

USE OF ESSENTIAL OILS FOR THE MANAGEMENT OF DIFFERENT SPECIES OF DENGUE MOSQUITOE.

Authors: S. Bushra* and M. Tariq¹

*Ph.D Scholar, Department of Entomology, Pir Mehr Ali Shah, Arid Agriculture University, Rawalpindi, Pakistan.

¹Assistant Professor, Department of Entomology, Pir Mehr Ali Shah, Arid Agriculture University, Rawalpindi, Pakistan.

Abstract

Mosquitoes are responsible for human and animal health problems in tropical and subtropical areas of the world. They transmit malaria, filariasis, chikungunya, yellow fever, Japanese encephalitis and dengue fever. Personal protection can prevent the victims from mosquito bites. After application of synthetic mosquitocides, several allergies like contact urticarial, skin eruption and encephalopathy is reported. To overcome the adverse effects of pesticide use, there is a need to develop mosquito repellents derived from plant extracts. Plant essential oils are plant derivatives. Some of essential oils possess mosquitocidal properties. They are target selective, biodegrade to non-toxic products and have few effects on non-target organisms and our environment. In this paper, several essential oils are reported to control the mosquito species. These plant essential oils will help the scientists to develop botanical and environment friendly insect repellents.

INTRODUCTION

Dengue has global health concern in the present era. Dengue fever is one of the most rapidly spreading insect-borne diseases. There are two types of dengue fever. Dengue fever and Dengue hemorrhagic fever (DHF). The Dengue hemorrhagic fever (DHF) is potentially lethal version of the disease. These both diseases been detected in almost all the countries. Nowadays, major cause of hospitalization and death among children and older people is dengue. It is estimated that about 2500 million people of world's population are at risk from dengue [1]. There are more than 50 million cases of Dengue infection worldwide every year [2].

The insecticide resistance in mosquito vector, *Aedes aegypti* is growing day by day. Dengue infects an estimated 50 million people in 100 countries annually [3]. Dengue hemorrhagic fever affects about 500,000 patients worldwide [4]. This resistance limits the effectiveness of vector control around the world. The need of day is the use of alternative approaches to control *A. aegypti* [5].

There is currently no available vaccine, so control of mosquito vectors is still the main tool to reduce incidence of this disease. However, insecticide resistance in the primary dengue vector *Aedes aegypti* [6-8] increasingly limits the utility of chemical insecticides, therefore alternative methods of control are needed. For this purpose, a great attention has been paid to attractants and repellents. Sometimes, volatile organic compounds attract or repel mosquito species [9]. It is

reported that combined application of attractants and repellents can efficiently manage the *Ae. aegypti* [10].

Essential oils are volatile in nature. They are found in a number of plants. They are extracted from different parts of plant. On the basis of chemical nature, they are mixture of many compounds including isoprenoids mono- and sesquiterpenes [11]. These chemicals act as carriers of the smell which found chiefly in the aromatic plants [12]. They possess antibacterial, antifungal, antimitic, antitermite and insecticidal properties. For this reason, essential oils are used as pharmaceuticals, flavor enhancers in food products, odorants in fragrances, and insecticides [13]. All around the world, organophosphates such as temephos and fenthion and insect growth regulators such as diflubenzuron and methoprene are chiefly used to control *A. aegypti*. These synthetic chemicals has spoiled our natural biological control systems and resulted widespread development of resistance in insect pests [14].

A personal protection like, use repellent products can minimize the risk of infection by *A. aegypti*. The repellents can be applied on exposed skin or to clothing. Commercial repellents often contain DEET *N,N*-diethyl-3-methylbenzamide (DEET), 3-(*N*-acetyl-*N*-butyl)-aminopropionic acid ethyl ester (IR3535), 1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-1-methylpropylester (Icaridin) or *p*-menthane- 3,8-diol (PMD) [15,16]. Very limited information is available on the effectiveness of attractants and spatial repellents to *A. aegypti* and *A. albopictus* [17]. Therefore, there is a need of establishment of an effective olfactometer system, so that biting behavior of *A. aegypti* can be analysed in presence of several attractants [18]. The exploration of bioactive chemical compounds such as essential oils from indigenous plants will help us in mosquito control [19]. In this review, we have highlighted the use and efficiency of different essential oils to repel and control *A. aegypti* (Table 1).

(Table 1): Showing the repellency of different essential oils against different mosquito species.

S. N.	PEST STUDIED	ESSENTIAL OIL USED	REFERENCES
1.	<i>Aedes aegypti</i> , <i>Ae. albopictus</i> , <i>Anopheles dirus</i> and <i>Culex quinquefasciatus</i> .	<i>Eleutherococcus trifolius</i> , <i>Schefflera leucantha</i> , <i>Ocimum sanctum</i> , <i>Vitex trifolia</i> , <i>Litsea cubeba</i> , <i>Manglietia garrettii</i> , <i>Aglaia odorata</i> , <i>Myristica fragrans</i> , <i>Melaleuca cajuputi</i> , <i>Psidium guajava</i> , <i>Piper betle</i> , <i>Piper nigrum</i> , <i>Murraya paniculata</i> , <i>Houttuynia cordata</i> , <i>Zingiber officinale</i> , <i>Alpinia galangal</i> , <i>Curcuma longa</i> and <i>Hedychium coronarium</i> .	(27)
2	<i>Aedes aegypti</i> .	<i>Ocimum gratissimum</i> , <i>Cymbopogon citratus</i> and <i>Ageratum conyzoides</i> .	(31)
3	<i>Aedes aegypti</i> , <i>Culex quinquefasciatus</i> and <i>Anopheles dirus</i>	<i>Cananga odorata</i> , <i>Citrus sinensis</i> , <i>Cymbopogon citratus</i> , <i>Cymbopogon nardus</i> , <i>Eucalyptus citriodora</i> , <i>Ocimum basilicum</i> and	(32)

