stat graphics Plus v5.1 (Statistical Graphics Corporation) software. Sex ratio was calculated using the following formula: (nFemale/nFemale + nMale) (Rodrigues, 2004).

RESULTS AND DISCUSSION

C. citratus essential oil from Brazil and Cuba obtained with the Clevenger apparatus yielded 0.25 and 0.28% (v/w, volume/dry-weight), respectively. According to Cuéllar et al. (2009), same species of plants contain different quantities of yield of essential oil, but could be more or less similar. As Table 1 summarizes, blowflies treated with *C. citrates* essential oil (Brazil/Cuba) show statistical differences regarding the control groups. The main susceptibility was observed in

Table 2. Duration (days) of post embryonic development of *C. megacephala*, *C. putoria* and *L. prina* (Diptera: Calliphoridae), treated with monoterpenecitral under laboratory conditions.

Species	Treatments	Larval stage (days)	Pupal stage (days)	Newly-hatched larvae to adult (days)
		X ± DP#	X ± DP#	X ± DP#
C. megacephala	C	3.96 ± 0.19[a]	5.23 ± 0.43[a]	9.23 ± 0.43[a]
	DMSO	3.96 ± 0.20[a]	5.20 ± 0.41[a]	9.20 ± 0.41[a]
	citral	3.12 ± 0.35[b]	5.57 ± 0.53[a]	8.57 ± 0.53[a]
C. putoria	C	3.04 ± 0.19[a]	4.26 ± 0.45[a,b]	7.31 ± 0.47[a]
	DMSO	3.17 ± 0.27[a]	4.08 ± 0.28[a]	7.16 ± 0.37[a]
	citral	3.22 ± 0.43[a]	5.00 ± 0.52[b]	8.08 ± 0.64[a]
L. prina	C	3.11 ± 0.32[a]	4.73 ± 0.45[a]	7.80 ± 0.40[a]
	DMSO	3.15 ± 0.36[a]	4.71 ± 0.49[a]	7.76 ± 0.43[a]
	citral	3.07 ± 0.27[a]	4.45 ± 0.52[a]	7.62 ± 0.51[a]

[#]Values within a column followed by the same letter is not significantly difference at the 5% level according to Tukey's LSD. DMSO = dimetilsufoxide, C = control.

*L. cuprina*with notorious increments in the periods of times of the three phases, but especially at pupal and newly-hatched larvae stages. In *C. megacephala,* also all stages (larval, pupal and newly-hatched larvae) suffer a significant increment in the mean time when compared with the control groups (with/without DMSO), being more evident in newly-hatched larvae stage, but those increments are lower than that observed for *L. cuprina*. In the case of *C. putoria,* this species shows only statistical differences at larval stage becoming the most resistant fly to *C. citratus* essential oil, at least when the stages' duration times are considered. No great difference was seen when considering both essential oils, neither was it possible to observe a regular tendency behavior through the concentrations used. Other plant extracts have exhibited the same property to prolong the flies' stages duration on these biological models (Mendonça et al., 2011).

As was declared before, the monoterpenecitral is the main constituent of the lemongrass essential oil, and its influence over the flies is summarized in Table 2. As can be appreciated in this table, the levels of susceptibility among flies' species proved to be different. The effect of this compound is only statistically different regarding the control groups for *C. megacephala*at larval stage. The rest of the stages and biological models remain with the same statistical behavior than the control groups, being classified as inactive at the evaluated concentration.

Larval weight (mg) and sex ratio were also studied, and these data are showed in Table 3. The larval weight of *C. megacephala*was significantly affected in the experimental groups. This characteristic is very important due to the fact that less weighed larvae could mean a weakness to reach the adult stage, therefore vulnerability to the environment influence and to its natural predators. Nevertheless, the values observed are far from the 30.1 mg fixed as the minimum weight for *C. megacephala* to become pupa (Von Zuben, 1998). On the other two flies, this behavior was non-regular; hence no clear information was extracted. Regarding the sex ratio, no significant differences between the experimental groups was observed.

Table 4 shows larval weight (mg) and sex ratio variables obtained when flies were treated with citral. In this case, only in *C. putoria*was statistical differences observed. The increment of the larval weight should be interpreted as an element that favors the development of the fly, but other elements are necessary to support this hypothesis. In any case, this is additional evidence that not necessarily the behavior of a plant extract has to be in line with those exhibited by its main compound.

