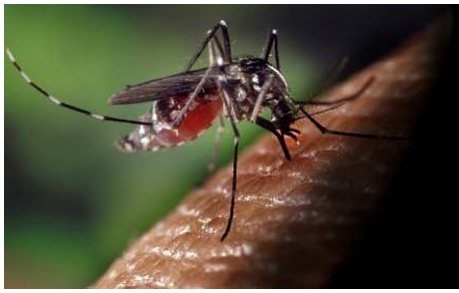

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Insect growth regulatory activity of *Acalypha alnifolia* (Euphorbiaceae) and *Vitex negundo* (Verbenaceae) leaf extracts against *Aedes aegypti* (Diptera: Culicidae)

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Abstract
The methanolic leaf extracts of *Acalypha alnifolia* and *Vitex negundo* were studied for IGRs activity against *Aedes aegypti*. The developmental bioassay using standard WHO protocol was tested for larval, pupal and adult development activity against freshly emerged first instar larvae of *A. aegypti* followed by the standard procedure. Effect of *A. alnifolia* showed (L1 to L4) prolonged larval duration and up to 8 days at 500 ppm. Whereas, control was reached up to 4 day of complete larval development. Effect of *A. alnifolia* showed (L1 to L4) prolonged larval duration and up to 8 days at 500 ppm. Whereas, in control complete larval development was completed in 4 days. Similarly, methonolic extract of *V. negundo* was prolonged larval (L_1 to L_4) duration up to 3 to 5 days at 500 ppm concentration than the control. Combined treatment of both extracts showed higher significant prolongation of larval, pupal and adult duration and was reached up to 7 days. The adult emergence period was extended to 28 days, whereas, in control it was reached to 12 days. Hence, the present study clearly showed that larval, pupal and adult emergence was greatly inhibited by the present active compound in the plants.

Keywords: *Acalypha alnifolia*, *Vitex negundo*, *Aedes aegypti*, IGR activity

1. Introduction
Vector - borne diseases still represent a significant threat to human health although mosquito borne diseases are under considerable national and international control efforts. Therefore, there is a need to develop novel insecticides to replace existing products to which mosquito larvae have developed resistance and has rendered these obsolete or incorporate novel insecticides in to resistant management program in order to extend the operational lifespan of existing insecticides. *Aedes aegypti*, the primary carrier for viruses that cause dengue and dengue hemorrhagic fever, yellow fever and chikungunya, are widespread over large areas of the tropic and subtropics. At present, no effective vaccine is available for dengue; therefore, the only way of reducing the incidence of this disease is by mosquito control, which is frequently dependent on applications of conventional synthetic insecticides [1].
Phytochemicals obtained from plants with proven mosquito control potential can be used as an alternative to synthetic insecticides or along with other insecticides under the integrated vector control. Plant products can be used, either as insecticides for killing larvae or adult mosquitoes for protection against mosquito bites, depending on the type of activity they possess. Large number of plant extracts have been reported to have mosquitocidal insect growth regulator (IGR) activity against mosquito vectors but very few plant products have shown practical utility for mosquito control. Plant products can be obtained either from the whole plant or from a specific part by extraction with different types of solvent such as aqueous, methanol, chloroform, hexane, etc., depending on the polarity of the phytochemicals [2].
Vitex negundo (Verbenaceae) is an important source of such natural drugs. It is a reputed medicinal herb and its parts have been employed as a traditional cure in Asian systems of medicine (Indian, Chinese and Malaysian) for a variety of disease conditions. A number of pharmacological activities have been attributed to *V. negundo*, such as: analgesic and anti-inflammatory activity [3], enzymes inhibition [4], nitric oxide scavenging activity [5], snake venom neutralization activity [6], antifeeding activity [7], antiradical and antilipoperoxidative [8], CNS activity [9], hepatoprotective activity [10], anti-bacterial activity [11], anti-fungal [12], larvicidal activity [13] antiandrogenic effects [14] and mosquito repellent activity [15]. Similarly, *Acalypha alnifolia* (Euphorbiaceae) is an indigenous plant. In Southern Kallar region, Nilgiris tribal people used this plant leaves as a smoke repellent. This plant contains phenolic, tannin And flavonoid compound [16].

