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**Research Article**

# *AEGLE MARMELOS* AS AN EFFECTIVE AGENT AGAINST THE FOURTH INSTAR LARVAE OF *CULEX QUINQUEFASCIATUS* (DIPTERA: CULICIDAE)

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**Abstract**: Effect of *Aegle marmelos* extract was evaluated on the fourth instars of *Culex quinquefasciatus.* Larval mortality was observed at higher doses ranging from 80ppm-120ppm in petroleum ether, chloroform and ethanol extracts of leaf and seed. However, 100% mortality was recorded at 48h in ethanol leaf extract.

Key**words:** *Aegle marmelos*, chloroform extract, *Culex quinquefasciatus***,** Larval mortality

## Introduction

Rapid urbanization and industrialization has led to unplanned town expansions with no sanitary facilities and proper wastewater disposal arrangements. This results in the creation of water bodies which are highly conducive for the breeding of mosquitoes. Use of biologically active plant materials with insecticidal properties has gained great impetus as they are biodegradable and relatively safe to human beings and non-target organisms present in the environment. Owing to this fact, an extensive survey of the flora was undertaken to search for potential plant extracts that could be used for the effective control of mosquitoes.

Petroleum ether extract of the leaves and tubers of *Curcuma raktakanda* exhibited toxicity against the early fourth instars of *Culex quinquefasciatus, C. sitiens, Aedes aegypti* and *A. stephensi*.[1] The larvicidal effects of essential oils extracted from the leaves of four plants namely, *Tagetes erecta, Ocimum sanctum, Mentha piperita & Murraya koenigii* were evaluated against *A. stephensi, C. quinquefasciatus* and *A. aegypti* where *T. erecta* was found to be most toxic against *C. quinquefasciatus* followed by *A. stephensi* and *A. aegypti*.[2]

Methanol fruit extract of *Piper longum* (10 mg/ml) was active against larvae of *C. pipiens pallens* after 24 h. A piperidine alkaloid, piperenon alanine, was responsible for this activity with 24 h $LD_{50}$ value of 21 mg/litre.[3] Insecticidal activity of 11 extracts from nine South American medicinal plants against *A. aegypti* larvae.. Eight of the 11 plant extracts studied showed toxicity against the *A. aegypti* larvae ($LC_{50}$ < 500 µg/ml).[4]The methanol extracts of the seed kernels of *Neem, Pongamia* and *Leucas aspera* were tested against the late third instar larvae of *C. quinquefasciatus* to observe the mortality and behavioral responses of the larvae.[5]

## Materials and Methods

### Laboratory culture of larvae

Hay infusion method was adopted for culturing mosquito larvae. Hay was taken, cut into small pieces and boiled in 5 litres of water for 20 minutes. After cooling, this water was poured into buckets and kept in different areas where mosquitoes were abundant. After one or two days eggs were laid by female mosquitoes in clusters forming an egg raft. The egg rafts were collected and maintained in the laboratory. The third instar larvae were collected, reared in enamel trays containing culture medium and provided with powdered dog biscuits and yeast in the ratio of 3:1 as the nutrient source. Immediately after moulting, the fourth instar larvae were introduced into beakers containing 200 ml of water and used for the bioassay studies.

### Preparation of leaf and seed extracts

Fresh leaves and seeds were collected (Fig 1), washed in water and air dried under shade. Dried leaves and seeds were powdered using an electric pulverizer. 10g of the seed powder was weighed and subjected to extraction with 500 ml of solvents such as petroleum ether, chloroform, and ethanol for 8h using a Soxhlet apparatus. Petroleum ether (60-80°C) extraction was followed by chloroform and ethanol extraction. The leaf and seed extract thus obtained was concentrated by distillation and dried by evaporation at 40°C.

### Bioassay studies

A pilot study was conducted to find out the effective doses of both leaf and seed extracts that produced mortality in larvae of *C. quinquefasciatus.* After determining the effective doses, the detailed investigations were undertaken. Concentrations ranging from 20 – 120 ppm which produced larval mortality at or after 24 h treatment were used for the bioassay studies.

**Experimental design**

The experiment was laid down in a Completely Randomized Design (CRD). The experimental set up consisted of two treatments, each with three replicates, and one set for leaf extract and another for seed extract. Twenty newly emerged fourth instar larvae of *C. quinquefasciatus* were introduced into the beakers for bio-assay studies. Control group with three replicates were also maintained simultaneously. 250 ml beakers each containing 200ml of water were used for the experiments. The following parameters like larval mortality at 24, 48, 72 and 96 h of treatment, larval and pupal behaviour, pupal mortality, total developmental duration, morphological changes were observed to assess the effective dose. Incidences of malformations and adult emergence were also observed.

