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EFFECT OF GERANYL ACETATE ON THE THIRD INSTAR LARVAE OF LATRINE BLOW FLY, *CHRYSOMYA MEGACEPHALA* (FABRICIUS, 1794) (DIPTERA : CALLIPHORIDAE)

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ABSTRACT : *Chrysomya megacephala* (Fabricius, 1794) acts as a vector for the propagation of many pathogens and causes myiasis in vertebrates. In the present investigation, essential oil of *Lantana camara* was analyzed by GC-MS to determine its' chemical composition. Further, geranyl acetate was tested against the last instar larvae of *C. megacephala* to examine its' larvicidal effect. Compositional analysis of EO revealed major components present in the EO were α- pinene, caryophyllene, geranyl acetate and eucalyptol. Effects due to topical application of geranyl acetate included larval mortality, abnormal pupariation, eclosion failure, low percentage of normal adult emergence. Our result on the significant negative effects of geranyl acetate on *C. megacephala* larval survival suggests that it can be considered in future designs of green pesticides against the dipteran population.

Key words : *Chrysomya megacephala*, myiasis, geranyl acetate, green pesticide, GC-MS.

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INTRODUCTION

The oriental latrine blowfly, *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) is a serious public health hazard (Mondal *et al*, 2016). It has been observed that these blowflies, which are synanthropic insects, cause myiasis in vertebrates (Badenhorst and Villet, 2018). They spread infection to freshly developed wounds in livestock. They also serve as a mechanical vector for the spread of parasitic microorganisms (Sontigun *et al*, 2018). Therefore, it is desirable to manage the dipteran insect's population and lessen the threat that they pose.

Since decades, certain pests that have shown to be harmful to the environment have been controlled with chemical pesticides (Mishra *et al*, 2020; Mishra *et al*, 2021; Maddheshiya and Singh, 2021; Rani *et al*, 2021). Because of these factors, scientists are concentrating their efforts on creating natural substitutes for chemical pesticides, such as plant extracts, essential oils, or their constituent parts (Suwannayod *et al*, 2019; Maddheshiya *et al*, 2021). Essential oils, which are known as a safe bio-pesticide and key plant-derived volatile components that are used to control pest populations have recently received a lot of attention.

A herb from the Verbenaceae family called *Lantana camara* is used medicinally to treat a number of illnesses (Sharma *et al*, 2007). A thorough review of the literature reveals a gap, nevertheless, because geranyl acetate, a volatile found in *L. camara* EO, kills the larvae of this blowfly's last instar. In light of this, the current work set out to identify the main chemical components in commercial-grade L. camera EO before looking at the larvicidal effects of geranyl acetate on *Chrysomya megacephala* final instar.

MATERIALS AND METHODS

Chrysomya megacephala was collected from the colony developed in the laboratory and reared in a plastic cage at 27 ± 1^{0}C and humidity (75± 5%). Adults were fed 20% honey + water solution, molasses and fresh pieces of liver were provided for oviposition. The eggs from single batch were collected, and the progeny that emerged was reared on the pieces of goat liver (Maddheshiya, 2021).

The Agilent (HP 7890) gas chromatography (GC) analyses were carried out using a fused silica capillary HP-5 column (30m × 0.25 mm; film thickness 0.25 m) and a flame ionization detector. Temperature settings for the injector and detector were 210 and 230 C, respectively. Agilent's model 5975C mass spectrometer and DB5 column gas chromatograph were used to conduct mass spectrometry (MS) analyses (60 m 9 0.32 mm; film thickness 0.25 lm).Identification of individual compounds was made by comparing their mass spectra with those of the internal reference mass spectra library (NIST2000/WILEY).

The essential oil (EO) of *L. camara* and geranyl acetate *was* purchased from Surajbala Exports (P) Ltd., New Delhi. The desired dose of 1, 2 and 4µl concentrations were obtained by dissolving the known quantity of geranyl acetate in 1 ml of acetone.

