Chapter 18

Larvicidal, Insecticidal and Mosquito repellent activity of Scented Geranium (*Pelargonium species*) essential oil against Malarial Vector; *Anopheles stephensi*

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ABSTRACT

The primary malaria vector in Asia is *Anopheles stephensi*, which comes in three distinct biotypes. It has terrible effects on newborns and early children in endemic areas and breeds in different habitats. Typically, microbiological agents, insect growth regulators, and pesticides are used to target mosquitoes. Insecticide-treated bed netting and indoor residual spraying have both been used. On the other hand, these tactics cause resistance in certain species and have detrimental impacts on the environment and human health. Plant-based pesticides are among the environmentally benign methods that have been used recently to combat mosquito vectors. Essential oils (EOs) are highly scented aromatic oils that are produced by plants as secondary metabolites. These oils have a variety of biological characteristics, such as repellant and larvicidal effects. For a very long time, people have employed Scented geranium (*Pelargonium graveolens*) essential oils or their extractions as insect or mosquito repellents. Research has shown that the EO increases the % repellency against Anopheles species and has insecticidal action. Because they are widely accessible, reasonably priced, and safe, plant oils could eventually replace synthetic repellents in many areas of the world.

KEYWORDS
Malaria, *Anopheles stephensi*, Essential oils, *Pelargonium graveolens*, Larvicidal, Repellency

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INTRODUCTION

The world's deadliest creatures are thought to be mosquitoes (Diptera: Culicidae), as they transmit and carry several diseases that kill millions of people every year, including Lymphatic filariasis, Zika, Chikungunya, Malaria, Dengue, Encephalitis, Yellow fever, and Rift valley fever (Lee et al., 2018). In endemic areas, malaria, in particular, still has a terrible effect on newborns and early children. Malaria claimed the lives of 405,000 individuals worldwide in 2018, down from an estimated 416,000 in 2017 and 585,000 in 2010 (Al-Awadhi et al., 2021; Patel et al., 2024). A substantial risk to human life, malaria has been reported to have caused 247 million cases and 619,000 deaths in 2021 (WHO, 2022). The primary method of transmission is by the bites of female Anopheles Meigen mosquitoes carrying the parasite, which act as vectors for the infection (Lefevre et al., 2018). Preventing mosquito bites is one of the best strategies to reduce the incidence of malaria. A complete strategy for managing diseases spread by insects must include the use of repellents to protect people from mosquito bites (Aregawi et al., 2009; Patel et al., 2024). Chemicals including dimethyl phthalate, allethrin, N, N-diethyl mendelic acid amide, and N, N-diethyl-metatoluamide (DEET) are used in the production of most commercial repellents (Patel et al., 2012). It has allegedly been demonstrated that these chemical repellents are unhealthy for the general public and ought to be applied sparingly. The reason for this is their harmful effects on plastic and synthetic fabrics, in addition to their toxic responses that can cause dermatitis, allergies, and adverse effects on the nervous system and cardiovascular system (Patel et al., 2012). The extended and regular application of artificial repellents to manage mosquito populations has led to pesticide resistance, resurgence in mosquito populations, and detrimental effects on non-target creatures (Hemingway et al., 2002; Weill et al., 2003; Liu et al., 2006; Liu, 2015). By substituting natural insect repellent products, it would be able to make new eco-friendly repellents that counterbalance the harmful effects on the environment and human health. The availability of safe, bioactive phytochemicals found in plants that can be examined for their insecticidal and mosquito-repelling qualities has made using them popular once more in recent times. These phytochemicals also biodegrade into harmless byproducts (Nerio et al., 2010; Benelli et al., 2014; Palanisami et al., 2014; Pavela, 2015). Numerous research has demonstrated the global malaria vector-repelling abilities of plant extracts or essential oils. Rose-scented geranium, a member of the *Geraniaceae* family, is one of them (*Pelargonium* species). The insecticidal properties of geranium oil and its constituents, primarily citronellol and geraniol, are widely recognized (Dale and Saradamma, 1981) and these effects are discussed in this chapter.

