

Analysis of the chemical composition (GC–MS) of *Lantana camara* (Verbenaceae) essential oil and its insecticidal effect on the post-embryonic development of *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae)

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ABSTRACT

The chemical composition of *Lantana camara* essential oil (EO), its insecticidal activities, and its impact on some biological parameters of the oriental latrine blowfly *Chrysomya megacephala* was assessed. The chemical composition of the tested EO was analyzed by gas chromatography and mass spectroscopy. Totally, 58 compounds corresponding to 99.8% of the total oil were identified, and major constituents of EO were α -pinene (15.3%), caryophyllene (15.28%), eucalyptol (7.8%), camphene (6.05%), caryophyllene oxide (5.33%), β -pinene (4.8%), and geranyl acetate (4.3%), citral (1.36%), α -terpineol (1.23%), tricyclene (0.613%), and linalool (0.62%). The dip toxicity test against the larvae of *C. megacephala* resulted in the disruption of metamorphosis, leading to developmental deformities, reduced pupariation rate, and inhibition of adult emergence. *Lantana camara* EO also resulted in larval and pupal toxicity, abnormal pupariation, non-viable pupal-adult intermediates production, and adultoid formation. Our result manifests that this Eo is acting as a juvenoid. This work supports the use of biological control methods for the control of medico-veterinary important insects.

1. INTRODUCTION

Chrysomya megacephala (Fabricius, 1794) is recognized as an important medico-veterinary pest distributed in Oriental and Australian regions [1]. It is a synanthropic insect and transmits various deadly pathogens and helminth eggs from the filth substrates to the human body, causing a lot of nuisance [2–4]. The oriental latrine blowfly is of great importance because it causes myiasis in agronomic livestock and humans [5,6]. It also causes a significant loss to the fish and meat industries every year [7]. Thus, *C. megacephala* has a negative adverse impact on human and veterinary livestock; so, it is necessary to control its population growth.

Several methods were implemented to curb the menace caused by the insects. One such control measure included implementing chemical pesticides like dichlorodiphenyltrichloroethane, Permethrin, etc. The imprudent use of these pesticides proved hazardous because of their non-biodegradable nature and retention in plants and animals, mammalian toxicity, and the development of resistant strains of insects [8–13]. Therefore, a novel concept of insect growth regulator (IGR) came into existence [14], which included green insecticides as hormonal analog (fenoxycarb) [15,16] and plant essential oils (EOs) [17]. These IGRs are benign for the environment and non-targeted organisms [18,19].

Plants are biological factories that produce various chemicals collectively known as secondary metabolites [20–23]. Plant oils are rich in terpenes, enabling them to create a web of target sites to exhibit the unique insecticidal activity against different pathogens [24,25]. These EOs are used as a protective measure to counter pathogens as a deterrent, growth inhibitor [26], as a juvenile hormone (JH) analog (JHA) [27], or as anti-JH [28,29].

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The EOs biological properties can be because of the synergistic effect of the major active components present in it [30]. These oils are generally safe for mammals at low concentrations [31–33]. The beneficial effects of EOs over other chemical insecticides make them potentially a preferable candidate for integrating pest management programs [34–35].

Previous studies revealed that a range of plant EOs, such as Piper betel, *Melaleuca alternifolia*, *Carapa guianensis*, *Boesenbergia rotunda*, *Curcuma longa*, *Citrus hystrix*, *Ocimum gratissimum*, and *Zanthoxylum limonella*, was successfully employed for the control of blowflies, *C. megacephala* [36,37]. Studies on insects' post-embryonic development using various EOs are available [38,39]. Still, an extensive review of the literature showed a dearth of studies on the application of *Lantana camara* EO on larvae of the latrine blowfly *C. megacephala*. Therefore, the current study determines the toxic effects of *L. Camara* Eo on the third instar (0 and 1-day-old) larvae of the blowfly, *C. megacephala*.