Insect growth regulators (IGR) have been found to be highly active against mosquitoes. Most of the compounds evaluated to date fall into the groups of juvenoids (juvenile hormone analogs), benzamides, carbamates and urea- type compounds. Other types of compounds have also been found to manifest growth modifying or inhibiting properties. Some IGRS show ovistatic or ovicidal activities and sterilizing effects. In general, these types of compounds are known as juvenoids or juvenoid analogues and mimics and have been designated as 3rd generation insecticides. They induce a variety of morphogenetic aberrations.

In mosquito control, most IGRs are evaluated and applied against the aquatic stages of mosquitoes. Mosquito larvae are ideal targets for IGR activity as most IGRs have delayed activity, inducing mortality or morphogenetic anomalies in stages beyond the one treated. In other words, IGRs do not induce immediate mortality in the stage (larvae in particular) treated. This is quite a plus point for IGRs, since mosquito larvae are beneficial, providing a ready source of food for invertebrate and vertebrate predators. Mortality in the various stages is dosage dependent, rapid mortality occurring in the treated stage at higher dosages. But at lower dosages the time of mortality is delayed and effects are materialized in later stages or instars. Hence, the present study has evaluated the interaction of *Acalypha alnifolia* and *Vitex negundo* for the growth regulatory effect against *Aedes aegypti*.

2. Materials and Methods

2.1 Mosquito rearing

Aedes aegypti eggs were collected from wild populations in and around Bharathiar University Campus, Coimbatore, TN, India. Rearing conditions for all mosquitoes were 27±2 °C, 75–85% RH, and a L14: D10 photoperiod. Larvae were reared in 2 L of water in enamel trays (30 cm long×25 cm wide×6 cm deep) where they were provided a daily food mix comprising 3 parts dog biscuit and 1 part brewer's yeast. Pupae were placed into screened cages (23 cm long×23 cm wide×23 cm deep). When adults emerged after 24 h, they were transferred to glass cages (30 cm long×30 cm wide×30 cm deep) and provided with 10% sucrose solution (in water) via cotton wick. Five days after emergence, female mosquitoes were allowed access to a restrained 1 week-old chick for blood feeding.

2.2. Plant specimens and preparation of extracts

Specimens of *Acalypha alnifolia* Klein ex Willd. (Family: Euphorbiaceae) and *Vitex negundo* Linn. (Verbenaceae) were collected near the Bharathiar University campus during April to June 2010. These were identified to species by personnel at the Botanical Survey of India (BSI) in Coimbatore and voucher specimens deposited with BSI. In the laboratory, ½ kg of leaves of *A. alnifolia* and *V. negundo* were rinsed with tap water and shade-dried at room temperature (27 °C). These were pulverized into a powder (50-100 µm dia) using an electric blender. Half kg of powder was extracted using 2 L of methanol in a Soxhlet apparatus for 8 h [17]. The extract was then concentrated in a rotary vacuum evaporator to remove the solvent. The yield of crude obtained from these plants was about approximately 50 g of each plant extract.

2.3 Phytochemical Screening

The aqueous and methanolic extracts of leaf of *A.alnifolia* and *Vitex negundo*, were analyzed for the qualitative determination of phytochemical constituents (Table 1 and 2) as described by Sofowora [18].

2.4 Quantitative Phytochemicals determination of plant specimens

Preparation of fat free Sample: 5 g of the sample were defatted with 100 ml of diethyl ether using a soxhlet apparatus for 4 h as described by Evangeline and Natarajan [15].

