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Efficacy of certain plant essential oils as larvicides and repellents on mosquitoes

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Abstract

Mosquitoes are noxious nature Dipteran insect vectors responsible for transmitting the life threatening pathogenic organisms and connected diseases in humans such as malaria, dengue, filariasis, chikungunya, Japanese encephalitis etc. The female mosquito bite, by piercing and sucking type mouth parts, causes localized skin irritation and injects pathogenic organism in to the skin through its saliva. The mosquito borne diseases chances and cases are showing increasing trend regularly in India and across the world due to some adverse environmental changes as global warming and climate change. Due to the lack of proper sanitary conditions, cleanliness and presence of open stagnant water sites and drainage, the mosquitoes population is increasing. The stagnant water is the breeding place for mosquitoes, as its eggs and immature stages survive in water. In India, a big number of people suffer from mosquitoes borne diseases every year, causing disease burden in public. The present study is focused on the control strategies of mosquito population in aquatic environment by aqueous solution technique, using some plant essential oils, EO, in certain concentrations mixed in water, such as Peppermint, Lemongrass, Citronella, Neem (*Azadirachta indica*), *Eucalyptus* sp. Essential oils etc. The taken essential oil formulations were applied to test their repellent efficacy for adult mosquitoes by using liquid vaporizer machine in experimental box and room. In aqueous solution technique, the larval mortality was found hundred percent in the combination of Neem EO 1%, lemongrass-1% and Eucalyptus-1% after 24 hours. The LC₅₀ assessed as 0.4738%. The combination of peppermint and neem EO in 0.1% each was observed potent controlling mixture for larvae after experimental time period of 24 hours, with LC₅₀ as 0.0521 percent. The lemongrass and peppermint EO in 0.1% separately were found quite effective to kill hundred percent the immature stages of mosquitoes after 24 hrs. Neem and peppermint EOs were found effective killing in 0.1% combination. The repellent efficacy of taken plant EOs was found effective by using liquid vaporizer, LV. On exposure to plant EO mixture solution, the restlessness, convulsions, tremors, nervous breakdown and knockdown responses were noticed in adult mosquitoes. The experimental formulations repelled and knock downed the adult mosquitoes in iron net-wired experimental box and room, preventing from the mosquito bites.

Keywords: Peppermint, lemongrass, citronella, neem, *Azadirachta indica*, repellent, larvicide, malaria, dengue

Introduction

Mosquitoes cause life threatening health impacts in human beings, by transmitting different pathogenic agents, responsible for certain diseases as malaria, filariasis, dengue, chikungunya, Japanese encephalitis, yellow fever etc. They also act as noxious creatures for humans by their biting activities, causing itching and irritations at the site of skin, whether at night or during day hours. This present study is designed to control the mosquito larvae population in water by plant based essential oils and to use the such oils as repellent for adult mosquitoes. The use of health friendly effective mosquito repellents keep mosquitoes away, preventing the chances of transmission of health threatening microbes spread by vectors like mosquitoes. The mosquito vector borne diseases cause a significant challenge for human health by menacing the public health. The different species of mosquitoes exist throughout the year in Indian subcontinent but due to favourable environmental conditions, as poor cleanliness management in urban, suburban and rural areas, water lodging at various places in human habitat areas as potential breeding environments available for mosquitoes, and rise in atmospheric temperature after winter months and onset of summers, the population of mosquitoes can be seen enormously increased, causing the probability of mosquito borne diseases. The open drainage system, stagnant water bodies are the best available conditions for breeding and development of

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mosquitoes species in India. In such sites, the female mosquitoes prefer to lay their eggs and further developments take place in aquatic realms, as larval and pupal stage developments.

Thousands people die every year due to mosquitoes as vectors borne diseases in India and worldwide. In India, there are plenty of vector borne diseases prone areas due to lack of proper sanitary conditions and awareness. Rising global and regional temperature is responsible for the expansion of geographical range of many key vector species, increasing the number of people at risk of contracting of these diseases [1-5]. The infected female mosquito individuals suck the human blood for feeding purpose for their metabolic need and at the time of feeding they transfer the microbial pathogenic agents with saliva into the human body by injecting the piercing and sucking type mouth parts in the exposed part of skin.

