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Larvicidal, Adulticidal and repellent activity of different essential oils.

¹Nilesh M.Jare, ²Dr.S.S.Khadabadi, ³Dr.S.L. Deore, ⁴Dipak Mane, ⁵Ashwini S.Meshram

¹PG Student, Government College of Pharmacy, Amravati, ²Principal, Government College of pharmacy Amravati. ³Associate professor, Government College of pharmacy, Amravati, ⁴Senior officer, Lupin pharmaceutical Ltd, Goa. ⁵ PG Student, Government College of Pharmacy, Amravati. India.

Abstract:

In the present study used natural oils such lemongrass oil, clove oil, ocimum oil, peppermint oil, neem oil and eucalyptus oil for the mosquito repellent. In these check larvicidal activity, adulticidal activity and repellent activity of all oils in 100% concentration. All these activity was checked individual oils and combinational oils.

In these method used for larvicidal activity and adulticidal activity was different and new. In these activity checked on aroma of oil than the application. Eucalyptus oil shows the better larvicidal activity within 4hr 10 min and in combination of peppermint and clove oils shows activity within 3hr 20min. lemongrass oil shows better adulticidal activity within 42 min and in combination lemongrass and peppermint oil shows activity within 27 min. In repellent activity lemongrass oil shows activity for 5 hrs 40min. and in combinational repellent activity the combination of lemongrass and eucalyptus oils, and lemongrass and ocimum oils shows activity for 6 hr.

Index Terms – Oil extraction, larvicidal Bioassay, Adulticidal Bioassay, repellent activity or arm in cage bioassay.

1. INTRODUCTION

Throughout the world mosquitoes represent one of the most significant threats to human and veterinary health. With over 3500 unique species of mosquito currently described having capacity to transmit diseases agent in the animal kingdom. [Norris E, 2017] Mosquito borne diseases are main problem in tropical and subtropical countries. Approximately 17% of the total burden of the whole infectious diseases are vector-borne diseases leading to greater than 1 million mortality per year and greater than 2.5 billion individuals contracting dengue and malaria leads to greater than 400,000 deaths per year internationally. [Mathew N, 2017] Major species of mosquitoes that act as vector for various diseases are: Culex causes Japanese encephalitis, west Nile, chikungunya, Anopheles causes filariasis, malaria, Aedes causes chikungunya, dengue, Yellow fever. [Naseem S et.al, 2016]

Dengue is mosquito borne disease caused by four dengue virus serotypes (DENV1–4) and transmitted by female Aedes mosquitoes. Dengue fever symptoms, including high fever, arthralgia, myalgia, anorexia, rash, and retro-orbital pain. [Katzelnick LC et.al, 2017] In the tropical and subtropical regions Aedes aegypti has been the most important epidemic vector. Other species such as Aedes albopictus, Aedes polynesiensis, and member of Aedes scutellaris is complex and Aedes niveus play a role as secondary vectors. However, Aedes niveus is a sylvatic vector. At room temperature the life cycle of Aedes mosquito depending upon the extent of feeding lasts for 8–10 days. It consists of two phases: aquatic phase (larvae, pupae) and terrestrial phase (eggs, adults). [Khetarpal N et.al, 2016]

In the world Malaria is the common parasitic disease transmitted by mosquitoes of the genus Anopheles to the human host. The interaction between the hosts, the vector and the parasite require for the transmission. The four species of parasites responsible for human malaria are: Plasmodium falciparum, Plasmodium ovale, Plasmodium malariae, Plasmodium vivax. [Rossati A et.al, 2016]

