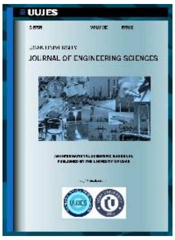

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Review article

A REVIEW OF PLANT-BASED INSECT REPELLENTS, THEIR APPLICATIONS ON TEXTILES AND REPELLENCY TEST METHODS

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Abstract

There are many life-threatening insects in nature and the most dangerous ones are mosquitoes. People have taken herbal precautions against them since ancient times. Although several chemical and synthetic insect repellents exist in the market, the plant-based protection methods have never been given up such that there is a trend towards producing and commercializing plant-based insect repellents in recent years. In the light of ethnobotanical studies, new insect repellent plants and formulations are being explored, developed and tested. In literature, there are a lot of research on plant-based repellents some of which are reviewed in this article. Information about the most frequently used plant-based repellents and related studies are presented. Different application methods of the plant-based repellents on the textiles are explained with their a few examples in literature. Also, common test methods used for determining the effectiveness of insect repellents and repellent treated textiles are stated and the importance of the standardization of these test methods is emphasized. It is understood that usage of plant-based insect repellents in practice can be beneficial and useful if stayed within their safe concentration limits.

Keywords: *Repellent; plant-based; natural; insect; mosquito; insecticide; pesticide.*

1. Introduction

Among insects found in nature, mosquitoes are dangerous insects for human being so that they cause diseases like malaria, dengue, yellow fever, chikungunya and filariasis which can result in death (Fig. 1) [1-4]. Raja et al. reported that Caraballo and King notified that over 700 million people are infected by mosquitoes worldwide and about one million of them are died. According to malaria fact sheet published by UNICEF in 2015, over 1,200 children die because of malaria just in a day [3]. Therefore, taking precautions against mosquito bites has been a critical issue from past to present. There are very limited

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opportunities of primary prevention against the diseases caused by insects such that there are no vaccines with a few exceptions which are mentioned in the followings: (i) Yellow fever vaccine in South America and Africa, (ii) Japanese encephalitis vaccine in Southeast Asia, (iii) Several regional tick-borne virus vaccines in Eastern Europe [2]. In this context, usage of the insect repellents and insecticides come to the forefront as a practical solution.

Fig. 1 Aedes aegypti (A) and Aedes albopictus (B) mosquitoes while feeding on a human, which are the vectors of life-threatening diseases, dengue and chikungunya [2]

Insect repellents generate a noxious atmosphere within 4 centimeters of human skin, which prevents insects to contact and bite. Insect repellents can be composed of chemical or organic substances. In case of insecticides, nerve tissue of the insects is damaged by a neurotoxin and insects die. Insecticides, which are generally derived from plants, can be chemical or organic agents. Besides, some substances like plant-derived permethrin and all other synthetic pyrethroids act as both insect repellents and insecticides [2].

Synthetic repellents have been widely used in the past. The most remarkable synthetic insect repellent is DEET (N,N-Diethyl-3methyl benzamide). DEET was found in 1946 by US Army, used to protect soldiers against insects of the jungles in World War II and introduced to the market by 1957 for civilians. Now it is the most widely used chemical against mosquitoes. It is reported that DEET is used by about 200 million people in the world, of which 15 million is from the U.K. and 78 million is from the U.S.A., in each year. It is available in forms of aerosol, cream, lotion, spray, gel, stick and wipe (towelette) with various formulas at concentrations between 5% and 100% (mostly 30-40%). It is a quite efficient substance but harmful for the human health [1,2,5,6]. In literature, six deaths are reported related to DEET poisoning. Three of these cases are occurred after intentional ingestions. In one case, a child died of ornithine transcarbamylase deficiency. In two cases, convulsive central nervous system reactions are seen in children due to repeated overapplications [2]. It is reported by Mishra et al. that Clem at al. revealed that DEET-based creams may show adverse effects such as skin reactions like black spot, rashes and itching [1]. Other synthetic repellents except DEET are IR3535 (3-[N-butyl-Nacetyl]-aminopropionic acid ethyl ester), Permethrin (3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate, Picaridin (US) and Icaridin (EU). Synthetic repellents cause many problems including urticaria syndrome, hypotension, encephalopathy in children, anaphylaxis, lethargy, confusion, disorientation, ataxia, tremors, seizures, decrease heart rate, breathing problems, eye irritation and headache [1,2]. The usage of chemical repellents is not safe for environment, animals and aquatic species though [3].

Mosquitoes have gained resistance against chemicals in time, so it’s needed to develop new repellents. When the environmental issues and the human health is taken into consideration, it's beneficial to tend toward nontoxic and eco-friendly plant-based materials [1].

Traditional usage of plant-based mosquito repellents by individuals exists in history. By the time of progress, very valuable knowledge on traditionally used plants is obtained by ethnobotanical researches. On the basis of the gained knowledge, several natural products have been developed and still there is an attempt to develop and commercialize new ones [6].

