Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2013, 3(5): 422-427

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Comparative analysis of larvicidal activity of essential oils of *Cymbopogon flexeous* (Lemon grass) and *Tagetes erecta* (Marigold) against *Aedes aegypti* larvae

Beena Joshi Bhatt

Department of Zoology, Dolphin (PG) Institute of Biomedical & Natural Sciences, Dehradun

---

ABSTRACT

In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, this study was undertaken to assess the larvicidal potential of the essential oil Cymbopogon flexeous (lemongrass) and Tagetes erecta (marigold) against medically important mosquito vector, Aedes aegypti. 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 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. Such findings would be useful in promoting research aiming at the development of new agent for mosquito control based on bioactive chemical compounds from indigenous plant sources as an alternative to chemical larvicides.

Key words: Essential oils, *Aedes aegypti*, Lethal concentration, Probit analysis, Relative potency.

INTRODUCTION

Mosquito-borne diseases cause significant morbidity, mortality and economic burden to humankind [1]. The mosquito, *Aedes aegypti* is the major vector of yellow fever, dengue and dengue hemorrhagic fever (DHF). These mosquito-borne infections are found in tropical and sub-tropical regions around the world, predominantly in urban areas and semi-urban areas. The global incidence of dengue has grown dramatically around the world in recent decades and there are approximately 2.5 billion people at risk [2]. One of the methods available for the control of mosquitoes is the use of insecticides. In last two decades, the use of chemical insecticides in mosquito control method has resulted in instability of the environment, mosquito resistance, mosquito resurgences and toxic to non-target organisms including natural enemies in the agriculture ecosystem [3]. Hence, it has now become important to find an alternative means of mosquito control method, which can eliminate the use of chemical pesticides.

Plants offer an alternative source of insect control agents because they contain a range of bioactive chemicals [4], many of which are selective and have little and no harmful effect on non-target organisms and the environment [5]. In this context, essential oils have received much attention as potentially useful bioactive compound against insects

[6] showing a broad spectrum of activity against insects, low mammalian toxicity and degrading rapidly in the environment.

The essential oil of lemongrass exhibited many activities like analgesic, anti-helminth, anti-inflammatory, anti-bacterial, anti-fungal, anti- malarial etc. One of the main constituents of the many different species of lemongrass (genus *Cymbopogon*) is citral (3,7-dimethyl-2,6-octadien-1-al). Lemongrass oil has been found to contain up to 75-85% citral. Lemongrass also contains z-citral, borneol, estragole, methyleugenol, geranyl acetate (3,7-dimethyl-2,6-octadiene-1-ol acetate), geraniol (some species higher in this compound than citral), beta-myrcene (MYR, 7-methyl-3-methylene-1,6 octadiene), limonene, piperitone, citronellal, carene-2, alpha-terpineole, pinene, farnesol, proximadiol, and (+)-cymbodiacetal [7].

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 [8], [9]. The main compound of the Tagetes erecta oil were piperitone (45.72%), D-limonene (9.67%) and piperitone (5.89%) [10].

In the present study the comparision of essential oils of *Cymbopogon flexeous* (Lemon grass) *Tagetes erecta* (Marigold) and were tested against third instar *Aedes aegypti* larvae in a search for effective and affordable natural products to be used in the control of dengue.

MATERIALS AND METHODS

The essential oil of *Cymbopogon flexeous* (lemongrass) and *Tagetes erecta* (marigold) used in the present study were procured from the Centre of Aromatic Plants (CAP), Selaqui. The larvae of *Aedes aegypti* 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 [11]. 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.

Calculation of LC_{50} and Statistical Analysis

Percent-corrected mortality was determined using Abbott's formula [12] LC_{50} values (the concentration at which 50% of the larvae were immobilized) was calculated by probit analysis using the PROBIT software [13] by log-probit regression using SPSS 16.0 for Windows/Microsoft Excel Programme.

Abbott's Formula

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

RESULTS AND DISCUSSION

Cymbopogon flexeous and *Tagetes erecta* essential oils exhibited toxicity to *Aedes aegypti* larvae. The statistical data are presented in Table 1.

