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# LARVICIDAL POTENTIAL OF *ZANTHOXYLUM RHETSA* (ROXB.)DC. CARPEL OIL AGAINST *AEDES AEGYPTI* (L.) (DIPTERA: CULICIDAE).

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## ABSTRACT

Mosquitoes plays a vital role in transmitting number of diseases that are harmful to human beings causing malaria, filariasis and viral diseases, such as Japanese encephalitis, dengue, and yellow fever. Mosquitoes have become resistant to traditional chemical pesticides and there is growing concern about the potential health and environmental degradation due to these products hence efforts have been taken to control the mosquito using biological substances. In the present study plant oil was used to control the mosquito larvae. The efficacy of different concentration of the plant oil viz 100.0 ppm, 200.0 ppm, 300.0 ppm, 400.0 ppm, 500.0 ppm and 1000 ppm for their larvicidal activity against *Aedes aegypti* (l.) was tested. However, the maximum larval mortality in minimum time was detected in 1000 ppm concentration, However, the highest concentration studied for the effective of extracted from Zanthoxyulum rhetsa Roxb Dc carpels oils was 500.0 ppm (Table 9, Graph 9 & Table 10, Graph 10) for dried carpel oils and 400 ppm (Table 7, Graph 7& Table 8 Graph 8) for fresh carpel oil. The results revealed that larvicidal properties of *Zanthoxylum rhetsa* (Roxb.)DC fruits encourages further effort to investigate the bioactive compounds that might possess good larvicidal properties when it will be isolated in pure form. These results obtained are promising in thus creating new effective and affordable approach to the control of vector mosquitoes.

**KEYWORDS:** *Zanthoxylum rhetsa* (Roxb.)DC, larvicidal, dengue vector mosquito.

## INTRODUCTION

Plants have been a rich source of alternative agents for control of mosquitoes, because they possess bioactive chemicals, which are eco-friendly (Sukumar K, 1991). For many centuries traditionally plant based products have been used in human communities as insecticides. Several secondary metabolites present in plants serve as a defence mechanism against insect attacks. Recently natural products of plants are under investigation for the bioactive chemical against insects due to their excellent properties like cheap, availability and renewable nature and their environmental safety nature (Saxena and Thikku, 1988 and 1990) presence of an array of characters like juvenile hormone mimics, antifeedants, oviposition deterrents, moulting hormones, repellents, growth inhibitors, antimoulting hormones as well as attractants. Nearly 2000 species of terrestrial plants have been reported for their insecticidal properties (Feinstein L, 1952).

Mosquitoes are vector causing malaria, yellow fever, filariasis Japanese encephalitis and chikungunya. Synthetic insecticides are available for controlling the mosquitoes is use of. Eventually mosquitoes develop genetic resistance to synthetic insecticides (Wattal BL et.al., 1981) and even to biopesticides such as *Bacillus sphaericus* (Tabashnik B. E, 1994). The need of the hour is to find plant based insecticides as alternatives to the synthetic insecticides which is more potent and low-cost. Mosquito control is essential to prevent proliferation of mosquito borne diseases and to improve quality of environment and public health.

The eradication of diseases such as malaria, filariasis, dengue, Japanese encephalitis, etc. largely relied on interruption of disease transmission cycle which can be controlled by either targeting the mosquito larvae by spraying on stagnant water breeding sites or by killing the adult mosquitoes by use of insecticides (Joseph et al., 2004).

An alternative approaches under the biological control programme is to explore the floral biodiversity and use safer insecticides of botanical origin for mosquito control. Conventional insecticides are based on a single active ingredient, plant derived insecticides comprise of botanical blends of bioactive compounds which act concertedly on both behavioural and physiological processes there being a little chance of pests developing resistance. Identifying bio-insecticides that are efficient and suitable and adaptive to ecological conditions, is imperative for effective vector control management. Botanicals have widespread insecticidal properties and will work as a new weapon in the arsenal of synthetic insecticides and be an alternative product to fight against mosquito borne diseases.

Larvicidial is a very successful way of reducing mosquito population in their breeding places and is the most effective way to fight with this mosquito importunation. For the last 30 years synthetic insecticides have been used as larvicide. (Chavasse and Yap, 1997) are toxic to human, animal and plant life. The need for development of effective insecticides considering the toxicity problems and insect resistance.