In spite of the previous parameters informed before, the most important characteristic of a bio-pesticide is the mortality index all over the flies' cycle of live. Figures 1 to 3 shows the mortality of larval, pupal and newly-hatched larvae for *C. megacephala, C. putoria* and *L. cuprina*, respectively, after the exposure to different

Table 3. Larval weight (mg) and sex ratio of *C. megacephala, C. putoria and L. cuprina* (Diptera: Calliphoridae) treated with different concentrations of *C. citratus* (DC) Stapf. from Brazil and Cuba, under laboratory conditions.

Species	Treatments	Brazil		Cuba		Brazil	Cuba
		(Mean ± SD)#	Variation Interval	(Mean ± SD)#	Variation Interval	Sex ratio	Sex ratio
C. megacephala	Control	74.51 ± 4.11^{a}	69.20 - 85.60	74.51 ± 4.11^{a}	69.20 - 85.60	0.51	0.51
	DMSO	74.27 ± 4.05^{a}	68.00 - 84.00	74.27 ±.4.05^{a}	68.00 - 84.00	0.53	0.53
	5%	57.57 ± 2.63$^{b.c}$	50.00 - 62.10	57.35 ± 2.80^{b}	51.50 - 62.50	0.50	0.52
	10%	60.08 ± 5.86$^{b.c}$	52.00 - 60.90	59.84 ± 5.91^{b}	52.00 - 70.60	0.47	0.52
	25%	50.28 ± 5.28^{c}	43.30 - 69.50	55.87 ± 5.86^{b}	42.70 - 68.50	0.48	0.47
	50%	59.93 ± 3.53$^{b.c}$	53.30 - 96.00	59.45 ± 3.45^{b}	53.30 - 96.00	0.40	0.46
	75%	61.71 ± 8.93^{b}	51.70 - 81.80	52.52 ± 9.55^{b}	52.70 - 82.70	0.47	0.53
	100%	56.62 ± 9.02$^{b.c}$	33.00 - 67.04	56.88 ± 9.16^{b}	31.00 - 67.10	0.46	0.52
C. putoria	Control	40.15 ± 6.74^{a}	30.00 - 65.00	40.15 ± 6.74^{a}	30.00 - 65.00	0.50	0.50
	DMSO	45.29 ± 4.40$^{a.b}$	30.70 - 49.90	45.29 ± 4.40$^{a.b}$	30.70 - 49.90	0.49	0.49
	5%	47.37 ± 3.60$^{a.b}$	42.25 - 55.50	48.28 ± 2.31$^{a.b}$	45.25 - 55.50	0.57	0.51
	10%	53.00 ±5.14^{b}	37.40 - 65.40	52.28 ± 5.43^{b}	38.40 - 66.40	0.51	0.49
	25%	46.10 ± 6.90$^{a.b}$	37.80 - 72.90	45.45 ± 6.28$^{a.b}$	35.80 - 73.80	0.52	0.50
	50%	46.13 ± 2.12$^{a.b}$	41.80 - 48.80	45.59 ± 2.13$^{a.b}$	42.50 - 49.80	0.48	0.52
	75%	47.62 ± 5.02$^{a.b}$	42.40 - 68.10	48.72 ± 4.89$^{a.b}$	43.40 - 66.10	0.44	0.46
	100%	50.40 ± 5.65^{b}	40.60 - 66.20	51.21 ± 5.42^{b}	41.60 - 67.20	0.50	0.53
L. cuprina	Control	33.19 ± 3.41^{b}	26.87 - 37.37	33.19±3.41^{b,c}	26.87 - 37.37	0.52	0.52
	DMSO	32.45 ± 2.47^{a,b}	28.20 - 36.83	32.45 ± 2.47^{a,b,c}	28.20 - 36.83	0.52	0.52
	5%	35.31 ± 3.74^{b}	28.00 - 38.60	34.79±3.87^{c}	27.30 - 38.40	0.53	0.49
	10%	26.85 ± 2.94^{a}	21.60 - 32.40	27.49±3.09^{a,b,c}	21.60 - 33.40	0.49	0.52
	25%	31.91 ± 4.61^{a,b}	11.00 - 36.60	32.21±4.79^{a,b,c}	13.00 - 37.20	0.48	0.52
	50%	27.26 ± 2.84^{a}	24.20 - 32.40	26.72±2.67^{a}	23.20 - 31.40	0.48	0.52
	75%	32.53 ± 3.16^{a,b}	19.00 - 39.00	33.44±3.19^{b,c}	21.00 - 39.00	0.49	0.51
	100%	30.96 ± 3.12^{a,b}	26.25 - 35.20	31.88±3.51^{a,b,c}	26.25 - 36.20	0.49	0.51