Table 1. Larvicidal activity of essential oil of *Cymbopogon flexeous* and *Tagetes erecta* against *Aedes aegypti*

Hours	Conc. (ppm)	*Cymbopogon flexeous*		*Tagetes erecta*	
		LC_{50}	Regression Equation	LC_{50}	Regression Equation
12	6.25 12.5 25 50 100 200	136.8	y=0.061x+1	81.765	y=0.062x+2.492
24	6.25 12.5 25 50 100 200	52.736	y=0.077x+3.075	48.951	y=0.069x+3.657
48	6.25 12.5 25 50 100 200	24.056	y=0.079x+5.688	17.729	y=0.072x+6.797

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. Even though, the essential oils of both showed low LC_{50} (24.056 and 17.729ppm) after 48 h of exposure period. Acetone and water showed no mortality after 24 and 48 hours of exposure period. The larval mortality rate of the essential oil was entirely time and dose dependent. The most active essential oil against third instar larvae of *Aedes aegypti* was *Cymbopogon flexeous* with LC_{50} value of 136.8ppm (12h), 52.736ppm (24h) and 24.056ppm (48h) (Fig.1,2 &3).

Fig.1: Comparision of larvicidal activity of essential oils of *Tagetes erecta* (Marigold) & *Cymbopogon flexeous* (Lemon grass) at 12h

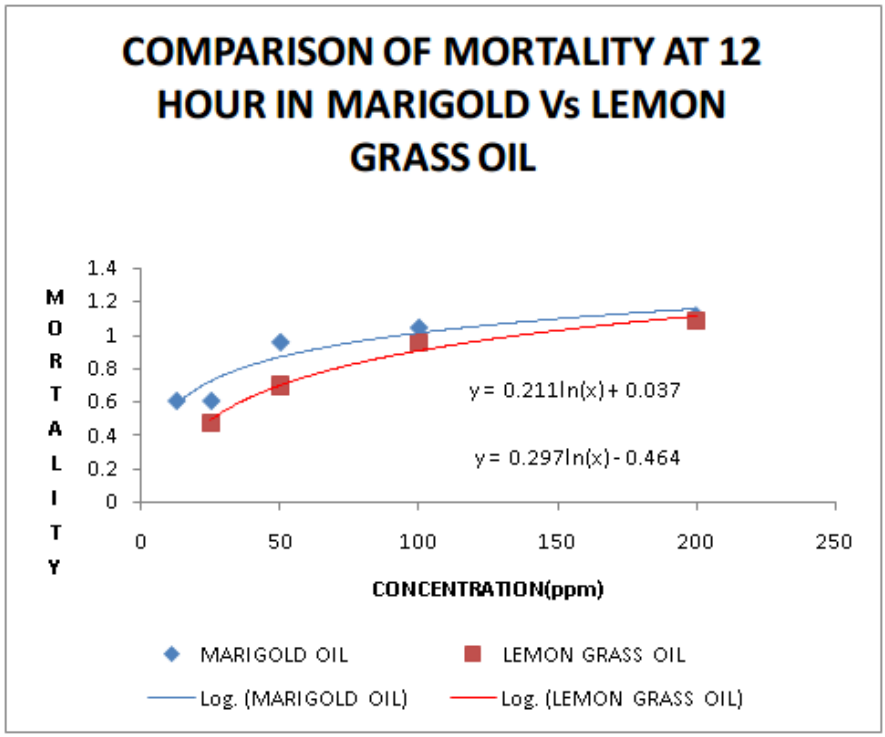


Fig.2: Comparision of larvicidal activity of essential oils of *Tagetes erecta* (Marigold) & *Cymbopogon flexeous* (Lemon grass) at 24h