2. MATERIAL AND METHODS

2.1. Insect Rearing

The colony of the blowfly, *C. megacephala*, was obtained from the laboratory maintained in the Department of Zoology, University of Allahabad, India. The adults were reared in the cage (30 × 30 × 30 cm). Molasses was given for feeding, and cotton soaked in distilled water was provided for drinking. A piece of goat liver (fresh) was also provided because it acts as an excellent protein-rich diet source and an oviposition site. After oviposition, a batch of the eggs was separated, and the first instar larvae that emerged were kept in a separate beaker with a fresh liver piece.

2.2. Gas chromatography and mass spectroscopy (GC–MS)

GC–MS analysis of *L. camara* EO was carried out using an Agilent 7890B GC connected to 5977A mass selective detector equipped with an HP-5 MS capillary column (30 m × 250 × 0.25 μm). The carrier gas used in the process was helium with a flow rate of 1 ml/minute. Exactly 1 μl of the sample was injected into the column (Split-less). The GC oven temperature was adjusted from 60°C to 325°C at the rate of 10°C/minute and held at 280°C for 10 minutes. The injector temperature was 250°C, and the detector temperature was 280°C. The percentage composition of each component was

calculated by integrating the GC peak area normalization. This analysis of MS parameters was an ionization voltage (EI) of 70 eV and a mass range of 15–500 *m/z*. Analytic profiles were characterized from their mass spectral data using the NIST MS2011 library.

Lantana camara Eo was purchased from Surajbala Exports Pvt. Ltd. For the dip-assay method, 1 ml of the EO was dissolved in 1 ml of pure acetone to get the concentration.

2.3. Procedure

Last instar larvae (0 and 1-day-old) of *C. megacephala* were separated from the stock and divided into three batches (20 larvae in each). Larvae were dipped in the prepared solution for 15, 30, 45, 60, and 120 seconds [39]. Small pouches of 90 mm diameter were made with the filter paper, and in each pocket, 20 larvae were placed. Each pouch containing larvae was dipped in the prepared tested EO solution for a specific duration of time (Fig. 1a–e). Larvae in control groups were also treated similarly with pure acetone only. After administering EO, larvae were transferred to a filter paper to eliminate the EO's excess quantity. Later, larvae were transferred in a glass beaker of 250 ml capacity containing a piece of goat liver. After the fourth day, as larvae started to wander, they were transferred in beakers (250 ml) containing sterilized sawdust for pupation [16].

Observations were recorded carefully regarding larval and pupal mortality, abnormal pupariation, total pupation, intermediates formation, total adult emergence, and adultoid formation. Larvae were considered dead if they were fully immobile or flaccid [40]. Dead specimens were preserved in Bouins' solution for a day and later fixed in 70% ethanol for the morphological study. A day after emergence in control groups, the pupae from which adults could not emerge were dissected and fixed in Bouins' solution and further preserved in 70% ethanol for morphological study.

Inhibition of adult emergence was calculated using the method of Kumar *et al.* [41]. Percentage inhibition rate (PIR) was calculated as follows:

$$\text{PIR} = \{[C_{\%} - T_{\%}] \times 100\} / C_{\%}$$

where $C_{\%}$ is the percentage of newly emerged adults in control groups and $T_{\%}$ is the percentage of newly emerged adults in treated groups.