Under present environmental conditions, the cases of dengue virus infections are being noticed in great numbers in Muzaffarnagar and adjacent region in western Uttar Pradesh, India, affecting the health of people in a serious way. The muscular weakness, joint related problems, taste related disorder and nasal bleeding are main disease symptoms in dengue infected people. So mosquitoes as vectors of different pathogenic agents are posing a serious threat in human health area.

The present study is concerned to the use of plant based essential oils and extracts to control the aquatic larval stages of mosquitoes and adults by using essential oils vapour to repel the mosquitoes away as these are safe for health purpose and environment. Understanding the mode of action of mosquito repellents has been a big study area in vector biology community for decades and has only been partially explained for some mosquito repellent active ingredients [6-12]. The mosquito repellents act on chemo-receptors associated with olfactory or gustatory organs and other body appendages having chemo-receptive sensillae as the tarsi and wings [13-19]. The various modes of action of repellents on chemo-receptors elicit a repellent response in mosquitoes as including over-stimulation or hyper-stimulation and by blocking specific chemo-receptors or by masking the odors [7, 20-24]. The mosquito repellents are categorized in two types based on the application or use of repellent as available in studies and literature [25-30]. These are spatial and contact repellents. A mosquito repellent can have either one type or both types of repellency nature [11, 16, 31]. In case of the spatial nature repellents, the applications include the topical treatments like lotions, cream and sprays or as devices that vaporize or aerosolize repellent ingredients into the proximity area. The spatial repellency is generally observed by the absence of mosquitoes in the vicinity or by decrease in mosquito numbers in a significant level to touch or visit a treated object or individual [32, 33].

The other repellent category is the contact repellent, in which the repellent repel mosquitoes that come into direct contact with the product and are generally applied topically through spray or lotions. Contact repellency is typically observed when mosquitoes land on a treated host or object without proceeding to initiate feeding behaviour, like probing and instead fly away immediately [19].

The repellents available commercially in markets now, contain active ingredients such as DEET (N, N- diethylemeta-toluamide), Picaridin, IR3535, or para-methane-diol (PMD). [34, 35].

There is a general concern in public connected to the health

safety issues due to the impacts of active ingredients present in the long lasting synthetic repellents [36, 37]. The concern persists even with reports that say, the active ingredients are safe when used as recommended and directed [38-45]. Due to the health safety issue, the focus of recent researches is on natural ingredients and products as the experimental work on the repellent efficacy of certain natural essential oil ingredients, which are considered in this present investigation. Presently we use insecticide based mosquito repellents as DEET and pyrethroid based synthetic formulations, such as Transfluthrin 1.6% w/w Liquid vaporizer (Household insecticide) in vaporizer electric machines in homes. Such formulations cause health risks. A study results showed that transfluthrin, the active insecticide induces oxidative stress, morphological defects and embryonic lethality in zebrafish. [46] In aquatic bodies, the chemical insecticides are in use. The essential oils of Neem (*Azadirachta indica*), Eucalyptus, Lemon grass, Citronella, Peppermint EO (containing menthol and menthone and menthyl acetate, menthofuran etc.) were tested to assess their efficacy as larvicide and repellent actions against adult mosquitoes in Muzaffarnagar, India. Rose and night jasmine essential oils were also tested in combination to assess the repellent actions.

According to National Centre for Vector-Borne Diseases Control (NCVBD), about 95% of the population in India resides in malaria-endemic areas and 80% of malaria reported in the country is confined to areas consisting of 20 percent of population residing in tribal, hilly, difficult and inaccessible areas.