There has been a Search for eco-safe, low cost and a highly potential insecticide for the control of mosquitoes which needs the preliminary screening of plants to evaluate their insecticidal activities. Recent research has proved that effectiveness of plant derived compounds that have potential larvicidal activity. The present investigation was carried out to validate the larvicidal potential of *Zanthoxylum rhetsa* (Roxb.)DC medicinal plants against *Aedes aegypti* (l.) larvae.

**Nature of the active Plant ingredients responsible for larval toxicity**

Plants have a rich untapped pool of phytochemicals that may be used in place of synthetic insecticides in the mosquito control programme (Kishore et al) The efficacy of phytochemicals against mosquito larvae depend on their chemical nature such as, alkenes alkanes, alkyne, alkaloids, essential oils and simple aromatics, lactones, and fatty acids, terpenes, isoflavonoids,steroids, pterocarpans and lignans.

## 2. MATERIALS AND METHODS

### 2.1. Plant Material

The fruits were collected from Sindhudurg Dist, Western Ghats of Maharashtra, India and authenticated at Blatter's Herbaria, Mumbai (accession no. 9046 /2010).

### 2.2. Plant Processing and Extraction of the Essential Oils

The essential oils were extracted via steam distillation and Hydro Distillation of fresh green carpels and dried carpels at KET's (Kelkar Scientific Research Centre, Mulund)

**A. Carried by two methods**

- Steam distillation ( fresh green carpels)
- Hydro distillation (dried carpels)

**B. Essential Oils-Composition Analyses**

- Steam distillation (fresh green carpels): Amount of sample processed 4 kilograms, Moisture content 36.2% at 100 degrees Celsius till constant weight.

- Hydro distillation (dried carpels): Amount of sample processed 4 kilograms, Moisture content: 11 % at 100 degrees Celsius till constant weight.

**2.3 Gas Chromatography Mass Spectroscopy** for the identification of biochemical components present in the carpels. GC-MS was carried out at SAIF Department, IIT Bombay.

**2.4 Larvicidal bioassay**

Larvae of *Aedes aegypti* (l.) were collected from stock culture. The Plant Oil Formulation (POF) was tested for its larvicidal activity against 25 numbers of fourth instar larvae of Aedes aegyti by the standard procedure of WHO (1996). The Plant Oil Formulation was volumetrically diluted to 500 ml with dechlorinated water to obtain the test solution of 1000, 500.0, 400.0, 300.0, 200.0, 100.0 ppm and Tween20 served as a control. The experiments were carried out 28 ± 2 $^{o}$C, 85% relative humidity (RH), with a photoperiod of 14:10 LD for the larval growth. Late third instars to early fourth instars larva were used for larval bioassay which obtained from the stock culture maintained at Zoonosis department at Haffkine Institute for Training, Research & Testing, Mumbai. For each dose four replicates were maintained.

**3. RESULT**

**3.1Essential Oils-Composition Analyses**

The oil obtained from steam distillation of fresh green carpels was. 1.75 gm% and dried carpels was 3.18 gm %.

**3.2Gas Chromatography Mass Spectroscopy**

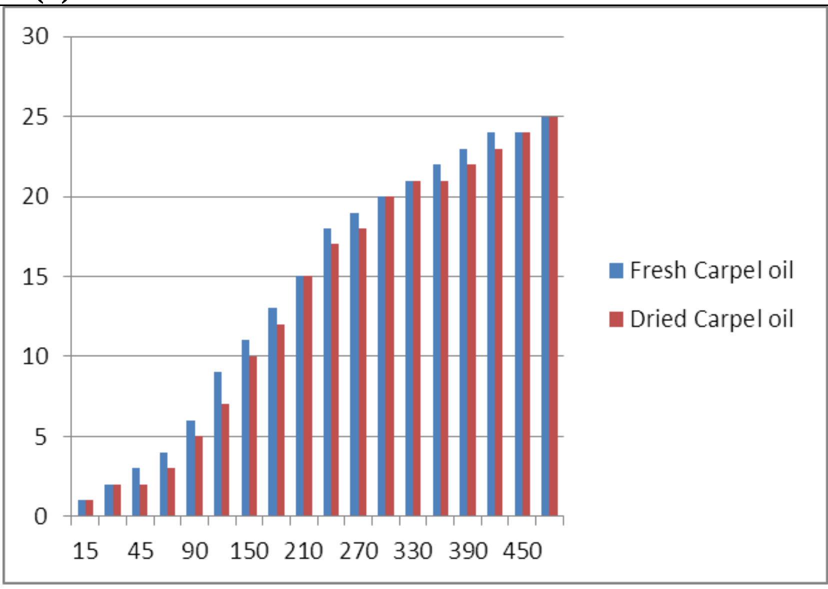
The fresh carpel oil sample showed the presence of pinene, Phellendrene, Benzene, 1-methyl-3-methylethyl, 3- cyclohexane-1-ol, 4 – mehyl-1-(1-methylethyl) and Cyclohexane, 1-ethenyl-1-methyl 2-4bis (1 – methylethenyl). The dried carpel oil sample showed the presence Phellendrene, 3- cyclohexane-1-ol, 4 – mehyl-1-(1-methylethyl) and 4 – hydroxyl-4- methylhen-5-enoic acid tert-butyl ester.

