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Field evaluation of repellency of a polyherbal essential oil against blackflies and its dermal toxicity using rat model

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Abstract. In the present study we have evaluated the repellent activity of mixture of *Curcuma longa*, *Zanthoxylum limonella* and *Pogostemon heyneanus* essential oils in 1:1:2 ratio at 5%, 10% and 20% concentration against blackflies in northeastern India. Initially the essential oil mixture tested here has been found effective against *Aedes albopictus* mosquitoes. The average protection recorded in 20% concentration (170.56±4.0; 95% CI = 162.09-179.02) was higher as compared to other two concentrations ($F = 90.2$; $p<0.0001$; $df = 53$). Percentage repellency and repellency index was found to be higher in 20% concentration ($p\leq0.017$). No appreciable clinical and behavioral signs were observed in the acute dermal toxicity using rat model. No changes were observed in biochemical profiles of treatment group animals. Similarly, no prominent lesions were observed in vital organs of treatment in both the sexes. The study concludes that tested repellent is safe for use and has multi-insects repellent property.

INTRODUCTION

Insect borne infectious diseases pose prime health risk to over billions of people primarily in the tropical countries. Use of personal protection against insect bites and control interventions are currently the most important measures to control insect vector borne diseases (Tawatsin *et al.*, 2001; Hazarika *et al.*, 2012). The repellents are practical and economical means of preventing the transmission of these diseases to humans. The synthetic repellents have been proven effective against the insect bites, however these are less accepted due to the toxicity reactions after the topical applications (Yap, 1986; Qiu *et al.*, 1998; Fradin *et al.*, 2002; Malau & James, 2008). Studies on plant derived repellents have demonstrated their repellency potential in order to replace the existing synthetic repellents (Tawatsin *et al.*, 2001; Aisen *et al.*, 2004; Kamraj *et al.*, 2010; Hazarika *et al.*, 2012). The plant based repellents have mostly been evaluated under laboratory conditions using olfactometer and animal model, however evaluation activity is preferred to be carried out using human volunteers rather than animal model, which is inadequate to simulate the human skin conditions onto which the repellents will be applied ultimately (WHO, 1996; Tawatsin *et al.*, 2001; Hazarika *et al.*, 2012).

In the recent years, many studies have used plant derivatives for the control of various insect vectors of disease agents (Tawatsin *et al.*, 2001; Aisen *et al.*, 2004; Zahir *et al.*, 2009; Kamraj *et al.*, 2010; Hazarika *et al.*, 2012). Plant material contains many bioactive chemicals which are selective in action and have insignificant harmful effect on non-target flora and fauna. The essential oils have shown a broad spectrum of activity against variety of

insects and low level of mammalian toxicity (Cheng *et al.*, 2003; Hazarika *et al.*, 2012). Studies have been conducted on the joint, antagonistic, additive and synergistic toxic effect of mixture of plants derivatives on insect pests (Kalyanasundaram & Babu, 1982; Shaalan *et al.*, 2005). The joint toxicity of various insecticide mixtures was initially investigated to differentiate between synergism, antagonism and potentiation (Sun & Johnson, 1960). Thangam & Kathiresan (1991b) suggested that synergism may be due to phytochemicals inhibiting a mosquito larva's ability to employ detoxifying enzymes against synthetic chemicals. Mixtures of plant extracts showing synergistic interactions between them have been considered to have greater and long-lasting effect (Chockalingam *et al.*, 1990).

In the present study, we have investigated the repellent activity of a mixture of some indigenous plants based essential oils against medically important *Simulium* (blackflies) species under field conditions. The tested mixture composition was found effective as repellent against *Aedes albopictus* mosquitoes and its patent has been applied in the Indian Patent Office (Application No 735/DEL/2008). Results of present study indicate that the bioactive chemical constituents from plant material have multi insect repellent property.

