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Synergistic mosquito-repellent activity of *Curcuma longa*, *Pogostemon heyneanus* and *Zanthoxylum limonella* essential oils

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Summary Mosquito repellents play an important role in preventing man—mosquito contact. In the present study, we evaluated the synergistic mosquito-repellent activity of *Curcuma longa*, *Pogostemon heyneanus* and *Zanthoxylum limonella* essential oils. The mosquito repellent efficacies of three essential oils were evaluated separately and in combination under laboratory and field conditions. N,N-Diethylphenylacetamide (DEPA) and dimethylphthalate (DMP) were used for comparison of the protection time of the mixture of essential oils. At an optimum concentration of 20%, the essential oils of *C. longa*, *Z. limonella* and *P. heyneanus* provided complete protection times (CPTs) of 96.2, 91.4 and 123.4 min, respectively, against *Aedes albopictus* mosquitoes in the laboratory. The 1:1:2 mixture of the essential oils provided 329.4 and 391.0 min of CPT in the laboratory and field trials, respectively. The percent increases in CPTs for the essential oil mixture were 30 for DMP and 55 for N,N-diethylphenylacetamide (DEPA). The synergistic repellent activity of the essential oils used in the present study might be useful for developing safer alternatives to synthetic repellents for personal protection against mosquitoes.

Introduction

Mosquito-borne diseases, such as malaria, filariasis, dengue and encephalitis, are major causes of illness and death worldwide [1]. Reducing disease transmission by vector management is

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highly significant in the context of the control of mosquito-borne diseases. Mosquito repellents thus play a major role in preventing man—mosquito contact and thereby minimize the chance of infections. Synthetic repellents, such as DEET (N,N-diethyl-3-methylbenzamide), are used worldwide for protection against mosquito-borne diseases. However, DEET has an unpleasant odor, can damage plastics and synthetic rubber and exhibits a high level of skin penetration [2]. Moreover, concerns have been raised over the safety of DEET and other synthetic compounds [3]; hence, plant-based products have become increasingly popular as safe and biodegradable mosquito repellents [4].

The use of plant-based repellents for protection against mosquitoes has a long history. Plants with mosquito-repellent properties are well known among various communities, and plant products have been used traditionally all over the world to ward off mosquitoes [5]. The plant products that are in use include a wide range of substances from crude plant extracts to essential oils and isolated compounds. Synthetic derivatives of many plant compounds with repellent properties are also in use. It is well known that the essential oils from plants are potential sources of compounds with bioactivities against vector mosquitoes [6]. The advantage of these essential oil repellents is that they are generally considered to be safe to human health and the environment. These natural oils are easily biodegradable and do not contaminate the environment, making them suitable candidates for the development of mosquito repellents.

The northeastern region of India, which is a biodiversity hot spot, is highly prone to the incidence and transmission of mosquito-borne diseases. There is a need for the development and evaluation of safe alternatives to synthetic repellents to combat mosquito-borne diseases in this part of the country. Efforts are being made to explore the flora of this region for natural products with bioactivity against mosquitoes. Although many studies have been performed in the past regarding the repellent activity of essential oils, the synergistic activities of essential oils have yet to be evaluated. The present study was an attempt to evaluate the repellent activity of mixtures of essential oils from commonly available plants in northeastern India with the goal of developing an herbal mosquito repellent formulation. The field evaluations of complete protection time were performed using synthetic repellents DMP (dimethylphthalate) and DEPA (N,N-diethylphenylacetamide).

Materials and methods

Test materials

The essential oils of *Curcuma longa* (Zingiberaceae) rhizomes, *Pogostemon heyneanus* (Lamiaceae) leaves and *Zanthoxylum limonella* (Rutaceae) fruits were obtained from a commercial oil extraction plant in Assam, India. DMP was supplied by High Purity Chemicals (New Delhi, India), and DEPA was obtained from Defence Research and Development Establishment (Gwalior, India). Four concentrations (5, 10, 20 and 30%) of the essential oils, their mixtures, DEPA and DMP were prepared in sunflower oil, which has no mosquito repellency based on laboratory trials. The laboratory trials involved mixtures of essential oils that were prepared at different ratios based on the results of preliminary trials.

Laboratory trials

Repellent test chambers ($30 \times 30 \times 62.5$ cm) were used for the repellent trials in the laboratory. *Aedes albopictus* mosquitoes that were maintained in the laboratory at $28 \pm 2\,^{\circ}C$ and 75—80% humidity were used for the test. Approximately 60 starved (for 12 h) adult female mosquitoes (3 days old) were released into the test chamber. The test materials were applied at the rate of 0.3 ml to the hands (wrist to fingertip) of volunteers ($n = 3$). A hand was inserted into the test chamber in intervals of 30 min until it received two bites within a period of 30 min, and each treatment was replicated five times.

Field trials

The field trials were conducted in the Solmara village (Assam), India by applying the test materials on to the hands (elbow to fingertips) and legs (knees to toe tips) of volunteers in volumes rates of 0.75 and 1.25 ml, respectively. Each treatment was applied to five volunteers along with one control (sunflower oil in a volume of 1.25 ml), and the volunteers were made to sit at distances of 3 m from each other. The treatments were repeated thrice, and the repellents were applied on to different subjects on successive days to avoid subject preference. The study procedure was approved by the institutional ethical committee, and informed consent was obtained from the volunteers who participated in the testing.

Data analysis

The complete protection time was recorded as the time elapsed between the application of the