#Values within a column followed by the same letter is not significantly difference at the 5% level according to Tukey's LSD. DMSO = dimetilsulfoxide, C = control.

concentrations of *C. citratus* essential oil from Brazil and Cuba, under laboratory conditions. As it can be seen in Figure 1, independently of the concentration level the newly-hatched larvae stage results to be the most susceptible to the lemongrass essential oil action, being in coincidence with the most affected stage regarding the necessary time for their development. At concentration of 100%, the mortality index reaches 80%, higher than the 61 and 28% reached for the larval and pupal stages, respectively. Mortality of *C. megacephala* in larval, pupal and newly hatched larvae to adult periods was concentration dependent for oils (Brazil/Cuba), being estimated as the LC_{50} of Brazil and Cuba oil in 47.89 and 46.18%, respectively. The adjustment of the points to the equations render determination coefficients superior to 0.87 (R^2>0.

Table 4. Larval weight (mg) and sex ratio of *C. megacephala, C. putoria* and *L.cuprina* (Diptera:Calliphoridae) treated with monoterpenecitral, under laboratory conditions.

Species	Treatments	X ± DP#	Sex Ratio
C. megacephala	C	75.75 ± 4.51[a]	0.46
	DMSO	75.78 ± 4.06[a]	0.55
	Citral	69.49 ± 10.96[a]	0.51
C. putoria	C	40.83 ± 0.80[a]	0.54
	DMSO	45.94 ± 2.51[b]	0.48
	Citral	59.33 ± 2.58[c]	0.54
L. cuprina	C	41.93 ± 2.62[a]	0.42
	DMSO	41.96 ± 2.59[a]	0.44
	Citral	38.81 ± 0.61[a]	0.50

#Values within a column followed by the same letter is not significantly difference at the 5% level according to Tukey's LSD. DMSO = dimetilsulfoxide, C = control.

