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Comparative study of essential oil of *tagetes erecta* (Asteraceae) against third instar larvae of *Aedes aegypti* (Linnaeus, 1762) and *anopheles stephensi* (Liston, 1902)

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Abstract
The objective of the present study was the comparision of essential oil of *Tagetes erecta* (Asteraceae) against third instar larvae of *Aedes aegypti* (Linnaeus, 1762) and *Anopheles stephensi* (Liston, 1902). Essential oil was hydro distilled in the laboratory from the plants obtained from the CAP. Bioefficacy of the essential oil was evaluated under laboratory conditions using III instar mosquito larvae. The number of dead larvae in each beaker was counted after 12, 24 and 48h. Percent-corrected mortality was determined using Abbott's formula LC_{50} values (the concentration at which 50% of the larvae were immobilized) was calculated by probit analysis using the PROBIT software by log-probit regression using SPSS 16.0 for Windows/Microsoft Excel Programme. The LC_{50} values of essential oil of *Tagetes erecta* for *Aedes aegypti* were 81.765, 48.951 and 17.729ppm and for *Anopheles stephensi*, the values were 85.671, 53.451 and 26.484ppm after 12, 24 and 48 hours of exposure respectively. Chi-square values were significant at $p<0.05$ level. Even though, the essential oil showed low LC_{50} 17.729 ppm in *Aedes aegypti* and 26.484 ppm in *Anopheles stephensi* after 48 hours of exposure period. The larval mortality rate of essential oil was entirely time and dose dependent. The essential oil of *Tagetes erecta* showed comparatively higher mortality to the larvae of *Anopheles stephensi*.

Keywords: *Tagetes erecta, Aedes aegypti*, *Anopheles stephensi*, Relative Potency, Chi-square, LC_{50}

Introduction
The mosquito-borne diseases, dengue fever, malaria, encephalitis, yellow fever, chikungunya, filariasis, are causing havoc in many countries, and loss in terms of human lives is irreversible (ICMR, 2007) [1]. *Aedes aegypti* (*Ae*. *aegypti*), the primary vector for dengue fever, dengue haemorrhagic fever and yellow fever is widespread over large areas of the tropics and subtropics; and is reported to infect more than 100 million people every year in more than 110 countries in the tropics (Yang *et al.*, 2009) [2]. According to WHO (2011) [3] about two fifths of the world's population are now at risk of dengue and the only way to prevent dengue virus transmission is to combat the disease-carrying mosquitoes. In the Indian subcontinent, cases of dengue fever are on the rise and, therefore, the control of dengue vector needs immediate attention. In the South East Asian Region, out of about 1.4 billion people living in 11 countries, 1.2 billion (85.7%) are exposed to the risk of malaria and most of whom live in India. Among the 109 malaria endemic countries, India had 1.5 million confirmed malaria cases in 2009 with over 1,000 deaths (NVBDCP, 2011) [4]. *Anopheles stephensi* transmits malaria in the plains of rural and urban areas of India. *A. stephensi* plays a major role in malaria transmission because of its predominantly domestic habits and preference for human blood.

Over the last five decades the indiscriminate and frequent use of synthetic insecticides in agriculture and public health programs has caused multifarious problems viz. insecticide resistance, environmental pollution, destabilization of the ecosystem and toxic hazards to human and non-target organisms (Jirakanjanakit, 2007; Sarwar *et al.*, 2009) [5, 6]. These problems have necessitated the need for search and development of alternative strategies using eco-friendly, environmentally-safe, biodegradable and low cost natural products as mosquito larvicides.

Plants offer an alternative source of insect control agents because they contain a range of bioactive chemicals (Heldin *et al.*, 1997), many of which are selective and have little and no harmful effect on non-target organisms and the environment (Amason *et al.*, 1989) [8]. In this context, essential oils have received much attention as potentially useful bioactive compound against insects (Cheng *et al.*, 2003) [9] showing a broad spectrum of activity against insects, low mammalian toxicity and degrading rapidly in the environment.

