A number of deadly diseases such as malaria, dengue, lymphatic filariasis, yellow fever, encephalitis, chikungunya, etc. among man and animals are transmitted by mosquitoes especially in tropical and subtropical areas. Every year millions of people are infected and killed by these deadly diseases. Emerging diseases like dengue fever, dengue hemorrhagic fever (DHF) and chikungunya are mosquito borne viral diseases and are spread by infected Aedes aegypti female mosquitoes among a wide range of people in the world. Personal protection by using repellents is one of the effective methods to reduce man mosquito contact to minimize disease transmission.

Essential oils are natural scented plant products obtained from different plant species. Essential oil is a combination of highly volatile chemical constituents obtained from different parts of plants with the help of various distillation methods. Plant essential oils from families such as Apiaceae, Asteraceae, Burseraceae Cupressaceae Geraniaceae, Labiatae, Lamiacea, Lamicae, Lauraceae, Myrtleaceae Myrtaceae, Oleaceae, Piperaceae, Poaceae and Rutaceae have shown different types of biological properties like insecticidal, repellent, antifeedent and growth inhibition against insects including mosquitoes. The best known natural insect repellents come from the plant families such as Lamiaceae, Myrtaceae and Poaceae. Examples of effective natural mosquito repellents are p- Menthone-3, 8- diol (PMD) from Eucalyptus maculate citrodon, lemon eucalyptus and citronella oil from Cymbopogon nardus. Essential oils from plants such as andiroba, basil, catnip, cedar, citronella grass, clove, lemon scented eucalyptus, garlic, geranium, neem, rosemary and thyme have all been identified as mosquito repellents but they have very feeble repellency and protection time against
mosquitoes is very short. Isolated studies have been made on mosquito repellency of plants, their extracts and essential oils, on the basis of which it is difficult to draw definite conclusions and recommendations. Hence they are of little practical utility. Though some comprehensive studies on natural on natural mosquitoes have been made, but they were limited to preliminary screening of crude preparations. Moreover, for comparison, no simultaneous experiments have been conducted with well known synthetic mosquito repellents like DEET and DEPA. Therefore it was decided to carry out a detailed study on mosquito repellency of 23 essential oils and their combinations.

The main objective of the present study was to find out an effective mosquito repellent amongst the essential oils or to make such a formulation by testing different combinations of essential oils or their active constituents. For this purpose 23 essential oils from plants namely amyris, basil, black pepper, catnip, camphor, chamomile, cinnamon, citronella, dill, frankincense, galbanum, geranium, jasmine, juniper, lavender, lemon grass, lemon scented, litsea, pepper mint, tagetes, thyme, rosewood and rosemary were selected and they were procured from Fragrance and Flavour Development Center (India), Kannauj. Two synthetic insect repellents, N, N-diethyal-3-methylbenzamide or N, N-diethyl-m-toluamide (DEET) and N, N-diethyl phenyl acetamide (DEPA) were also employed in the study as positive controls.

The repellency experiments were conducted on adult mosquitoes, *Ae. aegypti* (unfed 5 to 6 days old) which were drawn from the standard laboratory culture maintained at 27 ± 2 °C and 70 ± 5 % relative humidity and 12:12 dark: light photocycle.
A variety of methods are available for testing repellency of volatile compounds against mosquitoes. For the present study K & D module (Klun and Debboun 2000) was considered to be an excellent tool for fast screening as it is easy to use in laboratory conditions, it can be used for qualitative and quantitative measurement of a large number of replicates of various doses over single species and single doses over various species.