2.5 Estimation total Phenolics determination of plant specimens

The phenolic content in the plant material was estimated by the method of Okwu [19]. For the extraction of the phenolic component, the fat free sample was boiled with 50 ml of ether for 15 minutes. To this, 5.0 ml of the extract, 10.0 ml of distilled water, 2.0 ml of ammonium hydroxide and 5.0 ml of concentrated amyl alcohol were added. The sample was left to react for 30 minutes for color development. The absorbance of the solution was read using a spectrophotometer (at 760 nm) wavelength. The results were expressed as mg of phenol/ gm of dried sample.

2.6 Estimation total Flavonoid determination of plant specimens

The total flavonoid content in the sample was estimated by the method of Chang [20]. The extract prepared for the estimation of total phenolics was used as sample for this assay. Sample at 0.25 ml was diluted to 1.25 ml of distilled water and then sodium nitrite (5%) at 75 µl, 0.15 ml of aluminium chloride solution, 0.5 ml of 0.1M NaOH was added to the sample simultaneously, after 5 minutes the sample one more added with 2.5 ml distilled water. The final solution was mixed well and the absorbance was read (at 510 nm) in comparison with standard quercetin at 5-25 µg concentration. The results are expressed as mg of flavonoids as quercetin equivalent/ g of dried sample.

2.7 Test for Insect Growth Regulatory Activity (IGR)

The methanolic extract of *A. alnifolia* and *V. negundo* were tested for freshly cached early L1 instar of *A. aegypti* larvae up to adult development followed by the standard procedure [21]. Tests of the Plant products for development activity were done at different concentration ranging from 25 to 500 ppm. For each test we made three replicates for each concentration was set up. Larval stages were monitored till to adult emergence and were provided with larval food (dog biscuit and yeast 3:1 ratio). The control was set by control I and control II. The control I was set without the plant extract concentration and control II was set with synthetic IGRs (2.5% Methoprene) compound. The larval, pupal duration and adult emergence were observed in experimental set up and in control at 24h intervals. The dead larvae were daily removed and counted. The developmental stages of larvae (L1 to L4 Instar), pupae and adults were recorded. The emergence inhibition concentration (EI_{50}), (EI_{90}) was derived from the experimental data through probit analysis [22].

2.8. Statistical Analysis

The DMRT and t- test were applied in this experimental data. The analytical data together with tables are presented in appropriate places in the report. SPSS software package was used for computing all the data including probit analysis, SE and mean of the sample.

3. Results

Table 3 showed the effect of *A. alnifolia* on IGR activity against *A. aegypti*. One percent concentration of *A. alnifolia* treated *A. aegypti* extended the larval (L1 to L4 instar)

duration and adult emerged on 17.12 (mean) day and at 500 ppm concentration, the larvae to adult duration was extended on 30.48 (mean) days; The emergence inhibition EI_{50} and EI_{90} values were observed to be 0.45 and 4.16 respectively. The larval, pupal and adult emergence duration was greatly extended in the treated group than in control group (without plant ingredient). We set two control groups; control 1 was set without plant ingredient and control 2 was set synthetic IGR compound (concentration of Methoprene). The control 1 (without plant ingredient) and control 2 (synthetic IGR) group were compared with experimental setup. Methoprene 50 ppm (control 2) leads to 15% adult emergence, whereas, combined plant extracts at 50 ppm leads to 10% adult emergence was observed (Table 5). The larval and pupal prolongation (days) was noted control was 12.58, synthetic IGR was 26.17 and combined plant extracts was 24.55 days at 50 ppm and larval and pupal prolongation also increased with increasing concentration. The DMRT was significant different at 5% level.

Table 4 provides the effect of *V.negundo* on IGR activity against *A. aegypti*. The adult emergence rate was 18.31 days at 25 ppm concentration and larval to adult emergence duration was extended to 23.98 days at 500 ppm. The emergence inhibition EI_{50} (EI_{90}) values are 0.51 (3.92). Similarly, table 3 showed the effect of *A.alnifolia* on IGR activity of *A.aegypti*. The adult emergence rate was 17.12 days at 25 ppm concentration and larval to adult emergence duration was extended to 25.48 days at 500 ppm. The emergence inhibition EI_{50} (EI_{90}) values are 1.45 (4.16).