**3.3 Larvicidal bioassay**

The highest concentration studied for the effective of extracted from Zanthoxyulum rhetsa Roxb Dc carpels oils was 500.0 ppm (Table 9, Graph 9 & Table 10, Graph 10) for dried carpel oils and 400 ppm ( Table 7, Graph 7& Table 8 Graph 8) for fresh carpel oil. However, the maximum larval mortality in minimum time was detected in 1000 ppm concentration The larvae subjected to this concentration agitated during the first 15 min of exposure; following this, the larvae showed slowed and abnormal movements, including tremors and convulsions. The larvae were afflicted with paralysis and floated to the bottom of the containers prior to death. The mortality rate was observed to be directly proportional to the tested concentrations.

**Table 1 & Graph 1: Larvicidal activity of Oils (100.0ppm) against third instars larvae of *Aedes aegypti* (l.)**

No Of DEATH

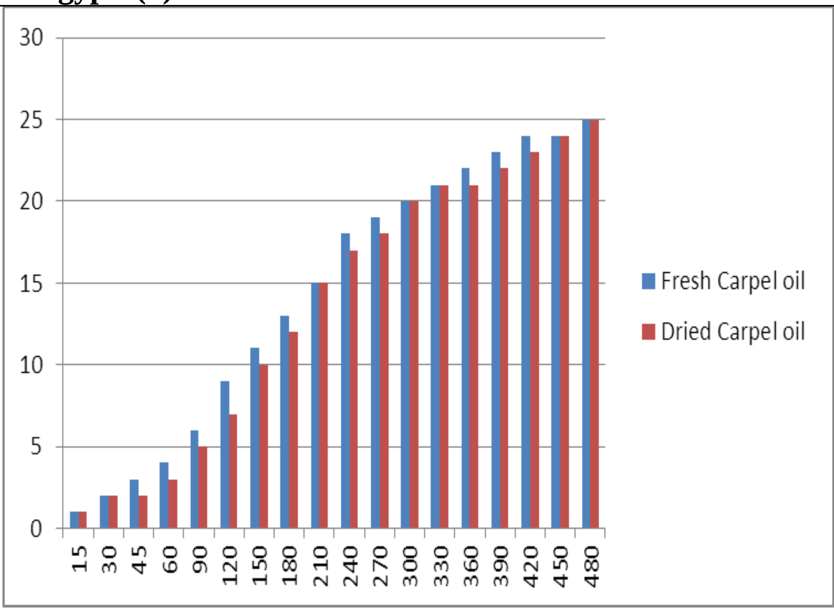



Time in mins

| Time (Mins) | Fresh Carpel oil | Dried Carpel oil |
|---|---|---|
| 15 | 1 | 1 |
| 30 | 2 | 2 |
| 45 | 4 | 3 |
| 60 | 5 | 6 |
| 90 | 7 | 7 |
| 120 | 9 | 7 |
| 150 | 11 | 10 |
| 180 | 13 | 12 |
| 210 | 15 | 15 |
| 240 | 18 | 17 |
| 270 | 20 | 18 |
| 300 | 20 | 20 |
| 330 | 22 | 21 |
| 360 | 23 | 22 |
| 390 | 24 | 23 |
| 420 | 24 | 23 |
| 450 | 24 | 24 |
| 480 | 25 | 25 |