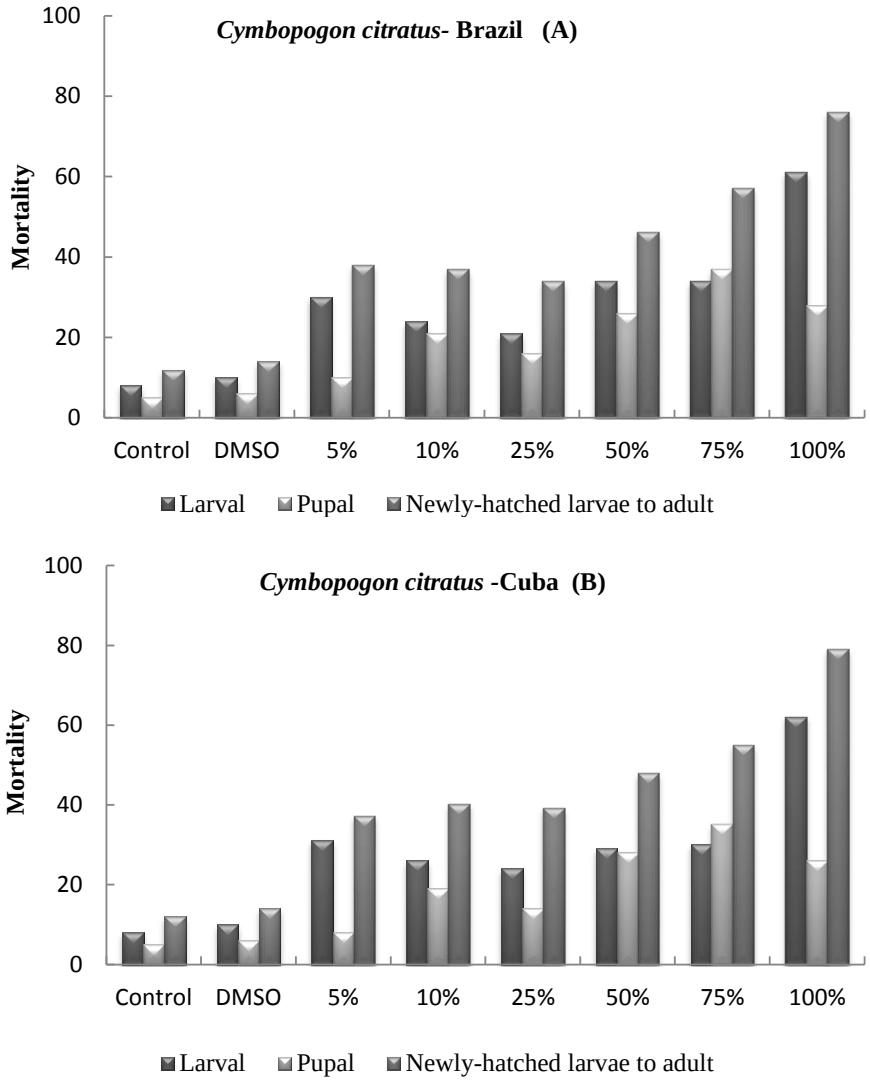

Figure 1. Mortality of larval, pupal and newly-hatched larvae to adult stage of *C. megacephala* after exposure to different concentrations of *C. citratus* oil from Brazil and Cuba, under laboratory conditions.

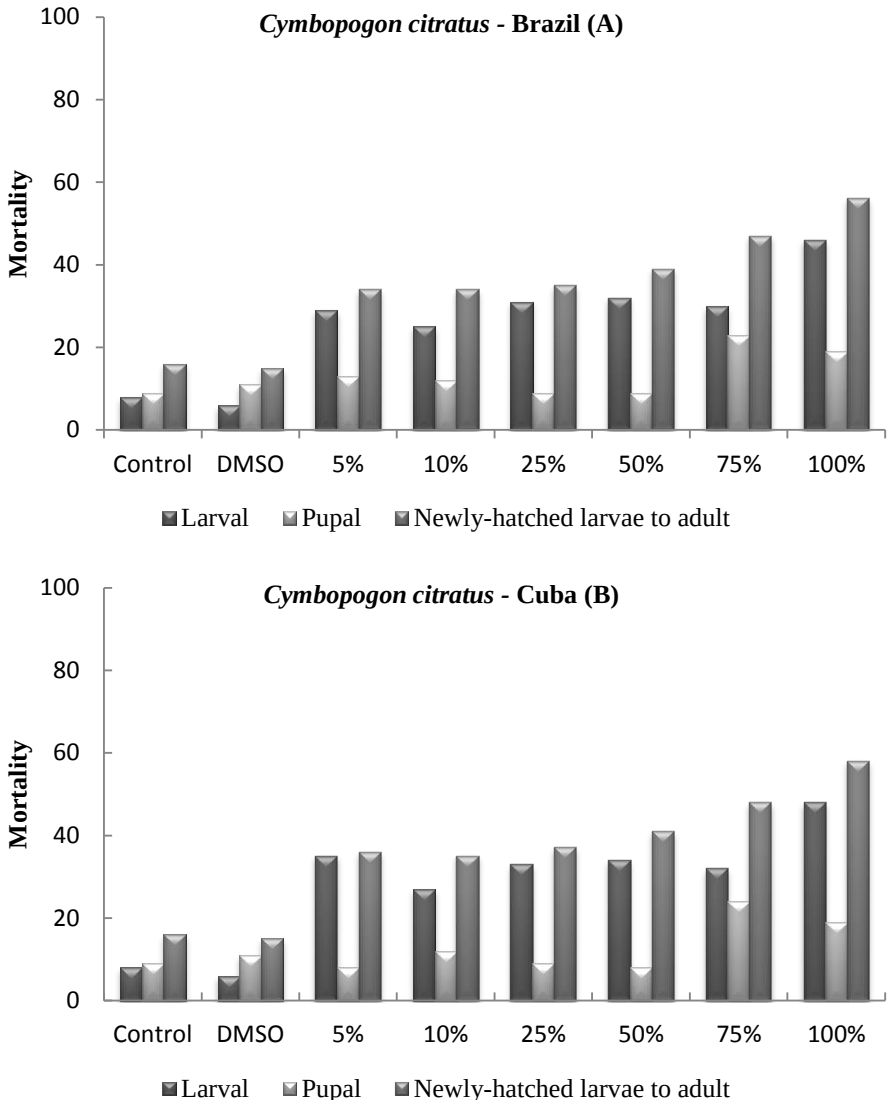

Figure2. Mortality of larval, pupal and newly-hatched larvae to adult stage of *C. putoria* after exposure to different concentrations of *C. citratus* oil from Brazil and Cuba, under laboratory conditions.