The results of the skin repellency experiment at four doses 0.01% (0.2µg/cm²), 0.1% (2µg/cm²), 1% (20µg/cm²) and 10% (200µg/cm²), litsea exhibited 43%, 86%, 93% and 100% effective repellency against *Ae. aegypti* female mosquitoes over 22 essential oils. However, the percentage repellency of 14 essential oils litsea, rosewood, lemon scented, lemon grass, camphor, galbanum, citronella, rosemary, geranium, cinnamon, peppermint, dill, frankincense and basil at 0.01% showed 13% to 43% repellency over technical DEPA (13%) and DEET (20%). Ten essential oils litsea, rosewood, lemon scented, lemon grass, camphor, citronella, rosemary, geranium, cinnamon and peppermint gave 56% to 86% effective repellency over DEPA (50%) and DEET (60%) at 0.1% concentration. However, at 1% concentration only nine oils litsea, rosewood, lemon scented, lemon grass, camphor, citronella, rosemary, cinnamon and peppermint showed 90% to 96% repellency over DEPA (90%) and DEET (90%). At 10% concentration, out of 23 essential oils only chamomile, tagetes and juniper showed lower than 80% repellency and other 20 oils showed 100% repellency similar to DEPA (100%) and DEET (100%). The repellent action of the tested 23 oils showed that percent repellency of 10 essential oils of litsea, rosewood, lemon scented, lemon grass, camphor, citronella, rosemary, geranium, cinnamon, pepper mint was higher than other 13 essential oils tested
and also higher than technical DEET and DEPA. Based on the observations of repellency of the tested essential oils, the ED$_{50}$ (0.012 to 0.060) of these oils was found to be lower than DEPA (0.074) and DEET (0.067). The other 13 essential oils ED$_{50}$ (0.128 to 1.627) were found to be higher than DEET and DEPA.

The protection time of these 23 essential oils was determined at 20% (2mg/cm$^2$) dose level. 22 essential oils showed complete protection and only juniper gives 66% repellency against _Ae. aegypti_ for a freshly applied oils soon after the time of application. At same formulation litsea provided the highest and the longest duration of protection. It’s showed more than 80% repellency up to 2 hours and reduced gradually 66%, 50% repellency at 4, 6 hours respectively. Next strongest oils, lemon scented and lemongrass oils showed complete protection up to 2 hours at the same concentration. The other four oils rosewood, geranium, camphor and dill gave more the 80% repellency for 2 hours. However, citronella, galbanum, cinnamon, black pepper and thyme provided complete protection up to 1 hours. Frankincense, basil, jasmine and rosemary showed more than 80% repellency up to 1 hour against _Ae. aegypti_ and remaining six essential oils gave below 63% repellency for 1 hour.

It was further observed that selective blending of effective ten oils namely litsea, rosewood, geranium, lemon scented, lemongrass, citronella, galbanum, cinnamon, camphor and dill 10% concentration (1:1 ratio) showed complete protection soon after application against _Ae. aegypti_. The combination of litsea oil with rosewood, geranium, lemon scented and lemongrass provided 73% to 86% protection for 4 hours and decline
50% to 56% at 6 hours. The combination of lemon scented and lemongrass is the strongest combination gave complete protection at 2 hours and gradually reduced 73%, 30% in 4, 6 hours respectively. The other 12 combinations such as litsea + citronella, litsea + dill, litsea + galbanum, rosewood + geranium, dill + lemongrass, dill + galbanum, lemon scented + cinnamon, lemon scented + galbanum, lemongrass + galbanum, galbanum + cinnamon, citronella + lemongrass, citronella + cinnamon provided more than 80% up to 2 hours. The other 29 combination provides less than 80 % protection after 2 hours of application against *Ae. aegypti*. The maximum protection time shown by essential oils was up to 2 hours, whereas in case of DEET and DEPA it was more than 5 hours against *Ae. aegypti*. The K & D module bioassay is a kind of human skin bioassay in which the influence of test compounds (essential oils) might be influenced by human odour component and skin emanations.

In cage bioassay, all the essential oils at 20% (1mg/cm²) dose of repellent were applied on the external surface of the fist of human hand over an area of about 150 cm². Essential oil of litsea was found effective till 2 hrs while other oils namely rosewood, lemon scented, geranium, lemongrass and dill were effective till 1.5 hrs. Three oils namely cinnamon, galbanum and catnip were effective till 1 hrs. While other 12 oils provided complete protection up to 30 minutes but in black pepper and juniper no protection observed. The technical DEET and DEPA provided complete protection ranging from 5 to 6 hrs against *Ae. aegypti* mosquito.
In second set of experiment the behavioural response (repellents and attractants) of mosquitoes was recorded using Y-maze olfactometer in which there is no involvement of skin factors. It also provides quick and effective results. The spatial repellency of selected essential oils was determined at three different concentrations i.e., 1ppm, 10ppm and 100ppm in comparison with DEET and DEPA against Ae. aegypti. However, litsea, rosewood and geranium showed effective repellency and the behavioural response of Ae. aegypti mosquitoes towards these samples was similar to that of DEET and DEPA. Litsea oil showed 50%, 60%, and 77% repellency at 1ppm, 10ppm and 100ppm respectively and other two oils rosewood and geranium gave more than 46%, 56%, 66% repellency at the same concentrations. The other twenty oils showed 13% to 33% at 1ppm, 16% to 45% at 10ppm and 30% to 57% at repellency 100ppm against Ae. aegypti mosquitoes. While technical DEET and DEPA showed 57% to 59% at 1ppm, 65% to 68% at 10ppm, 80% to 85% repellency at 100ppm. For all the essential oils tested, the repellent activity gradually increased with increasing concentrations. According to the result of experiments on behavioural response of Ae. aegypti mosquitoes using Y-maze olfactometer towards all the 23 essential oils have shown significant repellent activity. However, due to highly volatile property of essential oils exhibited varying degree of repellency and it was not possible to get correlative results with skin bioassay and the Y-maze olfactometer.