**Test for larvicidal activity**

The larval mortality in both treatment and control was recorded at 24h of treatment and the percentage of mortality was calculated using Abbott's formula.[6]

$$\text{Corrected Mortality \%} = \frac{\text{Mortality in treatment (\%)} - \text{Mortality in control (\%)}}{100 - \text{Mortality in control (\%)}} \times 100$$

**Statistical analysis**

The data on bioassay studies were subjected to one way ANOVA as described by (Panse & Sukhatme, 1985). Further $LC_{50}$, $LC_{70}$, $LC_{90}$, Regression equation and 95 percent Upper Confidence Limit (UCL) and Lower Confidence Limit (LCL) and Chi-square values were calculated by using probit analysis.[7]

**RESULTS**

***Leaf extracts***

A dose dependent increase in larval mortality was observed in all the three treatments (Table I). The mortality percentage recorded at 24 h was 45% in 120 ppm of chloroform extract followed by 40% in 120 ppm of ethanol extract. At 48 h, 50% and >50% larval mortality was observed in higher doses ranging from 80 ppm – 120 ppm in all the three extracts. However, acute toxicity resulting in 100% mortality was recorded at 48 h in ethanol extract. Similar acute toxicity was observed in petroleum ether and chloroform extracts at 72 h in higher concentrations.

The result of studies on lethal concentrations is presented in Table II. The 24 h $LC_{50}$ ranged from 159.40 ppm 389.43 ppm. High larvicidal activity was found in ethanol extract (159.40 ppm) at 24 h, regression equation being Y=1.64+1.52X, 95% confidence limit UCL $LC_{50}$ was 217.86 ppm and LCL $LC_{50}$ was 116.62 ppm. However, 96h $LC_{50}$ value was minimum in chloroform extract (2.13 ppm) with the regression equation of Y=4.61+1.19X and 95% confidence limits UCL $LC_{50}$ 26.64 ppm and LCL $LC_{50}$ 0.17 ppm.

No pupation was seen at higher doses ranging from 80 ppm to 120 ppm in all the three leaf extracts while in lower concentrations pupation ranging from 5 to 35% was observed. Pupal mortality ranging from 5 to 30% was recorded in lower concentrations. Minimum adult emergence (5%) was observed in 40 ppm concentration in both chloroform and ethanol extracts. The highest percent of adult emergence was 20% in 20 ppm petroleum ether extract. Owing to 100% larval mortality in all higher concentrations, no adult emergence could be observed. At lower concentration such as 60 ppm despite 5% pupation there was no adult emergence due to pupal mortality.

**Seed extracts**

Larval mortality of 50% and >50% was recorded at 48 h in higher concentrations of all the three seed extracts. By 72 h, 100% mortality was obtained in 100 ppm and 120 ppm treatments of petroleum ether and chloroform extracts and in 120 ppm of ethanol extracts(TableII). Higher concentrations of all the three extracts produced 100% mortality at 72 h.

The 24 h $LC_{50}$ value was comparatively less in ethanol treatment (492.35 ppm) than in petroleum ether (1150.18 ppm) and chloroform (797.84 ppm) showing the effectiveness of ethanol extract over that of petroleum ether and chloroform, 24 h regression equation for ethanol extract was Y=2.30+1.00X and 95% confidence limits were UCL $LC_{50}$ 1361.40 ppm and LCL $LC_{50}$ 178.06 ppm (Table IV).

Owing to 100% mortality, no pupation and no adult emergence were recorded in higher concentrations ranging from 60 – 120 ppm in all the three treatments. Of the three extracts, pupation was minimum in 40 ppm chloroform seed extract (5%) and pupal mortality ranging from 5 to 15% was observed in lower concentrations of all the three extracts. Adult emergence was totally inhibited in all the concentration of chloroform seed extracts while 10 to 15% adult emergence was recorded in lower concentrations of petroleum ether and ethanol extracts.

**Abnormal Formations**

Abnormalities were observed in larval, pupal and adult stages in both leaf and seed treatments. The dead larvae were found to be darker in colour and thoracic cuticle was split on the dorsal side. Abnormalities observed in pupal stages were the formation of non-melanized, a few partially melanized, and many hyper melanized pupae (Plate I). Such abnormalities during metamorphosis could be due to an imbalance in the hormonal activity.

