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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 ± 2 °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 test material and the receipt of two bites within a period of 30 min. The mean of five replicates was calculated with 95% confidence intervals, and one-way ANOVA was used to identify the significant differences between the treatment means. The data were analyzed using the SPSS Statistics 19 computer program.

Results

The laboratory trials of the repellent efficacies of the individual essential oils indicated that the essential oil from *C. longa* provided complete protection times (CPTs) of 22.4, 43.8, 96.2 and 104.8 min at concentrations of 5, 10, 20 and 30%, respectively (Table 1). The CPTs produced by *P. heyneanus* were 23.2 (5%), 49.2 (10%), 123.4 (20%) and 125.2 (30%) minutes, and the corresponding values for *Z. limonella* were 19.6, 40.8, 91.4 and 103.2. Although the *P. heyneanus* essential oil provided slightly greater complete protection times (CPTs) compared with *C. longa* and *Z. limonella*, this increase in protection time was not statistically significant ($p > 0.05$).

Among the tested mixtures of essential oils, the *C. longa*—*P. heyneanus* mixture (1:2) provided 48.4 (5%), 133.4 (10%), 244.6 (20%) and 246.6 (30%) min of CPT, and the mixture of *C. longa* and *Z. limonella* (1:1) provided 114.4 (5%), 203.6 (10%), 308.8 (20%) and 325.6 (30%) min of CPT (Table 1). The *Z. limonella*—*P. heyneanus* mixture (1:2) produced 46.6, 120.2, 228.4 and 243.4 min of CPT at the tested concentrations (5—30%). These findings clearly indicated that the protection provided by the *C. longa*—*Z. limonella* mixture was significantly greater at all concentrations ($p < 0.001$). Further evaluation of the mixtures of *C. longa*, *Z. limonella* and *P. heyneanus* indicated that the mixture in a ratio of 1:1:2 offered the longest duration of protection. This mixture provided 117.2, 219.2, 329.4 and 344.8 min of CPT at the 5, 10, 20 and 30% concentrations, respectively. However, the CPT provided by the mixture of all three essential oils was not significantly different than that provided by the mixture of *C. longa* and *Z. limonella* ($p > 0.05$).

The field trials preformed with the 1:1:2 mixture of essential oils provided 135.8 (5%), 272.6 (10%), 391 (20%) and 396.2 (30%) minutes of CPT, and DEPA provided 175.6 (5%), 319.6 (10%), 463.8 (20%) and 472.2 (30%) minutes of CPT (Table 2). DMP, which was used as the positive control, produced relatively low CPTs of 76.4, 201.8, 302.0 and 309.6 min at the 5, 10, 20 and 30% concentrations, respectively. The field efficacies of the essential oil

Table 1 Laboratory evaluations of the synergistic repellencies of essential plant oils against *Aedes albopictus* mosquitoes.

Essential oil/mixture	Mean complete protection time in minutes (95% CI)			
	5%	10%	20%	30%
Curcuma longa (CL)	22.4^a (17.2—27.6)	43.8^a (27.9—59.7)	96.2^a (71.8—120.6)	104.8^a (87.5—122)
Pogostemon heyneanus (PH)	23.2^a (17—29.4)	49.2^a (29.6—68.8)	123.4^a (109.1—137.7)	125.2^a (105—145.4)
Zanthoxylum limonella (ZL)	19.6^a (15.1—24.1)	40.8^a (25.3—56.3)	91.4^a (67.3—115.4)	103.2^a (82.1—124.3)
CL + PH (1:2)	48.4^b (34.7—62)	133.4^b (111.4—155.4)	244.6^b (226—263.2)	246.6^b (224.5—268.7)
CL + ZL (1:1)	114.4^c (92.7—136.1)	203.6^c (180.9—226.3)	308.8^c (285.3—332.3)	325.6^c (303.3—347.9)
ZL + PH (1:2)	46.6^b (31.2—62)	120.2^b (98.2—142.2)	228.4^b (207.8—249)	243.4^b (222.4—264.3)
CL + ZL + PH (1:1:2)	117.2^c (95.2—139.2)	219.2^c (196—242.4)	329.4^c (311.6—347.2)	344.8^c (324.7—364.8)

ANOVA followed by Tukey's HSD; $p = 0.000$. The values in columns followed by same letters are not significantly different ($p > 0.05$).

Table 2 Field evaluations of the repellencies of the essential oil mixtures compared with synthetic repellents (DMP and DEPA).

Essential oils/repellent	Mean complete protection time in minutes (95% CI)			
	5%	10%	20%	30%
CL + ZL + PH (1:1:2)	135.8^{b} (115.4–156.2)	272.6^{b} (250.8–294.4)	391^{b} (367.2–414.7)	396.2^{b} (371.8–420.6)
DEPA	175.6^{c} (156.3–194.8)	319.6^{c} (296.9–342.3)	463.8^{c} (439.2–488.4)	472.2^{c} (447.2–497.2)
DMP	76.4^{a} (59.8–93)	201.8^{a} (179.5–224.1)	302^{a} (278.1–325.9)	309.6^{a} (285.3–333.9)

ANOVA followed by Tukey's HSD; $p = 0.000$. The values in the columns followed by same letters are not significantly different ($p > 0.05$).