Quick screening of mosquito repellent and behavioural response studies using skin bioassay and olfactometer bioassays respectively provide useful information to identify effective mosquito repellent compounds. But crude material (essential oils) may
have a large number of active ingredients or components which are responsible of repellency with different modes of activity against mosquitoes. It depends on the combined effect of different chemical components present in essential oils and different plants have different compounds with different volatile properties. The repellency of essential oils, as detected in the present study, was quite significant. But as compared to the repellency of DEET and DEPA, at comparable doses, the response not very impressive. They can provide protection only of shorter durations. For a variety of reasons these oils cannot be used at very high doses and their repeated applications cannot be recommended. It is also not 100% true that the use of natural plant products is totally safe. In fact their overdose and or continuous internal and external use may some side effects and may cause health problems. Therefore it was felt necessary to separate and identify mosquito repelling constituents of the essential oils that have shown their superiority in repellency screening.

The oils that showed good repellent properties in the first and second stages were transferred to the third stage for identification of effective components detected by insect antennae within complex volatile essential oils by using gas chromatography-electroantennogram detection (GC-EAD). This technique is useful for identification of particular volatile components which are responsible for the excitation of sensory receptor neurons of the antennae. In the study of GC-EAD, antenna of female Ae. aegypti responded to 25 components in alone 10 essential oils and 20 compounds, isolated by GC from a mixture of 10 essential oils namely litsea, geranium, rosewood, cinnamon,
citronella, lemongrass, lemon scented, camphor, galbanum and dill, from 6 different plant families could generate an antennal response at the rate of 1µl dose as recorded by EEG.

In electroantennogram study predominant component of litsea oil is Z-Citral a mixture of geranial and neral elicit a spick response in the antenna of *Ae. aegypti* female mosquito and the other components were identified as Cis-Geraniol also called Nerol and Geranyl acetate. However, the antenna stimulatory component of rosewood oil is Linalool and Linalool oxide elicits a spick response and the active component of geranium oil is β-Citronellol, Menthone, p-Menthane and Trans-Caryophyllene. Moreover, the EAD active major component of lemon scented oil is citronellal, β-Citronellol, Geraniol and lemongrass oil is Citral, Linalool, Camphene elicited a strong response of the antenna of *Ae. aegypti* female mosquito. However, an electroantennogram active component of cinnamon oils includes Cinnamaldehyde, β-Caryophyllene, Methoxycinnamaldehyde and camphor oil includes p-Menthane, Cinnamaldehyde. Camphene and Dillapole was the only component of galbanum and dill oil that elicited antennal response respectively. Two components of citronella oil, citronellal and β-Citronellol were EAD active. The blend of 10 oils chemical eluting Linalool, Linalool oxide, Citronellal, Menthone, p-Menthane, β-Citronellol, Z-Citral, Cis-Geraniol, Citral, Cinnamaldehyde, Camphene, Geraniol, Citronellal, Geranyl acetate, β-Caryophyllene, Methoxycinnamaldehyde, Caryophyllene, Dillapole, Trans-Caryophyllene and β-Citronellol in the antenna of *Ae. aegypti* female mosquito. The EAG amplitude response and the repellent activity of essential oils studied with the help of various bioassay methods for comparison provide information of the effective repellent property.
At fourth set of experiment, the chemical constituents of all the tested oils were identified by GC/MS. Essential oil is a natural plant product and mixture of highly volatile compounds mainly monoterpenoids and sesquiterpenoids, benzenoids, phenylpropanoids, etc. The major constituent of amaryis, basil, black pepper, catnip, chamomile, camphor, cinnamon, citronella, dill, frankincense, galbanum, geranium, jasmine, juniper, lavender, lemon-scented, lemongrass, litsea, peppermint, tagetes, thyme, rosemary, rosewood contain Cadinene (\(\beta\)-Cadinene), Linalool, \(\beta\)-Caryophyllene, Caryophyllene, 1, 8-Cineole, Camphor, Cinnamaldehyde, Citronella, DL-Limonen, \(\alpha\)-Pinene, DL-Limonene, \(\beta\)-Citronellol, Eugenol, \(\alpha\)-Pinene, Linalool, Citronella, Citral, Z-Citral, Menthol, DL-Limonene, m-Thymol, Camphene, 1,8-Cineole respectively. The percentages, molecular weights, and retention times of the main constituents of the essential oil group compared with their repellency and behavioural responses of \(Ae.\ aegypti\) mosquito may be useful in the quality control of the final product.