MATERIALS AND METHODS

Testing site and insects

Repellent trials were carried out in Tenga valley in Arunachal Pradesh of north-east India during Mar–Apr 2010.Tenga is a military cantonment located at an altitude of around 5,000 ft (mean sea level) and has fast flowing fresh water stream. The military-men engaged in routine activity are prone to the *Simulium* bites during entire day. The *Simuliidae* are small insects measuring 1 to 5 mm in length and have shiny thorax. The colour varies from dark black to various shades of grey and yellow. These are notorious pests of northeastern India for biting nuisance and may cause mild to severe allergic reactions to the human. Repeated biting can cause a medically recognized syndrome called Black Fly Fever and is characterized by symptoms like headache, fever, lymphadenitis and psychological depression in human (Singh & Tripathi 2003). In the present study both pupal and adult stages were collected from the study area and identified using morphological characters (Puri 1932a, b, c, d; Puri 1933 a, b, c; Datta 1983; Lewis 1974; Das *et al.*, 1990).

Essential oils and test procedure

Essential oils of *Curcuma longa* Linnaeus (Zingibereae: Zingiberaceae) rhizomes, *Pogostemon heyneanus* Bentham (Lamiales: Lamiaceae) leaves and *Zanthoxylum-limonella* Alston (Zanthoxyleae: Rutaceae) fruits were procured from a local oil extraction plant in Assam, India. The mixture of *C. longa*, *Z. limonella* and *P. heyneanus* essential oils in 1:1:2 ratio was prepared, of which 5%, 10% and 20% concentrations (v/v) were made in *Helianthus annus* Linnaeus (Asterales: Asteraceae) oil for field testing. Each concentration was tested three times in three different locations in the valley and *H. annus* Linnaeus (Asterales: Asteraceae) oil was used in control test. Each concentration was tested using six volunteers each on three different days (referred to as test 1, test 2 and test 3). Volunteers were aged between 21 to 45 years and had no allergy to insect bites and herbal oils. The informed consents were obtained from volunteers. Testing was essentially carried out following Hazarika *et al.*, 2012.

Acute dermal toxicity study

This test was conducted as per the OECD guideline 402. (Ref. OECD. OECD Guideline for Testing of Chemicals: Acute Dermal Toxicity. France: OECD; 1987. p. 402.) Healthy adult Wistar albino rats, females weighing 150-160 g (4-6 week) and males weighing between 200 and 250 g (4-6 week) were used in this study. The females used were nulliparous and non-pregnant. Animals were acclimatized to the experimental conditions for 1 week. They were maintained under standard laboratory hygienic conditions, providing laboratory animal feed and water *ad libitum*. All experimental

protocols using animals were performed according to the "Principles of Laboratory Animal care" (NIH publication 85-23, revised 1985) and approved by the institutional ethical committee. Animals were divided into four groups (n = 6) as follows: group I (control group male rats), group II (20% polyherbal formulation in ointment base), group III (control group female rats) and group IV (20% polyherbal formulation in ointment base). Approximately 24 h before the test, the hair coat was removed by closely clipping the dorsal area of trunk of the tested animals. Care was taken to avoid abrading the skin and only animals with intact skin were used. Films under study were moistened with physiological saline and applied on the shaved area. They were held in contact with the skin using porous gauze dressing and non-irritating tape for a period of 24 h, whereas for the control groups, gauze moistened with physiological saline was applied and held in contact in a similar way as the treatment groups. Animals were observed within 30 min, 4 and 24 h after the removal of films and kept under observation for 48 h. Individual weight of animals was determined on the day of application of film and weekly thereafter. They were observed for the changes in skin, eyes and mucous membranes, behavioral patterns, diarrhea, salivation and tremors. Mortality was recorded during the course of study. At the end of the study, survived animals were weighed, sacrificed and subjected to gross necropsy. In rats, the blood samples were collected on day 0, 48 h by retro-orbital plexus puncture method and serum biochemical parameters were estimated from the serum samples using clinical chemistry analyzer.

Data analysis

The data obtained were tested for normality using Kolmogorov and Smirnov method (Stephens, 1979). Percent repellency and repellency index (RI) were determined following the methods of Sharma & Ansari (1994) and Yap *et al.* (1998). Results obtained were compared using Student's t-test and analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests at 95% confidence interval using Graph Pad In Statsoftware.

