LABORATORY EVALUATION OF TWO COMMERCIAL REPELLANTS AGAINST *LEPTOTROMBIDIUM FLETCHERI* (ACARI : TROMBICULIDAE)

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Abstract. Two commercial repellants were evaluated in the laboratory against *Leptotrombidium fletcheri* chiggers. The active ingredient in one was DEET and in the other was citrus oil. Excito-toxicity effect was studied and it was determined by the time ("escape time") chiggers took to move off filter papers treated with the repellants. All chiggers exposed on filter papers treated with DEET died and did not move off the treated papers. None of the chiggers that were placed on papers treated with citrus oil were killed. Escape times on papers treated with a 2-sec spray of citrus oil were longer than those for the 4- and 8-sec sprays. The weights of citrus oil deposited increased with increasing spray times. Electron microscopy showed that the repellants had no effect on the texture of the filter papers. It was concluded that the spray containing DEET was more effective; however, both repellants should be further evaluated under field conditions for protection against chigger bites.

INTRODUCTION

Scrub typhus is a rickettsial disease that is endemic in many parts of Southeast Asia (Oaks *et al,* 1983). The disease is transmitted by the bite of an infected larval trombiculid mite which is also referred to as a chigger. There are three known vectors of scrub typhus in Malaysia; one of these is *Leptotrombidium fletcheri* (Womersley and Heaslip) which is mainly found inhabiting areas covered with long grasses locally known as "lalang".

Based on serological surveys, it was estimated that as many as 500,000 cases of scrub typhus per year may occur in Malaysia (Saunders *et al,* 1980). In view of that, the control of the disease is vital. Vector contral has mainly depended on the use of chemical compounds either as insecticides or repellants (Traub and Wisseman, 1968). For certain areas where human activity is sporadic, it is not economically justified to apply insecticides before every human intrusion into the affected area. In such instances, the use of repellants for peronal protection is highly recommended. There have been earlier reports of laboratory evaluation of repellants against chiggers (Kulkarni, 1977; Buescher *et al,* 1984). There is another effect which has not been evaluated much and that is the toxicity of the compounds. The term "excito-toxicity" was coined in this study to describe the combined repellancy and toxicity of such compounds. In addition, little is known about the effectiveness of commercial repellants which are formulated against mosquitos in general and not against chiggers. The objectives of this study were thus to investigate a technique for evaluation of excito-toxicity of those commercial products and to use the technique to evaluate two repellants that are available in Malaysia.

MATERIALS AND METHODS

Chiggers

Colonies of noninfected *L. fletcheri* established in the Division of Acarology, Institure for Medical Research, Kuala Lumpur, were used. These mites were maintained at room temperature and were not exposed to any insecticide or repellant prior to the study. Unfed chiggers which were 2 weeks old were used.

Repellants

Two commercial repellants were evaluated. These were CITRUS (Tetra Arm Co Ltd, Bangkok) and TABARD (Tiram Kimia Private Ltd, Kuala Lumpur). CITRUS contained pure natural citrus oil as active ingredient whereas that in TABARD was 20% w/w diethyltoluamide (DEET). The

amount of citrus oil contained in each can of CITRUS is not stated by the manufacturer. New cans of the two repellants werepurchased off the shelf from a department store. There was no mention on both cans whether they were effective against chiggers.

Bioassays

Whatman no. 1 filter papers which were 5 cm in diameter were placed flat on a table. The repellant spray was held in a horizontal position, 0.5 m above each filter paper. The nozzle of the spray was directed towards each paper and depressed for a specific period (to be referred to as "spray time"). The following spray times were evaluated: 2, 4, and 8 seconds Untreated filter papers were used as controls. The weights of repellants deposited on the papers were determined from the difference between the pre- and post-spray weights of the papers. Treated papers were allowed to dry for periods of 1, 5, 10, 30 or 60 minutes After drying, treated papers were placed individually in a Petri dish which in turn was placed in an enamel tray containing water (Fig 1). A chigger was placed on each treated paper and the time for it to move off the top surface of the paper was recorded as "escape time". The chigger was then removed and placed individually in a glass vial with a moist Plaster-of-Paris base. The chigger was observed for 24 hours and any mortality was recorded. A total of 15 chiggers was used for each parameter studied. The whole study was repeated twice. Differences of means were analysed by student's *t*-test at 95% significance.