**Table 2 & Graph 2: Larvicidal activity of Oils (100.0ppm) against fourth instars larvae of Aedes Aegypti (l.)**

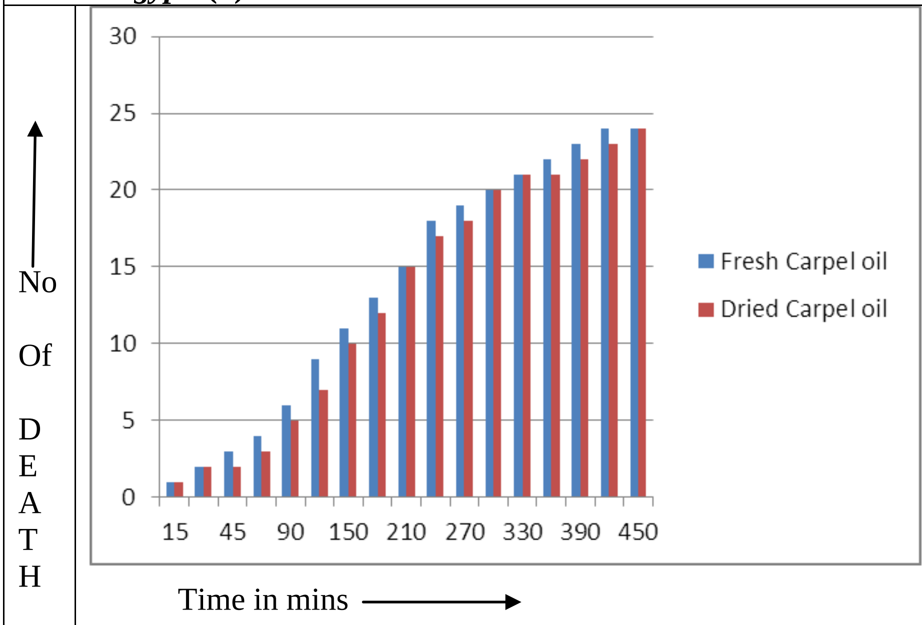
No Of DEATH




Time in mins

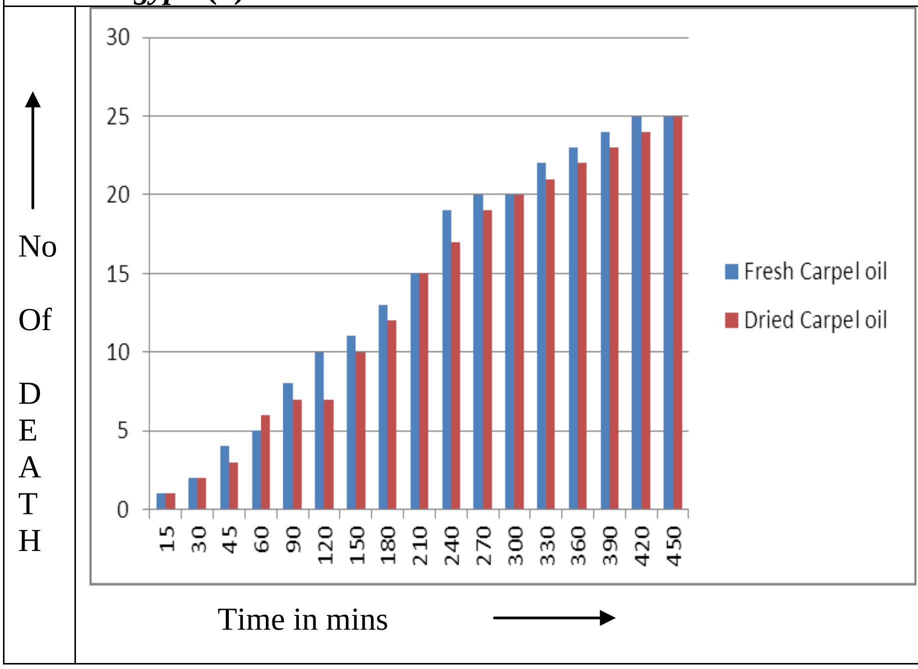
| Time (Mins) | Fresh Carpel oil | Dried Carpel oil |
|---|---|---|
| 15 | 1 | 1 |
| 30 | 2 | 2 |
| 45 | 3 | 2 |
| 60 | 4 | 3 |
| 90 | 6 | 5 |
| 120 | 9 | 7 |
| 150 | 11 | 10 |
| 180 | 13 | 12 |
| 210 | 15 | 15 |
| 240 | 18 | 17 |
| 270 | 19 | 18 |
| 300 | 20 | 20 |
| 330 | 21 | 21 |
| 360 | 22 | 21 |
| 390 | 23 | 22 |
| 420 | 24 | 23 |
| 450 | 24 | 24 |
| 480 | 25 | 25 |