Malaria: A Deadly Threat to Humanity

A female *Anopheles* mosquito carrying the *Plasmodium* parasite causes malaria, a deadly disease that infects humans when it bites. Malaria is the leading cause of mortality globally, but unfavourable outcomes can be prevented with early identification and timely intervention. In Africa and several Asian countries, malaria is the most prevalent disease; in the industrialized world, malaria is imported from endemic regions. As far back as the second century BC, Chinese medicine employed the sweet sagewort plant to treat malaria. Quinine became known for its antimalarial properties much later. In 1955, the global campaign to eradicate malaria began, and in 1964, Croatia proclaimed it to be eradicated forever (Talapko et al., 2019).

Malaria is caused by a small protozoon with several subspecies that belongs to the *Plasmodium* species group. Certain *Plasmodium* species cause illnesses in humans (Walker et al., 2014; White et al., 2014). Malaria pigment is an insoluble metabolite of hemoglobin that is acquired by amoeboid intracellular parasites belonging to the genus *Plasmodium*. There are several different vertebrates that these parasites infect, and some of them are present in tissue and red blood cells. There are known to be five human-infecting *Plasmodium* species out of 172. These are *P. malariae*, *P. falciparum*, *P. vivax*, *P. ovale*, and *P. knowlesi*. South-East Asia is home to *P. knowlesi*, a zoonotic malaria parasite. Infections of humans by other species are rare (Antinori et al., 2012; Singh and Daneshvar, 2013; Walker et al., 2014; Ashley, 2018). One of the most common diseases produced by any of the aforementioned *Plasmodium* species is malaria (Latin *Malus aer*, meaning "Filthy-air"). All species share comparable morphologies and biological characteristics (Vuk et al., 2008).

Revealing *Anopheles Stephensi*: An Up-Close Look at the Vector of Malaria

Approximately forty species of Anopheles are known to be the primary malaria vectors (Hay et al., 2010). *An. stephensi*, the Asian malaria mosquito, is one of these species and is found over much of Asia, ranging from the Middle East to the Indian subcontinent and Southeast Asia (Krishnan, 1961; Manouchehri et al., 1976; Vatandoost et al., 2006; Karimian et al., 2014; Hoosh-Deghati et al., 2017). Three biological forms (BFs) of *An. stephensi* have been identified based on egg phenotypes. The "Type" form of malaria is supposed to be more adept at spreading the disease throughout urban areas and to be anthropophilic, whereas the "Mysorensis" and "Intermediate" forms are thought to be more zoophilic and less successful in spreading the disease throughout rural areas. *An. stephensi* is known to be an effective vector for the malaria parasites *Plasmodium falciparum* and *Plasmodium vivax*, which cause clinically severe malaria (Sinka et al., 2011), additionally for *Plasmodium* species, the organisms that cause malaria in rodents (Matsuoka et al., 2002). A wide variety of fresh and brackish waters in rural, coastal, and urban environments are home to *A. stephensi* larvae throughout their breeding season. In rural areas, the larvae feed on surrounding water storage containers, wells, catch basins, seepage canals, freshwater pools, and stream walls and bottoms. They readily proliferate in urban settings in a range of man-made containers found in houses and businesses, both inside and outside of them (Zaini et al., 1975; Sinka et al., 2011; Hanafi-Bojd et al., 2012).

Bioluminescent Revelations: Shedding Light on Plasmodium's Life Cycle

Plasmodium Mosquito Stages

The term "sporogonic cycle" describes the parasites' capacity to proliferate inside of mosquitoes (Figure 1). The microgametes in the mosquito's stomach pierce the macrogametes, creating zygotes. The ookinetes are elongated, motile zygotes that enter the mosquito's midgut wall and develop into oocysts. As the oocysts mature and rupture, sporozoites are released and make their way to the mosquito's salivary glands. The malaria life cycle is maintained by injecting the sporozoites into a new human host (Tuteja, 2007).

Plasmodium Human Stages

During a blood meal, sporozoites from a female Anopheles mosquito carrying the malaria virus are injected into the human host. Sporozites grow into schizonts, which rupture and release merozoites, once they infect liver cells. After first replicating in the liver (exo-erythrocytic schizogony), the parasites then reproduce asexually in the erythrocytes (erythrocytic schizogony). Merozoites cause infections in blood cells. During the ring stage, trophozoites transform into schizonts, which then rupture to release merozoites. Blood-stage parasites are responsible for the disease's clinical manifestations. The gametocytes are consumed by Anopheles mosquitoes (Figure 2) during a blood meal. The gametocytes are classified as male (microgametocytes) and female (macrogametocytes). Injecting sporozoites into a newly recruited human host initiates the malarial life cycle (Meibalan and Marti, 2017).