Tagetes genus belongs to the family Asteraceae; comprises about 55 species distributed around the world. Phytochemical studies carried out to different species of *Tagetes* have revealed the presence of flavonoids and terpenes displaying pharmacological and insecticidal properties. The main compound of the *Tagetes erecta* oil were piperitone (45.72%), D-limonene (9.67%) and piperitone (5.89%) (Perich *et al.*, 1995) [12].

In the present study the comparision of essential oil of *Tagetes erecta* (Asteraceae) was tested against third instar larvae of *Aedes aegypti* (Linnaeus, 1762) and *Anopheles stephensi* (Liston, 1902) in a search for effective and affordable natural products to be used in the control of dengue and malaria.

Materials and Methods

The essential oil of *Tagetes erecta* (marigold) used in the present study was procured from the Centre of Aromatic Plants (CAP), Selaqui. The larvae of *Aedes aegypti* and *Anopheles stephensi* were obtained from the cyclic colonies of mosquitoes maintained in PG Lab of Department of Zoology, Dolphin (PG) Institute of Biomedical and Natural Sciences, Dehradun.

Preparation of the Oil Solution

The larvicidal activity was analyzed as per the standard procedures recommended by the world Health Organization (1996). The larvicidal activities of these oils were determined against Aedes aegypti after making stock solution by serial dilutions- 5, 2, 1, 0.5, 0.25 and 0.125% in acetone. Later 1ml of the stock solution was made up to 250ml with distilled water to obtain a final concentration ranging 200ppm, 100ppm, 50ppm, 25ppm, 12.5ppm, 12.5ppm and 6.25ppm. A control was maintained with acetone water mixture.

Bioassay of Oil Solution

Each replicate containing 250ml of the described oil solution was placed in a 500ml glass beaker. Then third-instar larvae of the target mosquitoes were transferred in to each beaker. After that, the beakers were left on the laboratory table for 48h. The number of dead larvae in each beaker was counted after 12, 24 and 48h.

Table 1: Larvicidal activity of *Tagetes erecta* against *Aedes aegypti* and *Anopheles stephensi*

Time (Hrs)	Conc. (ppm)	*Aedes aegypti*		*Anopheles stephensi*	
		LC_{50}	Regression Equation	LC_{50}	Regression Equation
12	6.25 12.5 25 50 100 200	81.765	y= 0.061x+2.492	85.671	y= 0.059x+3.711
24	6.25 12.5 25 50 100 200	48.951	y=0.077x+3.075	53.451	y= 0.067x+6.1015
48	6.25 12.5 25 50 100 200	17.729	y= 0.079x+5.688	26.484	y= 0.065x+8.6429

Table 2: Relative potency

Mosquito Species	12 hrs	24 hrs	48 hrs
Aedes - Anopheles	1.007	0.997	0.946
Anopheles- Aedes	0.9931	1.002	1.0568

Calculation of LC $_{50}$ and Statistical Analysis

Percent-corrected mortality was determined using Abbott's formula (Abbott, 1995) LC_{50} values (the concentration at which 50% of the larvae were immobilized) was calculated by probit analysis using the PROBIT software (Finney, 1971) by log-probit regression using SPSS 16.0 for Windows/Microsoft Excel Programme.

$$\text{Abbott's Formula Pecentage (\%) Mortality} = \frac{\text{\% Test Mortality- \% Control Mortality x100}}{\text{100- Control Mortality}}$$

Results and Discussion

The LC_{50} values of essential oil of *Tagetes erecta* for *Aedes aegypti* were 81.765, 48.951 and 17.729ppm and for *Anopheles stephensi*, the values were 85.671, 53.451 and 26.484ppm after 12, 24 and 48 hours of exposure respectively. Chi-square values were significant at $p<0.05$ level. Even though, the essential oil showed low LC_{50} 17.729 ppm in *Aedes aegypti* and 26.484 ppm in *Anopheles stephensi* after 48 hours of exposure period. The larval mortality rate of essential oil was entirely time and dose dependent. The essential oil of *Tagetes erecta* showed comparatively higher mortality to the larvae of *Anopheles stephensi*.