Every year 25 April is commemorated as World Malaria Day to raise awareness and highlight the need for continued investment and sustained political commitment to malaria prevention and control. It is preventable and treatable disease, yet it is responsible for the death of over 500,000 people per year around the globe. According to World Malaria Report 2019 by World Health Organization (WHO) [47], India represents 03 percent of the global malaria burden.

According to the World Malaria Report 2021 [48], there was an estimated 241 million malaria cases and 627000 malaria deaths worldwide in 2020 and it is about 14 million more cases in 2020 compared to 2019, and 69000 more deaths.

According to NCBVD, the malaria caseload, though steady at around 2 million cases annually in the late nineties, has shown a declining trend since 2002. Meanwhile, the annual parasite incidence, API, has consistently declined from 2.12 per thousand in 2001 to 0.05 per thousand in 2019. Government of India is working to eradicate malaria by 2030, but there are several challenges to India's ambition of eradicating malaria by 2030 and forest malaria is one of them. India has hills, plains, coasts and deserts areas with different environmental conditions, facilitating the spread and transmission of vector-borne diseases.

India is a multi-ethnicity wide variety of vectors, transmitting different malaria species in rural, urban and especially hilly and forest areas leading to multi-organ failure, posing an uphill task for the public health system in India. In 2016, Sri Lanka was declared malaria-free by WHO and according to experts, this can be a model for India.

In recent years, the rate of infection spread has increased dramatically, and a growing number of scientists are concerned because global warming could lead to the explosive growth of the mosquitoes-borne diseases worldwide. These are some of the most common diseases spread around the world by mosquito bites ; Zika virus, West

Nile virus and chicken guinea virus infections, dengue fever and malaria. There is no vaccine to prevent or medicine to treat most of these diseases [49].

Materials and Methods

Several plant essential oils, EO, were arranged from local market in pure state as lemongrass, citronella, peppermint, neem, eucalyptus, clove, and rose. The experimental set-ups were designed as to assess larvicidal response in aquatic medium. The efficacy of essential oils as repellents in designed iron net-wired wooden box and open room atmosphere were also determined. For assessment of larvicidal activity, several essential oils were mixed in water in specific concentrations. Mosquito larvae were collected from open water bodies and drainage. The mosquito larvae were reared in water in round shaped open glass jars, kept in a wired open able ironed-net wooden box of a size 30cm³ for experimental purpose. Last and previous instar healthy larvae were released in water for 24 hours, containing specific concentrations of essential oils. The larvae, 20 in number, were released. The toxicity response and mortality were recorded using aqueous solution technique. The data were analyzed to calculate LC₅₀ value by using online tool for LC₅₀ calculation "Quest Graph TM LC₅₀ calculator" Bioquest, Inc. (Ref) Control experiment was also set-up. For repellent actions of plant essential oils, the single and mixture of oil concentrations, were mixed in absolute ethanol, as loader or vaporizer agent for essential oils and tested in electrically operated vaporizing device, available in market, installed in iron net-wired wooden box. The adult mosquitoes 20 in number were released to test the repellent efficacy of different essential oils, EOs. The knockdown response was assessed. Open testing in rooms was also done and repellent action was assessed. As per the guidelines for repellent efficacy against mosquitoes as to assess mosquito biting inhibition in open condition in indoor household environment, the health safety measures for personal protection were maintained [50]. The test solution was evaluated for biting inability and inhibition by observing the paralyzing and fled away behaviour in mosquitoes. The vaporized test solution containing concentration of essential oil/s in ethanol was experimented in 30 m² room having air changes/ half hour to mimic open window or door condition. The size of experimental study area/ chamber was kept as 30m² with several required ventilation so as to simulate the indoor room size and conditions in order to have more realistic scenario of how the product performs in these settings. The study areas/ chambers were maintained at 27⁺20 °C and 80⁺10% relative humidity. The female adults of mosquitoes of different types as mixed number of *Aedes aegypti*, *Culex Sp.* and *Anopheles Sp.* were taken for study purpose.