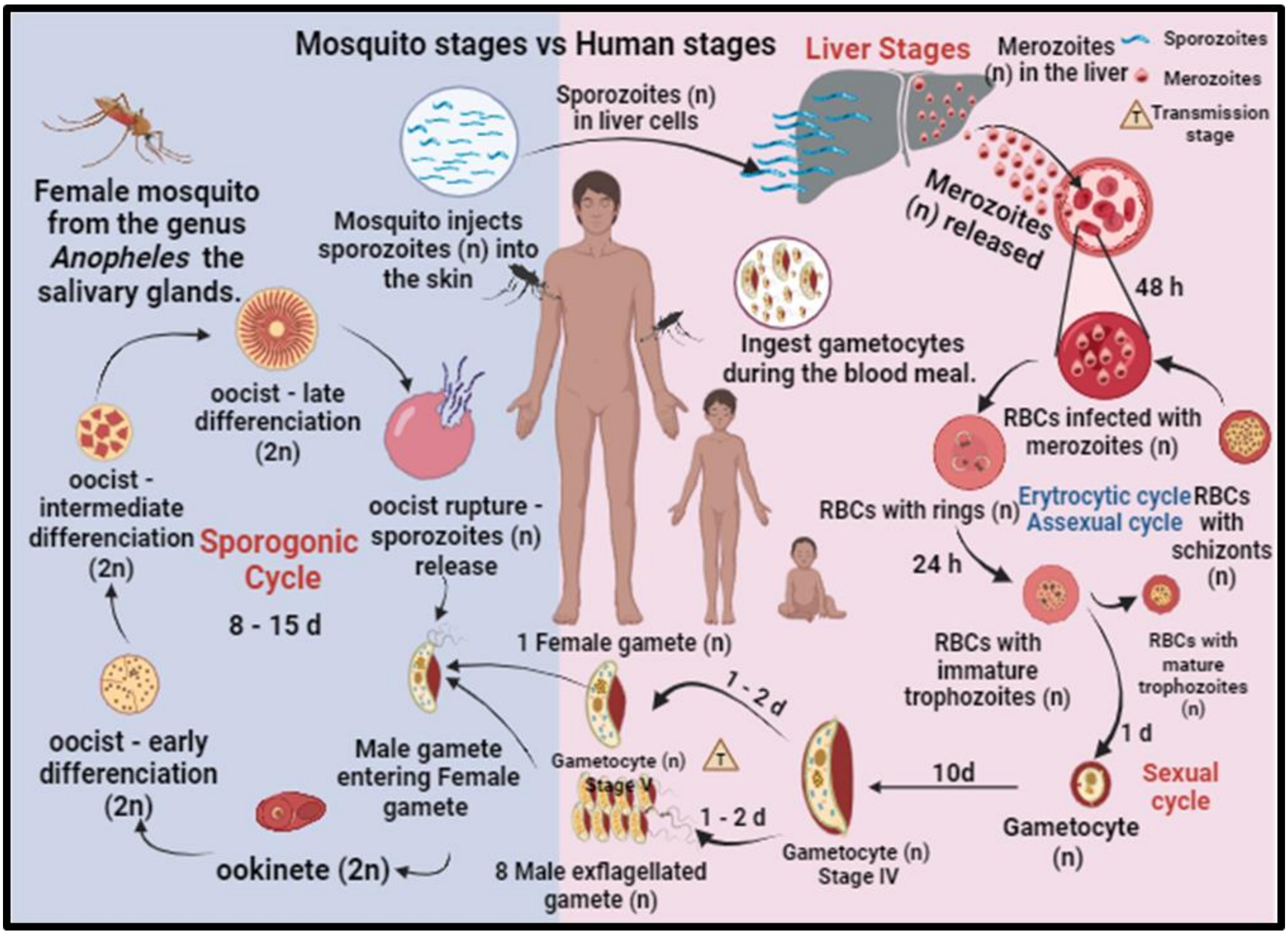

Fig. 1: Life cycle of Malaria

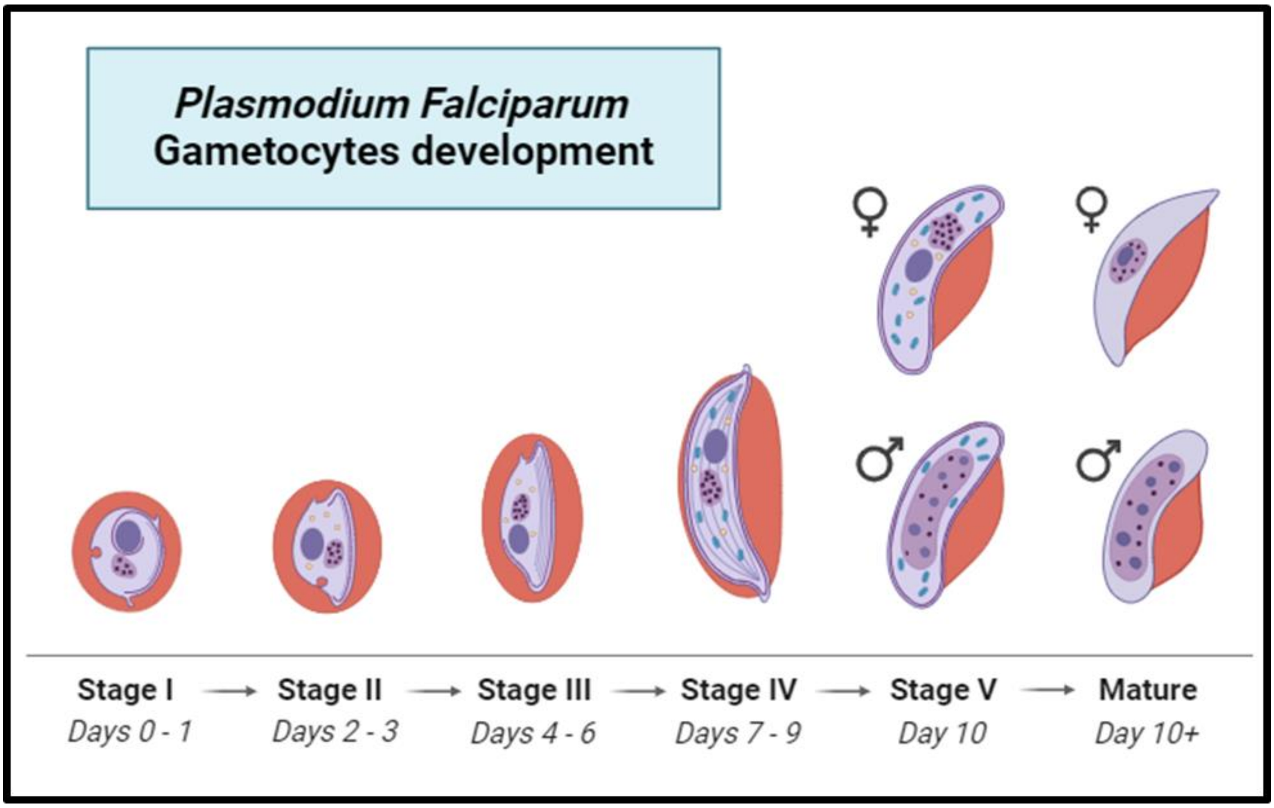

Fig. 2: Plasmodium Gametocytes Development from Dormancy to Fertility in erythrocytes

Mapping Malaria's Asexual Reproduction

When sporozoites from infected mosquitoes enter the human bloodstream and infiltrate hepatocytes, the asexual phase of the Plasmodium life cycle begins (Figure 3). Sporozoites mature into schizonts within hepatocytes, and those schizonts go through several rounds of replication via schizogony. Merozoites are created as a result, and when a hepatocyte ruptures, they are released into the bloodstream. After that, erythrocytes are invaded by merozoites, starting the erythrocytic cycle of infection. Erythrocytic schizonts are formed when merozoites proliferate asexually inside erythrocytes during the erythrocytic cycle. These schizonts divide into several daughter merozoites during schizogony. After erythrocyte lysis and merozoite release from infected erythrocytes, merozoites are released into the bloodstream. The typical symptoms of malaria are partly caused by this cycle of invasion, replication, and erythrocyte rupture (Cowman et al., 2016).