		Syzygium aromaticum	
4	Aedes aegypti.	Cymbopogon nardus and C. citratus	(33)
5	Aedes aegypti.	Citronella, linalool, and geraniol	(37, 38)
6	Aedes aegypti.	Cinnamomum osmophloeum	(47)
7	Culex quinquefasciatus, Anopheles tessellatus and A. aegypti	Cinnamomum zeylanicum	(48)
8	Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus.	Cinnamomum zeylanicum, Juniperus macropoda and Pimpinella anisum	(49)
9	Aedes aegypti.	Linalool	(51)
10	Aedes gardnerii, Anopheles barbirostris, Armigeres subalbatus, Culex tritaeniorhynchus, Culex gelidus, Culex vishnui and Mansonia uniformis.	Zanthoxylum piperitum	(53)
11	Aedes albopictus.	Zanthoxylum limonella, Citrus aurantifolia and Z. limonella	(54)
12	Aedes aegypti.	Amomum xanthioides, Curcuma zedoaria, Kaempferia galanga, Anethum graveolens, Apium graveolens, Carum carvi, Foeniculum vulgare, Piper longum, Zanthoxylum limonella and Zanthoxylum piperitum	(55)
13	Aedes aegypti.	Cinnamomum osmophleum, Taiwania cryptomerioides Cunninghamia lanceolata, Cryptomeria japonica and Calocedrus formosana	(11)
14	Aedes aegypti.	Hyptis fruticosa, H. pectinata and Lippia gracilis	(58)
15	Aedes aegypti.	Citrus sinensis and Hyptis suaveolens	(59)
16	Aedes aegypti.	Lavendula stoechas, Helichrysum italicum and Laurus nobilis.	(64)

17	Anopheles stephensi, Culex quinquefasciatus and Aedes aegypti.	Azadirachta indica	(70)
18	Culex quinquefasciatus, Aedes aegypti and Anopheles stephens	Azadirachta indica and Pongamia glabra	(71)

1.USE OF TURMERIC ESSENTIAL OIL:

Turmeric, *Curcuma longa* belongs to Zingiberaceae, contains a variety of monoterpenoids, sesquiterpenoids, and curcuminoids. Its powder has medicinal properties for the treatment of menstrual disorders, rheumatism, and traumatic diseases [20]. It constitutes insect repellent and antifeeding components i.e. turmerone and arturnerone [21, 22] curcuminoids [23]. Its extract has insecticidal [24], repellent [24-25], and antifeedant [25] against a variety of insect pests [23]. In the greenhouse studies, it was found that *C. longa* derived synthetic fungicides chlo-rothalonil and dichlofluanid are significantly effective against six phytopathogenic fungi [26].

In an experiment, the efficacy of 18 plant essential oils against *Aedes aegypti*, *Ae. albopictus*, *Anopheles dirus* and *Culex quinquefasciatus* under laboratory conditions using human volunteers was tested. For this purpose *Eleutherococcus trifoliatus*, *Schefflera leucantha*, *Ocimum sanctum*, *Vitex trifolia*, *Litsea cubeba*, *Manglietia garrettii*, *Aglaiia odorata*, *Myristica fragrans*, *Melaleuca cajuputi*, *Psidium guajava*, *Piper betle*, *Piper nigrum*, *Murraya paniculata*, *Houttuynia cordata*, *Zingiber officinale*, *Alpinia galangal*, *Curcuma longa* and *Hedychium coronarium* essential oils were prepared with 10% absolute ethanol. These essential oils were compared with chemical repellents, DEET and IR3535. It was found that repellency of *An. dirus* *Cx. quinquefasciatus* and *Ae. albopictus* was 4.5-8hrs and repellency of *Ae. aegypti* was 0.3-2.8 hrs. It was found that oviposition deterrent activity of *C. longa* against *Ae. aegypti* was 94.7%, whereas deet and IR3535 had no repellency. The results revealed that *C. longa* essential oil is an efficient mosquito repellents and oviposition deterrent [27].

2.USE OF CITRONELLA AND LEMONGRASS ESSENTIAL OILS:

Plant essential oils of neem (*Azadirachta indica*), basil (*Ocimum basilicum*, *O. basilicum*, *O. citratum*, *O. gratissimum*, *O. americanum*, *O. tenuiflorum*, citronella grass (*Cymbopogon nardus*), galingale (*Alpinia galanga* L.), clove (*Syzygium aromaticum* L.) and thyme (*Thymus vulgaris* L.), have mosquito repellents properties [28-29]. Some of the plant essential oils were reported that can only be effective by olfactometry or by using laboratory mice as hosts of *Aedes aegypti* (L.) under laboratory conditions [30].

In an experiment, three plant essential oils namely *Ocimum gratissimum*, *Cymbopogon citratus* and *Ageratum conyzoides* against *Aedes aegypti* under laboratory conditions were tested. For this purpose, 100 ml solution of each concentration was used against 4th larval instar and compared with a commercial insecticide namely endosulfan. It was found that after 24 hours of exposure, *A. conyzoides*, *C. citratus* and *O. gratissimum* gave 100% larval mortality at 120, 200 and 300 ppm

respectively. The results revealed that different formulations of plant essential oils can be effectively used for mosquito control programme strategies [31].

In an experiment, [32] tested the efficacy of essential oils of *Cananga odorata*, *Citrus sinensis*, *Cymbopogon citratus*, *Cymbopogon nardus*, *Eucalyptus citriodora*, *Ocimum basilicum* and *Syzygium aromaticum* against *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles dirus*. The concentrations of essential oil were prepared in soybean oil at 1%, 5% and 10% (w/v). By using knockdown LC₅₀ and KT₅₀ values against *Ae. aegypti*, *C. quinquefasciatus* and *An. dirus* were calculated. It was found that *C. citratus* oil was highly significant to control all tested mosquito species with LC₅₀ <0.1, 2.22 and <0.1%, respectively. At 10% concentration *C. citratus* resulted the 100% after 24 hrs of application. The results revealed that *C. citratus* is the most efficient compound to control *A. aegypti* among rest of the essential oils tested.