Falciparum malaria caused by *P. falciparum*. Tertian malaria caused by *P. vivax* and *P. ovale* and quartan malaria caused by *P. malariae*. Infections by *P. falciparum* and *P. malariae* can be severe complications, including kidney disease. *P. falciparum* has been increasing resistance to some drugs, such as chloroquine. Invade erythrocytes by parasite leading to high parasitemia levels, which correlate with mortality and disease severity. Malaria can be acute and chronic. Patients show primary symptom like asthenia, anorexia, headache, myalgia, nausea and vomiting. *P. vivax* and *P. ovale* cause to acute malaria and less frequently, by *P. malariae*. In acute malarial Patients present fever, with intervals from 1 to 4 days, with sweating, chills, anemia, splenomegaly and hepatomegaly. Chronic malaria is caused by *P. falciparum*, with anemia, jaundice, diarrhea, respiratory failure, acute kidney injury, hydroelectrolytic disturbances, shock and coma, disseminated intravascular coagulation. [Silva Junior GB et.al, 2017]

Chikungunya virus is reemerging arbovirus. It causes crippling musculoskeletal inflammatory disease in humans and also symptoms like fever, headache, polyarthralgia, myalgia and rash. *Aedes* species of mosquitoes transmit the Chikungunya virus and is capable of an epidemic, high rate of infection urban transmission cycle. There is no licensed vaccines or antiviral therapies are available. [8]

The personal protection from mosquito bites by application of mosquito repellents is most adopted method. Repellent are substance that act locally are distance, deterring an arthropod from flying, landing on or biting human skin. Mosquito repellent works by providing a vapour barrier deterring the arthropod coming into contact with human skin and animal skin. [Nerio LS et.al, 2010]

There are different commercial formulations in different forms. The N,N-diethyl-methylbenzamide (DEET), the main active ingredient of the commercial formulations. DEET has an effective mosquito repellent activity 1 to 8 hrs. But it is known for its toxic reactions and adverse effects on plastic and synthetic fabric. Hence, over this problem recently, there is an increased interest in natural product based mosquito repellents. [Naseem S et.al, 2016]

Controlling mosquitoes is most importance in the present day with rising number of mosquito borne diseases. Due to deforestation, industrialized farming and stagnant water increase in the range of mosquitoes. Thus, special products like mosquito repellents to repel mosquitoes. The products used for mosquito control have varying degrees of effectiveness. In sweat of warm-blooded animals present Carbon dioxide and lactic acid act as an attractive substance for mosquitoes. Perception of the odor is due to chemoreceptor's present in the antennae of mosquitoes. Mosquito repellents based on chemicals has a safety profile, but they are toxic against the skin and nervous system like rashes, swelling, eye irritation, and worse problems including anaphylactic shock, and low blood pressure. Hence, nowadays natural mosquito repellents were preferred. [Rani N et.al, 2013]

The natural repellents from essential oils of various representatives: (1) Myrtaceae: e.g.eucalyptus, cloves, and tea tree. (2) Lamiaceae: e.g. basil, mint, lavender, sage or thyme. (3) Graminae: e.g.citronella and lemongrass. (4) Araliaceae. (5) Labitae. (6) Rutaceae. (7) Compositae or Astaraceae. [Tisgratog R et.al, 2016]

2. Material and methods:

2.1 Materials:

2.1.1 Collection of Mosquito Larvae

Mosquito larvae was collected from outside Amravati (Maharashtra). These larvae collected and cultured in laboratory condition. With proper feeding and aeration in container.

2.1.2 Identification and Authentication of Larvae and Adult Mosquitoes Method

Mosquito's larvae and adult mosquitoes identified and authenticated from the zoology department of Sant Gadge Baba University Amravati-444602.

2.1.3 Plant Material

Lemongrass oil, Eucalyptus oil, Peppermint oil, Clove oil, Neem oil collected from yucca enterprises ltd Mumbai

2.2 Method

2.2.1 Ocimum Oil Extraction.

Ocimum oil extracted in lab.The fresh leaves of *Ocimum sanctum* were collected from the medicinal plant garden Government College of pharmacy Amravati and from outside Kathora nakka region Amravati. The leaves were washed, dried in the shade, and chopped. The essential oil was isolated using the Clevenger apparatus through water distillation for about 4 to 5 hours. The prepared essential oil was dried under anhydrous sodium sulfate and stored in the dark at 4°C until use.