The IGR activity was generally apparent after 72 hrs of treatment and exhibited by the appearance of larval- pupal intermediate and pupal-adult intermediate of various stages. A variety of delayed toxic effect was observed and recorded in different categories according to the stage of larvae to pupae moulting (reached when death occurred. Plants leaf extract showed larval-pupal malformation resulting in death at an early stage of pupation and at late stage. Morphological deformities due to leaf extracts resulted in appearance of larval-pupal malformation at various, death has occurred at an early stage of pupation. The abdomen was retracted to at least halfway along the larval abdominal skin and adopted the characteristic pupal shape. The leaf extract of *A.alnifolia* and *V.negundo* seems to have more IGR activity then in synthetic compound and it's caused more profound harmful effects in larvae and pupae during moulting.

4. Discussion

Combined effect of *A. alnifolia* and *V. negundo* had shown synergistic effect and also observed higher mortality then Methoprene was evident in this experiment. *V. negundo* extract showed promising IGR activity against *A. aegypti* and remarkable activity against dipteran and lepidoptera insects [15]. The extraction of *V. negundo* and *A. alnifolia* leaves offered IGR activity for 72 h against mosquito such as *A. aegypti*. In our study interesting to note that there was apparent difference in the nature and extent of deleterious effects on growth, moulting and metamorphosis. Five percent concentration of combined plant extracts showed the death occurred in mostly larval stage whereas low concentration (25 ppm) stimulated the morphological abnormalities in the larvae, pupae and adult. Some scientists reported that azadirachtin from chinaberry and neem has an effect on growth disruption [23, 24]. It was reported that the treatment of larvae of *A. aegypti* and *C. quinquefasciatus* with ethanol extracts of karanja (*Pongamia glabra*) seed coat significantly increased larval mortality and developmental period extended with increasing concentration [25]. *Mentha piperita* was found to be highly effective in controlling the larvae of *C. quinquefasciatus* [26]. A piperidine alkaloid from *Piper longum* fruit was found to be active against mosquito larvae of *C. pipiens* [27]. A butanol extract of soapberry plant, *Phytolacca dodecandra*, was very toxic to 2nd and 3rd instar larvae of *A. aegypti*, *C. pipiens* and *An. quadrimaculatus*; but the eggs and pupae were unaffected, and adults died only after ingestion of the 5% concentrated extract [28]. Similarly, the larvicidal activity of commercial bark saponin extract from *Quillaja saponaria*, was toxic to the 3rd and 4th instar larvae of *Ae. Aegypti* and *C. quinquefasciatus*, but did not affect egg hatching ability in either species [29].

Over one thousand plant species contain bioactive substance with many of these containing phytoecdysones, phytojuvenoids and anti-juvenile hormones, which act as IGRs [30]. The use of ajugarins, isolated from *Ajuga remota* by Marcard [31] against mosquitoes is an example. The previous studies have shown insect growth regulatory activity of crude petroleum ether–acetone extracts of 25 angiosperm plants on *Cx. quinquefasciatus* larvae [32]. In this case, 40% of the extracts possessed promising bioactivity. But progress is slow and no promising botanical IGRs have been commercialized to replace current synthetic IGRs such as methoprene.