In an experiment, the repellency of *Cymbopogon* essential oils against *A. aegypti* by using Y-tube olfactometer was tested. It was found that Citronella grass (*Cymbopogon nardus*), lemon grass (*Cymbopogon citratus*), citral and myrcene produced a low active response towards repellents @400 µL concentration. Similarly, a combined mixture of citral, myrcene, and citronellal oil in (6:4:1) significantly inhibited host-seeking behavior (76% active response; 26% treatment response with 40 µL; 42.5%, 18% with 400 µL; and 19%, 23% with 1000 µL). When the same experiment was performed with N,N-diethyl-3-methylbenzamide (DEET), active response was 44%, and treatment response 22% @ 400 µL concentration. The results revealed that citronella oil is effective repellent against *A. aegypti* as compared to DEET [33].

3.USE OF GERANIOL ESSENTIAL OIL:

Geraniol (3,7-dimethylocta-trans-2,6-dien-1-ol) have the chemical formula C₁₀H₁₈O. It is a pale-yellow oil which is soluble in organic solvents. It is present inside vegetative tissues of many herbs and emitted from floral parts [34]. It exhibits biochemical and pharmacological properties. It is an effective insect repellent [35]. It is an alcohol which occurs in essential oils of several aromatic plants. It has pleasant odour so it is widely used in flavour and fragrance industries as a common ingredient. It has insecticidal and repellent properties. It is an effective chemoprevention agents for cancer patients. It is antimicrobial, antioxidant, anti-inflammatory in nature [36].

In an experiment, the repellency of citronella, linalool, and geraniol essential oils against *A. aegypti* was tested. The essential oils at 5% were used in the form of diffusers (20g) and candles (88g) in this experiment. It was found that essential oils tested in form of continuous release diffuser have more significant repellency as compared to candle form. In Indoor, citronella linalool, and geraniol candles repelled the *A. aegypti* 14%, 0% and 50% respectively. While citronella linalool, and geraniol diffusers repelled the *A. aegypti* 68%, 93% and 97% respectively. In outdoor experiment, continuous release diffusers were placed 6m far away from mosquito traps. It was found that citronella, linalool and geraniol essential oils repelled the female mosquitoes at 22%, 58%, and 75% respectively. The results revealed that geraniol essential oil was more effective against mosquitoes as compared to citronella and linalool in indoor and outdoor experiments as well [37].

In an experiment, the repellency of 3 essential oils against mosquitoes was tested. For this purpose citronella, linalool and geraniol essential oils in candle form were used. It was found that the repellency rate of 5% citronella, linalool and geraniol was 29.0%, 71.1%, and 85.4%

respectively. The geraniol was twice effective as compared to linalool. But geraniol was 5 times as effective as compared to citronella. The results revealed that geraniol essential oil was more effective against mosquitoes as compared to citronella and linalool [38].

4. USE OF CINNAMOMUM ESSENTIAL OILS

The *Cinnamomum zeylanicum* extract have antibacterial [39-40], antifungal (41-42) and insecticidal against insect pests (43). Its oil extract contain 50-75% cinnamaldehyde, eugenol (5-18%), cinnamyl acetate, linalool, 1,8-cineole, α -caryophyllene and benzyl benzoate. Its leaf oil contains 70-95% eugenol, cinnamaldehyde (5%), benzyl benzoate, linalool and α -caryophyllene. It is reported that Cinnamaldehyde and eugenol are more active against *C. quinquefasciatus*, *A. tessellatus* and *A. aegypti* than the bark and leaf oil [44]. The leaf essential oil of the *C. osmophloeum* can induce certain inhibitory effects against many pathogens including bacteria, termites, mites, mildew and fungi [45-46].

The efficacy of 5 leaf essential oils of *Cinnamomum osmophloeum* was tested against *A. aegypti*. The leaf essential oils were classified into chemotypes-cinnamaldehyde type, linalool type, camphor type, cinnamaldehyde/cinnamyl acetate type, and mixed type. In the mosquito larvicidal assay, it was found that leaf essential oils of cinnamaldehyde type and cinnamaldehyde/cinnamyl acetate type have an excellent inhibitory effect against *A. aegypti* with LC₅₀ for cinnamaldehyde type and cinnamaldehyde/cinnamyl acetate type 36 ppm, LC₉₀ (79 ppm and 44 ppm) LC₉₀ (85 ppm), respectively. Cinnamaldehyde exhibited the strongest mosquito larvicidal activity with LC₅₀ (29 ppm) and LC₉₀ (48 ppm). The results revealed the components of *C. osmophloeum* can effectively control the *A. aegypti* [47].

The efficacy of Ceylon *Cinnamomum zeylanicum* against *Culex quinquefasciatus*, *Anopheles tessellatus* and *A. aegypti* was tested. It was found that *Cinnamomum zeylanicum* bark oil have significant mortality against *A. tessellatus* (LD₅₀ 0.33 μ g/mL) and *C. quinquefasciatus* (LD₅₀ 0.66 μ g/mL) [48].

In an experiment, the efficacy of essential oils extracted from 10 medicinal plants was tested against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. It was found that the essential oils of *Juniperus macropoda* and *Pimpinella anisum* were significantly toxic to eggs and larvae. The essential oil of *P. anisum* was toxic to 4th instar larvae of *A. stephensi* and *A. aegypti* with equivalent LD₉₅ values of 115.7 μ g/ml. The results revealed that essential oil of *Cinnamomum zeylanicum* have highest repellency LD₉₅ i.e. 49.6, 53.9 and 44.2 mg/mat against *A. stephensi*, *A. aegypti* and *C. quinquefasciatus* [49].