Figure.1 Photograph of Extraction of Oil Form Ocimum

2.2.2 Larvicidal Bioassay

For the bioassay test, larvae were taken in dechlorinated 30 ml water in 100ml containers. Then 10 fourth instar larvae placed in the container with the help of a dropper. Then these containers close with aluminium foil with inner side cotton ball applied with oil were a stick. For aerations or O₂ supply small 4-5 small pin hole was created. Then check the larval mortality every 30 minutes for 72 hrs. Repeated these studies thrice for individual's oil. The control was blank without any application.

2.2.3 Adulticidal Bioassay

10-20 adult mosquitoes 2-5 day's old blown into container (659.4cm²) with help of aspirator. Before the study, these mosquitoes fed with 10% sugar solution for 24 hr. This container was closed with aluminium foil. The treatment solution was applied on the cotton ball then these cotton balls stick to the inner side of aluminium foil. For aeration small 4-5 pinhole on aluminium foil. Mortality was observed every 10 minutes for 3 hours. Repeated this activity thrice for individual treatment solutions. The control was blank without any application.

2.2.4 Repellent activity/Arm in cage Bioassay

Took 45cm×30cm×15cm cage and were made of mosquito net with an opening at the front side for the only introduction of hand. At the bottom of the cage was mirror positioned allowing for observation of mosquitoes landing on the lower side of the arm. Took 100-300 host-seeking 5-10 days old mosquitos of species *Culex quinquefasciatus*. These mosquitoes were fed with 10% sucrose solution. Then these starved for 24 hours before the study. Before study treating hand or forearm washed with water. Before start activity without any application hand was introduced in a cage for 5 min. When a minimum of 5 mosquito land or bite on hand then studies were started. Then applied a 100% concentration of 1ml treatment solution on hand up to the elbow. Then recorded landing and biting of mosquitoes on hand after placing in a cage for 5 min. Mosquitoes were given a 1 hour an interval then place hand in cage for 5 min. A record number of mosquitoes biting and landing. The repeated above procedure for all treatment solutions. Repeated these activities thrice for individual treatment solutions. The control was blank without any application



Figure.2 Photograph of Cage for the Repellent Activity Testing (45cm×30cm×15cm).

3. Result and discussion

3.1 Larvicidal activity:

Larvicidal activity of individual oil was observed in these eucalyptus oil shows better larvicidal activity within 4 hours 10 min. Neem oil doesn't show larvicidal activity within 72 hours.

In combinational larvicidal activity was observed in these study combination of 100% concentration of lemongrass oil, eucalyptus oil, peppermint oil, ocimum oil and clove oil shows 100% larvicidal activity within 3 hours which was the best combination and combination of eucalyptus oil and clove oil took greater time for larvicidal activity it gives 4 hours 30 minute

Table 1. Larvicidal activity of individual oil in triplicate

Sr.no	Oil name	100% Larvicidal activity in hours			
		A	B	C	Mean
1.	Lemongrass oil	4:30 minute	4	4:30 minute	4:20 minute
2.	Eucalyptus oil	4	4	4:30 minute	4:10 minute
3.	Ocimum oil	24	30	30	28
4.	Peppermint oil	5	4:30 minute	4:30 minute	4:40 minute
5.	Clove oil	30	24	24	26
6.	Neem oil	Nil	Nil	Nil	Nil