Despite toxic effects and reductions in adult emergence, some phytochemicals such as those from *Annona squamosa* do not alter the larval developmental period. Many botanicals exhibit combined effects on the developmental period and adult emergence which occasionally extend to the progeny of exposed larvae. Decreasing mosquito fecundity caused by exposure of larvae to plant extracts has been studied by few researchers. More commonly, phytochemicals can produce morphological abnormalities indifferent developmental stages of mosquitoes. Abnormalities, such as lacking of melanization in larval and pupal stages, dead larval-pupal intermediate stage mostly with the head of a pupa and the abdomen of a larva, dead adults with folded wings in the pupal exuvium and emerged adults unable to escape the pupal exoskeleton, and half-ecdysis adults, indicate a metamorphosis-inhibiting effect of the plant extract, which is probably due to disturbance of hormonal control and/or interference in chitin synthesis during the molting process [33].

In the present study plant extracts toxicity is due to active chemical present in the leaves of *Acalypha alnifolia* phytochemicals such as saponins, flavonoids, terpenes and acalyphin and *Vitex negundo* contain active phytochemicals like, alkaloids, flavonoids, saponins, phenols, terpenoids, anthr aquinones. Sublethal concentrations of botanical extracts greatly affect mosquito development and this effect is dose dependant [34, 35, 36] and factors that affecting the bioactivity of essential oils include plant species (variety), cultivating conditions, maturation of harvested plants, plant storage, plant preparation and methods of extraction [37, 38].

In contrast to previous study against *A. Aegypti* [36, 39] these mixtures were highly active against *A. caspius* whereas white camphor and wintergreen mixture largely reduced emergence of *A. caspius* mosquitoes whilst cinnamon and wintergreen mixture completely stopped mosquito emergence. Similarly, a mixture of the peel oils extract of three citrus species (lemon, orange, and bitter orange) was much more effective than for the peel oils extract for the individual species [40]. Generally speaking, mixtures are always stronger and better than their single oils [34, 35, 38] and this strong activity is due to synergistic action of these mixtures. The mechanism of synergism is not known, but it might be due to phytochemicals inhibiting the

ability of mosquito larvae to employ detoxifying enzymes against synthetic chemicals [41].

Synergism between synthetic insecticides and phyto-chemicals appears to be more common than between different phytochemicals although this may be the result of increased focus in this area. In an attempt to explain synergistic activity involving phytochemicals, Thangam and Kathiresan [41] surmised that synergism may be due to phytochemicals inhibiting a mosquito larva's ability to employ detoxifying enzymes against synthetic chemicals. Mixtures of plant extracts with compounds showing synergistic or potentiating interactions between them are considered to have a higher and longer-lasting effect [42]. Identifying these synergist compounds within mixtures may lead to the development of more effective mosquitocides as well as the use of smaller amounts in the mixture to achieve satisfactory levels of efficacy.

The results of our study indicate that further evaluation of the combined *A. alnifolia* + *V. negundo* extract as a mosquito IGRs is warranted. Treatments with biopesticides should be timed in such a manner that they provide maximum population control of pests while having a minimum sub lethal effect on non-target organism) of the beneficial communities. Thus the timing of treatments depends on the knowledge of the biology of targets as well as non-target species. The application of plant compound to kill larva, pupa and adult dengue vector could significantly reduce parasite transmission and therefore lead to reduced dengue risk.

A large number of different plant species representing different geographical areas around the world have been shown to possess phytochemicals that are capable of causing a range of acute and chronic toxic effects. Not only have many botanical extracts been shown to cause remarkable deleterious effects on the fecundity and hatchability of mosquito eggs, but they have been shown to have significant and promising smoke repellent properties that include growth regulating effects. Any one of these effects taken alone is usually not impressive, but the combined IGR effects possessed by many phytochemicals can produce impressive results. When joint-action is considered, the application possibilities for vector control increase significantly. Indeed, joint-action may well prolong the usefulness of synthetic insecticides that will eventually be unusable due to resistance.