Asadollahi et al. stated that their review consists of the studies on the repellency effects of plants against Anopheles mosquitoes published between 1999 and 2018, in which the results are presented in terms of "percentage repellency" and "protection time". According to this review, Ligusticum sinense extract has the highest repellency effect and citronella, pine, Dalbergia sissoo, peppermint and Rhizophora mucronata oils follow it. The authors say that all these plant-based repellents' protection time range from 9.1 to 11.5 hours. Apart from these, it is reported that essential oils of several plants provide protection for 8 hours against different species of Anopheles. These plants are lavender, camphor, catnip, geranium, jasmine, broad-leaved eucalyptus, lemongrass, lemon-scented eucalyptus, amyris, narrow-leaved eucalyptus, carotin, cedarwood, chamomile, cinnamon oil, juniper, cajeput, soya bean, rosemary, niaouli, olive, tagetes, violet, sandalwood, litsea, galbanum, and Curcuma longa [7].

Essential oils which are obtained by distillation of the members of Lamiaceae (mint family including most culinary herbs), Poaceae (aromatic grasses) and Pinaceae (pine and cedar family) are generally used to repel insects worldwide [6]. Most of the commercial repellents contain several essential oils of peppermint, lemongrass, geraniol, pine oil, pennyroyal, cedar oil, thyme oil and patchouli for obtaining either good smell or repellent property. The duration of most of these oils is short because of their high volatility, but this issue can be solved with fixatives or carefully prepared formulas [6,7].

It is mentioned in several studies that the essential oils obtained from tea tree, neem [5], garlic [1,5], toothache tree (Zanthoxylum limonella) oil, kakronda (Blumea lacera) leaf extract [3], castor oil [4,8], geranium oil [4,7], pepper tree (Schinus molle) seed oil [4], the extract of Andrographis paniculate [9], oil solution of cinnamomum, tagetes and orange peel extract [7] are also effective against mosquitoes. Besides, Eyupoglu indicated that lavender, fennel and laurel oils have repellency effect against bees. It is especially mentioned that these oils do not kill bees [10].

Maia and Moore reported that several plant-based mosquito repellent oils are soybean oil, coconut oil, palm nut oil and andiroba oil and andiroba oil. These oils have a lot fewer effectiveness by comparison to DEET, but the usage of them as carriers for other active repellent substances can be beneficial because of both their low price and their content of unsaturated fatty acids and emulsifiers which enhance coverage of the repellent and slow evaporation of repellent volatiles [6].

Chattopadhyay et al. developed a mosquito repellent fast card with the usage of Duranta extract-algae mixture. The mortality percentage of the mosquitoes obtained by the developed card and commercially available card is 70% and 25%, respectively. The developed card is found to cause less gas emission than that of commercial one which is better for the human health. Also, it is revealed by the High Performance Liquid Chromatography (HPLC) analysis that propionic acid in Duranta is responsible for the mosquitocidal properties [11].

Asadollahi et al. reported that plant-based repellents attract attention recently because of their content in which there are a rich source of bioactive phytochemicals that are safe and biodegradable into non-toxic by-products [7]. On the other hand, Maia and Moore [6] stated that plant-based repellents are generally assumed to be safe because of their naturality, but this assumption cannot be accepted every time. The components of several plants may be hazardous for humans when a certain concentration percentage is exceeded. In relation to that, Maia and Moore presented a table (Table 1) in which there are safe concentrations of several ingredients which commonly exist in plant-based repellents. The authors emphasized that they reproduced this table with the permission of Strickman et al. [12].

Tracqui et al. notified that oleander (Nerium oleander L.) preparations have been used as insecticides for centuries [13]. Except from the information in Table 1, there have been fatal oleander (Nerium oleander L.) poisonings reported all throughout the world. Accidental poisonings occur in people when they chew the flowers, eat meat cooked over its branches or food including oleander stems [14].

Table 1 Safe concentrations of several ingredients which commonly exist in plant-based repellents [6,12]

Common Name	Scientific Name	Safe Conc. (%)	Hazard
Anise	Pimpinella anisum	3.6	Based on 0.11% methyl eugenol; carcinogen
Basil	Ocimum sp	0.07	Based on 6% methyl eugenol;carcinogen
Bergamot	Citrus aurantium bergamia	0.4	Sensitising and phototoxic; skin irritant
Cajeput	Melaleuca alternifolia	0.004	Based on 97% methyl eugenol, carcinogen
Cedar	Chamaecyparis nootkatensis	1	Likely allergenic contaminants if nootkatone not 98% pure
Cassia	Cinnaonium cassia	0.2 or 9	Sensiting skin irritant
Citronella	Cymbopon nardus	2	Safety is controversial; based on 0.2% methyl eugenol or 1.3% citral; sensitizing skin irritant
Citonella (java)	Cymbopogon winterianius	2	Based on 2% methyl eugenol, carcinogen
Citrus oils	Citrus up	16-25	Based on 0.005-0.0025% bergapten; phototoxic skin irritant
Clove	Syzyguim aromaticum	16-25	Based on 92 % methyl eugenol, sensitising skin irritant
Fewer tea, lemon bush	Lippie javanica	2	Based on 5% citral in related species; sensitising skin irritant
Geranium	Pelargonium graveolens	6	Based on 1.5% citral; sensitising skin irritant
Ginger	Zingiber sp	12	Based on 0.8% citral; sensitising skin irritant