**Table 3: & Graph 3: Larvicidal activity of Oils (200.0ppm) against third instars larvae of *Aedes aegypti* (l.)**




| Time (Mins) | Fresh Carpel oil | Dried Carpel oil |
|---|---|---|
| 15 | 1 | 1 |
| 30 | 3 | 2 |
| 45 | 5 | 3 |
| 60 | 5 | 6 |
| 90 | 8 | 7 |
| 120 | 9 | 7 |
| 150 | 11 | 10 |
| 180 | 13 | 12 |
| 210 | 15 | 15 |
| 240 | 18 | 17 |
| 270 | 20 | 19 |
| 300 | 21 | 20 |
| 330 | 22 | 21 |
| 360 | 23 | 22 |
| 390 | 24 | 23 |
| 420 | 24 | 21 |
| 450 | 25 | 25 |

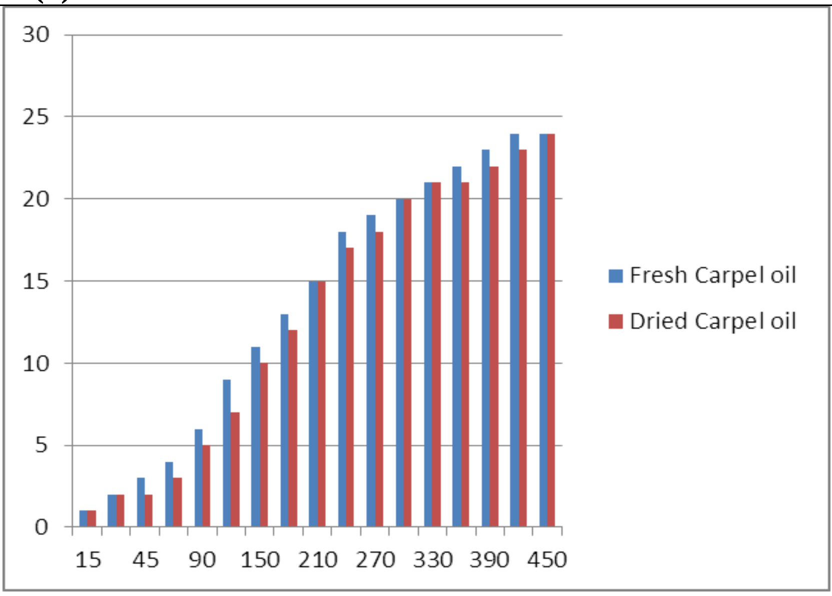
**Table 4 & Graph 4: Larvicidal activity of Oils (200.0ppm) against fourth instars larvae of *Aedes aegypti* (l.)**




| Time (Mins) | Fresh Carpel oil | Dried Carpel oil |
|---|---|---|
| 15 | 1 | 1 |
| 30 | 2 | 2 |
| 45 | 4 | 3 |
| 60 | 5 | 6 |
| 90 | 8 | 7 |
| 120 | 10 | 7 |
| 150 | 11 | 10 |
| 180 | 13 | 12 |
| 210 | 15 | 15 |
| 240 | 19 | 17 |
| 270 | 20 | 19 |
| 300 | 20 | 20 |
| 330 | 22 | 21 |
| 360 | 23 | 22 |
| 390 | 24 | 23 |
| 420 | 25 | 24 |
| 450 | 25 | 25 |