Table 1: Qualitative phytochemical analysis of leaf extracts of *Acalypha alnifolia* and *Vitex negundo*

S. No.	Test	*Acalypha alnifolia*		*Vitex negundo*	
		Methanolic	Aqueous	Methanolic	Aqueous
1.	Test for carbohydrates a. Molisch's test	+	+	+	+
2.	Test for Glycosides a. Keller-Killiani test	+	+	+	+
3.	Test for Saponins a. Foam test	+	-	+	-
4.	Test for Alkaloids a. Mayer's test	+	+	+	+
5.	Test for Flavonoids a. Alkaline reagent test	+	+	+	+
6.	Test for Phenolics and Tannins b. Test for Tannins	+	+	+	+
7.	Test for Phytosterols and Triterpenoids a. Leiberman-Bucharat test	+	+	+	-
8.	Test for fixed oils and fats a. oily spot test	-	-	+	-

(+) Present, (-) Absent

Table 2: Quantitative phytochemical analysis of leaf extracts of *Acalypha alnifolia* and *Vitex negundo*

sample	Methanolic*			Aqueous*		
	Phenol	Flovonoid	Protein	Phenol	Flovonoid	Protein
A. alnifolia	16.27 ± 0.05	6.00 ± 0.04	15.13±0.08	15.12±0.48	3.45±0.44	11.97±0.32
V. negundo	17.21 ± 0.08	8.00 ± 0.06	16.11±0.07	14.32±0.54	4.45±0.38	10.90±0.22

Expressed in terms of standards *mg/ml

Table 3: Effect of *Acalypha alnifolia* on the biology of dengue vector, *Aedes aegypti*

Conc. (ppm)	Mean duration of each instars (Days) Mean ± SE (100 larvae / expt.)					Total Number of days	Mortality (%)	Emergence (%)	EI_{50} EI_{90} (%)
	L_1- L_2	L_2-L_3	L_3-L_4	Pupa	Adult				
25	2.19±0.3^{c}	3.23±0.31^{e}	6.18±0.31^{d}	2.64±0.31^{d}	3.77±0.43^{d}	17.12±1.6^{c}	72	28	
50	2.12±0.3^{c}	4.16±0.31^{cd}	7.16±0.31^{bc}	3.33±0.31^{c}	4.33±0.31^{c}	21.31±1.5^{d}	83	17	
100	3.14±0.3^{ab}	4.83±0.31^{c}	7.16±0.43^{b}	3.50±0.4^{c}	4.5±0.53^{c}	23.65±1.9^{c}	85	15	
250	3.33±0.3^{b}	6.16±0.31^{b}	6.83±0.31^{de}	5.33±0.31^{a}	6.33±0.31^{a}	27.98±1.5^{b}	96	4	**1.45**
500	3.16±0.3^{a}	6.33±0.31^{a}	7.33±0.31^{a}	4.83±0.5^{b}	5.83±0.31^{b}	25.48±1.7^{a}	98	2	**(4.16)***
Control 1	1.12 ±0.3^{d}	2.14 ±0.3^{f}	3.13 ±0.3^{f}	4.6 ±0.3^{b}	1.5 ±0.3^{e}	12.51 ±1.5^{f}	9	91	
***Control 2**	**3.47±0.2^{b}**	**6.45± 0.12^{b}**	**6.81±0.5^{de}**	**5.41±0.6^{a}**	**6.61±0.8^{a}**	**26.17±1.14^{b}**	**85**	**15**	

Within the column means followed by the same letter(s) are not significantly different at 5% level of DMRT

L_1, L_2, L_3, L_4 – Larval Instars

EI – Emergency Inhibition

Control 1= without plant extracts

Control 2 = Methoprene (synthetic IGR compound)

Table 4: Effect of *Vitex negundo* on the biology of dengue vector, *Aedes aegypti*