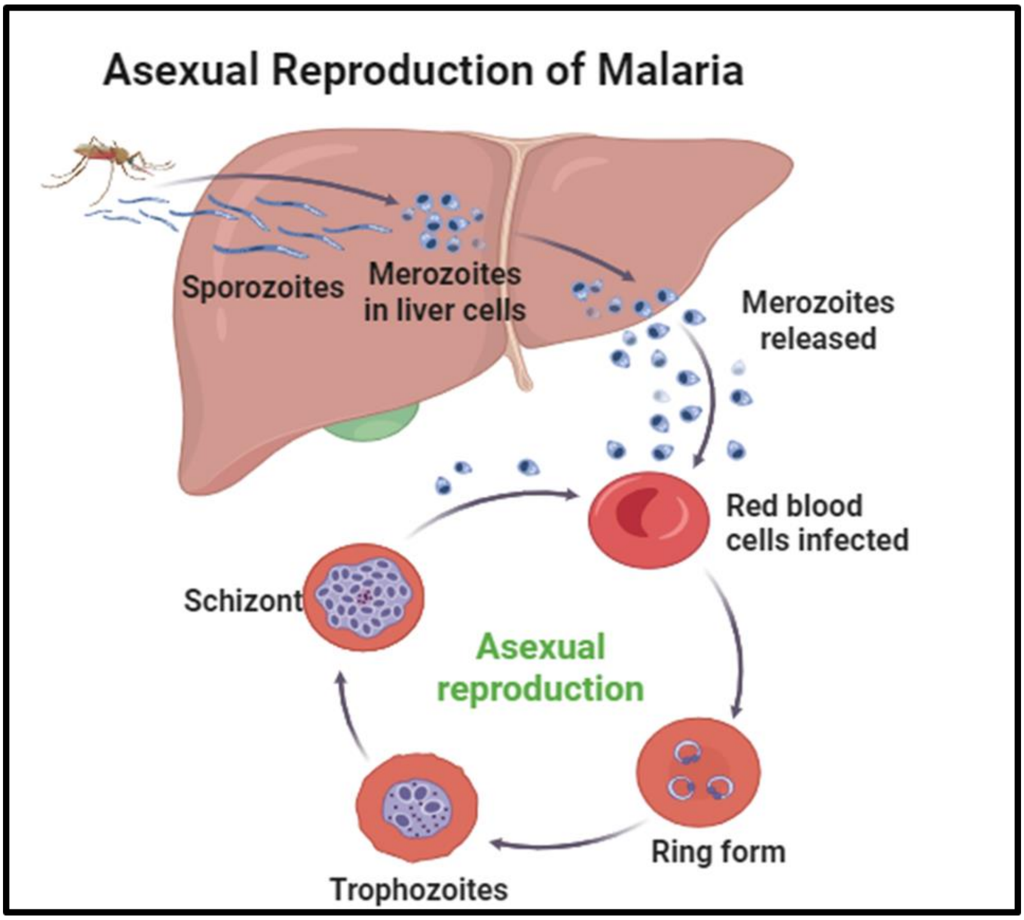

Fig. 3: Malarial Asexual Reproduction in Host

Spotting Malaria: Recognizing the Signs and Symptoms

The breakdown of erythrocytes and schizont rupture are the main causes of malaria's clinical symptoms. Malaria can present with vague symptoms and either a slow or rapid progression. Malaria frequently presents like other common viral diseases, which might cause a delay in diagnosis (Murphy and Oldfield, 1996). Fever (>92% of instances), chills (79%), headaches (70%), and diaphoresis (64%) are experienced by most patients (Genton and D'Acremont, 2001). Additional typical symptoms include dry cough, moderate diarrhoea, nausea, vomiting, myalgia, dizziness, and malaise (Figure 4). Among the physical signs are hepatomegaly, splenomegaly, tachycardia, pallor, jaundice, and temperature. Even in the absence of a fever, a non-immune person's clinical evaluation may be entirely ordinary (Trampuz et al., 2003).

