LABORATORY EVALUATION OF FOUR COMMERCIAL REPELLENTS AGAINST LARVAL *LEPTOTROMBIDIUM DELIENSE (*ACARI: TROMBICULIDAE)

Azima Laili Hanifah, Siti Hazar Awang Ismail and Ho Tze Ming

Acarology Unit, Infectious Disease Research Center, Institute for Medical Research, Kuala Lumpur, Malaysia

Abstract. Four commercial repellents were evaluated in the laboratory against *Leptotrombidium deliense* chiggers. Both *in vitro* and *in vivo* methods were used to determine repellency of the compounds. The repellents were Kellis® (containing citronella oil, jojoba oil and tea tree oil), Kaps® (containing citronella oil), BioZ® (containing citronella oil, geranium oil and lemon grass oil) and Off® (containing DEET). The combination of three active ingredients: citronella oil, geranium oil, lemon grass oil gave the highest repellency (87%) followed by DEET (84%). *In vitro* repellencies ranged from 73% to 87%. There was no significant difference between the four products. All the repellents had 100% *in vivo* repellency compared to 41-57% for the controls.

Key words: *Leptotrombidium deliense*, repellency, chigger, bioassay

INTRODUCTION

Scrub typhus is a zoonotic disease resulting from an infection with the gram-negative intracellular bacterium *Orientia* (formerly *Rickettsia*) *tsutsugamushi* (Seong *et al*, 2001). Scrub typhus is transmitted by several species of larval trombiculid mites, also referred to as chiggers (Tanskul *et al*, 1998). All known vectors of this disease belong to the genus and subgenus *Leptotrombidium*.

Repellents provide an effective method for protecting individuals from biting arthropods (Gupta and Rutledge, 1994). Diethyltolumide (DEET) is a common, effective topical insect repellent used since 1957 (Gilbert *et al*, 1957). In recent years, several essential plant oils had been found to have repellent properties. Such plants included citronella, cedar, verbena, pennyroyal, geranium, lavender, pine, cinnamon, rosemary, basil, thyme, allspice, garlic and peppermint (Mohinder, 2001; Rim and Jee, 2006). Eamsobhana *et al* (2009) tested aromatic essential oils from 13 plants species and found four of them to be effective as repellents against *Leptotrombidium imphalum* chiggers. Besides repellents containing DEET, many repellents with plant oils as active ingredients are now commercially available. These commercial products target various insects, especially mosquitoes. None have been recommended by their manufacturers for protection against chigger bites. The manufacturers may have no information

Correspondence: Azima Laili Hanifah, Acarology Unit, Infectious Disease Research Center, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia.
Tel: +603 2616 2695; Fax: +603 2693 5928
E-mail: azima@imr.gov.my

regarding the effectiveness of these repellents against chiggers.

The purpose of this study was to evaluate the efficacy of four commercial insect repellents against larval *Leptotrombidium deliense*.

MATERIALS AND METHODS

Chiggers

Unfed, uninfected 20-30 day old *Leptotrombidium deliense* larvae used for this study were obtained from laboratory colonies maintained at the Acarology Unit, Institute for Medical Research, Kuala Lumpur, Malaysia. These colonies are maintained at room temperature and have not been previously exposed to pesticides or repellents prior to this study.

Repellents

Four commercial repellents were evaluated: Kellis® natural insect repellent cream (Bodibasixs Manufacturing, Malaysia), Kaps® natural insects repellent stick (Bodibasixs Manufacturing, Malaysia), BioZ® natural insects repellent stick (Bodibasixs Manufacturing, Malaysia) and Off® lotion cream repellent [S.C. Johnson & Son (M), Malaysia Selangor]. The active ingredients of the repellents are shown in Table 1.

Table 1
Active ingredients of commercial repellents.