Huon oil, Macquarie pine	Langarostrobus franklini	0.004	Based on 98 methyl eugenol; carcinogen
Lemongrass	Cymbopogon citratus	0.1	Based on 90 citral; sensitising skin irritant
Lime	Citrus aurantifolia	0.7	Phototoxic skin irritant
Litsea	Litsea cubeba	0.1	Based on 78% citral; sensitising skin irritant
Marigold	Tagates minuta	0.01	Phototoxic skin irritant
Mexican tea, American wormseed	Chenopodium ambrosioides	Prohibited	Toxic
Mint	Menthe piperata and spicata	2	Based on 0.1% trans-2-hexenal; sensitising skin irritant
Nutmeg	Myristica fragrans	0.4	Based on 1% methyl eugenol; carcinogen
Palmarosa	Cymbopogon martini	16	Based on 1.2% farnesol; sensitising skin irritant
Pennyroyal	Menthe pulegium or Hedeoma pulegioides	Prohibited	Toxic
Pine	Pinus sylvestris	Prepare with antioxidants	Oxidation creates phototoxic skin irritants
Rosemary	Rosemarinus officinalis	36	Based on 0.011% methyl eugenol; carcinogen
Rue	Ruta chalepensis	0.15	Based on presence of psoralenes; phototoxic skin irritant
Thyme	Thymus vulgaris	2	Based on 0.1% trans-2-hexenal; sensitising skin irritant
Violet	Viola odorata	2	Based on 0.1% trans-2-hexenal; sensitising skin irritant
Ylang-ylang	Canagium odoratum	2	Based on 4% farnesol; sensitising skin irritant

Maia and Moore reported that very few number of adverse effects have been reported in relation to the usage of DEET since 1946 and these cases mostly occurred when the repellent is used excessively or anomalously. The toxicity of DEET is examined closely when compared to all other repellents and it has been regarded safe for humans, even for children, pregnant and lactating women. On the other hand, plant-based repellents are assumed to be safe with the thought that they are natural and have been used for centuries, but people must keep in mind that they have not been examined heedfully and it's better to use them cautiously [6].

It is reported by Nwabor that main phytochemicals found in plants are saponin, flavonoid, tannin, alkaloids, glycoside, steroids and terpenoid [15]. Active mosquito repellent ingredients of many plant extracts and oils are generally volatiles [6]. Plants consist of volatile organic compounds of which the main groups are monoterpenes, sesquiterpenes and phenols. The mixture of these compounds constitutes essential oils. The repellency effect of many essential oils may be related to the existence of lower isoprenoids. Monoterpenes like α-pinene, limonene, terpinolene, citronellol, citronellal, camphor and thymol are seen commonly in several mosquito repellent essential oils [3]. In fact, most of the plants use their compounds to prevent the attack of plant eating insects. It is reported by Maia and Moore that Gatehouse mentioned that in case of the leaf damage, plants generate volatile "green leaf volatiles" to repel them [6]. However, plants also repel the mosquitoes by attacking their olfactory nerve [1]. Maia and Moore reported that Carey et al. and Logan et al. observed that odour receptors of mosquitoes responded this type of volatiles including geranyl acetate, citronellal, 6-methyl-5- hepten-2-one and geranylacetone strongly. Syed and Leal found that the odour receptors of Culex quinquefasciatus, which are affected by DEET, are also affected by thujone eucalyptol and linalool. Kwon et al. indicated that citronellal stimulates the odour receptor "OR83b" which is mentioned as DEET receptor in Anopheles gambiae. On the other hand, TRPA1 cation channel modulates it. Besides, Gershenzon and Dudareva and Lee et al. stated that the insect deterrence or repellence of many plant volatiles is most likely because of their high vapour toxicity [6].

2. Most Frequent Insect Repellents

There are quite a few insect repellants that are extracted from plants and used. It is known that many plants are subject to research as insect repellents in the regional and laboratory environment. In the studies carried out, the effect time of the extracts or essential oils obtained from many endemic plants, depending on the concentration, on insects and the effect of repelling insects were evaluated. The plants most frequently used as insect repellents by 2020 are summarized in the Table 2.

Table 2 Plants with natural ingredients with mosquito-repellent properties

Plant	Study
Citronella (lemongrass)	[16-22]
Peppermint	[17, 20, 23]
Basil	[17-20, 24-25]
Neem	[24, 26-27]
Rosemary	[17, 28]
Clove	[19, 22, 29-31]
Turmeric	[18-19, 32]
Thyme	[17, 29]
Eucalyptus	[17, 19-20]
Nerium oleander	[33-36]

While essential oils obtained from plants were used extensively in the studies, plant extracts were also used in some of them.