Electron microscopy

Treated and control filter papers were examined under scanning electron microscopy to detect any changes in the texture of treated papers. The papers were first cut into small pieces with a diameter of approximately 1.0 cm. There were then afixed to metal stubs using double-sided sticky tape. The papers were then coated with a 20 nm layer of gold. Observations were then made through a scanning electron microscope (Hitachi model S430).

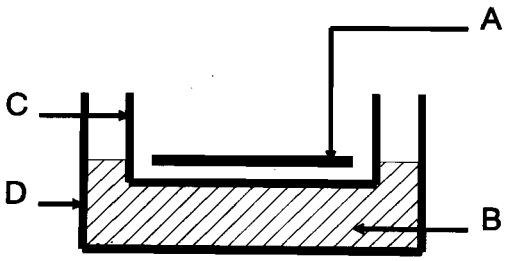

Fig 1—Apparatus used for laboratory bioassays of repellants against scrub typhus vectors; (A) Whatman filter paper, (B) water, (C) 5.5 cm diameter Petri dish, (D) 9.0 cm diameter Petri dish.

RESULTS

Chiggers released onto all papers treated with DEET became immobile and did not move off the papers even after a period of 1 hour. These chiggers were then collected and observed over 24 hours. At the end of the observation period, they were still immobile and were thus presumed dead.

Escape times from paper treated with citrus oil and dried at the various time periods were not significantly different ($p > 0.05$) within each of the spray times (Fig 2). These escape times were then combined for each spray time and their means determined (Table 1). The mean escape times for treated papers irrespective of the spray times were all significantly shorter than that of control

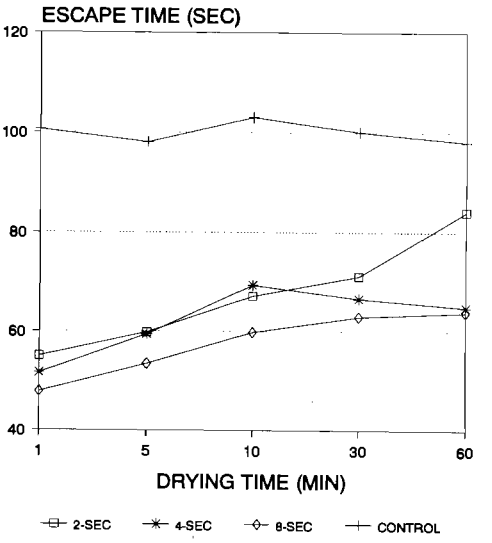

Fig 2—Escape times of *L. fletcheri* on dried citrus oil treated filter papers for three different spray times.

Table 1

Combined escape times of *L. fletcheri* chiggers placed on filter papers sprayed for various periods with citrus oil.

Spray time (sec)	No. chiggers tested	Escape time (sec) Mean ± SD
0	225	99.9 ± 18.7
2	225	67.5 ± 23.8
4	225	62.4 ± 22.3
8	225	59.1 ± 22.8

papers ($p < 0.05$). Papers treated with the 2-second spray had significantly longer mean escape times than that for the 4- ($p = 0.01$) and 8-second ($p < 0.01$) sprays; there was however no significant difference between the 4- and 6-second sprays ($p > 0.05$). None of the chiggers died 24 hours after exposure on papers treated with citrus oil.

The weights of papers treated with citrus oil and dried for the various periods were not significantly different ($p > 0.05$) for each spray times (Fig 3). The weights for the different drying times were then combined for each spray time (Table 2). As

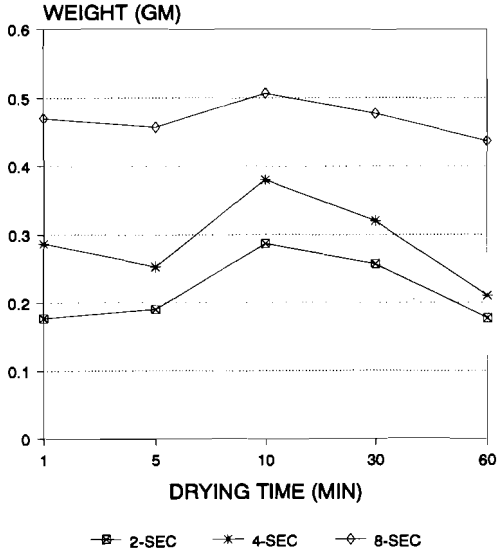

Fig 3—Weight of citrus oil repellant on dried citrus oil treated filter papers for three different spray times.