5. USE OF LINALOOL ESSENTIAL OIL:

Linalool is a monoterpene compound which is plant essential oil of several aromatic species. Linalool is used to relieve acute and chronic ailments [51]. It is toxic against several insect pests [50].

The repellency activity of DEET, dehydrolinalool and linalool against *Aedes aegypti* was tested by using olfactometer. The repellency of *A. aegypti* to each of the three chemicals, in the presence of human attractant mixture was observed. The lowest repellency (7.3%) was found using human attractant. While, highest repellency (33.6%) was found by using combination of linalool and

dehydrolinalool. The results revealed that linalool essential oil is the most efficient compound to repel *A. aegypti* among rest of the chemicals tested [51].

6.USE OF ZANTHOXYLUM PIPERITUM ESSENTIAL OILS:

Most of the plant essential oils are used as fragrances and flavoring agents in food products. They constitute numerous bioactive phytochemicals which can be used for insect pest control [52].

In an experiment, the efficacy of *Zanthoxylum piperitum* was tested against *Aedes gardnerii*, *Anopheles barbirostris*, *Armigeres subalbatus*, *Culex tritaeniorhynchus*, *Culex gelidus*, *Culex vishnui* and *Mansonia uniformis* under laboratory and field conditions. The *Z. piperitum* essential oil was compared with a synthetic repellent, namely N,N-diethyl-3-methylbenzamide (DEET). It was found that *Z. piperitum* alone and with 5% vanillin gave the repellency time up to 1.5 and 2.5 h, respectively. But the repellency time for 25% DEET and 25% DEET with 5% vanillin was 3.5 and 5.5 h, respectively. In field, *Z. piperitum* with 5% vanillin gave more significant results as compared to 25% DEET with 5% vanillin. The *Z. piperitum* resulted 100% protection against biting rate while DEET gave 99.7% protection. The results revealed that *Z. piperitum* can be used in combination with repellents as synergist against *Ae. gardnerii*, *An. barbirostris*, *Ar. subalbatus*, *C. tritaeniorhynchus*, *C. gelidus*, *C. vishnui* and *M. uniformis* [53].

In an experiment, the repellency activity of three plant essential oils, namely *Zanthoxylum limonella*, *Citrus aurantifolia* and *Z. limonella* was tested against *Aedes albopictus*. The formulations were prepared with mustard and coconut oil base in (10, 20 and 30%) concentrations and experiment was performed under laboratory conditions. It was found that formulations prepared with coconut oil gave the significant repellency activity as compared to mustard oil against the bites of *Ae. albopictus*. The results revealed that *Z. limonella* gave the highest repellency activity against the bites of *Ae. albopictus* at all the concentrations tested in both formulations [54].

The repellency of 10 plant essential oils, namely *Amomum xanthioides*, *Curcuma zedoaria*, *Kaempferia galanga*, *Anethum graveolens*, *Apium graveolens*, *Carum carvi*, *Foeniculum vulgare*, *Piper longum*, *Zanthoxylum limonella* and *Zanthoxylum piperitum* was tested against *Ae. aegypti*. It was found that *Z. piperitum*, *A. graveolens* and *K. galangal* essential oils gave the significant repellency after 1, 0.5 and 0.25 hr of treatment, respectively. But the degree of essential oil efficiency can be increased by addition of 10% vanillin. The *Z. piperitum* essential oil formulation with 10% vanillin gave the repellency for 2.5 h. The results revealed that *Z. piperitum* is a useful repellents against *Ae. aegypti* as compared to rest of the essential oils tested [55].

7.USE OF CRYPTOMERIA JAPONICA ESSENTIAL OILS:

In an experiment, the bioactivity of 14 essential oils against *A. aegypti* larvae was tested. These essential oils were extracted from 5 plant species, namely *Cinnamomum osmophleum*, *Taiwania cryptomerioides*, *Cunninghamia lanceolata*, *Cryptomeria japonica* and *Calocedrus formosana*. After 24 hrs of treatment, 9 of tested essential oils exhibited significant larvicidal activity against *A. aegypti* in 4th instar at 100µg/ml concentration. It was found that among the tested essential oils, *C. japonica* essential oil exhibited highest larvicidal activity, with a LC₅₀ =48.1 µg/ml (LC₉₀

=130.3 µg/ml). The results revealed that leaf and bark essential oils of *C. japonica* are significant larvicides against *A. aegypti* [11].

8. USE OF TEA ESSENTIAL OILS:

Hyptis suaveolens belongs to Lamiaceae. It is a native to America, but now it is widespread Africa, Asia and Australia. It can grow under a variety of soil and climate. It has medicinal value for a long time. Its essential oil possess insecticidal properties [56].

It is reported that *Citrus sinensis* peel extract have insecticidal properties. It exhibits very low toxicity to mammals and environment. It constitutes d-limonene and linalool. The odour and consistency of citrus appeals for its wider use as larvicides against mosquitoes [57].

In an experiment, the effectiveness of essential oils of *Hyptis fruticosa*, *H. pectinata* and *Lippia gracilis* was studied against *A. aegypti*. It was found that, 59 compounds represented 91.28-98.39% of the essential oils. A standard solution of 20 mL was used between (30-2000) ppm. Twenty samples at between 3rd and 4th larval stages were subjected to prepared essential oil solutions at different concentrations. After 24 h interval of treatment, mortality LC₅₀ at 95% probability was calculated. It was found that Carvacrol compound present in *H. fruticosa*, and *L. gracilis* have the larvicidal activity against *A. aegypti*. The results revealed that *H. fruticosa*, and *L. gracilis* are significantly effective larvicides against *A. aegypti* [58].