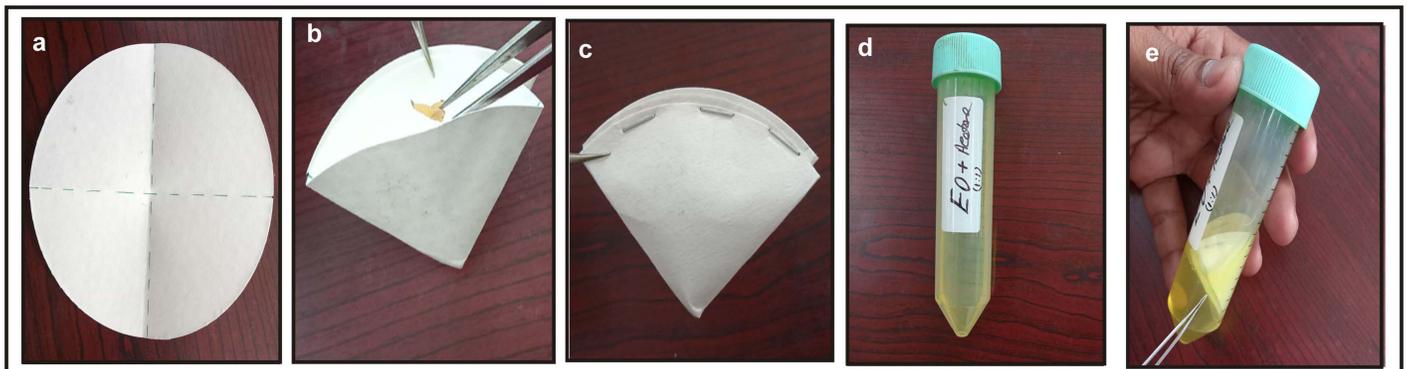


Figure 1: Procedure and preparation of the pouch used in the dip method. (a) Filter paper, (b,c) filter paper molded in a conical form and larvae were placed inside the cavity, (d) centrifugation tube 50 ml containing solution of EO + acetone, (e) pouch containing larvae dipped in the EO + acetone solution.

Photography was carried out using Nikon SMZ 1000 Binocular fitted with Nikon Digital Sight DS-U2 microscope and NIS software (Nikon Corp., Japan).

2.4. Statistical Analysis

All measures were expressed as mean \pm standard error of mean and analyzed by one-way analysis of variance, followed by *post-hoc* Dunnett's multifactorial test. The coefficient of correlation was calculated using GraphPad Prism 5.0. The alpha significance was set at $p < 0.05$.

3. RESULTS

Major compositions of the commercial *L. camara* EO analyzed by GC–MS are shown in Table 1. Totally, 58 volatile compounds were identified that constituted 99.8% of the total oil. The major volatile constituents analyzed were α -pinene (15.3%), caryophyllene (15.28%), eucalyptol (7.8%), camphene (6.05%), caryophyllene oxide (5.33%), β -pinene (4.8%), and α -terpineol (1.23%).

Topical application of EO on the larvae (0 and 1-day-old) of *C. megacephala* disrupted growth, molting, and metamorphosis. The developmental abnormalities resulting from the tested EO comprised mortality, formation of abnormal puparium, and pupal–adult intermediates, reduced pupariation, adult emergence, the appearance of adultoids, and adult emergence inhibition (Table 2).

3.1. Mortality

Treatment of larvae (0 and 1-day-old) of blowfly with the tested EO resulted in the larval mortality on both the days of treatment ($F = 61$, $R^2 = 0.96$, $p \leq 0.0001$, 0-days-old larvae) and ($F = 3$, $R^2 = 0.94$, $p \leq 0.0001$, 1-day-old larvae), respectively. The larval

mortality increased in a time-dependent manner (except at 30 second treatment, 1-day-old larvae) (Figs. 2 and 3). Tested EO also caused pupal mortality on both the days of larval treatment ($F = 1.8$, $R^2 = 0.43$, $p = 0.2$, 0-days-old larvae and $F = 14$, $R^2 = 0.86$, $p = 0.0001$, 1-day-old larvae) (Figs. 2, 3 and 4o).

3.2. Abnormal Pupariation

Administration of *L. camara* EO on the larvae (0 and 1-day-old) of *C. megacephala* caused formation of abnormal puparia at both larval durations ($F = 2.2$, $R^2 = 0.5$, $p = 0.12$, 0-days-old larvae and $F = 3.4$, $R^2 = 0.6$, $p = 0.04$, 1-day-old larvae) (Figs. 2 and 3).