87) being considered statically as good. Related to *C. putoria*, it can be noted (Figure 2) that even for the 100% of concentration, the mortality index does not get to surpass the 60%. This fly was already signed as the most resistant to the *C. citratus* essential oil in the previous variables discussed; and this is also reflected in the mortality index. In this biological model, the order of stages' susceptibilities is the same one, that is, *C. megacephala* being the most affected newly-hatched larvae, larval and finally pupal stage, respectively. This behavior is in coincidence with that observed in *C. megacephala.*Mortality of *C. putoria* in larval, pupal and newly hatched larvae to adult periods was concentration dependent for oils (Brazil/Cuba), being estimated as the LC_{50} of Brazil and Cuba oil in 85.48 and 78.19%, respectively. The equations computed to determine those values exhibit determination coefficients superior to 0.9 (R^2>0.9).

For the last biological model (*L. cuprina*), the mortality index reaches almost 80% at the maximum concentration used, but at the minimum level (5% essential oil), it already achieves the 60% of the flies' deceases (Figure 3). This fact again indicates *L.cuprina*as the most sensible fly to the *C. citratus* essential oil insecticidal effect. It was impossible to compute the LC_{50} for this fly considering that we did not find a dependent relation between concentration and mortality.

When citral is used, the same susceptibility tendency (Figure 1 to 3) is observed (Figure 4), being once again *C. putoria* the most resistant fly. Larval and newly hatched larvae to adult periods of flies appear to be highly sensitive to citral effect. In spite of this similarity in