**Table 5 & Graph 5: Larvicidal activity of Oils (300.0ppm) against fourth instars larvae of *Aedes aegypti* (l.)**

No Of DEATH

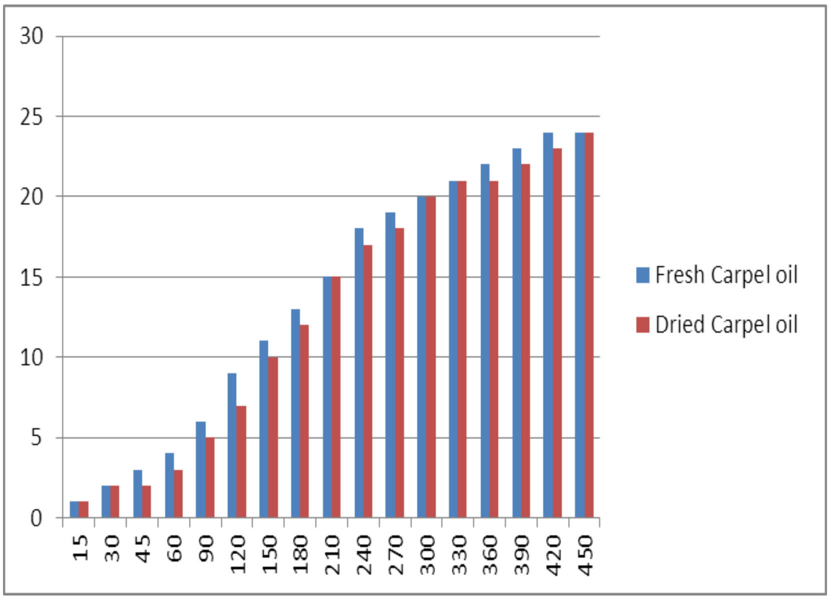



Time In mins

| Time (Mins) | Fresh Carpel oil | Dried Carpel oil |
|---|---|---|
| 15 | 2 | 1 |
| 30 | 3 | 2 |
| 45 | 4 | 3 |
| 60 | 5 | 6 |
| 90 | 9 | 7 |
| 120 | 10 | 7 |
| 150 | 12 | 11 |
| 180 | 13 | 12 |
| 210 | 16 | 15 |
| 240 | 19 | 17 |
| 270 | 20 | 19 |
| 300 | 22 | 20 |
| 330 | 23 | 21 |
| 360 | 24 | 22 |
| 390 | 24 | 23 |
| 420 | 25 | 24 |
| 450 | 25 | 25 |

**Table 6 & Graph 6: Larvicidal activity of Oils (300.0ppm) against fourth instars larvae of *Aedes aegypti* (l.)**

No Of DEATH

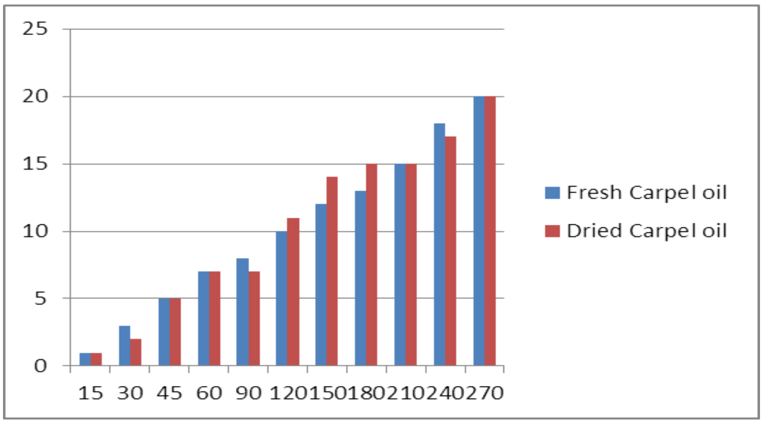



Time in mins

| Time (Mins) | Fresh Carpel oil | Dried Carpel oil |
|---|---|---|
| 15 | 2 | 1 |
| 30 | 3 | 2 |
| 45 | 5 | 3 |
| 60 | 6 | 6 |
| 90 | 8 | 7 |
| 120 | 9 | 7 |
| 150 | 11 | 10 |
| 180 | 12 | 11 |
| 210 | 15 | 14 |
| 240 | 17 | 16 |
| 270 | 18 | 17 |
| 300 | 20 | 18 |
| 330 | 22 | 21 |
| 360 | 23 | 22 |
| 390 | 24 | 23 |
| 420 | 25 | 24 |
| 450 | 25 | 25 |

**Table 7 & Graph7: Larvicidal activity of Oils (400.0ppm) against third instars larvae of *Aedes aegypti* (l.)**

No Of DEATH

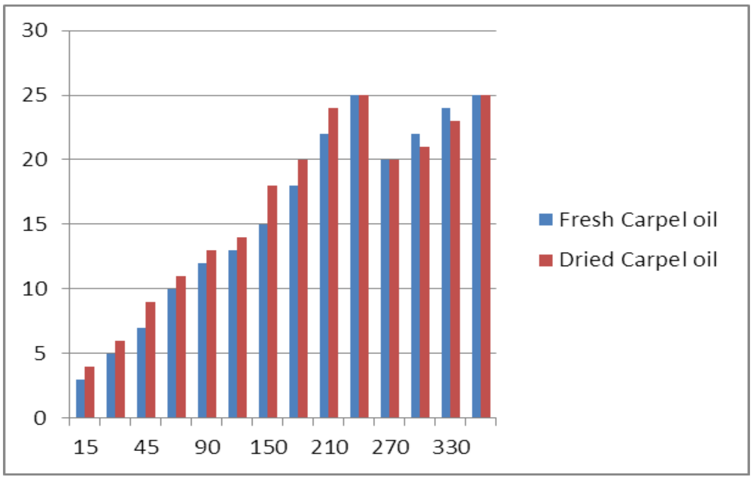



Time in mins

| Time (Mins) | Fresh Carpel oil | Dried Carpel oil |
|---|---|---|
| 15 | 1 | 2 |
| 30 | 3 | 3 |
| 45 | 5 | 7 |
| 60 | 10 | 9 |
| 90 | 12 | 11 |
| 120 | 14 | 12 |
| 150 | 15 | 14 |
| 180 | 16 | 15 |
| 210 | 18 | 18 |
| 240 | 22 | 20 |
| 270 | 25 | 25 |