Product	Active ingredients
Kellis®	2.0% w/w Citronella oil, 1.5% w/w Jojoba oil, 0.2% w/w Tea tree oil
Kaps®	1.5% w/w Citronella oil
BioZ®	3.5% w/w Citronella oil, 0.5% w/w Lemon grass oil, 0.5% w/w Geranium oil
Off®	7.5% w/w DEET

In vitro bioassay

The *in vitro* procedure used to evaluate repellency was based on the behavior of unfed chiggers in nature to climb upwards to wait for a passing host (Oaks *et al*, 1983), and was a modification of the technique developed by Kriangkrai *et al* (2003). Commercial cotton buds with plastic shafts were used to hold the test repellent. The shafts of the cotton buds were cut 2.5 cm from the base. Approximately 0.02 g of test repellent was placed on a glass slide. The cotton bud was used to absorb as much repellent as possible. Untreated cotton buds were used as controls. Each shaft was then embedded 0.5 cm into a round piece of plasticine placed in the middle of a 9 cm diameter Petri dish. The dish was filled with water to the base of the plastic shaft just covering the plasticine. This was to prevent the chiggers from escaping. A single chigger was placed at the bottom of the plastic shaft just above the water level and observed for 5 minutes. A chigger that climbed to the top of the cotton bud was considered as not repelled by the test repellent, but a chigger that did not climb the base of the cotton bud was considered repelled. A total of 30 chiggers were tested for each type of repellent.

In vivo bioassay

Five week old ICR strain laboratory white mice were used as subjects. Each mouse was anesthetized with an injection of 0.02 ml of Zolatile® (25% w/v Tiletamine, 25% w/v Zolazepam) and the inside of one earlobe was smeared with 0.02 g of test repellent. The actual weight of repellent absorbed was determined by the weight of each treated cotton bud before and after application on the mouse, as shown in

Table 2
Amount of repellent deposited on the ear lobes of each mouse for the *in vivo* bioassay.

Product	Set	Weight (g)	*p*-value
Kellis	1	0.0022	
Kellis	2	0.0024	0.368
Kellis	3	0.0023	
Kaps	1	0.0054	
Kaps	2	0.0032	0.368
Kaps	3	0.0069	
BioZ	1	0.0079	
BioZ	2	0.0031	0.368
BioZ	3	0.0043	
Off	1	0.0057	
Off	2	0.0020	0.368
Off	3	0.0042	

Table 3
In vitro bioassay repellency rates of 4 commercial repellents against *Leptotrombidium deliense* larvae.

Product	Total no. of chiggers applied	No. of chiggers repelled	Percent repelled
Kellis	30	24	80
Kaps	30	22	73.3
BioZ	30	26	86.7
Off	30	25	83.3
Control	30	0	0

Table 4
In vivo bioassay repellency rates of 4 commercial repellents against *Leptotrombidium deliense* larvae.

Product	Total no. of chiggers	No. (%) of chiggers repelled	No. (%) repelled by control
Kellis	90	90 (100)	40 (44.4%)
Kaps	90	90 (100)	37 (41%)
BioZ	90	90 (100)	51 (56.6%)
Off	90	90 (100)	40 (44.4%)

Table 2. Every test had a control. Untreated mice were used as controls. A total of 30 chiggers were placed inside the treated ear lobe. The mice with attached chiggers were placed individually in cages and observed after 24 hours. The mice were then anesthetized a second time to check for attached chiggers. The number of chiggers remaining inside the earlobe was counted. Repellency was defined as the percentage of chiggers not remaining in the earlobe. By the end each repellent was tested 3 times with 90 chiggers total.

Analysis of data

The results were analyzed by chi-square and non-parametric (Kruskal-Wallis) test using SPSS version 13.0 (SPSS, Chicago, USA).

RESULTS

***In vitro* repellency**

The larvae of *Leptotrombidium deliense* exhibited different sensitivities to the repellents. The repellency rates are shown in Table 3. The repellencies ranged from 73 to 87%. BioZ had the highest repellency rate compared to the other products, but there was no significant difference in repellencies among the products evaluated ($p>0.05$). The herbal products were as effective as the product containing DEET.