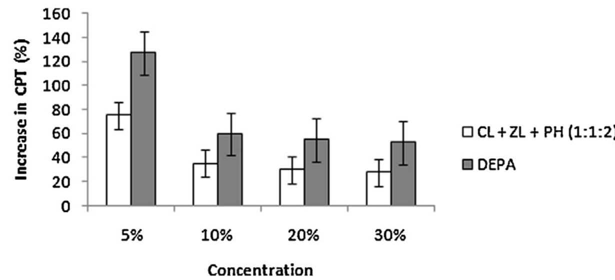

Figure 1 Repellent efficacy of essential oil mixture and DEPA in the field in comparison to DMP. CL — *Curcuma longa*; ZL — *Zanthoxylum limonella*; PH — *Pogostemon heyneanus*; CPT — mean complete protection time; Error bars are standard error of mean.

mixture and DEPA were evaluated as the percent increases in CPT over DMP (Fig. 1). The percent increases in CPT for the 1:1:2 mixture were 75 (5%), 35 (10%), 30 (20%) and 28 (30%), and those for DEPA were 127 (5%), 60 (10%), 55 (20%) and 53 (30%). It was evident that the lowest concentration (5%) of the essential oil mixture and DEPA yielded the greatest incremental protections of 75 and 127%, respectively, relative to DMP. The essential oil mixture was found to provide a better CPT in the field conditions than in the laboratory conditions; the percent increases in CPT were 15 (5%), 24 (10%), 18 (20%) and 15 (30%).

The mosquito bite rates recorded for the control subjects ranged from 35 to 45 bites per man per hour, and the mosquito species that landed on the control subjects were identified as *Anopheles maculatus*, *A. annularis*, *A. philippinensis*, *A. crawfordi*, *A. barbirostris*, *A. vagus*, *A. kochi*, *A. aconitus*, *Culex quinquefasciatus*, *C. vishnui gr.*, *C. malayi*, *C. gelidus*, *Mansonia uniformis* and *M. annulifera*.

Discussion

Repellents play an important role in preventing or reducing the incidence of vector-borne diseases by preventing man—vector contact. However, the continued use of synthetic repellents, such as DEET might lead to adverse effects in human beings as reported in previous studies [7]. Plants are rich sources of many compounds, such as alkaloids, terpenoids and phenolics, which can be utilized for the development of alternatives to commercially available synthetic repellents [8]. Repellents based on natural oils generally do not provide protection from mosquito bites for as long DEET, which provides 6 h of protection [9]. In the present study, combinations of essential oils in different ratios were evaluated to improve the protection time, and these combinations were compared with synthetic repellents.

The optimum concentrations of the essential oils and their mixtures were found to be 20% because the protection times produced by 20 and 30% were not significantly different. At 20%, the oils provided 1.5—2 h of complete protection under laboratory conditions. The mixtures of two oils resulted in protection times of up to 5 h. The mixture of all three of the essential oils at a ratio of 1:1:2 provided approximately 5.5 h of CPT and was thus selected for the field trials along with the synthetic repellents. Under the field conditions, DMP provided 5 h of protection, and this CPT was considered to be the standard. The essential oil mixture produced 6.5 h of protection, whereas the synthetic repellent DEPA produced more than 7.5 h of protection.

The essential oil mixture (1:1:2) was found to provide better protection against mosquitos than the seed extract of *Tephrosia purpurea* Linn. [10]. Rajnikant and Bhatt [11] reported more than 75% protection against culicine and 89—98% protection against *A. culicifacies* and *A. fluviatilis* with 2% neem oil, and Mishra et al. [12] reported 81—91% protection for 12 h with 3 and 4% neem oil in a coconut oil base. Similarly, Das et al. [13], reported 7.4, 6.5 and 4.4 h of protection against mosquitoes with 60% (0.57 mg/cm^2) concentrations of essential oils from *Zanthoxylum armatum* (fruits), *Curcuma aromatica* (rhizomes) and *Azadirachta indica* (seed), respectively, in mustard oil in field conditions. The petroleum ether extracts of *Vicoa indica*, *Buddleja asiatica*, *Chenopodium ambrosoides*, *Clerodendrum inerme* and the methanol extract of *Solanum erainthum* resulted in 3 h of protection against mosquitoes at 9% concentration [14]. Young-Cheol et al. [15] reported that the methanol extracts of *Cinnamomum cassia* bark, *Nardostachys chinensis* rhizome, *Paeonia suffruticosa* root bark and a steam distillate of *Cinnamomum camphora* produced 91, 81, 80 and 94% protection, respectively, against bites from *Aedes aegypti* at doses of 0.1 mg/cm^2. These results were comparable to those of DEET (82%), but the duration of repellency produced by the extracts from *C. cassia* bark and *N. chinensis* rhizome was approximately 1 h, and a relatively short duration of repellency (30 min) was observed for *P. suffruticosa* root bark extract with a *C. camphora* steam distillate. *Lantana camara* L. flower extract in coconut oil provided 94.5% protection from *A. albopictus* and *A. aegypti* without adverse effects on the human volunteers over a 3-month period after the application [16]. Various formulations for controlled release have been developed to increase the protection time provided by repellents [17]. For example, Sharma and Ansari [18] reported that a 1% neem oil—kerosene mixture provided economical personal protection from mosquito bites.

Plant-derived insect repellent agents are selective, have few or no harmful effects on non-target organisms or the environment and can be applied to human skin and clothing in the same manner as commercial repellents [19—22]. Many extracts and volatile oils that are highly volatile, such as alkanes, terpenoids, alcohols and aldehydes, are repellent to mosquitoes for periods ranging from 15 min to 10 h [21—23]. Although essential oils could be an alternative source for mosquito repellents, their efficacies and practical use are limited by their lower persistence on the applied surface. The results of this study indicated that the duration of protection provided by essential oils can be increased via the use of two or more oils in combination. Controlled release formulations developed using these essential oils could lead to viable alternatives to synthetic repellents.

Conflict of interests

None declared.

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