Larvae in their last instar were taken from the stock at 0, 1 and 2 days old or 0–2 hours old and divided into batches of 20 larvae each. In order to avoid any age differences, larvae from a single batch were chosen. With the aid of a micropipette, geranyl acetate doses of 1, 2 and 4 g/larvae were topically administered to the larvae on the dorsal surface of the abdomen (final 4 tergites) (Sigma-Aldrich). Only pure acetone was utilized in the control. Both the control and treated larvae were then moved to sterile beakers and fed with fresh goat liver after treatment. Larvae were moved to beakers containing sawdust for pupariation during the wandering stage (Maddheshiya and Singh, 2021). A day after the emergence of adults in the control groups the pupae were dissected from which adults failed to emerge. The dead specimens (samples) were fixed in Bouins' fluid for a day and then preserved in 70% ethanol for morphological study permanently.

Pearson Correlation coefficient (r) was used to examine the correlation between the doses administered and the various deformities using Graph Pad Prism 2007, 5.01 software (San Diago, California).

RESULTS

The GC/MS analysis revealed the presence of 58 compounds in the EO. The major volatiles were α- pinene, caryophyllene, Geranyl acetate, eucalyptol, camphene, β-himachalene, caryophyllene oxide, β-pinene, Artumerone, α -curcumene, α- verbenol, geraniol, citral, α-terpineol, β-elemene, tricyclene and linalool.

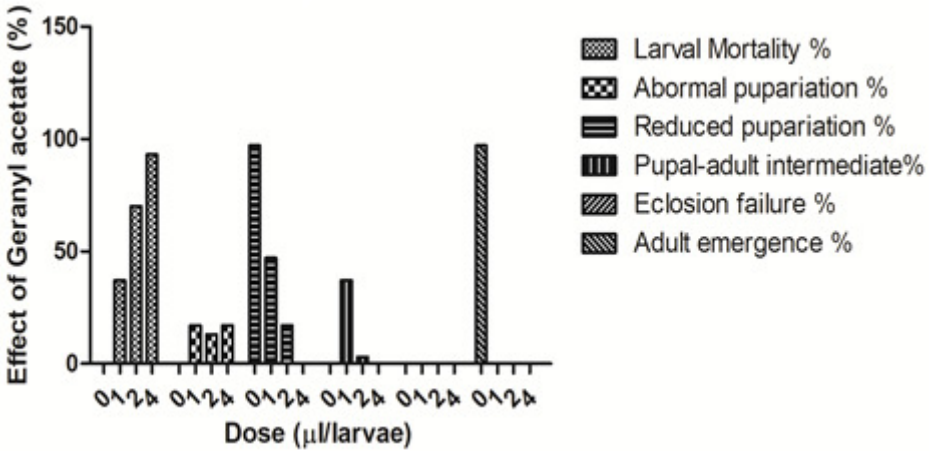

Fig. 1 : Showing effect of geranyl acetate on the 0-day old last instar larvae of *C. megacephala.*

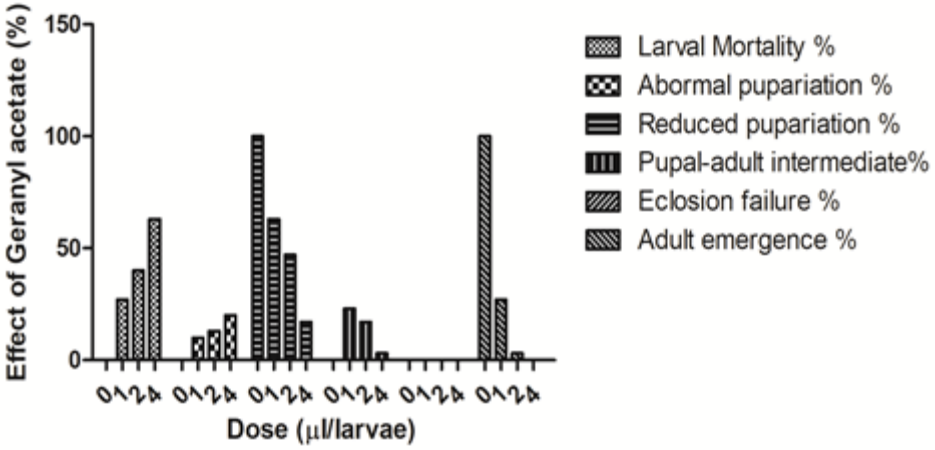

Fig. 2 : Showing effect of geranyl acetate on the 0-day old last instar larvae of *C. megacephala.*