***In vivo* repellency**

There was a wide range in the weight of repellent deposited on the ears of the mice (Table 2) for the *in vivo* bioassay but the difference was not significant ($p>0.05$).

The results of the *in vivo* bioassay are shown in Table 4. The test products had 100% repellency compared to their respective controls which had repellency rates of 41.1-56%.

DISCUSSION

The *in vitro* test procedure used in this study was based on the behavior of unfed chiggers to climb upwards on the surrounding vegetation to await a passing host. It was inexpensive and easy to perform. Many chiggers could be tested per day. The time required per test was less than 5 minutes from the time of soaking a repellent on the cotton bud to the time of recording whether a chigger was repelled. Some skill was needed to place the chigger at the bottom of the shaft just above the water level. After a few attempts, this could be done quite easily with the aid of a magnifying glass. In the controls, all the chiggers reached the top of the cotton buds during the 5 minute test period.

The *in vitro* bioassay results clearly demonstrated that repellents containing essential plant oils are just as effective as those containing DEET against chiggers. The commercial repellents tested in this study were formulated for protection against mosquitoes. Various formulations of essential plant oils have been tested against mosquitoes. A study investigating a mosquito repellent containing jojoba oil, application of 1.2 g of the product offered complete protection for three to four hours post-application (Govere *et al*, 2000). A cream containing a combination of 2.5% citronella oil, 5% galingale oil and 0.5% vanillin was reported to prevent biting by *Anopheles minimus* mosquitoes for at least six hours (Tawatsin *et al*, 2001). One percent lemon grass oil (*Cymbopogon nardus*) and 0.05% geranium oil (*Pelargonium graveolens*) gave 19 minutes complete protection against the bites of *Aedes aegypti* mosquitoes (Fradin and Day 2002). Wasuwat *et al* (1990) demonstrated under laboratory conditions a cream containing 14% citronella oil (*Cymbopogon nardus*) repelled *Ae. aegypti* for about two hours. Melaleuca oil is a parasiticide and has been used by many as a flea, head louse and insect repellent (http://www.pharmainfo.net). Suzann *et al* (2009) demonstrated that 5% essential oil of *Melaleuca alternifolia* provided at least some protection, less than 110 minutes, against *Ae. aegypti*, *Culex quinquefasciatus* and *Cx. annulirostris*. Laboratory tests have been carried out against the chigger *L. imphalum* using the essential oils, of 13 different plant species, 4 of them were effective as a repellent. *Syzygium aromaticum* (clove) exhibited 100% repellency at a 5% concentration, *Melaleuca alternifolia* (tea tree) oil exhibited 100% repellency at a 40% concentration and the undiluted oils of *Zingiber cassumunar* (plai) and *Eucalyptus globules* (blue gum) exhibited 100% repellency (Eamsobhana *et al*, 2009). The findings of this study demonstrate essential plant oils may be as effective against chiggers as against mosquitoes.

A human finger bioassay was used to evaluate the ability of DEET to repel *Ixodes scapularis* and *Amblyomma americanum* nymphal ticks (Schreck *et al*, 1998; Carroll *et al*, 2004). Human bioassays are the most useful as they allow arthropods to display normal host-seeking behavior and allow evaluation of the level of protection in humans. However, there are ethical concerns with the use of human subjects. In this study, a laboratory white mouse model was used. It is a routine practice as part of the colonization procedure in our laboratory to attach unfed chiggers to the earlobes of such mice for feeding. The

mouse model was thus based on this feeding procedure. Application of repellent in the earlobe of a mouse with essential plant oils or DEET gave 100% repellency. None of the tested chiggers attached to the mouse earlobe treated with repellent, compared to 41.1 - 56.6% in controls. Some of the chiggers not attached were found in the water trap beneath the mouse. No further attempts were made to locate the other unattached chiggers. There was large, although not significant, variation in the amount of repellent applied to the mouse. However, the results indicate 100% repellency in spite of the different amounts of repellent applied. The *in vivo* results support the *in vitro* findings that all commercial repellents were effective in repelling chiggers. The repellents containing essential plant oils were as good as those containing DEET.