The adult mosquitoes emerged from pupae, reared in round glass jar containing water, were released, 50 in number inside the experimental room/chamber and were acclimatized for 1 hour. The liquid vaporizers (LV) were tested to assess the repellent efficacy against taken species of mosquitoes by using human volunteer. The electricity operated LV was installed in the centre of experimental space/chamber at a certain height from ground, and was switched on. The test with machine vaporizer was conducted with normal/regular mode. The volunteer sat on a stool 1 meter away from LV. The observations were taken for knock down response at 5 minutes interval till 60 minutes observing landing and probing inhibition. The repellent efficacy tests in confined ironed net-

wired box was conducted and knockdown response, NDR, was assessed in percent. In room testing for adult mosquitoes, the repellency and knockdown action was observed. The present study was carried in months of monsoon and winter in Muzaffarnagar, the region of Northern Indian Ganga-Yamuna Doab plains, situated 272 meters above the sea level at latitude and longitude 29.4727⁰N and 77.7085⁰E respectively. The atmospheric temperature touches approximately 43 °C in summers and a lowest as 7 °C approximately in winters. The region has a monsoon influenced humid subtropical climate and receives good rainfall in monsoon months.

In aqueous solution technique, *Azadirachta indica* (neem) oil-1%, eucalyptus oil-1%, lemongrass oil-1%, were mixed in water.

In another concentration, the neem oil 0.1% with peppermint oil, 0.1% were taken.

The next concentrations, the citronella essential oil only, lemongrass oil only, peppermint essential oil only, eucalyptus essential oil only in 0.1% and further increased to 1%, 2.0% and 5%, were prepared and tested separately.

In vaporizing bottle/device, the lemongrass essential oil-5%, eucalyptus-5%, neem oil-5%, peppermint-5%, clove oil-2.5% were dissolved in ethanol v/v in preparation.

Other preparations, for repellent actions, various combinations of essential plant oils in different concentrations dissolved in ethanol, were also tested.

Results and Discussion

The efficacy of certain essential oils, EO of different plants against mosquito's larvae and adults was tested and analyzed by aquatic solution technique and vaporizer repellent actions respectively. After observations, this was found that larval mortality, in combination of concentrations of *Azadirachta indica*, the Neem oil 1%, eucalyptus, 1% and lemongrass 1% in water, was noticed hundred percent after 24 hours. The pupae were also shown hundred percent mortality after 24 hours of experimental time period. The LC₅₀ value for this test in percent was assessed as 0.4738. The combination of concentrations of Neem and Peppermint essential oils in 0.1 percent each, in aqueous medium, the second and last instar larvae and pupae have shown hundred percent mortality after experimental time period of 24 hrs. The LC₅₀ value was calculated 0.0521 in percent. The larval mortality was found hundred percent in concentration of essential oils of citronella in 0.1 percent in aqueous medium after same experimental treatment time period. The LC₅₀ value was assessed 0.0466 in percent. The essential oils of lemongrass were also found potentially lethal for larval stages and pupae in 0.1 percent in aquatic form and had shown hundred percent mortality after treatment for toxicity response.

The LC₅₀ value was analyzed as 0.0427 in percent. The larval and pupal mortality in concentration of peppermint essential oils in 0.1 percent in water were detected hundred percent after toxicity treatment experimental time period. The LC₅₀ value was determined 0.0387 in percent. The larval mortality in concentration of eucalyptus essential oils in 2 percent were assessed lethal after experimental treatment time period with LC₅₀ value 2.5916 in percent. The toxic response of neem essential oils in 0.1 percent separately against larvae, was not found effective.

The comparative results of study showed that in a separated concentration of citronella essential oils, in 0.1 percent in aquatic medium, hundred percent mortality in larval a pupal

form, observed after experimental treatment time period. In a concentration of 0.1 percent of lemongrass essential oils, complete mortality was observed in larval and pupal developmental stages. In the same concentration, 0.1 percent of peppermint essential oils, the complete mortality was assessed for both the developmental stages in water. The concentration of eucalyptus essential oils in aqueous solution was found less toxic to larvae as compared the toxicity to citronella, lemongrass and peppermint. When a combination of concentrations of essential oils of neem and peppermint in 0.1 percent was tested for larval toxicity in aqueous solution for second instar, the hundred percent mortality was assessed after one hour of concentration treatment for toxicity response. The last instar larvae have shown full mortality after sixty minutes of treatment and pupae showed complete mortality in one hour. After 24 hours, hundred percent mortality was observed.