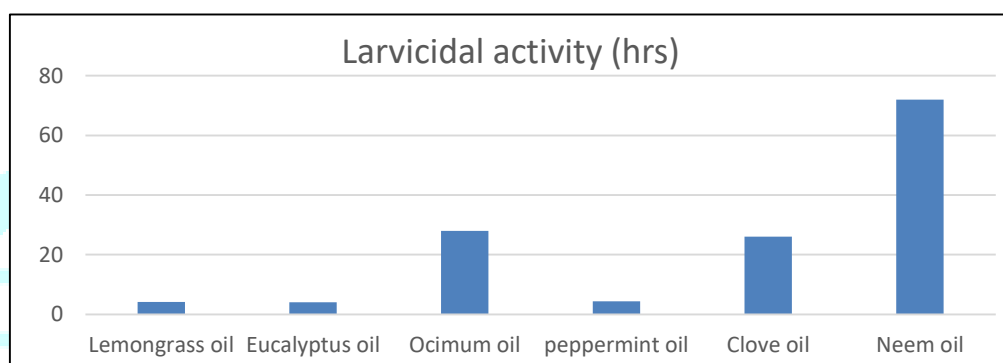


Figure 3. Larvicidal activity of individual oils in hours.

Table 2. Larvicidal activity of combinational oils in triplicate.

Sr.no	Oils name	100% Larvicidal activity in hours			
		A	B	C	Mean
1.	Lemongrass + Eucalyptus (L+E)	3:30 minute	3:30 minute	3:30 minute	3:30 minute
2.	Lemongrass + Peppermint (L+P)	4	4:30 minute	4	3:90 minute
3.	Lemongrass + Ocimum (L+O)	4:30 minute	4:30 minute	4	4:20 minute
4.	Lemongrass + Clove (L+C)	4:30 minute	3:30 minute	4	4
5.	Eucalyptus + Peppermint (E+P)	4	3:30 minute	3:30 minute	3:80 minute
6.	Eucalyptus + Clove (E+C)	4:30 minute	4:30 minute	4:30 minute	4:30 minute
7.	Eucalyptus + Ocimum (E+O)	4	4	4	4
8.	Peppermint + Clove (P+C)	3:30 minute	3	3:30 minute	3:20 minute
9.	Peppermint + Ocimum (P+O)	3:30 minute	3:30 minute	3:30 minute	3:30 minute
10.	Clove + Ocimum	4	4:30 minute	4	4:10 minute

	(C+O)				
11.	All oils(excluding neem oil) (L+E+P+O+C)	3	3	3	3

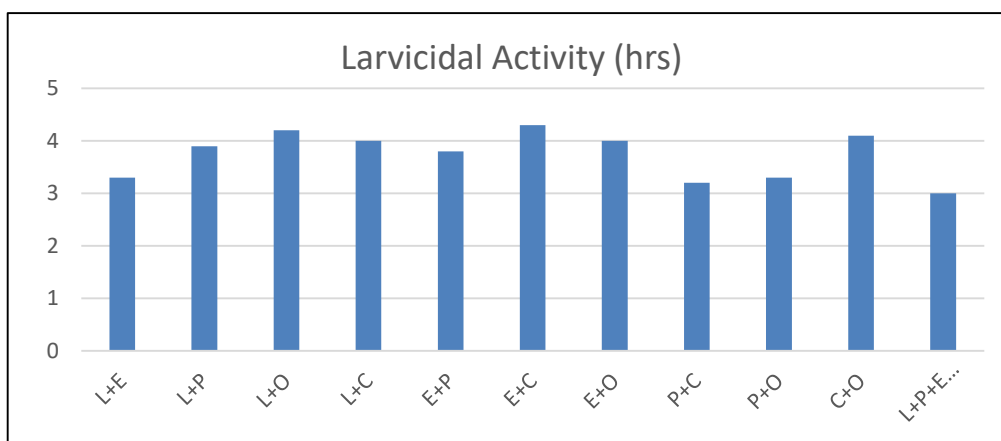


Figure 4. Larvicidal activity of combinational oils in hours

3.2 Adulticidal activity:

In adulticidal activity lemongrass oil shows 100% activity within 42 minute and greater time taken by neem oil its 140 minute for 100% activity. In Combinational adulticidal activity was observed in minutes. In these study combination of lemongrass oil and peppermint oil and combination of all oils in which lemongrass oil, eucalyptus oil, ocimum oil, peppermint oil and clove oil shows 100% activity within 27 minutes .these two combination shows best adulticidal activity in less time. The combination of peppermint oil and clove oil takes greater time than othe combination. These combination takes 50 minutes.