RESULTS

Simulium species and repellent activity: During the study *Simulium striatum*, *S. rufibasis*, *S. christophersi*, *S. novolineatum*, *S. indicum*, *S. himalayense and S. barraudi* were recorded. *S. rufibasis*, *S. indicum* and *S. barraudi* were recorded in large count in both pupal and adult stages, whereas the other species was recorded in comparatively low number. The repellent activity of the polyherbal repellent is shown in Table 1. The repellent activity was higher in 20% concentration (protection time in min = 170.56±4.0; 95% CI = 162.09–179.02) as compared to the other two concentrations ($F=90.2$; $p<0.0001$; df =53). The performance of each concentration of the repellent oil was found to be consistent during the entire study (Table 1). Biting activity of blackflies among the three trial locations (2.51±0.1; 95% CI = 2.06–2.95) was similar ($F = 0.47$; $p = 0.64$; df = 14). The protection time provided by control oil was 22.56±1.57 (protection time in min±SEM). The percent repellency (PR) and repellency index (RI) obtained in the present study has been depicted in figures 1 and 2 respectively. The PR after 4^{th} and 5^{th} h of the trial was higher in 20% than 5% and 10% concentrations ($p\leq0.017$), similarly the RI was also found to be higher in 20% after 4^{th} and 5^{th} h of the trial ($p\leq0.017$).

Dermal toxicology: No skin irritation, hot sensations or rashes were observed on the skin of the test volunteers treated with essential oil during two months after the study. In the acute dermal toxicity, there were no appreciable clinical signs observed throughout the observation period of 48h after the patch removal and there were no appreciable changes in skin, eyes, mucous membranes and behavioral pattern. There was no mortality seen throughout the observation period. Also, there was no significant ($P>0.05$) difference in body weight in the treatment group compared to the

Table 1. Repellent activity of poly-herbal essential oil at different concentrations in the field

Concentration	Test 1	Test 2	Test 3	p (df)	F
5% (95% CI)	94.5±4.6 (82.6–106.4)	96.7±4.7 (84.6–108.7)	99.7±4.1 (89.0–110.3)	0.72 (17)	0.33
10% (95% CI)	153.7±8.7 (131.3–176.1)	144.3±6.6 (127.5–161.2)	146.7±11.3 (117.6–175.7)	0.75 (17)	0.29
20% (95% CI)	173.2±7.9 (152.7–193.7)	171.7±6.1 (155.9–187.4)	166.8±7.7 (147.2–186.5)	0.82 (17)	0.20

P = non significant; df = degree of freedom

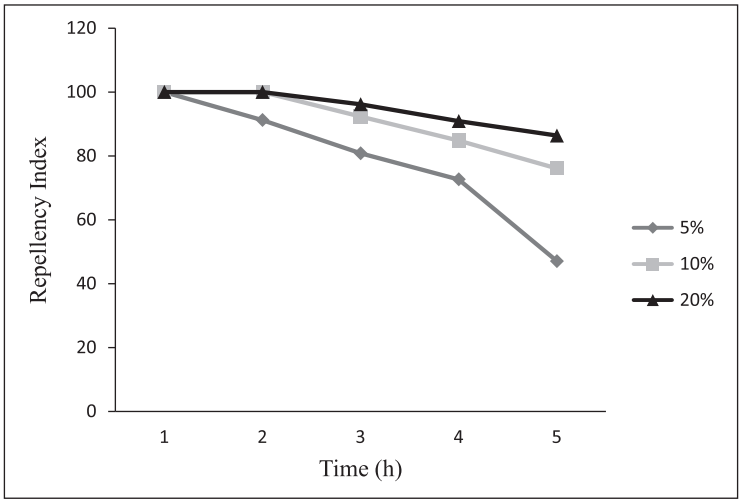

Figure 1. Percent repellency (PR) of tested oil after 5h at 5%, 10% and 20% concentrations