**Table 8 & Graph 8: Larvicidal activity of Oils (400.0ppm) against fourth instars larvae of *Aedes aegypti* (l.)**

No Of DEATH

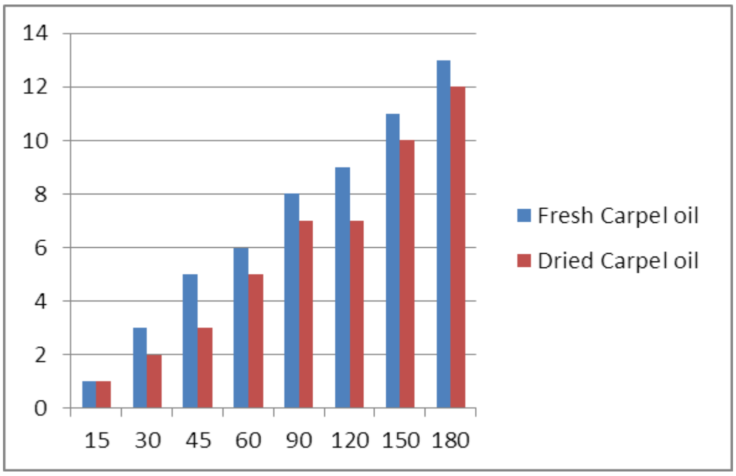



**Time in mins**

| Time (Mins) | Fresh Carpel oil | Dried Carpel oil |
|---|---|---|
| 15 | 1 | 1 |
| 30 | 2 | 3 |
| 45 | 3 | 5 |
| 60 | 7 | 7 |
| 90 | 8 | 7 |
| 120 | 10 | 11 |
| 150 | 12 | 14 |
| 180 | 13 | 15 |
| 210 | 15 | 15 |
| 240 | 18 | 17 |
| 270 | 20 | 20 |
| 300 | 22 | 21 |
| 330 | 24 | 23 |
| 360 | 25 | 25 |

**Table 9 & Graph 9: Larvicidal activity of Oils (500.0ppm) against third instars larvae of *Aedes aegypti* (l.)**

No Of DEATH

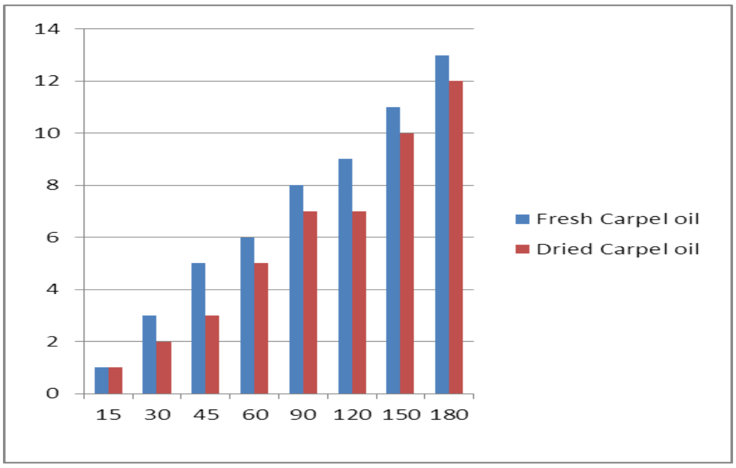



Time in mins

| Time (Mins) | Fresh Carpel oil | Dried Carpel oil |
|---|---|---|
| 15 | 3 | 4 |
| 30 | 5 | 6 |
| 45 | 7 | 9 |
| 60 | 10 | 11 |
| 90 | 12 | 12 |
| 120 | 14 | 13 |
| 150 | 15 | 16 |
| 180 | 18 | 17 |
| 210 | 23 | 22 |
| 240 | 25 | 25 |