2.1 Citronella

Citronella (Fig. 2) is one of the essential oils that is extracted from the stems and leaves of various types of lemongrass [7]. It is used widely in natural repellent products on the market, generally at 5-10% concentrations. These concentration values are lower than those of many commercial repellents, but citronella can lead to skin sensitivity at higher concentrations [6].


Fig. 2 Citronella

Components of the lemongrass are α-pinene, citronellal, silo from lemone grass, rosefuran epoxide, nerol, β-citronellol, neral, geraniol, geranial, 2-undecanone, geranyl acetate, (E)-caryophyllene, α-(E)-bergamotene, 2-tridecanone, δ-cadinene, 5-epi-7-epi-a-eudesmol, α-cadinol, 6-methyl-5-heptane-2-one, (Z)-β-ocimene, β-ocimene, myrcene, linalool, 6,7-epoxyocimene. Components of the citronella grass are Citronellal, Citronellol, Linalool, Neral, Geraniol, Geranial, Eugenol, Limonene, Ocimene [1].

Asadollahi et al. reported that Ansari et al. detected that citronella obtained from lemongrass is fully repellent against Anopheles culicifacies for 11 hours. Tawatsin et al. concluded that citronella could repel Anopheles dirus with the repellency rate of 100% for 6 hours [7].

2.2 Peppermint

Peppermint (Fig. 3) is obtained by hybridization of cross-breeding spearmint (Mentha spicata) and water mint (Mentha aquatica). The constituents of peppermint are biologically active. It has high menthone, menthol and methyl esters. Peppermint is native to Europe and the Middle East. It has been widespread over time and now it's grown in many regions of the world [7,37].


Fig. 3 Peppermint leaves

In literature, Farnad and Aslanipour exhibited an HPLC (High-performance Liquid Chromatography) chromatogram of the extract of peppermint at 280 nm. According to this chromatogram, the components which make peeks are mentioned as followings: ascorbic acid, rutin, gallic acid, chlorogenic acid, ferulic acid, 4-hydroxy benzoic acid, vanillic acid, caffeic acid and 1-naphthol [38].

Asadollahi et al. reported that Ansari et al. obtained that 1 ml peppermint oil without dilution repels Anopheles annularis with the repellency rate of 100% for 11 hours [7].

2.3 Eucalyptus

Eucalyptus (Fig. 4) which is a pulpy woody plant usually grows in tropical regions [7]. Diaz reported that it is mentioned in the study conducted by Carroll and Loye that the major repellent component of lemon eucalyptus (Corymbia citriodora) is para-menthane-3,8-diol (PMD) and it protects against a wide range of insects over several hours [2,6]. The authors concluded that the repellency effectiveness and duration of PMD against mosquitoes is equal to those of DEET [2]. Maia and Moore reported that Barasa et al. notified that many plant extracts and oils evaporate fast and makes the user vulnerable after a short period of time, but PMD constitutes an exception such that its vapour pressure is lower than those of volatile monoterpines of many plant oils [6]. PMD is used in pump sprays at 10-40% concentrations. On the other hand, Food and Drug Administration (FDA) recommended not to use PMD in children under 3 years old [2].

Fig. 4 Eucalyptus leaves

Maia and Moore reported that Phasomkusolsil and Soonwera stated that lemon eucalyptus essential oil is known to repel the mosquitoes for around one hour. However, Maia and Moore also reported that Barasa et al. found that the essential oil of the lemon eucalyptus was hydro-distillated and it is explored that the mosquito repellency rate of the waste distillate remaining is better than that of essential oil [6].

In literature, there are several studies conducted with the aim of determining the repellency effect of different sub-species of eucalyptus. In accordance with the information about the study of Amer et al., which is reported by Asadollahi et al., sub-species of eucalyptus show difference in terms of the repellency rate. Lemonscented eucalyptus, narrow-leaved eucalyptus and broad-leaved eucalyptus provide protection against An. stephensi for 8 hours with the repellency rate of 52.4%, 42.8% and 38.1%, respectively. 20% oil solution of the plant was used in all these cases [7].

It is also reported by Asadollahi et al. that Auysawasdi et al. observed that E. globulus essential oil completely protects against An. dirus between 1.7 and 3.4 hours. These protection time values depend on the concentration ratio ranging from 5% to %25 [7].

2.4 Basil

Basil (Fig. 5) is a member of the Lamiaceae family. It's an annual plant of the Ocimum genus and used to prepare traditional medicine in general [7]. Prakash and Gupta reported that people have used basil for centuries to enhance the immune system. Also, it has been used for treating malaria fever in ancient times [39].

Components of the basil are linalool, methyl chavicol, methyl cinamate, eucalyptol, α-terpineol, eugenol, methyl eugenol, camphor, α-caryophyllene, β-cadinene, α-bisabolol, β-farnesene, cubenol, β-elemene, germacrene D, α-bergamotene, α-guaiene, τ-cadinol, bornylacetate, α-caryophyllene, β-caryophyllene, elixen, α-copaene, epibiciclosesquiphelandrene, τ-muralol, δ-gurjunen [1].