A combination of concentrations in aquatic medium of neem, eucalyptus and lemongrass in 1% of each was also tested for toxicity response against last instar larvae and pupae of *Culex Sp.* and *Anopheles Sp.*, the hundred percent mortality was observed after a treatment for 24 hours. In conclusion, it can be determined that citronella, lemongrass and peppermint essential oils in aqueous solution in 0.1% constrain separately, found highly effective against larvae and pupae of mosquitoes and remained responsible for 100% mortality due to toxicity response. A combination of neem, and peppermint essential oils was found highly effective against last instar larvae just after one hour of exposure, leaving hundred percent mortality, and in second instar larval stage, a hundred percent mortality was also recorded in one hour exposure in constrain. After 24 hours of exposure time period, the hundred percent mortality was assessed. A combination of concentrations of neem, *Azadirachta indica*, Eucalyptus and lemongrass essential oils, in 1% each in water was also found effective against last instar larval stage of *Culex Sp.* *Anopheles Sp.* And *Aedes Sp.*, exhibiting hundred percent mortality after 24 hours of exposure time period. In control experiments in water no

mortality was recorded. The mortality was zero percent.

For repellent action study, a combination of lemongrass, citronella, peppermint, neem, eucalyptus essential oils, in 5% each and clove oil essential oils in 2.5%, was mixed in ethanol in v/v (NDR-I test solution), and tested for efficacy against adult mosquitoes by using electrical vaporizer device, arranged from market. The repellent action experiment was carried out in a wired open-able wooden box, in which glass jars were kept, containing mosquito larvae in collected water, and adult mosquitoes were emerged. The vaporizer electric machine was kept on to assess the repellent efficacy action, after 30 minutes, restlessness, compromised flight and sitting were noticed. The mosquitoes exhibited convulsions, restlessness, tremors, nervous breakdown and knockdown response on vapour exposure. The efficacy of combination was found quite effective in room test.

Repellent efficacy of a combination of citronella, peppermint and lemongrass essential oils in 10% v/v, (NDR-II test solution), each in ethanol in vaporizer device was tested in experimental box, it knock-downed the mosquitoes significantly. Room tests were also found effective. In this combination, the rose essential oil was added in 10% v/v concentration, a pleasant fragrance was appeared and this combination repelled mosquitoes significantly, leaving them paralyzed. This was also noticed that, they stay at a site calmly on the exposure of vapour for a longer time period without any movement, and restricted the flight. Citronella and rose essential oils, 10% v/v, (NDR-III test solution), constrain in ethanol, repelled mosquitoes with significant knockdown response. In above test observations, a repellent efficacy for 7 to 8 hours was assessed in room tests.

A combination of peppermint and night jasmine essential oils in 10 and 2.5% v/v, (NDR-IV test solution in box test) in ethanol, was also assessed quite effective overnight, leaving significant and effective knockdown response. Night jasmine essential oil in 10% in ethanol v/v, (NDR-V, box experiment) was also tested, exhibited significant repellent action up to 7 to 8 hours in room test.