Malaria and Pregnancy

According to (Chua et al., 2021) pregnancy-related malaria can cause a disease called placental malaria. Erythrocytes contaminated with *P. falciparum* adhere to placental receptors, inflaming the placenta and resulting in harm to both the mother and her fetus. Histopathological examinations of placentas infected with *P. falciparum* revealed a range of anomalies pertaining to the placenta, such as increased syncytial knotting, thickening of the trophoblast basal lamina, disruption of syncytiotrophoblast integrity, and accumulation of mononuclear immune cells within intervillous spaces (Figure 5). As a result, these events may impair the placenta's ability to grow and function, which could result in low birth weight, preterm delivery, intrauterine growth restriction, and placental weakness. Infected cells adhere to chondroitin sulfate A in syncytiotrophoblasts via VAR2CSA. The immune system reacts to a parasite's replication by releasing cytokines and activating complement, which compromises the placenta's ability to exchange nutrients and gases and modifies its structure.

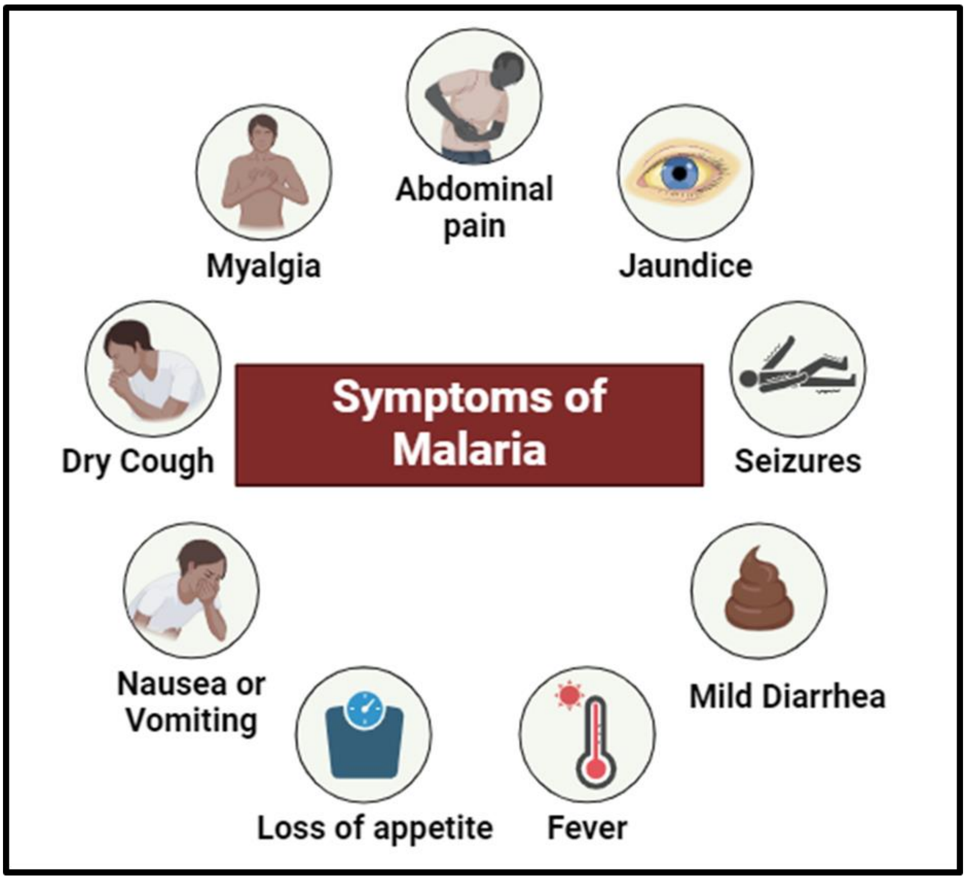

Fig. 4: Symptoms of Malaria

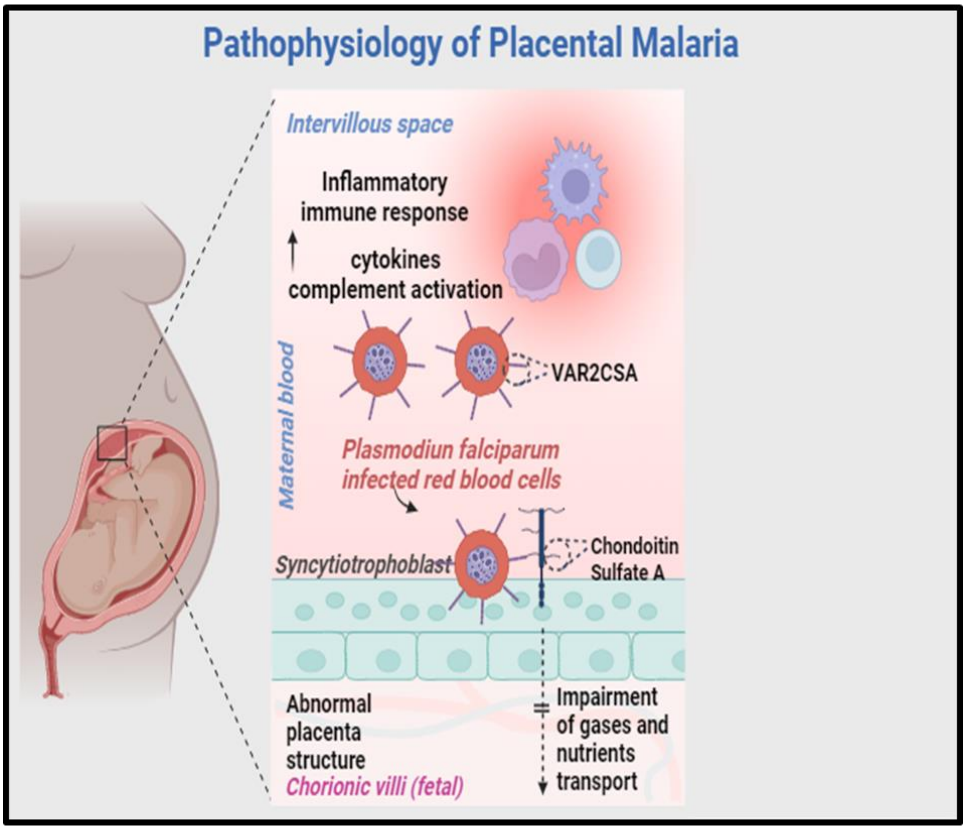

Fig. 5: An illustration of the pathophysiology of placental malaria that shows how red blood cells infected with *P. falciparum* sequester in the placental intervillous region.

Green Vector Control: Exploring the Efficacy of Plant Essential Oils against Malaria

A fundamental control strategy against major diseases carried by mosquitoes has traditionally been to target mosquito vectors to disrupt the circulation of infections (Niang et al., 2018). The mainstay of early mosquito control strategies was the management of the larval supply, which included environmental changes, larviciding, and biological control agents (Mulla, 1994; Tusting et al., 2013). The early 1940s saw the advent of the chemical age in vector control with the adoption of