Fig. 5 Basil leaves

Asadollahi et al. notified that Tawatsin et al. succeed to get 100% repellency rate against An. dirus for 6 hours with the usage of hairy basil oil at 3 ml concentration and Phasomkusolsil et al. revealed that O. basilicum oil at 0.21, 0.10 and 0.02 mg/cm^2 concentration provide repellency rate of 96%, 74% and 66%, respectively, against An. dirus [7].

2.5 Neem (Azadirachta indica)

Neem (Fig. 6) which is a versatile tropical tree generally grows in Indian subcontinent and most of the countries in Africa [7,40]. Among natural repellents, neem is mostly declared as an alternative of DEET. Maia and Moore reported that Reutemann and Ehrlich stated that the usage of neem as a topical insect repellent is not confirmed by EPA (Environmental Protection Agency). The dermal toxicity of neem is low, but it may lead to skin irritation like dermatitis in case of using without dilution [6].

Fig. 6 Neem leaves

Components of the neem are azadirachtin (the chief active component), nimbolinin, nimbin, nimbidin, nimbidol, gedunin, salannin, sodium nimbinate, gedunin, salannin, quercetin. Leaves of the plant contain nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol and amino acid, 7-desacetyl-7-benzoylazadiradione; 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione, nimbiol. Also, quercetin and ß-sitosterol which are polyphenolic flavonoids are obtained by purifying of fresh neem leaves and show antibacterial and antifungal properties [1,41].

Asadollahi et al. reported that Abiy et al. obtained the mean repellency rate of 71% against An. arabiensis for 3 hours by diluting neem and chinaberry oils to 20% using Niger seed (noog abyssinia) oil [7]. Mishra et al. reported that Wannang et al. found that the neem extract's repellency effectiveness on the female anopheles is better than that of culex mosquito species [1].

2.6 Rosemary (Rosmarinus officinalis)

Rosemary (Fig. 7) is a member of Lamiaceae (Labiatae) family and native to Mediterranean regions. It is an evergreen aromatic shrub [7].

Asadollahi et al. stated that Amer et al. achieved a complete protection for 8 hours when they used the 20% oil solution of rosemary (R. officinalis) as a repellent against An. stephensi [7]. Banupriya and Maheshwari applied rosemary extract to cotton plain woven fabrics. They carried out excito chamber test and determined the mosquito repellency rates of untreated and treated fabrics as 0% and 92%, respectively [42].

Fig. 7 Rosemary

2.7 Clove (Syzygium aromaticum)

Clove (Fig. 8) is an aromatic flower buds of a tree (Syzygium aromaticum) which belongs to the Myrtaceae family. When its anti-bacterial, anti-oxidant, antipyretic, anti-candidal and aphrodisiac properties are taken into consideration, clove is a valuable spice in nature [7,43].

Fig. 8 Cloves

Asadollahi et al. reported that 98%, 92% and 82% repellency ratios against An. dirus are obtained with the usage of S. aromaticum oil at 0.21, 0.10 and 0.02 mg/cm^2 concentrations, respectively, in the study conducted by Phasomkusolsil et al. [7].

Asadollahi et al. also reported that Barnard et al. got a complete protection against An. albimanus for 1.25, 1.5, 2.26 and 3.55 hours by using the clove essential oil at 25%, 50%, 75% and 100% concentrations. These results are consistent with the ones obtained in the study of Trongtokit et al, in which it is observed that the clove essential oil at 10%, 50% and 100% repels An. dirus for 1.33, 2.66 and 3.5 hours, respectively. In another study, Trongtokit et al. showed that 20% gel of clove oil and clove oil cream 20% provides repellency against An. dirus for 4.5 and 4.8 hours, respectively [7].

2.8 Turmeric (C. longa)

Turmeric (Fig. 9) which belongs to Zingiberaceae family is a perennial plant. It is generally used in human food as a spice. On the other hand, it is used for medicinal purposes [7].

In the study conducted by Auysawasdi et al., which is mentioned in the review article of Asadollahi et al., it is indicated that a complete protection against An. dirus for 4, 5, 5.5, 5.5 and 8 hours is obtained by using turmeric essential oil at 5%, 10%, 15%, 20% and 25% concentrations, respectively [7].

Fig. 9 Turmeric

Asadollahi et al. also reported that Tawatsin et al. used 3 ml turmeric volatile oil to repel An. dirus and got 100% repellency effect for 6 hours. In another study carried out by Phasomkusolsil and Soonwera, it is understood that 100 µl turmeric oil repels An. minimus for 1 hour [7].

2.9 Chrysanthemum

Chrysanthemum (Fig. 10) belongs to the family Asteraceae. They are generally found in East Asia, northeastern Europe and China. It has a lot of horticultural varieties and cultivars [44].

Bhatt and Kale applied finishing process to nylon 66 net fabrics with chrysanthemum oil nano emulsion at 50, 75 and 100 g/l concentrations by layer by layer (LBL) technique and obtained repellency rates of 55%, 75-80% and 95%, respectively [45].