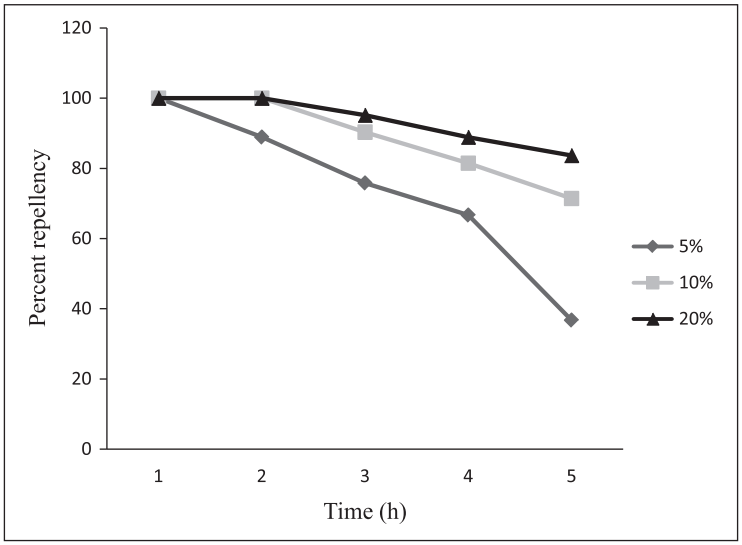

Figure 2. Repellency Index (RI) of tested oil after 5h at 5%, 10% and 20% concentrations

respective control group female rats. Serum obtained from blood samples collected on hours 0 and 48 of the study period was used to estimate aspartate transaminase (AST), alanine transaminase (ALT), creatinine and blood urea nitrogen (BUN) for all the groups respectively. None of the biochemical parameters differed significantly (P>0.05) in the treatment group from the control group in both male and female rats. Also, there were no prominent gross lesions observed in vital organs like liver, lung, heart, kidney and spleen of the treatment groups in both the sexes.

DISCUSSION

Identification and evaluation of effective repellent compounds is essential to combat increasing concern for the environment safety and the unacceptability of synthetic counterparts. Many studies have demonstrated the insecticidal and repellent properties of herbal derivatives (Tawatsin *et al.*, 2001; Aisen *et al.*, 2004; Usip *et al.*, 2006; Malau & James 2008; Zahir *et al.*, 2009; Kamaraj *et al.*, 2010). However, only a few reports present the repellent activity of plant products against blackflies (Aisen *et al.*, 2004; Hazarika *et al.*, 2012). In the present study, the mixture of plant based essential oils at 20% concentration was able to protect the human volunteers from black fly bites for ~ 3 h during the peak biting hours. In the previous study, more than 5 h protection time has been recorded against *Simulium* using single essential oil application in the field (Hazarika *et al.*, 2012). Other studies have shown that synthetic compound were able to provide protection to the human volunteers from mosquitoes upto 8–10 h, whereas plant based repellent were able to protect upto 2–3 h only (Fradin & Day 2002; Usip *et al.*, 2004; Trongtokit *et al.*, 2005; Zahir *et al.*, 2009; Kamaraj *et al.*, 2010). The repellency achieved in the present study was consistent throughout the trials indicating that the effectiveness of the tested repellent is reproducible. In addition to the essential oils, studies have shown that the extracts of some plants have been shown to have anti-mosquito properties (Kamaraj *et al.*, 2010). Many studies have demonstrated that essential oils used in combination have better insect repellent activity as compared to the pure oils. Protection time of >4 h with the essential oils of *Z. armatum*, *C. aromatica* and *A. indica* respectively has been reported against mosquitoes in field conditions (Das *et al.*, 2000). Young-Cheol *et al.* (2004) has reported that methanol extract and steam distillate of some plants materials provided 91, 81, 80 and 94% protection against *Aedes aegypti*. Studies have been carried out to increase the protection time provided by the repellents in field conditions (Sharma & Ansari, 1994).

The tested polyherbal repellent was initially developed for mosquito, however we have tested its activity against blackflies in the field. The present results evidence that the tested oil is effective not only against the mosquito but have repellent efficacy against blackflies also. The tested repellent was found safe to the skin as no adverse reaction could be observed in the test volunteers in the field and rat model in the laboratory. The findings advocate that the tested repellent has broad spectrum repellent activity and can be used against type of hematophagous insects.

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