Table 3. Adulticidal activity of individual oils in triplicate

Sr.no	Oil name	100% Adulticidal activity in minutes			
		A	B	C	Mean
1.	Lemongrass oil	40	40	45	42
2.	Eucalyptus oil	70	65	70	68
3.	Ocimum oil	70	70	70	70
4.	Peppermint oil	70	60	70	67
5.	Clove oil	80	85	85	83
6.	Neem oil	140	140	140	140

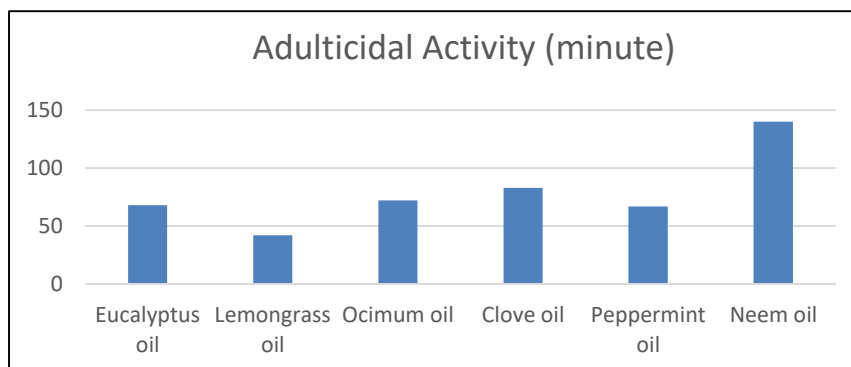
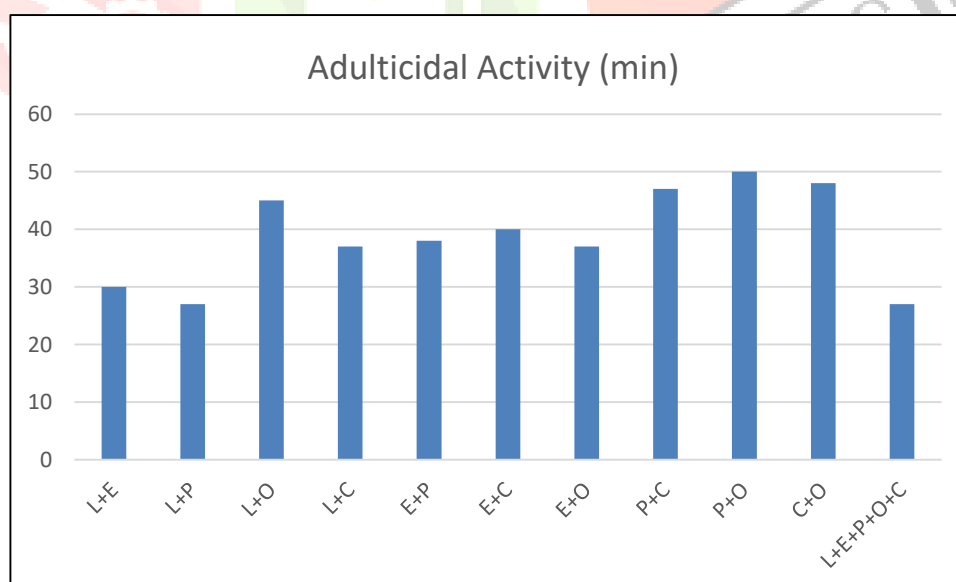


Figure 5. adulticidal activity of individual oils in minutes.

Table 4. Adulticidal activity of combinational oils in triplicate.