There are synthetic chemicals called pyrethroids which mimic natural extracts of the chrysanthemum flower. One of the famous synthetic insecticides, permethrin, is a member of the pyrethroid family [46].

Fig. 10 Chrysanthemum flower

2.10 Castor Oil Plant (Ricinus communis)

Castor oil plant (Fig. 11) is a kind of flowering plant which belongs to the spurge family called Euphorbiaceous. It is a member of the Ricinus genus and Ricininae subtribe [8].

Mature trees of castor oil repel flies and mosquitoes. Mole and insects cannot live in gardens which have this tree. In addition, castor oil is also used in the production of drugs that destroy other harmful insects and fungi [47].

Fig. 11 Castor oil plant

In the research conducted by Tseghai, 2% castor oil was applied to the cotton fabrics by padding technology in different conditions. Then the author carried out a cage test with 50 mosquitoes. Total number of mosquitoes that contact to the fabric in 30 minutes is determined for each fabric sample. The mosquito repellency of untreated and treated fabrics are specified as 10 and 1-5, respectively. The best result is obtained in case of 2% castor oil and 6% binder by pad-batch-dry method. However, it is observed that the washing resistance of the mosquito repellent finish is found to be poor [8].

2.11 Nerium oleander

It has been determined that Nerium oleander, known as a lethal, is a natural insect repellent especially for fly species that are resistant to synthetic insect repellents (33). It has been stated that flavonoids, sterols, terpenes and coumarin in the structure of Nerium oleander are effective on the repellent. Flavonoids are basically the main component that protects plants against bacterial, fungal or viral diseases. This component also provides a defense against insects. D-limonene, beta-nyrcene, durene, phytol, gamma-sitosterol, p-cresol, di-isopulegol, guaiol, phenyl ethyl alcohol, 8-quinolinol, 4-methyl, verbenone, 2(4H)-benzofuranone, squalene and caryophyllene oxide (34,35). However, these contents differ more or less in extracts obtained from plants grown in different regions (34).

Fig. 12 Nerium oleander plant

It has been determined that D-limonene, which is isolated from the Nerium oleander structure, acts by inhibiting the reproductive parameters of insects, while cresol acts by denaturing the cells of flies by respiration or contact (34,36). It is stated that polysaccharides and phospholipid fatty acids are also effective in insect cell transitions (34).

4. Application Methods of Repellents on Textiles

Insect repellents can be applied to the textiles in various forms. These forms are mentioned below.

4.1 Fiber and Yarn Form

Yuce reported that there are studies in literature, which indicates that permethrin, a plant-based insect repellent, can be incorporated in the fibers during the fiber extraction process. By this way, the protection time of the repellent textiles against mosquitoes becomes longer. Olyset® Net produced by Sumitomo Chemical (Japan) is an example of these textiles, which is a fly-repellent net composed of polyethylene monofilaments that are produced by incorporation of permethrin during the fiber extraction process [48].

4.2 Fabric Form

Repellents are applied to the fabrics by different methods. These are padding-drying, impregnation, spraying, encapsulation, coating and finishing [3,48].

Insect repellent textile products must be wash-resistant for multiple washes. Washing resistance of the repellent treatment can be enhanced by application of encapsulated insect repellents. It is important to make an optimum adjustment of the control of repellent release [48]. Also, Maia and Moore reported that Sakulku et al. stated that the release percentage of oils is decreased and longer protection time is provided by nanotechnological applications [6].

Yuce reported that Specos et al. applied the microencapsulated citronella oil to 100% cotton plain weave fabrics. They also treated the fabrics with ethanol solution of the volatile citronella oil by spraying. Then microencapsulated and sprayed fabrics are compared in terms of repellency effect. It is understood that microencapsulated fabrics has repellency rate of more than 90% for three weeks and these results are better than those of sprayed. Yuce also reported that 100% polyester fabrics are treated with the encapsulated lemongrass oil in the study conducted by Anitha et al. and a repellency rate of 92% is obtained [48].

4.3 Net Form

Bed nets which are treated with the insect repellents act as both physical barrier and insecticide against mosquito bites. One of the most remarkable insect repellent substances, the permethrin, can be attached to the nets during various phases such as fiber extraction, yarn spinning, net production or coating. Bed nets can be produced as woven or knitted fabrics with the pore size of 1.2-1.5 mm (If the pore size becomes larger than 2 mm, flies can pass through the net). Bed nets are generally composed of synthetic fibers such as polyester and polypropylene [3,48]. Natural fibers are preferred in cases of the allergic reactions of the users. However, the attachment of repellents to natural fibers becomes more difficult than to those of synthetic ones [48]. It is stated in the review of Raja et al. that the hydrophilic property of the natural fibers may prevent the pesticide binding by using polymeric binders to be strong. Raja et al. reported that Curtis et al. revealed that permethrin treated nylon or cotton nets, which are used in the house, protected against mosquitoes for 2-6 months. The protection time depends on the usage, wash cycle, etc. [3].