The following types of abnormal pupariation were seen:

- Some portions of the larval body were sclerotized (Fig. 4a).
- The whole body is barrel-shaped, but the mouth is everted out (Fig. 4b).
- Puparium abnormally formed without proper barrel-shaped formation and retraction of the mouth region (Fig. 4c–e).

3.3. Reduced Pupariation

Larvae (0 and 1-day-old) of *C. megacephala* treated with EO resulted in reduced pupariation ($F = 29$, $R^2 = 0.9$, $p \leq 0.0001$, 0-day-old larvae and $F = 31$, $R^2 = 0.93$, $p \leq 0.0001$, 1-day-old larvae) (Figs. 2 and 3).

3.4. Pupal–Adult Intermediates

Application of the treated EO on the last larval instar (0 and 1-day-old) of the oriental latrine blowfly has resulted in the formation of pupal–adult intermediates in both the treated groups ($F = 36$, $R^2 = 0.94$, $p \leq 0.0001$, 0-day-old larvae and $F = 13$, $R^2 = 0.84$, $p = 0.0002$, 1-day-old larvae) (Figs. 2 and 3).

The pupal–adult intermediates formed by the application of *L. camara* EO can be classified into the following types:

- Pupal–adult intermediates consisted of white, untanned bodies, pupal proboscis; wings are pupal white in appearance; eyes are white or unpigmented; pupal white abdomen; and undeveloped genitalia (Fig. 4f–g).
- Pupal–adult intermediates consisted of the tanned head and thorax but the abdomen is less developed and pupal in appearance; ptilinum is everted; and genitalia are not formed (Fig. 4h–j).
- Pupa–adult intermediates consisted of adequately developed head and thorax. The abdomen is elongated and the genitalia are not adequately formed (Fig. 4k).
- Pupal–adult intermediates consisted of deformed head, thorax, and abdomen. Eyes were showing improper pigmentation (Fig. 4l).
- Pupal–adult intermediates are showing a well-differentiated body into the head, thorax, and abdomen. The only deformity noticed was abnormal eye pigmentation (Fig. 4m) and improperly pigmented eyes (Fig. 4n).

Table 1: Chemical composition (%) of the *L. camara* EO by GC–MS analysis method.

Sl. No.	Compound name	Retention time (Minutes)	Molecular weight	Percentage (%)
1	α -Pinene	6.764	136	15.3
2	Caryophyllene (15.28%)	14.477	204	15.3
3	Eucalyptol	8.477	154	7.8
4	Camphene	7.010	136.12	6.05
5	Caryophyllene oxide	16.549	220	5.3
6	β -Pinene	7.523	136	4.8
7	α -Terpineol	11.205	154	1.23

Table 2: PIR of the *L. camara* essential oil against the third instar larvae (0 and 1-day-old) of *C. megacephala*.

Period of exposure (Seconds)	PIR (0-days-old larvae)	PIR (1-day-old larvae)
15	55.7	17.2
30	82.5	24.7
45	93	64.5
60	100	78.5
120	100	100
<i>r</i>	0.73	0.94*

R = coefficient of correlation; PIR = percentage inhibition rate.

*Correlation significant at $p \leq 0.01$.