Larval–pupal intermediates were recorded in leaf and seed extracts and Pupal – adult intermediates were observed in leaf extracts (Plate II). Lengthening of larval period was observed in the chloroform extract of leaf and petroleum ether of seed. The larvae remained as supernumerary larvae without undergoing further metamorphosis (Plate II). Only few of the pupae could attain adulthood and adults normally got drowned in water since they were unable to expand their wings. Partially emerged adults attached with pupal exuviae were observed in the petroleum ether extract of seed.

**Table-I: Lethal concentration of leaf extracts of *Aegle marmelos* against *Culex quinquefasciatus***

| Solvents used | Hours | $LC_{50}$ (ppm) | $LC_{70}$ (ppm) | $LC_{90}$ (ppm) | Regression equation | 95% confidence limits | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | UCL (ppm) | | LCL (ppm) | |
| | | | | | | $LC_{50}$ | $LC_{90}$ | $LC_{50}$ | $LC_{90}$ |
| Petroleum ether | 24 | 389.43 | 1259.38 | 6854.57 | Y = 2.33 + 1.03X | 918.73 | 59606.98 | 165.07 | 788.25 |
| | 48 | 64.51 | 139.35 | 423.59 | Y = 2.16 + 1.57X | 75.12 | 717.33 | 55.4 | 250.14 |
| | 72 | 25.24 | 48.37 | 123.69 | Y = 2.40 + 1.86X | 31.66 | 180.33 | 20.11 | 84.85 |
| Chloroform | 24 | 211.49 | 609.5 | 2809.04 | Y = 2.35 + 1.14X | 351.79 | 12447.79 | 127.15 | 633.91 |
| | 48 | 40.64 | 75.5 | 184.64 | Y = 1.86 + 1.95X | 61.74 | 409.9 | 26.75 | 83.17 |
| | 72 | 9.99 | 35.59 | 222.59 | Y = 4.05 + 0.95X | 29.06 | 1011.42 | 3.44 | 48.99 |
| Ethanol | 24 | 159.4 | 352.04 | 1105 | Y = 1.64 + 1.52X | 217.86 | 2663.26 | 116.62 | 458.47 |
| | 48 | 58.38 | 138.82 | 484.77 | Y = 2.54 + 1.39X | 73.55 | 1303.11 | 46.34 | 18.34 |
| | 72 | 22.82 | 41.14 | 96.33 | Y = 2.22 + 2.05X | 28.72 | 146.11 | 18.13 | 63.52 |

UCL – Upper Confidence Limit LCL – Lower Confidence Limit

**Table-II: Lethal concentration of seed extracts of *Aegle marmelos* against C*ulex quinquefasciatus***

| Solvents used | Hours | $LC_{50}$ (ppm) | $LC_{70}$ (ppm) | $LC_{90}$ (ppm) | Regression equation | 95% confidence limits | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | UCL (ppm) | | LCL (ppm) | |
| | | | | | | $LC_{50}$ | $LC_{90}$ | $LC_{50}$ | $LC_{90}$ |
| Petroleum ether | 24 | 1150.18 | 7055.95 | 96780.93 | Y=2.96+0.67X | 8802.8 | 1.55 | 150.28 | 603.68 |
| | 48 | 56.77 | 177.36 | 918.41 | Y=3.14+1.06X | 70.89 | 2582.46 | 45.46 | 326.62 |
| | 72 | 19.19 | 36.11 | 89.96 | Y=2.55+1.91X | 25.54 | 122.37 | 14.42 | 66.14 |
| Chloroform | 24 | 797.84 | 150055.5 | 102749.3 | Y=3.24+0.61X | 5094.54 | 2.02E+07 | 124.95 | 522.54 |
| | 48 | 50.01 | 150.24 | 735.24 | Y=3.13+1.10X | 62.53 | 1850.1 | 39.99 | 292.19 |
| | 72 | 13.74 | 35.99 | 144.52 | Y=3.57+1.25X | 139.14 | 1900.27 | 1.36 | 10.99 |
| Ethanol | 24 | 492.35 | 16.4 | 9315.73 | Y=2.30+1.00X | 1361.4 | 107507.7 | 178.06 | 807.22 |
| | 48 | 73.48 | 188.11 | 730.7 | Y=2.60+1.28X | 88.99 | 1624.66 | 60.66 | 328.64 |
| | 72 | 25.02 | 46.55 | 109.02 | Y=2.11+2.05X | 31.55 | 139.84 | 21.13 | 84.99 |