Sr.no	Oils name	100% Adulticidal activity in minutes			
		A	B	C	Mean
1.	Lemongrass + Eucalyptus (L+E)	30	30	30	30
2.	Lemongrass + Peppermint (L+P)	30	25	25	27
3.	Lemongrass + Ocimum (L+O)	45	45	45	45
4.	Lemongrass + Clove (L+C)	35	35	40	37
5.	Eucalyptus + Peppermint (E+P)	35	40	40	38
6.	Eucalyptus + Clove (E+C)	40	40	40	40
7.	Eucalyptus + Ocimum (E+O)	35	40	35	37
8.	Peppermint + Clove (P+C)	50	50	50	50
9.	Peppermint + Ocimum (P+O)	45	45	50	47
10.	Clove + Ocimum (C+O)	45	45	45	45
11.	All oils(excluding neem oil)(L+E+P+O+C)	25	25	30	27

**Figure 6. Adulticidal activity of combinational oils in minutes.****3.3 Repellent activity/ Arm in cage method:**

Repellent activity of lemongrass oil shows better activity up to 5 hour 40 minute no landing or biting of mosquitoes. Neem oil shows repellent activity upto 2 hours 40 minute.

In repellent activity study combination of lemongrass oil and eucalyptus oil which shows no biting and landing up to 6 hours and combination of all oils excepting neem oil which also shows the activity up to 6 hours. In these combination clove and ocimum oil

shows less repellent time up to 3 hours 20 minute. So combination of lemongrass oil and eucalyptus oil, lemongrass oil and ocimum oil, and the combination of all oils was best for formulation.

Table 5. Repellent activity of individual oil in triplicate.

Sr.no	Oil name	100% Repellent activity in hours			
		A	B	C	Mean
1.	Lemongrass oil	6	6	5	5:40 minute
2.	Eucalyptus oil	5	4	5	4:40 minute
3.	Ocimum oil	4	4	5	4:20 minute
4.	Peppermint oil	5	5	5	5
5.	Clove oil	4	3	3	3:20 minute
6.	Neem oil	3	2	3	2:40 minute

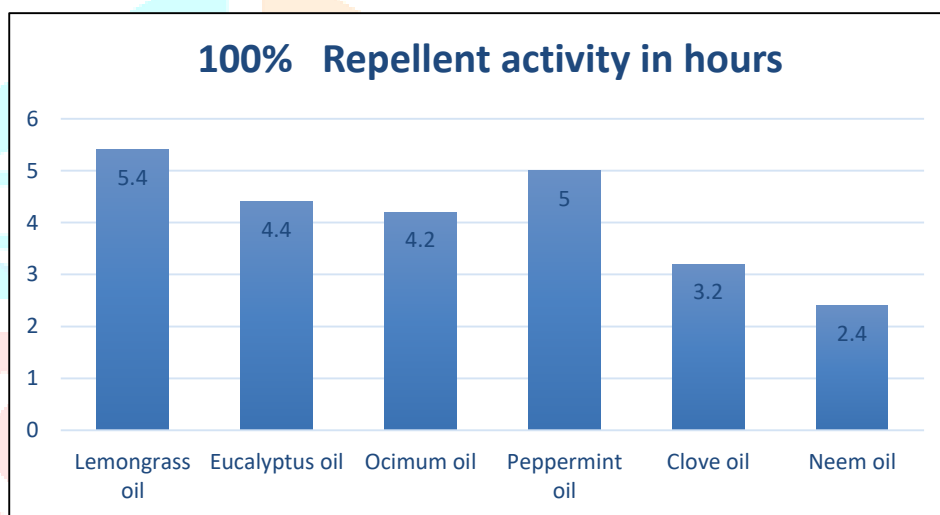


Figure 7. Repellent activity of of individual oils in hours.