**Table 10 & Graph 10: Larvicidal activity of Oils (500.0ppm) against third instars larvae of *Aedes aegypti* (l.)**

No Of DEATH

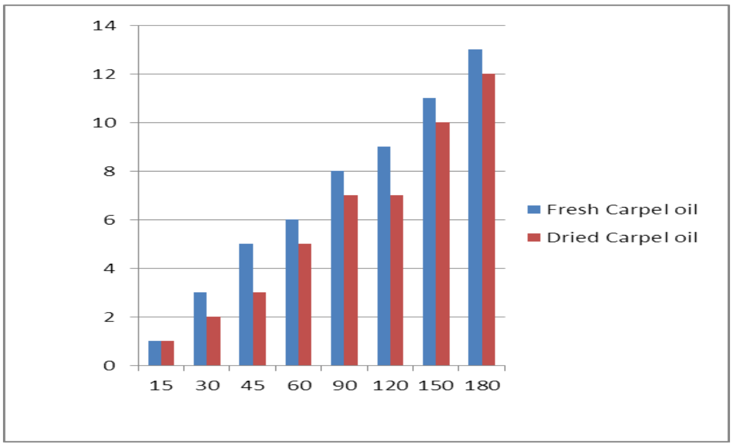



Time in mins

| Time (Mins) | Fresh Carpel oil | Dried Carpel oil |
|---|---|---|
| 15 | 3 | 3 |
| 30 | 5 | 5 |
| 45 | 8 | 7 |
| 60 | 9 | 8 |
| 90 | 12 | 11 |
| 120 | 15 | 14 |
| 150 | 18 | 16 |
| 180 | 20 | 20 |
| 210 | 24 | 23 |
| 240 | 25 | 25 |

**Table 11 & Graph 11: Larvicidal activity of Oils (1000ppm) against third instars larvae of *Aedes aegypti* (l.)**

NO Of DEATH

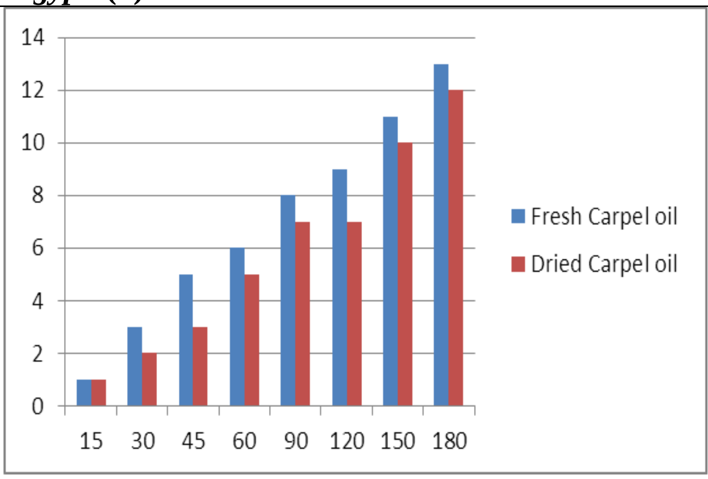



Time in mins

| Time (Mins) | Fresh Carpel oil | Dried Carpel oil |
|---|---|---|
| 15 | 7 | 6 |
| 30 | 11 | 10 |
| 45 | 14 | 12 |
| 60 | 16 | 15 |
| 90 | 18 | 18 |
| 120 | 21 | 21 |
| 150 | 24 | 23 |
| 180 | 25 | 25 |

**Table 12 & Graph 12: Larvicidal activity of Oils (1000ppm) against third instars larvae of *Aedes aegypti* (l.)**

No Of DEATH




Time in mins

| Time (Mins) | Fresh Carpel oil | Dried Carpel oil |
|---|---|---|
| 15 | 6 | 7 |
| 30 | 9 | 8 |
| 45 | 12 | 10 |
| 60 | 15 | 14 |
| 90 | 18 | 17 |
| 120 | 21 | 20 |
| 150 | 23 | 22 |
| 180 | 25 | 25 |

## CONCLUSIONS

Natural-derived products, such as essential oils, in the production of natural larvicidal insecticides, could be a promising tool to help reduce the spread of dengue and chikungunya. As these products are the natural sources of substances which display insecticidal activity against mosquito (affecting the different stages of mosquito development) they are biodegradable and express low toxicity and eco- friendly. It is important to standardize the procedures used for the determination of larvicidal activity.

The *Aedes aegypti* (l.) larvae were observed to be sensitive to the two oils fresh carpel oil and dried carpel oil tested in this study. The identification of the chemical composition and biological properties of essential oils will help in the development of products with larvicidal activities.

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