Essential oils have short duration of protection due to highly volatile chemical constituents. The oils that showed good repellent properties in all stages were transferred to the fifth and final stage. litsea, rosewood, geranium, lemon grass and lemon scented showed good repellent property against \(Ae.\ aegypti\) and then these oils were transferred to the final stage to test and all the five effective oils were prepared through emulsification methods. The complex formulation of active compounds/ingredients litsea, rosewood, geranium, lemon scented and lemongrass (in 10\%, 15\% and 20\% v/v, respectively) were incorporated to it Stearic acid, stearyl alcohol, acetyl alcohol and propyl paraben (lipid phase) were heated together at about 70°C and glycerin, potassium hydroxides, methyl
paraben (preservative) were mixed together which comprised the aqueous phase. However, the aqueous phase was heated at 70°C. Both two phases were gradually mixed and the cream was allowed to cool at room temperature. In the study of protection period at 2mg/cm² dose in three concentrations i.e. 10%, 15% and 20% using K & D module, the vanishing cream base of litsea showed a long lasting repellent effect against two mosquitos *Ae. aegypti* and *Ae. albopictus* over four oils. At 15% and 20% concentration, all five cream based oils provided complete protection up to 2 hours and 10% concentration of cream based oils provided 100% repellency up to one hour against *Ae. aegypti* and *Ae. albopictus*. The technical DEET and DEPA provided complete protection up to 5 hours against *Ae. aegypti* and *Ae. albopictus* mosquitoes at same concentrations.

In cage bioassay, 20% concentration of repellent were applied on the external surface of the fist of human hand over an area of about 150 cm² at the rate of 1mg/cm². The protection period of vanishing cream based litsea oil at 10%, 15% and 20% against *Ae. aegypti* and *Ae. albopictus* was found effective till 1.5 hrs, 2.5 hrs and 3.5 hrs respectively. While other vanishing cream based oils such as rosewood, geranium, lemon scented and lemongrass at same concentration were effective till 1.5 hrs, 2 hrs and 3 hrs against *Ae. aegypti* and *Ae. albopictus*. Technical DEET and DEPA provided 3.5 to 4 hrs protection at 10%, 4 to 4.5 hrs at 15% and 5 to 6 hrs at 20% against *Ae. aegypti* and *Ae. albopictus*.
The average protection time of vanishing cream based oil showed effective results due to the volatility of essential oils was reduced because formulated vanishing cream contain some fixation materials to fix the aromatic materials on the skin for as long as possible.

The evaluation of 23 essential oils for their repellency was done against *Ae. aegypti* a major vector of dengue and chikungunya in the laboratory condition. In this thesis, Essential oils play central role as a barrier to the insect, preventing either landing or penetration of the skin. The result obtained shows that essential oils can provide significant repellent activity against *Ae. aegypti* female mosquito. The repellent effect of volatile essential oils against mosquitoes is useful for developing slow release repellent and also increase in the duration of repellency.

The present study provides information for developing safe, biodegradable, eco-friendly and effective insect repellent formulation for topical application by using a non insecticidal chemical which can be used by individual and communities in specific situations to minimize the transmission of vector borne diseases. Further, it is based on this point of view that this study was conducted in the laboratory to evaluate the repellency of the essential oils.