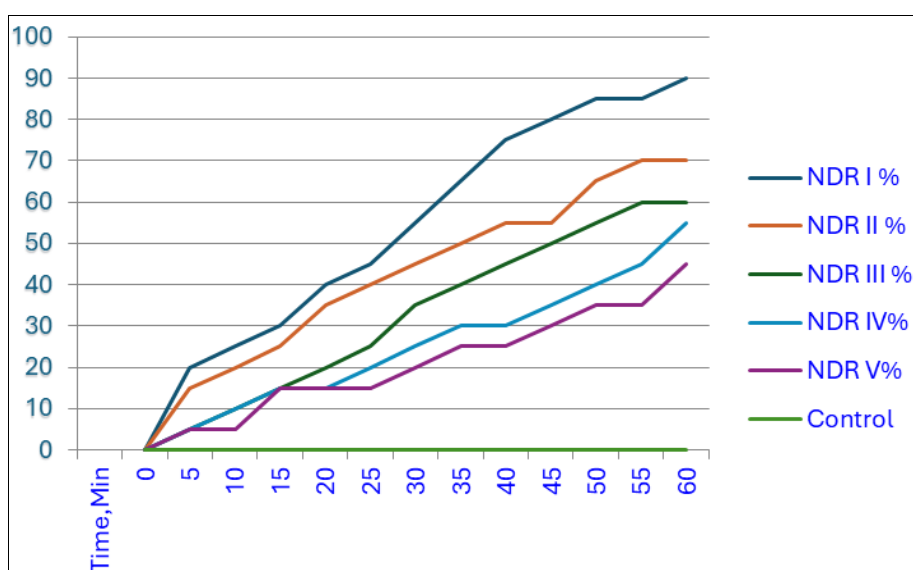


Fig 1: Percent Knockdown Response, NDR, in repellency test experiments in different plant essential oil concentrations (Iron wired-box experimental set-up).

A new compound named eucamalol and 4-isopropylbenzyl alcohol were tested as mosquito repellents from essential oils of *Eucalyptus camaldulensis*. Both compounds exhibited potent repellent activities against *Aedes aegypti* ^[51].

Effectiveness of plant based repellents against different *Anopheles* species, a systemic review study was carried out. ^[52]

The plant based insect repellents, a review study of their

efficacy, development and testing was studied [53]. A review study, performing a bibliographic survey of plants with repellent activity, evaluate the trends of natural repellent formulations in the scientific literature, those described in patents and commercially available products. Limonene 1, 8-lineole, geraniol, eugenol and citronella are the active compounds that mostly appear in the essential oils of plants with repellent activity. Future research must be conducted to the use of nanotechnology in the development of extended release system containing essential oils with repellent action produced from natural and biodegradable materials [54]. Efficacy and safety of repellents marketed in Brazil against bites from *Aedes aegypti* and *Aedes albopictus* were reviewed and reported no adverse effects. Against *Ae. aegypti*: protection time using DEET (10% and 20%-spray) was similar to IR 3535 (10% and 20%-spray) and longer than citronella (5%-spray). DEET 25% solution had longer protection time than eucalyptus 25% solution, while DEET 20% lotion had longer protection time than citronella 10%-solution. There was no difference in protection time between herbal repellents. DEET 7% and 15% spray had higher percentage repellency compared to both icaridin 7%-spray and IR 3535 20%-spray. Against *Ae. albopictus*: DEET 15%-spray had a similar protection time to icaridin 20%-spray, but longer than citronella 10%-spray. Therefore, DEET proved more effective than the other synthetic and natural repellents marketed in Brazil for protecting against bites from the mosquito species investigated. All repellents studied exhibited satisfactory safety profile [55].

An approach to natural insect repellent formulations from basic research to technological development was assessed [54]. In this review, a bibliographic survey of the plants with repellent activity, evaluate the trends of natural repellent formulations in the scientific literature, those described in patents and economically available products. Limonene, 1, 8-cineole, geraniol, eugenol and citronellal are the active compounds that mostly appear in the essential oils of plant with repellent activity. Future research must be conducted to the use of nanotechnology in the developmental of extended research system containing essential oils with repellent activity produced from plant origin.

On the basis of results of present investigation, it can be concluded that the taken essential oils of different plant origin can be used for the effective control of mosquito larvae population in an eco-friendly way in water with environment and health safety approach. The repellent responses of different plant essential oils were also found enthusiastic for indoor household use purposes.

Conflict of interest

The author has no conflict of interest.

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