The efficacy of orange peel, *C. sinensis* and *H. suaveolens* essential oils was tested against *Aedes aegypti* larvae. The essential oils were applied at (0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3 and 0.2ppm) concentrations. It was found that *C. sinensis* resulted mortality rate 95 and 90% at 0.8 and 0.3ppm concentration respectively. The *H. suaveolens* resulted mortality rate 80% at 0.9 and 0.3ppm. The results revealed that *C. sinensis* and *H. suaveolens* are significantly effective larvicides against *A. aegypti* [59].

9. USE OF LAURUS NOBILIS ESSENTIAL OILS:

Laurus nobilis belongs to Lauraceae. It is an evergreen shrub and widely used as ornamental. It grows in the humid and sub-humid areas [60]. It can grow up to 2.15 m in height [61]. It is widely cultivated in Europe and the USA [62]. It is used as a dried herb and its essential oil is used as spice and flavoring agent in food industries [61]. It has highly medicinal value in folk diseases i.e. gastrointestinal problems, rheumatism, diuretic, urinary problems and kidney stones [63].

In an experiment, 19 plant essential oils for its repellent potential were tested against *Aedes aegypti*. It was found that essential oil of *Lavendula stoechas*, *Helichrysum italicum* and *Laurus nobilis* resulted repellency of a human finger for yellow fever mosquitoes in olfactometer bioassays. In 'hedonic dimension' studies, it was found that three oils i.e. *Calamintha nepeta*, *Laurus nobilis* and *Rosmarinus officinalis* were most promising. By thermogravimetric analysis, it was found that *Calamintha nepeta* oil has significantly slower evaporation rate as compared to *Laurus nobilis* and *Rosmarinus officinalis*. It was found that *L. nobilis* oil have both properties: as a repellent for *Aedes aegypti* and acceptance by the volunteers for its integration as a repellent product. The results revealed that *L. nobilis* is an efficient repellent for *A. aegypti* [64].

10.USE OF NEEM ESSENTIAL OIL:

Neem plant, *Azadirachta indica* is native to India. It belongs to family Meliaceae. It can attain the maximum height 12–24m. It is found chiefly in tropical, subtropical semiarid and wet tropical regions of the world [65]. Neem seeds contain biological compounds namely azadirachtin, nimbin, nimbidin and nimbolides which have antifeedant, ovicidal, fecundity suppression, insect growth regulation and repellency properties against insect pests [66-67]. Neem formulations have low toxicity to mammals. It is unable to develop resistance in insect pests and it is considered to be more ecofriendly as compared to other synthetic insecticides. Neem is an effective insect growth regulator because it weakens the cuticle defence system at larval stage. The pesticidal efficacy, environmental safety and public acceptability of neem and its products for control of crop pests has led to its adoption into various mosquito control programmes [68-69].

The efficacy of neem oil formulation against 3rd and 4th larval instars of *Anopheles stephensi*, *Culex quinquefasciatus* and *Ae. aegypti* was tested. Neem oil formulation was prepared with polyoxyethylene ether, sorbitan dioleate and epichlorohydrin at different concentrations (0.5–5.0 ppm) along with untreated control. It was found that LC₅₀ against *An. stephensi*, *C. quinquefasciatus* and *Ae. aegypti* was 1.6, 1.8 and 1.7 ppm respectively. LC₅₀ of formulation stored at 26°C, 40°C and 45°C for 48 hours against *Ae. aegypti* were 1.7, 1.7, 1.8 ppm while LC₉₀ values were 3.7, 3.7 and 3.8 ppm respectively. The neem formulation at 140 mg A.I. /m² was applied in different mosquito breeding sites under field conditions. It was found that the maximum reduction rate of *Anopheles*, *Culex* and *Aedes* was 100%, 95.5% and 100% respectively. The results revealed that neem oil formulation was found to be very effective to control *Anopheles*, *Culex* and *Aedes* in different breeding sites under natural and field conditions. So, *Azadirachta indica* can be used as an alternative of synthetic pesticides for mosquito control [70].

The efficacy of neem (*Azadirachta indica*) and karanja (*Pongamia glabra*) oil cakes was tested against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*. Both the oil cakes were applied individually and in combination. It was found that combination of neem and karanja oil cakes showed significant results as compared to individual treatments. The LC₉₅ for combination of two oil cakes was 0.93, 0.54 and 0.77% respectively for *C. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* [71].

CONCLUSION

The use of synthetic chemicals to control mosquito result several human health concerns. However, use natural products i.e. plant essential oils having good efficacy is an alternative to synthetic mosquitocides. The repellent activities of essential oils and synergistic effects among their components are main reason to control mosquitoes. Basically, essential oils are volatile mixtures of hydrocarbons and their repellent activity has been linked to the presence of monoterpenes and sesquiterpenes. The essential oils commonly used as repellents are derived from *Cymbopogon spp.*, *Ocimum spp.* and *Eucalyptus spp.* From an economical view point, synthetic chemicals are still more frequently used but these natural products are more efficient and safer to our environment [73]. Further studies and field trials are needed to develop mosquitocidal formulations. Field trials will evaluate operational feasibility to its use and dermal toxicity.