The relative potency revealed statistically significant difference at all time period for the oil. Comparision between *Aedes aegypti* and *Anopheles stephensi* with *Tagetes erecta* essential oils LC_{50} values at 12, 24 and 48 hours using relative potency analysis revealed statistically significant difference at all time periods.

The results are comparable with earlier reports of the worker who observed larvicidal activity of *Pinus longifolia* oil against three vector mosquitoes namely *Aedes aegypti* (LC_{50} – 82.1 ppm), *Culex quinquefasciatus* (LC 50 – 85.7 ppm) and *Anopheles stephensi* (LC_{50} – 112.6 ppm) (Ansari *et al.*, 2005). One of the scientist reported that the larvicidal activity of essential oils of Brazilian plants against *Aedes aegypti* and observed the LC_{50} to range from 60 to 533 ppm (Cavalcanti *et al.*, 2004). The results of the present study are also comparable to the previous study made by some scientist on *P. arbonicus* (Cheng *et al.*, 2009) [18]. The essential of *P. arbonicus* showed larvicidal activity against *Anopheles stephensi* reared in laboratory with the LC_{50} values of 33.54 (after 12h) and 28.37ppm (after 24 h). Recently some workers reported larvicidal activity of essential oils of apiaceae plants against *Anopheles stephensi* with LC_{50} value of 20.10ppm (Senthikumar and Venkatesalu, 2010) [19]. Some of the workers estimated the larvicidal activity of essential oil of Indian borage on *Anopheles gambiae* (Sedaghat *et al.*, 2011) [20]. They calculated LC_{50} after 12, 24 and 48 h of exposure on laboratory colony and wild populations. The LC_{50} of the laboratory colony were 98.56 (after 12h), 55.20 (after 24 h) and 32.41ppm (after 48h) and the LC_{50} values for wild populations were 119.52 (after 12h), 67.53 (after 24h) and 25.51ppm (after 48h). They considered the larval mortality rate of the essential oil was entirely time and dose-dependent. In the past previous years some workers extracted

essential oils from nine plants widely found in Northeast of Brazil were analyzed by measurement of their LC_{50} (Kweka *et al.*, 2012) [21]. They reported that *Ocimum americanum* and *O. gratissimum* have LC_{50} of 67 ppm and 60 ppm respectively. Some of the workers extracted essential oils from the leaves of *Myrcia ovata*, *Psidium guajava* (L.), *Spondias purpurea* (L.) and *Plectranthus amboinicus* (Lour.) for larvicidal activity from Brazil against *Aedes aegypti* with LC_{50} values ranging from 24.7 to 192.1 µg/ml (Lima *et al.*, 2010). Some scientist reported larvicidal activity of *Piper betle* with 2h and LC_{50} value of 86 and 48 ppm respectively (Row and Ho, 2009) [23]. Some of the workers found larvicidal activity of hydrolates of *Z. officinale*, *C. longa* and *C. citrates* with LC_{50} of at 15.8, 24.7 and 33.7 (%v/v) respectively against *Aedes albopictus* and 21.8, 35.5 and 38.8 (%v/v) against *Culex quinquefasciatus* (Rabha *et al.*, 2012) [24]. In the few past years some workers analysed the larvicidal activity of essential oil of *Zanthoxylum armatum* DC (Rutaceae) against three mosquito species. They found *Culex quinquefasciatus* was the most sensitive (LC_{50} = 49 ppm) followed by *Aedes aegypti* (LC_{50} = 54 ppm) and *Anopheles* stephensi (LC_{50} = 58 ppm) (Tiwary *et al.*, 2007). Recently some worker analysed the comparative larvicidal activity of *Cymbopogon flexeous* and *Tagetes erecta* against *Aedes aegypti.* The LC_{50} values of *Cymbopogon flexeous* are 136.8, 52.736 and 24.056ppm after 12, 24 and 48 h of exposure respectively. The LC_{50} values of Tagetes erecta are 81.765, 48.951 and 17.729ppm after 12, 24 and 48 hours of exposure respectively. Chi-square values were significant at $p<0.05$ level. The essential oil of *Cymbopogon flexeous* found effective to control the larvae (Bhatt, 2013).