Conc. (ppm)	Mean duration of each instars (Days) Mean ± SE (100 larva/ expt.)					Total Number of days	Mortality (%)	Emergence (%)	EI_{50} EI_{90} (%)
	L_1- L_2	L_2-L_3	L_3-L_4	Pupa	Adult				
25	1.13±0.6^{c}	4.11±0.21^{b}	6.56±0.21^{b}	3.23±0.31^{c}	3.33±0.21^{b}	18.31±1.24^{d}	68	32	**1.51 (3.92)***
50	2.66±0.5^{b}	3.73±0.3^{c}	7.36±0.43^{a}	3.40±0.4^{c}	4.5±0.5^{a}	21.65±1.73^{c}	70	30	
100	2.18±0.6^{b}	2.40±0.31^{d}	6.23±0.31^{b}	2.56±0.31^{d}	2.16±0.21^{c}	15.41±1.44^{e}	75	25	
250	3.26±0.5^{a}	5.53±0.1^{a}	6.53±0.31^{b}	5.63±0.5^{a}	4.83±0.21^{a}	25.58±1.5^{a}	85	15	
500	3.43±0.4^{a}	5.22±0.1^{a}	5.63±0.31^{c}	5.43±0.41^{a}	3.33±0.21^{b}	23.98±1.42^{b}	92	8	
Control 1	1.16 ±0.3^{c}	2.16 ±0.3^{d}	3.16 ±0.3^{d}	4.8 ±0.3^{b}	1.3 ±0.3^{d}	12.58 ±1.5^{f}	10	90	
***Control 2**	**3.47±0.2^{b}**	**6.45±0.12^{b}**	**6.81±0.5^{de}**	**5.41±0.6^{a}**	**6.61±0.8^{a}**	**26.17±1.14^{b}**	**85**	**15**	

Within the column means followed by the same letter(s) are not significantly different at 5% level of DMRT
L_1, L_2, L_3, L_4 – Larval Instars
EI – Emergency Inhibition
Control 1= without plant extracts
Control 2 = Methoprene (synthetic IGR compound)

Table 5: Combined effect of *A. alnifolia* and *V. negundo* on the biology of dengue vector, *Aedes aegypti*

Conc. (ppm)	Mean duration of each instars (Days) Mean ± SE (100 larva / expt.)					Total Number of days	Mortality (%)	Emergence (%)	EI_{50} EI_{90} (%)
	L_1- L_2	L_2-L_3	L_3-L_4	Pupa	Adult				
12.5	4.11±0.6^{b}	3.2±0.22^{d}	5.13±0.5^{c}	4.60±0.53^{cd}	4.26±0.23^{c}	21.18± 2.16^{d}	83	17	**2.49 (5.24)***
25	4.32±0.4^{b}	4.13±0.3^{c}	7.16±0.21^{a}	3.31±0.34^{d}	5.13±0.37^{b}	24.34 ± 1.8^{c}	88	12	
50	3.15±0.4^{c}	4.85±0.3^{c}	7.60±0.30^{a}	3.30±0.7^{d}	5.6±0.54^{b}	24.55± 2.33^{c}	90	10	
125	3.30±0.5^{c}	6.14±0.3^{b}	6.82±0.32^{b}	6.33±0.31^{b}	8.35±0.31^{a}	27.90 ±1.25^{b}	95	5	
250	5.15±0.6^{a}	7.30±0.3^{a}	7.31±0.33^{a}	7.83±0.5^{a}	8.81±0.41^{a}	28.61±1.27^{a}	99	1	
Control	1.16 ±0.3^{d}	2.18 ±0.4^{e}	3.16 ±0.3^{d}	4.8 ±0.3^{c}	1.3 ±0.3^{d}	12.58±1.5^{e}	10	90	
***Control 2**	**3.47±0.2^{b}**	**6.41±0.12^{b}**	**6.81±0.5^{de}**	**5.41±0.6^{a}**	**6.61±0.8^{a}**	**26.17±1.14^{b}**	**85**	**15**	

Within the column means followed by the same letter(s) are not significantly different at 5% level of DMRT
L_1, L_2, L_3, L_4 – Larval Instars
EI – Emergency Inhibition
Control 1= without plant extracts
Control 2 = Methoprene (synthetic IGR compound)

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