organochlorine DDT as a larvicidal and adulticide (WHO, 2012). Organochlorines gradually supplanted two classes of powerful cholinesterase inhibitors, carbamates and organophosphates (Bird et al., 2014; Srivastava and Kesavachandran, 2014). Insecticides used for public health were expanded to include synthetic pyrethroid chemicals in the 1980s (Sougoufara et al., 2017). These substances are widely advised as contemporary pesticides for treating mosquito nets and other fabrics, as well as for controlling insects within the home (Zaim et al., 2000; Chrustek et al., 2018). Even while chemical pesticides are effective in controlling vectors, there are still problems that threaten the progress made in getting rid of or controlling serious diseases spread by mosquitoes. When people are exposed to chemicals, their health may suffer both immediate and long-term repercussions (Collotta et al., 2013). Furthermore, constant pesticide usage pollutes the environment and interferes with biological and natural control systems (Aktar et al., 2009; Roubos et al., 2014; Özkara et al., 2016). The development and dissemination of pesticide resistance are extremely concerning problems that have led to higher pesticide dosages and a search for more potent and secure substitutes (Karunamoorthi and Sabesan, 2013). The majority of methods that could lessen dependency on artificial pesticides are in the field of biological control (Benelli et al., 2016), both paratransgenic and transgenic techniques (Coutinho-Abreu et al., 2010; Maleki-Ravasan et al., 2015; Dehghan et al., 2017; Stigum et al., 2019) as well as plant-based essential oils (EOs), which have the potential to function as environmentally friendly insecticides (Mossa, 2016; Nollet and Rathore, 2017; Tahghighi et al., 2019). Pelargonium graveolens is one of these; studies have shown that its essential oil (EO) has some anti-mosquito efficacy against laboratory strains of malaria vectors (Tabari et al., 2017).