5. Test Methods for Determining Effectiveness of Insect Repellents and Repellent Treated Textiles

There are several methods for evaluating effectiveness of insect repellents and repellent treated textiles. Most used techniques are cage test, cone test and excito chamber test.

5.1 Cage Test

One of the most used methods for mosquito repellents is the cage test (Fig. 12). This method [4,49,50] is suitable for the evaluation of topical repellents (creams, lotions and spray formulations) and impregnated fabrics. In this method, mosquito behavior is observed in a cage. Subject wears treated and conventional fabric separately, and then mosquitoes are observed landing on the subject. Among the advantages of this method, mosquitoes can be seen landing and biting on humans, and at the same time, mosquito movement can be observed directly against the repellent-treated fabric. The disadvantage of the cage test is that it involves human subjects. In terms of human participation, the consent of the volunteers and the ethical approval of an authority should be sought. Mosquitoes used in the test must be free of pathogens. Thus, subjects can be assured that the test will not harm them [48].

Fig. 12 Cage test for measuring the effectiveness of mosquito repellents in a laboratory [50]

According to the standards of the World Health Organization (WHO 2009), 200 female mosquitoes are filled in square test cages measuring 40 x 40 x 40 cm. Mosquitoes should not have given birth and should be placed in the cage 1 day before the test. The test time is 3 minutes and mosquitoes landing on the arm are counted during this time. Test products are applied to the volunteers' forearms and repeated every 30 or 60 minutes. Mosquito protection activity of the fabric is calculated using Eq. 1:

$$\text{Mosquito protection percent (\%)}: (U - T)/U \times 100 \quad (1)$$

where U is the number of flies arriving in the untreated sample. T is the number of flies arriving on the treated fabric [48].

5.2 Cone Test

This method [51] provides an advantage over the cage test as no human subjects are used. However, this method is used less frequently. The test is performed at an exposure time of 3 minutes and at 27 °C. The cone test (Fig. 13) is a test performed according to WHO 1998 testing standards. Standard plastic sleeves are fixed to the treated test surface. Then, five female mosquitoes are transferred into the cone with an aspirator and exposed to the surface of the repellent-applied fabric for three minutes. Post-exposure mosquitoes are removed from the cones and placed in small cages for observation in drug-free air. The number of dormant mosquitoes is counted one hour after removal and the dead rate is determined after 24 hours. Each test surface is repeated ten times, with a total of 50 mosquitoes observed. In

Fig. 13 Apparatus for WHO cone bioassay test [30]

addition, the natural mortality rate is tested on 50 mosquitoes for another untreated fabric. The percentage of mosquito death as a result of the test is calculated using Eq. 2:

% Mosquito mortality: (MR-MC) / (100-MC) x 100 (2)

where MR and MC are the numbers of dead mosquitoes in cases of treated fabric exposure and control fabric exposure, respectively [48].

5.3 Excito Chamber Test

This method [9,42] is based on the principle of obtaining the difference between the mosquito treated material and the untreated material by observing the movement of the mosquitoes (Fig. 14). In this test, as in the cone test, humans are not used as subjects. Mosquitoes are fasted overnight or at least 4 hours before the test. The exposure time is between 10 and 30 minutes. The percentage of mosquito repellent was calculating using Eq. 3:

Fig. 14 Measurement of mosquito repellency by excito chamber test [21]

% Mosquito repellent: (NES + NDE)/(NEX) × 100 (3)

NES value corresponds to the number of mosquitoes that escaped, while the NDE value is the number of mosquitoes that have died. The NEX value is the number of mosquitoes exposed to the test. This method is more suitable for comparing products with and without mosquito repellent [48].

Besides, there is no available effective method for evaluating the insecticidal effect of textile products like uniforms after wear. Testing mosquito repellency of textiles along with the ones which are washed differently is usually made by bioassay method, knock test etc. within the conditions controlled [3].

5.4 World Health Organization (WHO) Repellency Test Standards

Maia and Moore [6] prepared a table of guidelines on repellent testing based upon the document published by WHO in 2009 [50]. The mentioned guidelines are given in Table 2.

It is very important that researches about plant-based repellents should be done with regard to WHO test standards and methodology for making a significant progress in this field. Always a DEET control should be included for comparing multiple studies and specifying standard errors. It will be possible to find out if the observed results are reliable when the issues mentioned above are taken into consideration [6].