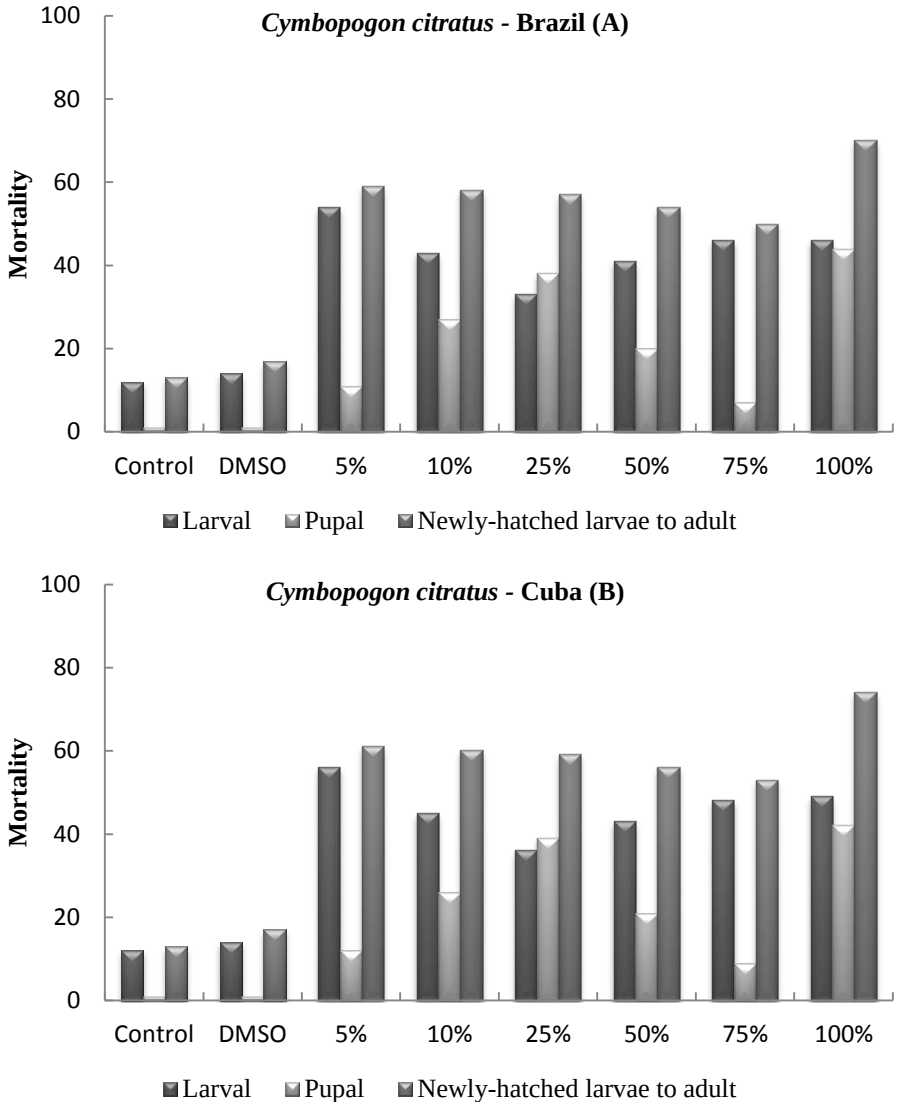

Figure 3. Mortality of larval, pupal and newly-hatched larvae to adult stage of *Luciliacuprina* after exposure to different concentrations of *Cymbopogoncitratus* oil from Brazil and Cuba, under laboratory conditions.

the behavior between citral and the essential oil of *C. citratus* from Cuba/Brazil, the values of mortality index for citral are lower than those computed by the action of lemongrass essential oil, demonstrating over again that the activity of the oil does not necessary have to be conditioned byits main constituent.

This research shows that essential oil of *C. citrates* (Brazil/Cuba) and the monoterpenecitral led to disruption of development of the treated blowfliesinducing different morphological abnormalities in adults (Tables 5 and 6). Malformations include defective wings, deformed abdomen and small-sized flies.The higher ratio of deformities was foundin adults of *C. putoria.* This evidence reveals that even when this species was the less susceptible at the previous parameters evaluated, a high number (more than 85%)of the flies that reached adult stage did it have any malformation giving them less probability to reach the maturity age with capacities to reproduce.Previous results suggest that essential oils are capable to produce such effects. The oils of *Menthapiperita* and *Lavandula angustifolia* induce deformities in larvae and pupae of *M. domestica* (Bolsy,2013).According to this author, deformities may have been caused by the oil that has the ability to inhibit metamorphosis, suggesting that the effect is similar to the insects treated with growth regulators (IGRs).

The present studyrevealed that topical treatment with essential oil of *C. citratus* (Brazil/Cuba) and monoterpenecitral induces alterations at different levels in *C. megacephala, C. putoria* and *L. cuprina* cycle's life,