Pelargonium graveolens

The genus *Pelargonium* (*Geraniaceae*) comprises over 280 species of evergreen perennial flowering plants. They are generally referred to as geraniums, pelargoniums, or storksbills (Albers and Van der Walt, 2007; Wagh et al., 2015). They can withstand heat and drought and are found throughout the world in temperate, tropical, and subtropical regions (Blerot et al., 2016). One variety of Pelargonium that is grown for both its fragrance and beauty as an ornamental plant is *Pelargonium roseum* (PRO), which is used as a key component in the food, beverage, and perfume industries (Szutt et al., 2020). Research has shown that essential oils and the main parts of geraniums can have some anti-mosquito effect on laboratory strains of malaria vectors (Gnankiné and Bassolé, 2017; Tabari et al., 2017; Tahghighi et al., 2019; Dehghankar et al., 2021).

Larvicidal Activity of *Pelargonium graveolens* EO and its Main Constituents

A larvicidal activity analysis reveals that the four main constituents are citronellol (21.34%), L-menthone (6.41%), linalool (4.214%), and geraniol (2.19%). Many plants use geraniol, an acyclic monoterpene alcohol, as the primary component in the synthesis of citronellol. A microbiological reduction process that creates citronellol or an ionization-dependent reaction can both make this chemical. Linalool is among geraniol's structural isomers. A minty-flavored monoterpene called L-menthone is found naturally in several essential oils (Chen and Viljoen, 2010). For *An. stephensi* intermediate, the LC50 and LC90 values are 12.55 and 47.69 ppm, respectively, but the values for *An. stephensi* mysorensis larvae are 11.44 and 42.42 ppm. Citronellol and linalool, in particular, are linked to the strongest and lowest larvicidal activity in both types of *An. stephensi*, respectively (Dehghankar et al., 2021). According to (Yohana et al., 2022) EO exhibits more larvicidal efficacy in the lab than in semi-field studies. *P. graveolens* has LC50 values of 7.13–0.9 ppm and 13.63–8.98 ppm in semi-field and laboratory settings, respectively, for exposure times of 24–72 hours.

Mosquito-repellent Activity of *Pelargonium graveolens* essential oil

According to (Moore et al., 2002) the EO of *P. graveolens* exhibits 96.88% mosquito protection for four hours. Geranium has the same repellent effectiveness as DEET, providing 140 ± 6 minutes of total protection (Sanei-Dehkordi et al., 2023). *An. stephensi* is repelled by *P. graveolens* 20% oil solution for eight hours with 61.9% efficacy (Asadollahi et al., 2019). The wild populations and laboratory strains of *An. stephensi* show mean percentage mortality rates of 98.13% and 87.5%, respectively, at 50 ppm concentration after being exposed to *P. graveolens* EO-treated papers (Yohana et al., 2022). At different *P. roseum* concentrations, the average survival rate is roughly 35%. When exposed to a 25% concentration, female mosquitoes have a mean survival rate that is twice as high as 100%. The statistical significance of this difference is $p<0.001$ (Alipour et al., 2015).

Conclusion

Pervasive illness and pest management are severely hampered by vector resistance to chemical control methods. It is critical to establish practical solutions to solve this issue. With the growing need for eco-friendly, safe, and enjoyable insect repellents, the field of herbal remedies is developing quickly. The use of local flora as repellents offers an appealing alternative because the high expense of manufacturing repellents could impede the creation and successful implementation of pest and vector control programs. Essential oils and plant extracts are becoming more and more popular as prospective treatments for controlling *Anopheles* spp. due to their risk-free, inexpensive, and simple-to-use qualities. Plant essential oils, including geranium, have demonstrated good repellency against various *Anopheles* species for up to eight hours.

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