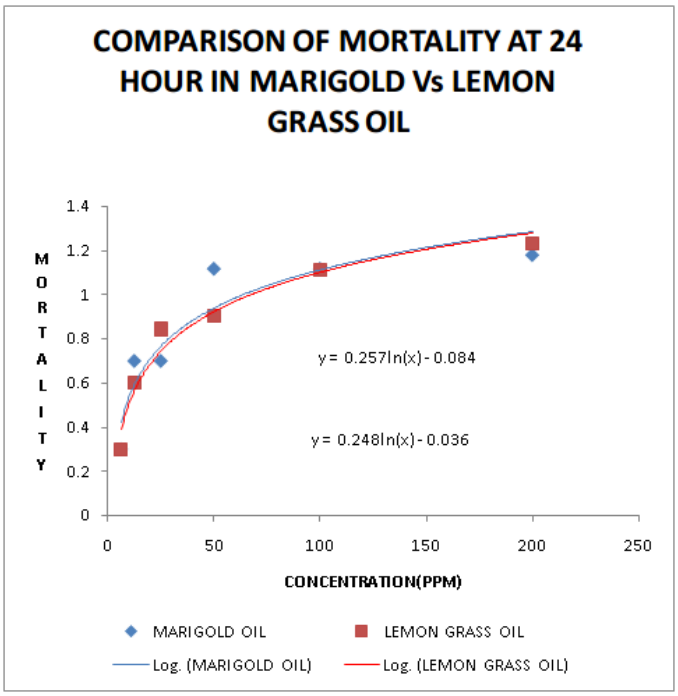
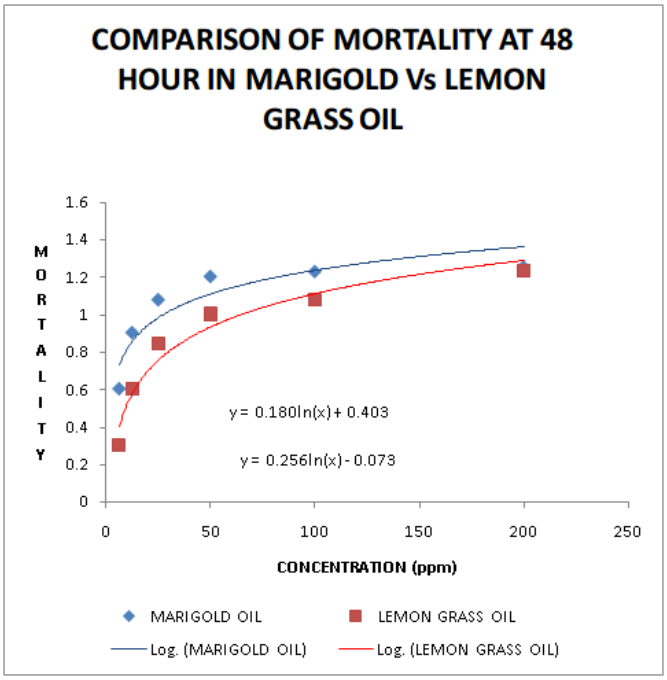


Fig.3: Comparision of larvicidal activity of essential oils of *Tagetes erecta* (Marigold) & *Cymbopogon flexeous* (Lemon grass) at 48h



The relative potency revealed statistically significant difference at all time period for both the oils. Comparision between *Cymbopogon flexeous* and *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 with *Cymbopogon flexeous* showing higher concentration compared to *Tagetes erecta*.

The relative potency of *Cymbopogon flexeous* and *Tagetes erecta* was given in Table 2.

Table 2. Relative potency of the essential oils

Cymbopogon flexeous -Tagetes erecta	**12 HRS**	**24 HRS**	**48 HRS**
	1.759	1.081	1.268
Tagetes erecta- Cymbopogon flexeous	0.568	0.925	0.789

The results are comparable with earlier reports of the worker who observed larvicidal activity of *Pinus longifolia* oil against three vector mosquitoes namely *Ae. aegypti* (LC_{50} – 82.1 ppm), *Cx. quinquefasciatus* (LC_{50} – 85.7 ppm) and *An. stephensi* (LC_{50} – 112.6 ppm) [14]. 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 [15]. Some workers observed insecticidal activities of leaf essential oils from *Cinnamomum osmophloeum* against *Aedes albopictus* larvae[16]. They observed LC_{50} value in 24h was 40.8 µg/ml. The results of the present study are also comparable to the previous study made by some scientist on *P. arbonicus* [17]. The essential of *P. arbonicus* showed larvicidal activity against *An. 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 *An. stephensi* with LC_{50} value of 20.10ppm[18]. Some of the workers estimated the larvicidal activity of essential oil of Indian borage on *An. gambiae* [19]. 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} [15]. 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 [20]. Some scientist reported larvicidal activity of *Piper betle* with 2h and LC_{50} value of 86 and 48 ppm respectively [21]. 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 *Ae. albopictus* and 21.8, 35.5 and 38.8 (%v/v) against *Cx. Quinquefasciatus* [22]. In the few past year Some workers analysed the larvicidal activity of essential oil of Zanthoxylum armatum DC (Rutaceae) against three mosquito species. They found *Cx. Quinquefasciatus* was the most sensitive (LC_{50} = 49 ppm) followed by *Ae. aegypti* (LC_{50} = 54 ppm) and An. stephensi (LC_{50} = 58 ppm) [23].