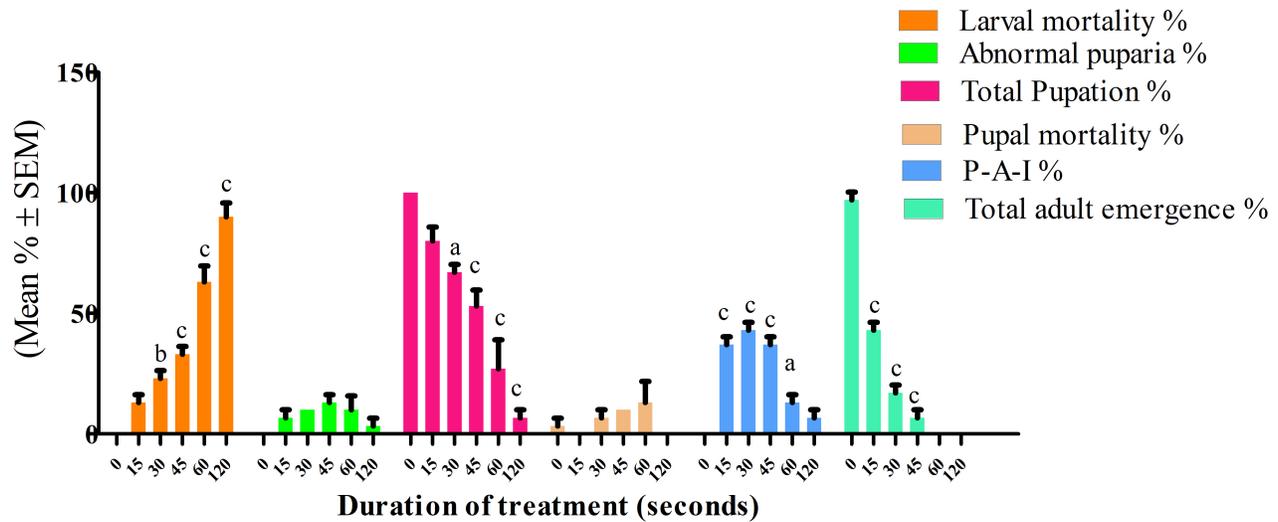


Figure 2: The effect of *L. camara* essential oil on the third instar larvae (0-days-old) of *C. megacephala*. (a) Correlation significant at $p \leq 0.05$; (b) correlation significant at $p \leq 0.01$; and (c) Correlation significant at $p \leq 0.001$.

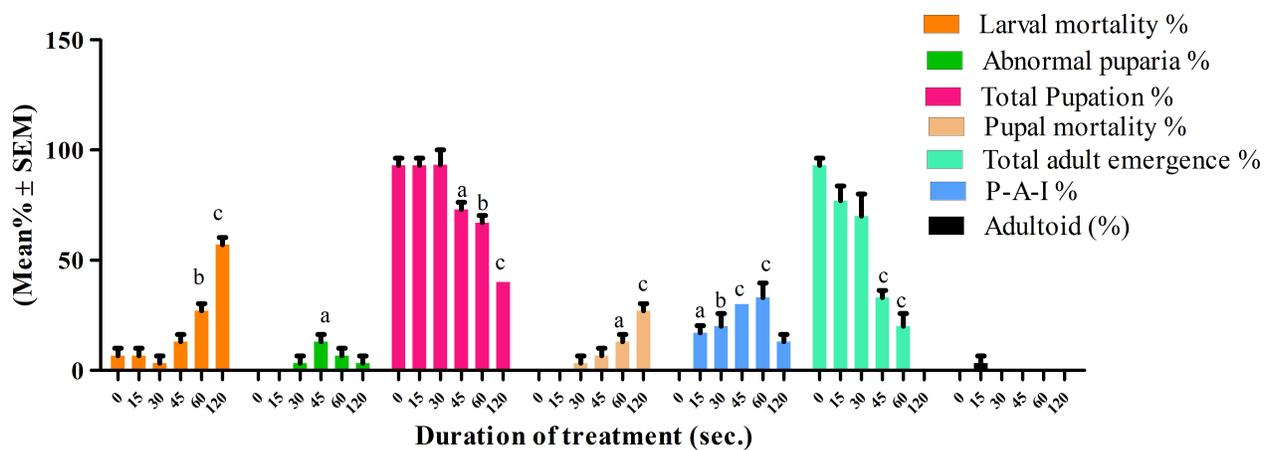


Figure 3: The effect of *L. camara* essential oil on the third instar larvae (1-day-old) of *C. megacephala*. (a) Correlation significant at $p \leq 0.05$; (b) correlation significant at $p \leq 0.01$; and (c) correlation significant at $p \leq 0.001$.