ACKNOWLEDGEMENTS

The authors are grateful to the Director-General, Ministry of Health, Malaysia, for permission to publish this article. We are grateful to Ms Wong Ah Leng from the Institute for Medical Research for providing the chiggers for testing.

REFERENCES

Carroll JF, Solberg VB, Klun JA, Kramer M, Debboun M. Comparative activity of DEET and AI3-37220 repellents against the ticks *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae) in laboratory bioassay. *J Med Entomol* 2004; 41: 249-54.

Eamsobhana P, Adisak Y, Wittaya K, *et al*. Laboratory evaluation of aromatic essential oils from thirteen plant species as candidate repellents against *Leptotrombidium* chiggers (Acari: Trombiculidae) the vector of scrub typhus. *Exp Appl Acarol* 2009; 47: 257-62.

Fradin MS, Day JF. Comparative efficacy of insect repellents against mosquito bites. *N Engl J Med* 2002; 347: 13-8.

Gilbert IH, Gouck HK, Smith CN. New mosquito repellents. *J Econ Entomol* 1957; 48: 741-3.

Govere J, Durrheim DN, Baker L, Hunt R, Coetzee M. Efficacy of three insect repellents against the malaria vector *Anopheles arabiensis*. *Med Vet Entomol* 2000; 14: 441-4.

Gupta RK, Rutledge LC. Role of repellents in vector control and disease presentation. *J Trop Med Hyg* 1994; 50 (6 suppl): 82-6.

Kriangkrai L, Nittaya K, Taweesak KM, *et al*. Development of *in vitro* method for the evaluation of candidate repellents against *Leptotrombidium* (Acari: Trombiculidae) chiggers. *J Med Entomol* 2003; 40: 64-7.

Mohinder SJ. Toxicity effect to garlic extracts on the eggs of *Aedes aegypti* (Diptera: Culicidae): A scanning electron microscopic study. *J Med Entomol* 2001; 38: 446-50.

Oaks SC Jr, Ridgway RL, Shirai AJ, Twartz C. Scrub typhus. *Inst Med Res Malaysia Bull* 1983; 21.

Rim IS, Jee CH. Acaricidal effects of herbal essential oils against *Dermatophagoides farinae* and *D. pteronyssinus* (Acari: Pyroglyphidae) and qualitative analysis of a herbal *Mentha pulegium* (Pennyroyal). *Korean J Parasitol* 2006; 44: 133-8.

Schreck CE, Fish D, McGovern TP. Activity of repellents applied to skin for protection against *Amblyomma americanum* and *Ixodes scapularis* ticks (Acari: Ixodidae). *J Am Mosq Control Assoc* 1998; 11: 136-40.

Seong SY, Choi MS, Kim IS. *Orientia tsutsugamushi* infection: Overview and immune responses. *Microbes Infect* 2001; 3: 11-21.

Suzann KM, Cameron EW, Sarah M, Richard CR. Are commercially available essential oils from Australian native plants repellent to mosquito? *J Am Mosq Control Assoc* 2009; 25: 292-300.

Tanskul P, Linthicum KJ, Watcharapichat P, *et al.* A new ecology of scrub typhus associated with a foci of antibiotic resistance in rice farmers in Thailand. *J Med Entomol* 1998; 35: 551-5.

Tawatsin AS, Wratten D, Scott RR, Thavara U, Techadamrongsin Y. Repellency of volatiles oils from plants against three mosquito vectors. *J Vector Ecol* 2001; 26: 76-82.

Wasuwat S, Sunthonthanasart T, Jarikasem S, *et al*. Mosquito repellent efficacy of citronella cream. *J Sci Tech* 1990; 5: 62-8.