Table 6. Repellent activity of combinational oils in triplicate

Sr.no	Oils name	100% Repellent activity in hours			
		A	B	C	Mean
1.	Lemongrass + Eucalyptus (L+E)	6	6	6	6
2.	Lemongrass + Peppermint (L+P)	5	5	4	4:40 min
3.	Lemongrass + Ocimum (L+O)	6	6	6	6
4.	Lemongrass + Clove (L+C)	4	4	3	3:40 min
5.	Eucalyptus + Peppermint (E+P)	4	4	5	4:20 min
6.	Eucalyptus + Clove (E+C)	4	4	4	4
7.	Eucalyptus + Ocimum	4	4	5	4:20 min

	(E+O)				
8	Peppermint + Clove (P+C)	4	3	4	3:40 min
9	Peppermint + Ocimum (P+O)	4	4	4	4
10.	Clove + Ocimum (C+O)	3	3	4	3:20 min
11.	All oils(excluding neem oil)(L+E+P+O+C)	6	6	6	6

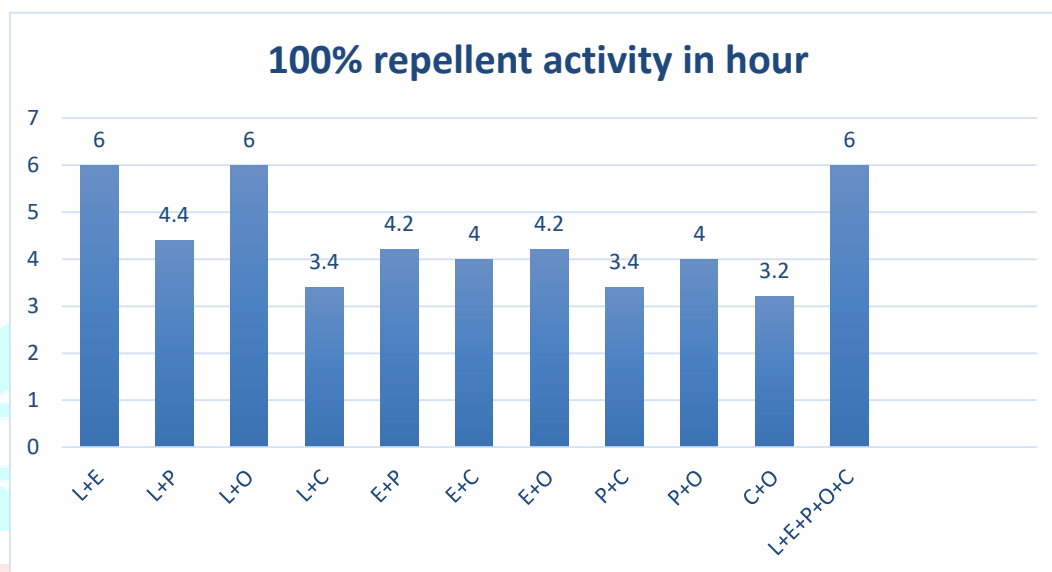


Figure 8. Repellent activity of combinational oils in hours.

Conclusion:

Chemical based mosquito repellents was safe, but they are toxic effect on the skin and nervous system like rashes, swelling, eye irritation, and worse problems including anaphylactic shock, and low blood pressure. Hence, natural mosquito repellents were preferred. 16 families, 35 genera, and 49 species such as Myrtaceae, Lamiaceae, and Rutaceae, etc. families being the one with most species show mosquito repellent activity. The composition of essential oil especially terpenes it shows the repellent activity. Extraction of essential oil especially with steam distillation and hydro distillation method. The production of natural insecticide from essential oil shows the less harmful effect on human and environment.

In these method used for larvicidal activity and aulticidal activity was different and new. In these activity checked on aroma of oil than the application. Eucalyptus oil shows the better larvicidal activity within 4hr 10 min and in combination of peppermint and clove oils shows activity within 3hr 20min. lemongrass oil shows better adulticidal activity within 42 min and in combination lemongrass and peppermint oil shows activity within 27 min. In repellent activity lemongrass oil shows activity for 5 hrs 40min. and in combinational repellent activity the combination of lemongrass and eucalyptus oils, and lemongrass and ocimum oils shows activity for 6 hr.

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