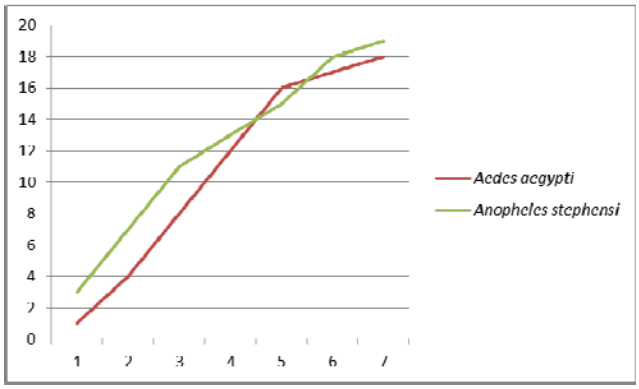

Fig 1.1: Comparision of mortality in *Aedes aegypti* and *Anopheles stephensi* in 12 hrs

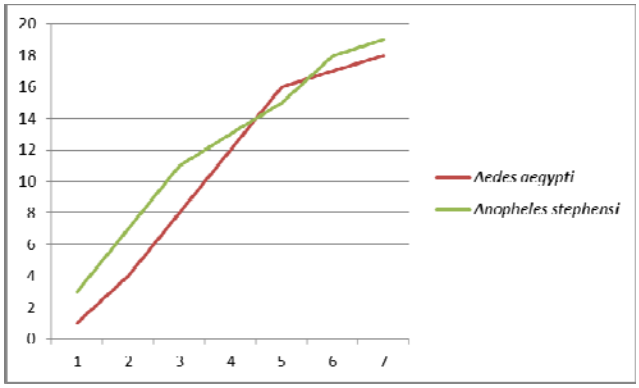

Fig 1.2: Comparision of mortality in *Aedes aegypti* and *Anopheles stephensi* in 24 hrs

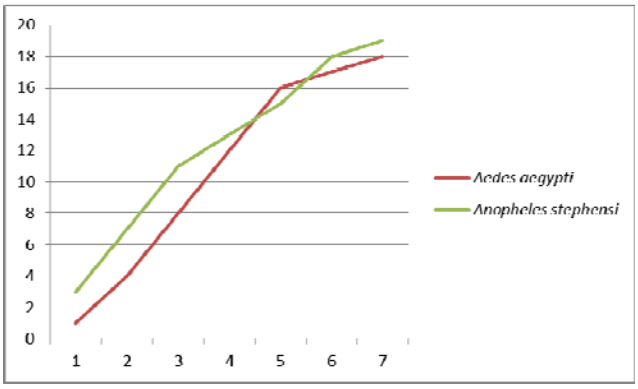

Fig 1.3: Comparision of mortality in *Aedes aegypti* and *Anopheles stephensi* in 48 hrs

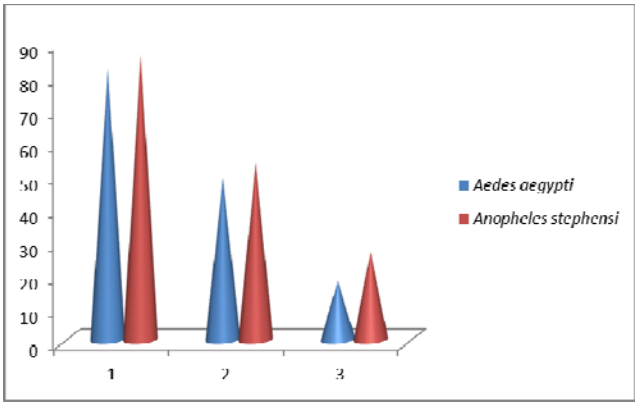

Fig 2: Comparision of LC_{50} Value of *Aedes aegypti* and *Anopheles stephensi*

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