REFERENCES

- [1] Rodrigues de Paula A, Brito, ES Pereira CR, Carrera MP and Samuels RI. 2008. Susceptibility of adult *Aedes aegypti* (Diptera: Culicidae) to infection by *Metarhizium anisopliae* and *Beauveria bassiana*: prospects for Dengue vector control. *Biocontrol Sci Tech.* 18(10): 1017-1025.
- [2] WHO (World Health Organization). 2008. www.who.int/mediacentre/factsheets/fs117/en/. (3) Anonymous. 2009. Second dengue fever outbreak declared in townsville. http://www.health.qld.gov.au/dengue/documents/media_release_091217.pdf, Accessed 11 May 2011.
- [4] WHO. 2011. Dengue and Dengue Hemorrhagic Fever. Available at <http://www.who.int/mediacentre/factsheets/fs117/en/index.html>. Accessed 1 August 2011.
- [5] Darbro JM, Grahamb RI, Kaya BH, Ryana PA and Thomas MB. 2011. Evaluation of entomopathogenic fungi as potential biological control agents of the dengue mosquito, *Aedes aegypti* (Diptera: Culicidae). *Biocontrol Sci Tech.* 21(9): 1027-1047.
- [6] Ponlawat A, Scott JG, and Harrington LC. 2005. Insecticide Susceptibility of *Aedes aegypti* and *Aedes albopictus* across Thailand. *J Med Entomol.* 42: 821-825.
- [7] Cui F, Raymond M and Qiao C-L. 2006. Insecticide Resistance in Vector Mosquitoes in China. *Pest Manag Sci.* 62: 1013-1022.
- [8] Garcí'a GP, Flores AE, Fernández-Salas I, Saaveda-Rodríguez K, Reyes-Solis G, Lozano-Fuentes S, Bond JG, Casas-Martí'nez M, Ramsey JM, Garcí'a-Rejo' n J, Domí'nguez-Galera M, Ranson H, Hemingway J, Eisen L and Black WC. 2009. Recent Rapid Rise of a Permethrin Knock Down Resistance Allele in *Aedes aegypti* in Me'xico. *Public Library of Science Neglected Tropical Diseases.* 3: 531p.
- [9] Fradin MS and J. F. Day. 2002. Comparative efficacy of insect repellents against mosquito bites. *New Engl J Med.* 347: 13-18.
- [10] Kline DL, Bernier UR, Posey KH, Branard DR. 2003. Olfactometric evaluation of spatial repellents for *Aedes aegypti*. *J Med Entomol.* 40: 463-467.
- [11] Cheng, S., H. Chang, S. Chang, K. Tsai and W. Chen. 2003. Bioactivity of selected plant essential oils against the yellow fever mosquito *Aedes aegypti* larvae. *Bioresource Technol.* 89: 99-102.
- [12] Debboun M. 2006. In *Insect Repellents—Principles, Methods, and Uses.* M. Debboun, S. Frances D. Strickman (eds). CRC Press: Fort Lauderdale, NJ. 311p.
- [13] Franzios G, Mirotson M, Hatzia Apostolou E, Kral J, Scouras ZG and Mavragani TP. 1997. Insecticidal and genotoxic activities of mint essential oils. *J Agric Food Chem.* 45 (7): 2690-2694.
- [14] Chang ST and Cheng SS. 2002. Antitermitic activity of leaf essential oils and components from *Cinnamomum osmophleum*. *J Agric Food Chem.* 50(5): 1389-1392.
- [15] Yang YC, Lee SG, Lee HK, Kim MK, Lee SH and Lee HS. 2002. A piperidine amide extracted from *Piper longum* L. fruit shows activity against *Aedes aegypti* mosquito larvae. *J Agric Food Chem.* 50(13): 3765-3767.
- [16] Costantini C, Badolo A and Ilboudo-Sanogo E. 2004. *Trans R Soc Trop Med Hyg.* 98: 644p.
- [17] Trexler JD, Apperson CS, Gemeno C, Perich MJ, Carlson D and Schal C. 2003. Field and laboratory evaluations of potential oviposition attractants for *Aedes albopictus* (Diptera: Culicidae). *J Am Mosquito Contr.* 19: 228-234.
- [18] Bernier UR, Kline DL, Posey KH, Booth MM, Yost RA and Barnard DR. 2003. Synergistic attraction of *Aedes aegypti* (L.) to binary blends of L-lactic and acetone, dichloromethane, or dimethyl disulfide. *J Med Entomol.* 40: 653-656.
- [19] Kim SI, Shin OK, Song C, Cho KY and Ahn YJ. 2001. Insecticidal activities of aromatic plant extracts against four agricultural insects. *Agric. Chem. Biotechnol.* 44(1): 23-26.
- [20] Tang W and Eisenbrand G. 1992. *Chinese Drugs of Plant Origin*; Springer-Verlag: Berlin and Heidelberg, Germany. pp 401-415.
- [24] Chander H, Kulkarni SG and Berry SK. 1992. Studies on turmeric and mustard oil as protectants against infestation of red flour beetle, *Tribolium castaneum* (Herbst) in stored rice. *J Insect Sci.* 5: 220-222.