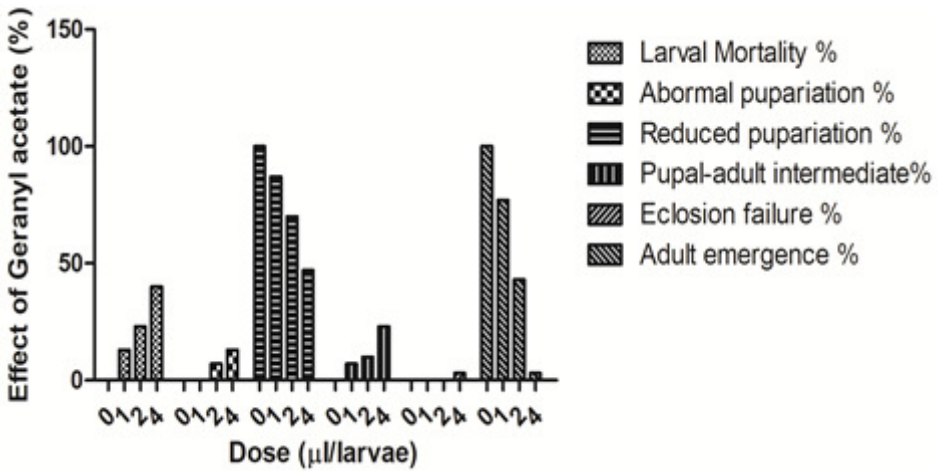

Fig. 3 : Showing effect of geranyl acetate on the 0-day old last instar larvae of *C. megacephala.*

Topical application of geranyl acetate to the last instar larvae of *Chrysomya megacephala* resulted in larval mortality, abnormal pupariation, reduced pupariation, formation of pupal-adult intermediates, eclosion failure and reduced adult emergence.

Geranyl acetate induced high larval mortality when treated topically on third instar (0, 1 and 2 days old; 0-2 hours old) larvae. Larval mortality occurred within 2 days of treatment without showing any morphological aberration. A significant positive correlation was observed as 0.8^{b}, 0.9^{c} and 0.8^{b} for 0, 1 and 2 days old larvae respectively ($p \leq 0.05$) (Figs. 1, 2, 3).

The last instar larvae exposed to geranyl acetate at doses of 0, 1 and 2 days and 0–2 hours experienced aberrant pupariation (Figs. 1, 2, 3). At 0 hours old larval treatment, abnormal pupariation was more frequently seen. In the case of 0 and 1 day old larvae treated with geranyl acetate, the coefficient of correlation was negligibly positive (0.2 and 0.7, respectively), whereas 2 day old larvae treated with geranyl acetate showed a considerably positive coefficient of correlation (r = 0.89; p 0.05).

A dose-dependent reduction in pupariation was seen after geranyl acetate was applied to final instar larvae that were 0, 1 and 2 days old as well as 0–2 hours old (Figs. 1, 2, 3). Significantly low correlation coefficients of -0.6, -0.9 and -0.8 were found, respectively (p 0.05). Its use on third instar larvae at 0 hours, 24 hours, and 48 hours produced pupal-adult intermediates as well. At 0, 24 and 48 hours after the larval treatment, a non-significant correlation of -0.6, -0.9, and 0.7 was seen, respectively (p 0.05).