Table 2 Repellent testing guidelines based on World Health Organization Pesticide Evaluation Scheme (WHOPES) [6,52]

WHOPES approved repellent testing methodology
Laboratory Testing
Use 20% DEET in ethanol as a positive comparison
Human subjects preferable to reflect the end user
Before the test, the test area of skin should be washed with unscented soap then rinsed with 70% ethanol / isopropyl alcohol
Mosquitoes should be reared under standard 27±2 °C temperature, ≥ 80±10% relative humidity, and a 12:12 (light:dark) photoperiod
Mosquitoes should be 3 to 5 days old, nulliparous females, starved for 12 hours preceding the test
Tests should be conducted with three or more species
40 x 40 x 40 cm cages with 50-100 mosquitoes for effective dose testing
40 x 40 x 40 cm cages with 200-250 mosquitoes for complete protection time testing
Control arms should be used to estimate mosquito readiness to feed
Treatment arms should be offered to mosquitoes after avidity has been measured
Field Testing
Use 20% DEET in ethanol as a positive comparison
Human subjects preferable to reflect the end user
Before the test, the test area of skin should be washed with unscented soap then rinsed with 70% ethanol / isopropyl alcohol
Volunteers should sit >20 meters apart
Design should be completely randomised
Trials should be conducted with medium biting pressures of representative vector species
All participants should be recruited on informed consent from the local area and be provided with malaria prophylaxis
In all testing monitoring of adverse effects should be carried out

6. Discussion and Conclusion

People have usually tried to protect themselves from dangerous insects, especially mosquitoes, since ancient times because they can make people have the diseases such as malaria, dengue, yellow fever, chikungunya and filariasis. These diseases are fatal and serious precautions must be taken against mosquitoes. There are different ways for protection but the most effective one is the usage of repellents. Various traditional and commercial repellents exist. They differ according to their content and grouped as synthetic and natural repellents.

The most famous synthetic repellent is DEET which has been used worldwide since 1946. DEET has overcome many strict tests by the time and deemed as safe for humans including children, pregnant and lactating women. Only few fatal cases are seen related to DEET and many of these cases are due to intentionally swallowing, overdosing and inappropriate usage. IR3535, Permethrin, Picaridin (US) and Icaridin (EU) are also other well-known synthetic repellents. These repellents can lead various disturbances in humans such as urticaria syndrome, hypotension, encephalopathy in children, anaphylaxis, lethargy, confusion, disorientation, ataxia, tremors, seizures, decrease heart rate, breathing problems, eye irritation and headache. Also, they are harmful for environment and animals, too. All these problems made people be in search of something natural and a trend towards plant-based repellents has begun.

In recent years, new plant-based natural insect repellent substances and formulas have been explored, developed and commercialized. There are a lot of mosquito repellent plants, plants-based materials (extracts, essential oils, oil solutions, seed oils, etc.), the formulas obtained by their mixture mentioned in literature. It is also seen that a mosquito repellent plant-based fast card is developed. Most frequently used plants are citronella, peppermint, eucalyptus, basil, neem (Azadirachta indica), rosemary (Rosmarinus officinalis), clove (Syzygium aromaticum), turmeric (C. longa), chrysanthemum and castor oil plant (Ricinus communis). Other mosquito repellent substances are mentioned in the introduction part of this review. The repellency effect of many plants come from their volatile organic compounds which can be grouped as monoterpenes, sesquiterpenes and phenols. The odour receptors of mosquitoes respond strongly to certain volatiles like geranyl acetate, citronellal, 6-methyl-5- hepten-2-one and geranylacetone. It is stated in literature that plant volatiles have high vapour toxicity. However, there is a problem with plant-based repellents that people become vulnerable when the volatiles evaporate. Hence, it is needed to decrease the evaporation rate and so the protection time will be increased. Also, there is a very important issue about plant-based repellents. It must be kept in mind that plants are natural but cannot be assumed as completely safe. Plants must be used with caution because they can be carcinogen, skin irritant, allergen and toxic when certain concentration limits are exceeded. Every plant has a safe concentration rate some of which are listed in Table 1.

There are various application methods for treating textiles with insect repellents. They can be added to the yarns during fiber extraction method. In case of fabrics, several practical methods exist like padding-drying, impregnation, spraying, encapsulation, coating and finishing. Among these, encapsulation technique is mentioned to be the best for improving washing resistance of the repellent treatment. Repellents can be incorporated to the textiles which are in net form at various production stages like fiber extraction, yarn spinning, net production or coating. It is also stated that the attachment of repellents is more difficult in case of natural fibers. This is because of their hydrophilic structure which prevents the repellent substance to bind strongly to the textile material.

Most frequently used test methods used for determining the repellency effectiveness of insect repellents are cage test, cone test and excito chamber test. Their settlement, testing details and related calculation formulas are explained in the related subtitle of this review. In cage test, behaviors of the mosquitoes can be seen clearly but it has a disadvantage that it is done with the participation of human subjects. In case of cone test and excito chamber test, there are no human subjects. Excito chamber test is mentioned to be useful for examining the difference between the treated and untreated materials. Apart from these, there are a few more methods like bioassay method, knock test, etc. These are used to determine the mosquito repellency effectiveness of textiles along with the ones washed differently. Unfortunately, the insecticidal effect of apparels like uniforms after wear cannot be evaluated because of the lack of available effective methods. There is a critical issue about the insect repellent testing that all studies should be done with regard to WHO guidelines and standards given in Table 2. In our literature search, we noticed that the test conditions of studies are quite different from each other. This situation generates a great confusion such that the test results cannot be compared properly. It should not be forgotten that standardization is very important for the future development of the plant-based insect repellents.

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