Table 2

Combined weight of citrus oil repellant deposited on filter papers after various spray times.

Spray time (sec)	Weight (mg) Mean ± SD
2	0.217 ± 0.087
4	0.314 ± 0.105
8	0.469 ± 0.125

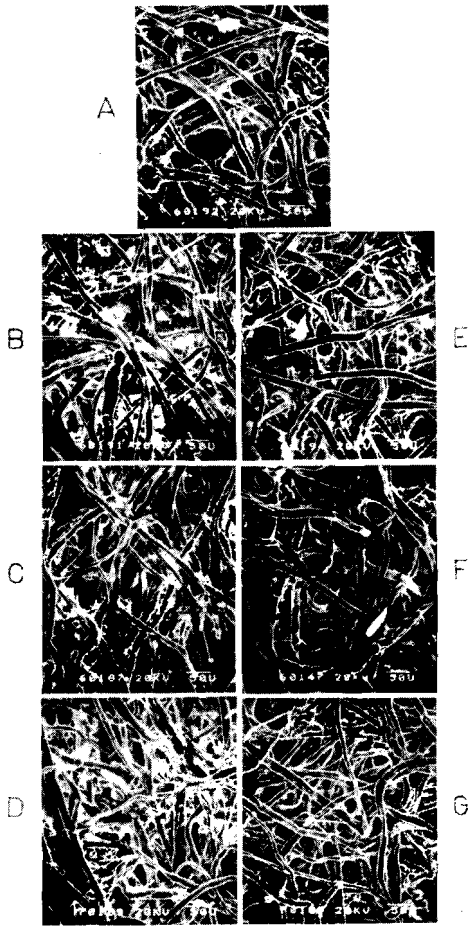

Fig 4—Electron micrographs of filter paper treated for different periods with two repellants (A, control; B, DEET 2-sec; C, DEET 4-sec, D, DEET 8-sec; E, citrus oil 2-sec; F, citrus oil 4-sec; G, citrus oil 8-sec).

expected, the weights of repellants deposited were significantly different between the three spray times ($p < 0.05$).

Electron microscopy of papers treated with the two repellants did not reveal any detectable differences in texture from that of control papers (Fig 4).

DISCUSSION

From the results, it is clear that the formulation containing DEET had a very marked effect on the chiggers compared to that of citrus oil. DEET is well-known for its effect against a number of arthropods (Grothaus *et al,* 1976; Schreck *et al,* 1978; Buescher *et al,* 1985). The acaricidal effect of DEET had been reported earlier by Kulkarni (1977). The commercial repellant containing DEET appears to be useful for prevention of chigger bites. Chiggers that land on a treated surface will be killed and thus lessen the risk of it transmitting scrub typhus. The optimum application rate will need to be determined by field trials.

Based on laboratory observations, chiggers from laboratory colonies require at least 10-15 minutes after attachment on a host before they start to feed. That time is considerably longer than the maximum escape time obtained with citrus oil. It is Thus expected that CITRUS can prevent chiggers that come into contact with it from feeding. This however need to be confirm with actual studies on a normal host. Citrus oil, although not as toxic as DEET, nevertheless appears to have the potential for use in personal protection against chigger bites. Further field studies are required to determine the effective application rates to be used. From the present results, a spray of at least 4 seconds for each area to be treated may be required to obtain maximum protection.

Citrus oil is unlikely to evaporate off the filter papers and is probably the reason for the similar weights of citrus oil deposited after various drying times. It is also apparent that the other components of the spray formulations may have negligible contribution to the total weight of the formulation after drying. The results also indicated that the rate of discharge for each of the three spray times was consistent. This is possible because new spray cans were purchased. If the can was used extensively prior to the study, it is likely that the amount of repellant remaining in the can may be insufficient to ensure consistency for each repeat of the study.

One possible reason for the longer escape times on treated papers could be the change in texture which hinders the movement of the chiggers. That however did not seem to be occurring in this study as shown by the electron micrographs of the various papers after treatment. Thus any increase of decrease in escape time is due to the repellants.

In conclusion, TABARD will kill any chigger that comes into contact with it and CITRUS will reduce the time a chigger stays on a surface treated with it and in the process possibly give protection against chigger bites. Both repellants should be further evaluated under field conditions to determine effective application rates.

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