Geranyl acetate topically applied to larvae in their last instar (0, 24 and 48 hours old) prevented eclosion (Figs. 1, 2, 3). Only third instar (2-day old) larvae were found to fail at eclosion, and they only displayed a non-significantly positive coefficient of association (r = 0.35; p 0.05). In the treatment of larvae that were 0 and 1 days old, no eclosion failure was noted.

When geranyl acetate was administered to larvae that were 0, 1 and 2 days old, it also reduced adult emergence (Figs. 1, 2, 3). It was discovered that the 0, 1 and 2 day old larval treatments had correlation coefficients of -0.6, -0.8 and 0.9 against the adult emergence, respectively.

DISCUSSION

Because of their efficacy across multiple functions, terpenoids, phenolics and alkaloids found in plants are emerging as possible sources for battling insect pests. The abundance of secondary metabolites secreted by humans is harvested in order to expand health nutrition pyramids and boost agricultural output. Insects exposed to these compounds have growth and developmental regression (Viegas-Junior, 2003). The results of the most recent GC-MS investigation of *Lantana camara* are consistent with the earlier discovery (Maddheshiya *et al*, 2021).

The results of the current investigation demonstrated *L. camara* EO's larvicidal ability against *C. megacephala* final instar larvae. Our findings are consistent with those of other researchers who have noted the larvicidal toxicity of plant volatiles against *Callosobruchus chinensis* (Coleoptera: Chrysomelidae) and *Corcyra cephalonica* (Lepidoptera: Pyralidae) (Dwivedi and Garg, 2003; Kathirvelu *et al*, 2019).

It has been documented that secondary metabolites in *L. camara* oil can be poisonous to insects at different stages of development. These alkaloids disrupt the neurological system, resulting in insect paralysis and eventual death at the post-embryonic larval stage. By docking several target sites as GABA and octopaminergic

receptors, Picollo *et al* (2008) demonstrated that 1, 8 cineole (eucalyptol), -pinene, Geranyl acetate, -pinene and linalool block acetylcholine esterase (AchE) activity. It is well known that Glutathione S-transferases (GSTs) play a role in the detoxification or neutralisation of foreign substances in insect bodies. *Atalantia monophylla* (Rutaceae) EO inhibits this crucial detoxifying esterase (GSTs), which causes the accumulation of harmful substances in the insect body (Nattudurai *et al*, 2017). As a result of their excitatory action and subsequent paralysis or full knockdown of the insect, EOs extracted from *Malva verticillata* (Malvales), *Heliomeris multiflora* (Asteraceae) and *Artemisia annua* (Asteraceae) all demonstrated neural toxicity (Palacios *et al*, 2009). Price and Berry (2006) reported that geraniol and citral have neurological effects on cockroaches. According to Politi *et al* (2017), active ingredients like 4-terpineol prevent the binding of choline substrate and act as a competitive inhibitor of acetylcholinesterase 1 (AChE1). This further causes an accumulation of acetylcholine at synaptic junctions, which causes the central nervous system to collapse. This provides an explanation for the mortality seen in the *in-vitro* assay.

Geranyl acetate treatment of blowfly larvae results in the formation of intermediates that are comparable to those created when the larvae are exposed to other volatile plant substances like pogostone and friedelin (Huang *et al*, 2004 and Baskar *et al*, 2014). *L. camara* EO also shown antimetamorphic activity, which led to the development of pupal-adult intermediates. It has been hypothesized that the plant's secondary metabolites function as a juvenile hormone analogue (JHA) (Jaipal *et al*, 1983; Bede and Tobe, 2000). It's interesting to note that *C. megacephala* larvae treated with JHAs pyriproxyfen and diofenolan also experienced pupal-adult mosaic development (Maddheshiya and Singh, 2021). The plant's volatile components function as JHA to disrupt the endogenous hormonal titer and cause the creation of non-viable intermediates (Sakthivadivel and Thilagavathy, 2003). Juvenoids are used to maintain the current state of the juvenile stages, which prevents transformation and the creation of adults (Maddheshiya, 2021).

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