UCL – Upper Confidence Limit LCL – Lower Confidence Limit


**Plate-I**

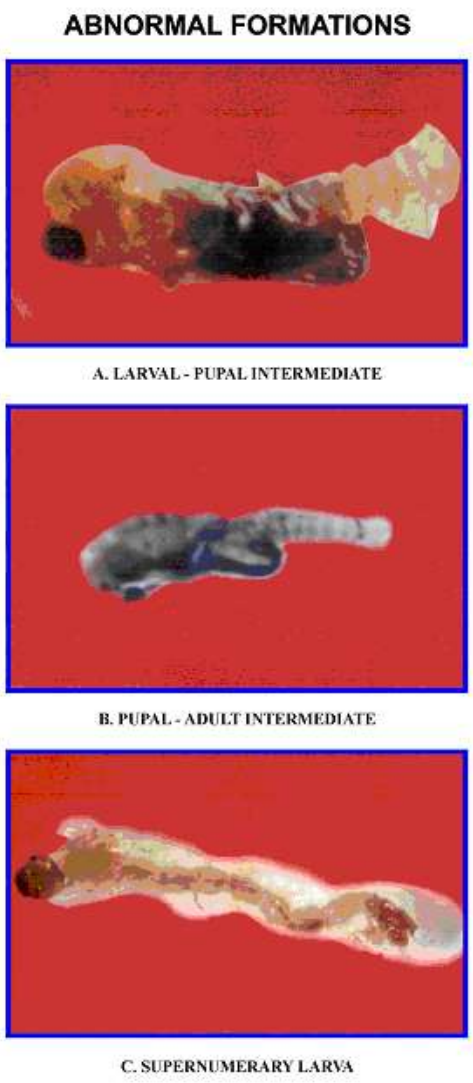


**Plate-II**

**Discussion**

Plants are rich sources of bioactive organic chemicals and synthesize a number of synthetic metabolites which serve as insecticides, antifeedants, oviposition-deterrents, repellents, growth inhibitors, juvenile hormone mimics, moulting hormones, antimoulting hormones as well as attractants. They offer an advantage over synthetic pesticides as they are less toxic, less prone to the development of resistance and easily biodegradable.[8] Therefore the search for insecticides of plant origin has gained great impetus in recent times.

Mosquito control is facing a threat due to the emergence of resistance to conventional synthetic insecticides warranting either counter measure or development of newer insecticides.[9] Botanical insecticides may serve as suitable alternative to synthetic insecticides in future as they are safe, easily degradable and are readily available in many areas of the world. Though several plants from different families have been reported to have mosquitocidal activity, only a very few botanicals have moved from the laboratory to field use, like neem based insecticides, which might be due to the light and heat instability of phytochemicals compared to synthetic insecticides.[10] The present investigation is an attempt to screen effective botanicals for the management / control of *C. quinquefasciatus*. Petroleum ether extract of both leaf and seed of *A. marmelos* was found to be the most effective one.

Acute toxicity causing 100% larval mortality was recorded in their studies using *Acalypha indica,* using *Pergularia extensa, A. mexicana* and *Withania somnifera* and using *Acacia nilotica* and *Citrullus colocynthus* against the larvae of *C. quinquefasciatus.* In the present observation 100% larval mortality was observed in higher concentrations of petroleum ether, chloroform and ethanol leaf extracts of *A. marmelos.* In the present study, 100% mortality was obtained in all the concentrations of chloroform extract and high percentage of mortality in petroleum ether and ethanol extracts.[11, 12, 13]

The effect of secondary metabolites, cardenolides isolated from *Calotropis gigantean* on *A.stephensi*, *A.aegypti* and *C.quinquefasciatus*. The LC50 value for *C.quinquefasciatus* was found to be 5mg/l at 24h. In the present study, 100% mortality was obtained in all the concentrations of chloroform extract and high percentage of mortality in petroleum ether and ethanol extracts. Similarly 100% larval mortality was recorded in higher concentrations of all the three solvents. Considering total mortality and adult emergence, petroleum ether and chloroform seed extracts of *A. marmelos* were very effective.[14]

**Conclusion**

From this study it could be concluded that the presence of phytochemicals in the leaf and seed of *Aegle marmelos* might be responsible for its larvicidal activity. The results of this experiment indicate that extracts of Aegle *marmelos* can be used as an environment- friendly sustainable insecticide for the control of mosquitoes.

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