3.5. Adult Emergence and Adultoid Formation

Administration of *L. camara* EO on the third instar larvae (0 and 1-day-old) of *C. megacephala* also resulted in the reduced adult emergence ($F = 190$, $R^2 = 0.98$, $p \leq 0.0001$, 0-days-old larvae) ($F = 40$, $R^2 = 0.94$, $p \leq 0.0001$, 1-day-old larvae) (Figs. 2 and 3). Treatment of last larval instar (0 and 1-day old) of *C. megacephala* resulted in adultoid formation. Only a single adultoid was formed in the larvae treated with EO (1-day-old) treated for 15 seconds ($F = 1$, $R^2 = 0.29$, $p = 0.045$, 1-day-old larvae) (Fig. 3). The adultoid form showed wrinkled wings.

4. DISCUSSION

The use of medicinal plants with insecticidal properties has various advantages in comparison to conventional pesticides. These botanicals are obtained from renewable resources and easily degrade. These botanical insecticides are less prone to

the development of insecticidal resistance [24]. Botanicals as insecticides are effective because they consist of many volatile constituents produced from secondary metabolism [20,30]. Major volatile components found in the *L. camara* EO were α -pinene, caryophyllene, eucalyptol, camphene, caryophyllene oxide, β -pinene, and α -terpineol. Alpha-pinene, caryophyllene, β -pinene, and camphene were also reported in *L. camara* [42]. Volatile constituents such as caryophyllene oxide, eucalyptol, caryophyllene, and α -terpineol are present in the EO of this medicinally important plant species [43–49].

The chemical composition of the EOs is quite complex mixtures of many components, and their insecticidal activity may be attributed to several active compounds or their synergistic activity [49]. Several volatile components present in the plant can cause a disturbance in the hormonal titer of the body, resulting in developmental deformities. Some of the major



Figure 4: The effect of the application of essential oil of *L. camara* by applying the dip method on the third instar larvae (0 and 1-day-old) of *C. megacephala*. (a) Abnormal puparium showing abnormal pigmentation (15 seconds); (b) bottle-shaped pupa showing everted mouthpart (30 seconds); (c–e) abnormal puparium (45, 60, and 120 seconds, respectively); (f) P-a-i showing white pupal body (120 seconds); (g) P-a-i showing pupal proboscis and leg along with a deformed abdomen (60 seconds); (h–j) P-a-i showing pupal abdomen, undeveloped genitalia, and everted ptilinum; (k) P-a-i abdomen elongated (60 seconds); (l) P-a-i showing improperly developed abdomen and improperly pigmented eyes (120 seconds); (m) P-a-i showing pupal abdomen with undeveloped genitalia (60 seconds); (n) P-a-i showing abnormal eye pigmentation (45 seconds); and (o) pupal death (120 seconds).

components identified in the present study have been reported to exhibit insecticidal effect, such as α -pinene, caryophyllene, β -caryophyllene, and eucalyptol [50–54].

The tested EO caused larval and pupal toxicity. This toxicity may be due to the specific biological compounds present in this EO [55,56]. Such a toxic effect has also been observed in treating the larvae of *Lasioderma serricorne* (F.), *Tropinota squalida*, *Anopheles Stephensi*, *Aedes aegypti*, *Culex Quinquefasciatus*, *Musca domestica*, and *A. aegypti* with the plant [39,57–62]. Toxicity due to JHA has also been reported on the larval stage of *Sarcophaga ruficornis* and *C. megacephala* [15–16].

Another apparent effect of the tested EO on larvae of blowfly is the formation of abnormal puparium. A similar result was also reported where the administration of EOs to the larvae of *M. domestica*, *Synthesiomyia nudiseta*, and *L. sericata* caused abnormal pupariation [63–67]. The abnormal pigmentation in the body may be due to the disorganization of the light and dark bands of the muscles or the inhibitory effect of the treated EO on melanin synthesis [64,68]. The third instar of *M. domestica* treated with the *P. nigra* volatile oil also caused abnormal puparium formation [65]. Similarly, topical treatment of third instar larvae of *C. megacephala* with JHA also caused abnormal pupariation [15].