- [25] Jilani G and Saxena RC. 1990. Repellent and feeding deterrent effects of turmeric oil, sweetflag oil, neem oil and a neem-based insecticide against lesser grain borer (Coleoptera: Bostrychidae). *J Econ Entomol.* 83: 629-634.
- [21] Su HCF, Horvat R and Jilani G. 1982. Isolation, purification, and characterization of insect repellents from *Curcuma longa* L. *J Agric Food Chem.* 30: 290-292.
- [23] Chowdhury H, Walia S and Saxena BS. 2000. Isolation, characterization and insect growth inhibitory activity of major turmeric constituents and their derivatives against *Schistocerca gregaria* (Forsk) and *Dysdercus koenigii* (Walk). *Pest Manage Sci.* 56: 1086-1092.
- [22] Lee HS, Shin WK, Song C, Cho KY and Ahn YJ. 2001. Insecticidal activities of ar-turmerone identified *Curcuma longa* rhizome against *Nilaparvata lugens* (Homoptera: Delphacidae) and *Plutella xyostella* (Lepidoptera: Yponomeutidae). *J Asia-Pacific Entomol.* 4: 181-185.
- [26] Gopalan B, Goto M, Kodama A and Hirose T. 2000. Supercritical carbon dioxide extraction of turmeric (*Curcuma longa*). *J Agric Food Chem.* 48: 2189-2192.
- [27] Tawatsin A, Asavadachanukorn P, Thavara U, Wongsinkongman P, Bansidhi J, Boonruad T, Chavalittumrong P, Soonthornchareonnon N, Komalamisra N and Mulla MS. 2006. Repellency of essential oils extracted from plants in Thailand against four mosquito vectors (Diptera: Culicidae) and oviposition deterrent effects against *Aedes aegypti* (Diptera: Culicidae). *Southeast Asian J Trop Med Public Health.* 37(5): 915-931.
- [28] Boonyabancha S, Suphaphom K and Srisurapat A. 1997. Repellent effect of volatile oils on *Aedes aegypti*. *Bull. Dept. Med. Sci.* 39: 61-66.
- [29] Barnard DR. 1999. Repellency of essential oils to mosquitoes (Diptera: Culicidae). *J. Med. Entomol.* 36: 625-629.
- [30] Tawatsin A, Wratten SD, Scott RR, Thavara U and Techadamrongsin Y. 2001. Repellency of volatile oils from plants against three mosquito vectors. *Journal of Vector Ecology.* 26(1):76-82.s
- [31] Sosan MB, Adewoyin FB and Adewunmi CO. 2001. Larvicidal properties of three indigenous plant oils on the mosquito *Aedes aegypti*. *Nig J Nat Prod And Med.* 5: 30-33.
- [32] Phasomkusolsil S and Soonwera M. 2011. Efficacy of herbal essential oils as insecticide against *Aedes aegypti* (Linn.), *Culex quinquefasciatus* (Say) and *Anopheles dirus* (Peyton and Harrison). *42(5): 1083-1092.*
- [33] Hsu W, Yen J and Wang Y. 2013. Formulas of components of Citronella oil against mosquitoes (*Aedes aegypti*). *J Environ Sci Health [B].* 48: 1014-1019.
- [34] Burdock GA. 2010. Geranio, Fenaroli's Handbook of Flavor Ingredients, 6th ed. CRC Press. pp: 733-734.
- [35] Barnard DR and Xue R. 2004. Laboratory evaluation of mosquito repellents against *Aedes albopictus*, *Culex nigripalpus*, and *Ochlerotatus triseriatus* (Diptera: Culicidae). *J Medical Entomol.* 41: 726-730.
- [36] Chen W and Viljoen AM. 2010. Geraniol-A review of a commercially important fragrance material. *South African Journal of Botany.* 76: 643-651.
- [37] Müller GC, Junnila A, Butler J, Kravchenko VD, Revay EE, Weiss RW and Schlein Y. 2009. Efficacy of the botanical repellents geraniol, linalool, and citronella against mosquitoes. *Journal of Vector Ecology.* 34 (1): 2-8.
- [38] Müller GC, Junnila A, Kravchenko VD, Revay EE, Butler J and Schlein Y. 2008. Indoor protection against mosquito and sand fly bites: a comparison between citronella, linalool, and geraniol candles. *Journal of the American Mosquito Control Association.* 24(1): 150-153.
- [39] Raharivelomanana PJ, Terrom GP, Bianchini JP and Coulanges P. 1989. Study of the antimicrobial action of various essential oils extracted from Malagasy plants, II: Lauraceae. *Arch. Inst. Pasteur Madagascar.* 56(1): 261-271.
- [40] Nir Y, Potasman I, Stermer E, Tabak M and Neeman I. 2000. Controlled trial of the effect of cinnamon extract on *Helicobacter pylori*. *Helicobacter.* 5(2): 94-97.
- [41] Samarasekera R, Wijendra WAS and Kalhari KS. Antifungal properties of essential oils of Cinnamon (*Cinnamomum zeylanicum*) against dermatophytes. Paper No.E2/ 246, Proceedings of the 57th Annual Session, Sri Lanka Association for Advancement of Science, Colombo, Sri Lanka.