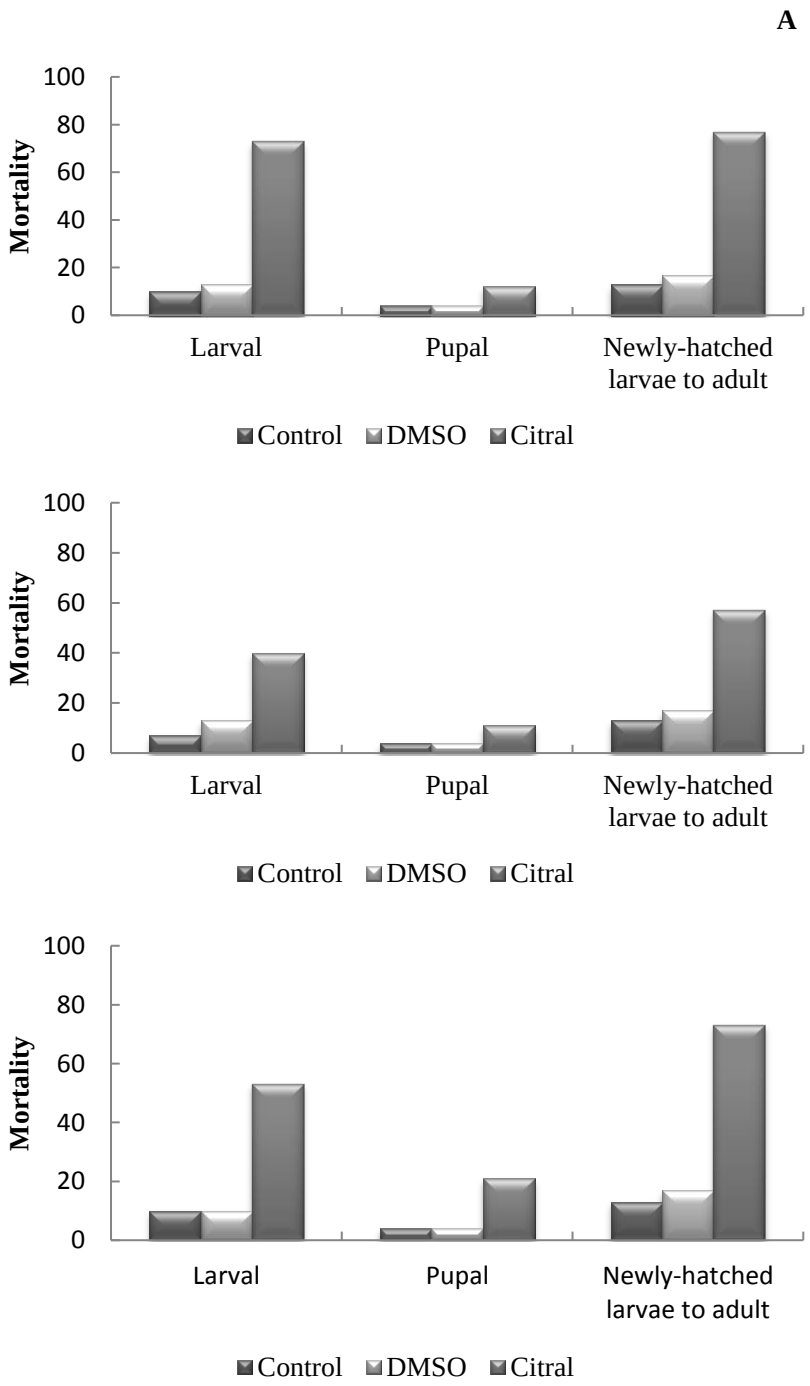

Figure 4. Mortality of larval, pupal and newly-hatched larvae to adult stage of (A) *C. megacephala,* (B) *C. putoria* and (C) *L. prina* (Diptera: Calliphoridae) after exposure to monoterpenecitral, under laboratory conditions.

emerging as a potential alternative in the control of these blowflies.

Conflict of interests

The author(s) did not declare any conflict of interest.

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Table 5. Percentage (%) of morphological deformities in adults of *C. megacephala, C. putoria and L. prina* (Diptera: Calliphoridae) treated with essential oil of *C. citratus* (DC) Stapf. from Brazil and Cuba, under laboratory conditions.

Species		Morphological deformities	
	Treatments	Brazil	Cuba
C. megacephala	C	0.00	0.00
	DMSO	0.00	0.00
	5 (%)	12.80	12.69
	10	15.83	15.70
	25	4.54	4.91
	50	4.81	4.11
	75	3.41	3.24
	100	10.42	10.28
C. aputoria	C	0.00	0.00
	DMSO	0.00	0.00
	5 (%)	30.09	35.16
	10	32.57	32.31
	25	46.15	48.82
	50	74.80	72.88
	75	60.75	62.50
	100	86.36	85.88
L. prina	C	0.00	0.00
	DMSO	0.00	0.00
	5 (%)	29.27	30.00
	10	26.83	27.16
	25	21.43	21,95
	50	16.30	16.67
	75	12.00	12.24
	100	43.33	44.83

Oils teste with four replication, N=50. DMSO = dimetilsulfoxide, C = control.

Table 6. Percentage (%) of morphological deformities in adults of *C. megacephala, C. putoria and L. prina* (Diptera: Calliphoridae) treated with monoterpenecitral, under laboratory conditions.

Species	Treatments	Morphological deformities (%)
C. megacephala	C	0,00
	DMSO	0.00
	Citral	28.57
C. putoria	C	0.00
	DMSO	0.00
	Citral	84.61
L. cuprina	C	0.00
	DMSO	0.00
	Citral	37.50

Oils tested with three replication N = 10. DMSO = dimetilsulfoxide, C = control.

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