The application of *L. camara* EO also resulted in pupal–adult intermediates' formation. The phytochemicals are responsible for disturbing the normal hormonal regulatory pathway [69]. Secondary metabolites from plants act as a juveno-mimic compound [27] and cause disturbance in the hormonal titer [70]. Surprisingly, the administration of juvenile hormone mimic (JHM/JHMs) to the larvae has also been observed to produce pupal–adult intermediates in *S. litura*, *C. megacephala*, and *S. ruficornis* [15,16,71,72]. The balanced titer of JH and ecdysone in the insect during a transformation stage determines the fate of metamorphosis. The excess of JH at a sensitive period prevents cellular differentiation, leading to the metamorphic catastrophe. Formation of intermediates is an actual juvenilizing effect caused due to the juvenoids/JHAs [73,74,75,86].

Administration of the tested EO has resulted in a reduced pupariation and adult emergence. These effects are similar to those observed by the application of EO on the larvae of *C. megacephala*, *L. sericata*, *S. nudiseta*, and *L. sericata* [57,66,67,71,76]. The JHA treatment has also observed similar effects in *Culex tarsalis*, *S. ruficornis*, *S. nudiseta*, and *C. megacephala* [15,16,66,77]. Several juvenoids are known to mimic the effects caused by the JH as suppression of pupation [78,79] and adult emergence due to the inhibition of the secretion of eclosion hormone [10]. These results infer that the EO of *L. camara* acts as a juvenoid.

The adultoid formation was not a very prominent effect induced by the *L. camara* EO. Adultoid formation has also been reported in *Schistocerca gregaria* and *M. domestica* [65,80]. Adultoid formation may be due to the plant oil's intervening effect in the ecdysteroid titer [70,81]. Application of JH/JHM or juvenoids during the acute phase of the development may result in adultoids' formation. During these sensitive periods in which most of the

imaginal disk cell proliferation occurs, any disturbance in the hormonal interplay may lead to abnormalities [72,82,83].

The insect's life cycle development comprises several metamorphic events, including de-differentiation and re-differentiation of the body tissues at each stage. During metamorphosis, neurosecretory cells secrete the prothoracico-tropic hormone. This hormone acts on the prothoracic gland, stimulating the molting hormone's release from corpora cardiaca [82]. The ecdysone hormone is responsible for the progression of a stage. In the final larval instar of holometabolous insects, the JH level is high during the initial stadium, which gradually declines as the pupal stage is about to commence to an undetectable titer in the hemolymph. A balanced titer of both JH and ecdysone (E) as a duo during larval–larval, larval–pupal, or pupal–adult ecdysis is stage-specific and varies from insect to insect. Any imbalance in the titer downregulates the principle metamorphosis pathway, leading to several developmental anomalies and aberrations [82,84]. Secondary metabolites from plants act as juveno-mimic compounds causing imbalance in principle hormonal titer [27,70].

Volatile terpenes act as pro-oxidants in mitochondria resulting in the interruption in the electron transport chain (ETC). This interruption in the ETC further results in the formation of reactive oxygen species [30]. High mortality, reduced pupariation, and adult emergence may be due to the oxidative stress caused by the *Lantana* EO. This oxidative stress results in the increase in the polyphenols and catalase activities in the body tissues [85]. The tested EO somehow disturbs the regulatory hormonal pathway, thus leading to various developmental abnormalities.

5. CONCLUSION

In this study, the tested EO of *L. camara* showed to be influential on the post-embryonic development in *C. megacephala* in the laboratory. This effect may be due to several volatile compounds that may have caused interference in the normal hormonal titer in the body of the blowfly. Therefore, *L. camara* EO can be used as an effective IGR against the larvae of *C. megacephala*. Our result manifests that this EO acts as a juvenoid.

6. ACKNOWLEDGMENTS

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7. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

8. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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