- [42] Ranasinghe L, Jayawardena B and Abeywickrama K. 2002. Fungicidal activity of essential oils of *Cinnamomum zeylanicum* (L) and *Syzygium aromaticum* (L) Merr et L. M. Perry against crown rot and anthracnose pathogens isolated from Banana. *Lett Appl Microbiol.* 35(3): 208-211.
- [43] Huang Y and Ho SH. 1998. Toxicity and antifeedant activities of cinnamaldehyde against grain storage insects, *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. *J Stored Prod Res.* 34(1): 11-17.
- [44] Spices. 1999. In: *Plant Resources of South-East Asia 13*. Edits., C.C. de. Guzman and J.S. Siemonsma, Backhuys Publ Leiden, Netherlands. pp 99-104.
- [45] Chang ST and Cheng SS. 2002. Antitermitic activity of leaf essential oils and components from *Cinnamomum osmophleum*. *J Agric Food Chem.* 50: 1389-1392.
- [46] Wang SY, Chen PF and Chang ST. 2005. Antifungal activities of essential oils and their constituents from indigenous cinnamon (*Cinnamomum osmophleum*) leaves against wood decay fungi. *Bioresour Technol.* 96(7): 813-818.
- [47] Cheng S, Liu J, Tsai K, Chen W and Chang S. 2004. Chemical composition and mosquito larvicidal activity of essential oils from leaves of different *Cinnamomum osmophleum* provenances. *J Agric Food Chem.* 52: 4395-4400.
- [48] Samarasekera R, Kalhari KS and Weerasinghe IS. 2005. Mosquitocidal Activity of Leaf and Bark Essential Oils of Ceylon *Cinnamomum zeylanicum*. *Journal of Essential Oil Research.* 17(3): 301-303.
- [49] Prajapati V, Tripathi AK, Aggarwal KK and Khanuja SPS. 2005. Insecticidal, repellent and oviposition-deterrent activity of selected essential oils against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. *Bioresour Technol.* 96: 1749-1757.
- [50] Lopez MD and Villalobo MJP. 2010. Mode of inhibition of acetylcholinesterase by monoterpenoids and implications for pest control. *Ind Crops Prod.* 31(2): 284-288.
- [51] Peana AT and Moretti MDL. 2002. Pharmacological activities and applications of *Salvia sclarea* and *Salvia desoleana* essential oils. *Studies Nat Prod Chem.* 26(7): 391-423.
- [52] Isman MB. 1999. Pesticides based on plant essential oils. *Pestic Outlook.* 10: 68-72.
- [53] Kamsuk K, Choochote W, & Chaithong U, Jitpakdi A, Tippawangkosol P, Riyong D and Pitasawat B. 2007. Effectiveness of *Zanthoxylum piperitum*-derived essential oil as an alternative repellent under laboratory and field applications. *Parasitol Res.* 100: 339-345.
- [54] Das NG, Baruah I, Talukdar PK and Das SC. 2003. Evaluation of botanicals as repellents against mosquitoes. *J Vect Borne Dis.* 40: 49-53.
- [55] Choochote WU, Chaithong K, Kamsuk A, Jitpakdi P, Tippawangkosol B, Tuetun D, Champakaew and Pitasawat B. 2007. Repellent activity of selected essential oils against *Aedes aegypti*. *Fitoterapia.* 78: 359-364.
- [56] Peerzada N. 1997. Chemical composition of the essential oil of *Hyptis suaveolens*. *Molecule.* 2: 165-168.
- [57] Mwaiko GL. 1992. Citrus peel oil extract as mosquito larvae insecticides. *E Afr Med J.* 69: 223.
- [58] Silva WJ, Do´ria GAA, Maia RT, Nunes RS, Carvalho GA, Blank AF, Alves PB, Marçal RM and Cavalcanti SCH. 2008. Effects of essential oils on *Aedes aegypti* larvae: Alternatives to environmentally safe insecticides. *Bioresour Technol.* 99: 3251-3255.
- [59] Amusan AAS, Idowu AB and Arowolo FS. 2005. Comparative toxicity effect of bush tea leaves (*Hyptis suaveolens*) and orange peel (*Citrus sinensis*) oil extract on larvae of the yellow fever mosquito *Aedes aegypti*. *Tanzania Health Research Bulletin.* 7: 174-178.
- [60] Conforti F, Statti G, Uzunov D and Menichini F. 2006. Comparative chemical composition and antioxidant activities of wild and cultivated *Laurus nobilis* L. leaves and *Foeniculum vulgare* sub sp. *piperitum* (Ucria) coutinho seeds. *Biol Pharm Bull.* 29: 2056-2064.
- [61] Amin G, Sourmaghi MHS, Jaafari S, Hadjagae R and Yazdinezhad A. 2007. Influence of phenological stages and method of distillation on Iranian cultivated bay leaves volatile oil. *Pak J Biol Sci.* 10: 2895-2899.
- [62] Sellami HI, Wannas A, Bettaieb W, Berrima I, Chahed ST, Marzouk TB, and Limam F. 2011. Qualitative and quantitative changes in the essential oil of *Laurus nobilis* L. leaves as affected by different drying methods. *Food Chem.* 126: 691-697.

- [63] Ali-Shtayeh MS, Yaniv Z and Mahajna J. 2000. Ethnobotanical survey in the Palestinian area: A classification of the healing potential of medicinal plants. *J Ethnopharmacol.* 73: 221-232. (64)
- Drapeau J, Fröhler C, Touraud D, Kröckel U, Geier M, Rosea A and Kunz W. 2009. Repellent studies with *Aedes aegypti* mosquitoes and human olfactory tests on 19 essential oils from Corsica, France. *Flavour Fragr J.* 24: 160–169.
- [65] National Research Council. 1992. *Neem: a tree for solving global problems*. In: Report of an adhoc panel of the Board on Science and Technology for International Development. National Academy Press, Washington, DC.
- [66] Isman MB. 2006. Botanical insecticides, deterrent and repellents in modern agriculture and an increasingly regulated world. *Ann Rev Entomic.* 51:45-66.
- [67] Schmutterer H. 2002. The neem tree (*Azadirachta indica*) and other Meliceous plants. In: *Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and other purposes*. 1st Ed. Mumbai: Neem Foundation.
- [68] Locantoni L, Guisti F, Cristofaro M, Pasqualini L, Esposito F, Lupetti P and Habluetzel A. 2006. Effect of neem extract on blood feeding oviposition and oocyte ultra structure in *Anopheles stephensi* Liston (Diptera: Culicidae). *Tissue Cell.* 38: 361-371.
- [69] Su T and Mulla MS. 1998. Ovicidal activity of neem products (Azadirachtin) against *Culex tarsalis* and *Culex quinquefasciatus* (Diptera; Culicidae). *J Am Mosq Control Assoc.* 14: 204-209.
- [70] Dua VK, Pandey AC, Raghavendra K, Gupta A, Sharma T and Fh AP. 2009. Larvicidal activity of neem oil (*Azadirachta indica*) formulation against mosquitoes. *Malaria Journal.* 8(124): 1-6.
- [71] Shanmugasundaram R, Jeyalakshmi T, Sunil DM and Balakrishna MP. 2008. Larvicidal activity of neem and karanja oil cakes against mosquito vectors, *Culex quinquefasciatus* (Say), *Aedes aegypti* (L.) and *Anopheles stephensi* (L.). *J Environ Biol.* 29(1): 43-45.
- [72] Nerio LS, Olivero-Verbel J and Stashenko E. 2010. Repellent activity of essential oils: A review. *Bioresource Technol.* 101: 372-378.