CONCLUSION

In the present study it was concluded that the essential oil of *Cymbopogon flexeous* and *Tagetes erecta* exhibited effective larvicidal activity. The essential oil of *Cymbopogon flexeous* (Lemon grass) is more effective than the essential oil of *Tagetes erecta* (Marigold). Further studies on identification of active compounds for larval control and commercial preparation of repellent products and field trials are needed to recommend the development of ecofriendly chemicals from this plant based oil for mosquito control and protection against the bites of haematophagous insects.

Acknowledgement
The author is grateful to Centre for Aromatic Plants (CAP) for providing essential oils. Principal, Director and management of the Dolphin (PG) Institute of Biomedical & Natural Sciences, Dehradun for providing necessary facilities. Dr. P. Patra for statistical analysis of the findings. Priya Ranjan Pati for his technical help during the experiment.

REFERENCES

[1] Massebo F, Tadesse M, Bekele T, Balkew M, Gebre-Michel T, *Afr J Biotechonol*, **2009**, 8: 4183-4188.
[2] World Health Organization, *Dengue and Dengue Haemorrhagic Fever. Fact sheet*. **2009** p. 11.
[3] Greenwood B, Mutabingwa T, *Nature*, **2002**, 415: 670-672.
[4] Heldlin PA, Holingworth RM, Masler EP, Miyamoto J, Thopson DG, *Chem Soc*, **1997**, 372.
[5] Arnason JT, Philogene BJR, Morand P, *Am Chem Soc*, **1989**, 13.
[6] Cheng SS, Chang HT, Chang ST, Tsai KH, Chen WJ, *Biores Technol*, **2003**, 89: 99-102.

[7] Bassole IH, Guelbeogo WM, Nebie R, Costantini C, Sagnon N, Kabore ZI, Traore SA, *Parassitologia*, **2003**, 45(1):23-26.
[8] Tereschuck M, Riera M, Castro G, Abdala L, *J Ethnopharmacol* **1997**, 56, 227-232.
[9] Perich M, Wells C, Bertsch W, Tredway K, J. *Am Mosq Contrl Asso*, **1995**, 11, 307-310
[10] Marques MM, morais SM, Vieira IG, Vieira MG, Raquel A, Silva A, De Almeida RR, Guedes MI, *J Am Mosq Control Assoc*, **2011**, 27(2): 156-8.
[11] WHO, *Report of the WHO informal consultation, on the evaluation and testing of insecticides*.**1996**, CTD/WHOPES/IC/ 96.1 p. 69.
[12] Abbott WS, *Journal of Economic Entomology*, **1925**, 18: 265-267.
[13] Finney DJ, *Third ed. Cambridge University Press*, **1971**, Cambridge, pp.68-72.
[14] Ansari MA, Mittal PK, Razdan RK, Sreehari U, *Journal of Vector Borne Diseases*, **2005,** 42: 95- 99.
[15] Cavalcanti ESP, Morais SM, Lima MA, Santana EW, *Memorias do Instituto Oswaldo Cruz*, **2004**, 99: 541-544.
[16] Cheng SS, Liu JY, Huang CG, Hsui YR, Chen WJ,Chang ST, *Biores Technol*, **2009**, 100: 457-464.
[17] Senthikumar A, Venkatesalu V, *Parasitol Res*, **2010**, 107: 1275-1278.
[18] Sedaghat MM, Dehkordi AS, Abai MR, Khanavi M, Mohtarami F, Abadi YS, Rafi F, Vetandoost H, *Iran J Arthropod-Borne Dis*, **2011**, 5(2): 1-9.
[19] Kweka EJ, Senthikumar A, Venkatesalu V, *Parasites & Vectors*, **2012**, 5:277.
[20] Lima MAA, Fabio F, Oliveira Mde, Gomes A, Lavor PL, Santiago GMP, Dias ATN, Arriaga AMC, Lemos TLG, Carvalho MGde, *African J Biotechnol*, **2011**, 10(55):11716-11720.
[21] Row LCM, Ho JC, *Journal of the Chinese Chemical Society*, **2009**, 56: 653-658.
[22] Rabha B, Gopalkrishnan R, Baruah I, Singh L, *Journal of Medical Research*, **2012**, 1 (1): 14-16.
[23] Tiwary M, Naik SN, Tewary DK, Mittal PK, Yadav S, *Journal of Vector Borne Diseases*, **2007**, 44:198-204.