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Research Note

Laboratory contact and topical evaluations of household disinfectants against house dust mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* (Acari: Pyroglyphidae)

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Abstract. The contact and topical activity of two household disinfectants containing chloroxylenol and benzyl chlorophenol against, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* mites were evaluated in the laboratory. For contact activity, 30 adult mites were placed for 24 hrs on Whatman No. 1 filter paper impregnated with disinfectant. For topical activity, each disinfectant was directly applied to individual body of all 30 dust mites and observed for 24hrs. All treatments were replicated 12 times. Chloroxylenol disinfectant was found to be more effective compared to benzyl chlorophenol disinfectant in killing pyroglyphid mites.

House dust mite is a source of allergen and a significant contributor to cause asthma, rhinitis atopic dermatitis, eczema and other allergic condition in sensitive individuals especially children. Current studies suggest that at least 45 % of young people with asthma are allergic to house dust mites, unlike "seasonal" allergies caused by molds and pollens, people who are allergic to dust mites often will have symptoms all year round (Dhruba *et al*., 2011). It was reported prevalence of allergic sensitization to house dust mite (HDM) species is high in tropical/ subtropical regions (Thomas, 2010). In Malaysia, the prevalence rate of allergies to dust mites in children and adults with asthma and allergic rhinitis are greater than 60% (Teo *et al*., 2015; Samiah *et al*., 2013; Lim *et al*., 2015). Dust mites have a cosmopolitan distribution and can be found in most human dwelling. Temperature and relative humidity inside the building are two main factors for the propagation and prevalence of dust mite populations. The most allergy-causing mites, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and *Blomia tropicalis* being the top three species in terms of global frequency and abundance (Colloff, 2009). Multiple approaches have been recommended to reduce dust mites and their allergen levels in homes through physical methods such as reducing indoor relative humidity to below 50%, regular vacuum cleaning the carpets, floors and mattresses with high-efficiency particulate air (HEPA) filter vacuum cleaner, replacement of carpets with hard floor coverings, use of bedding encasement (Micheal, 2013; Gehring *et al*., 2012). The application of chemicals with acaricidal activity is necessary if physical

methods are not able to ensure full mite and allergen control in homes. Several chemicals (acaricides) have been examined in laboratories and used in homes to kill dust mites such as benzyl benzoate, disodium octaborate tetrahydrate (DOT), permethrin, diethyl-m-toluamide (DEET) and tannic acid (Colloff, 2009). Among both methods, acaricides use seems to be the most effective to reduce house dust mite load (Nurmatov *et al*., 2012).

Chemical based disinfectants have the potential to be acaricides but only few have been studied and no related research has been conducted in Malaysia. Disinfectants are antimicrobial chemical agent used to destroy pathogenic microorganisms that are living on inanimate objects. In view of the fact that dust mites are invisible to naked eye, the researchers looked at logical and commercial point view to control bacteria simultaneously with killing or controlling dust mites. Commercially available disinfectants in foreign market such Paragerm AK (thymol) and Tymasil (natamycin) were highly effective acaricides to kill dust mites (Penaud *et al*., 1975; De Saint George-Gridelet, 1988). A number of household disinfectants found in Malaysian market are sold at the supermarket, pharmacies and through direct selling companies. Nevertheless, their potential as acaricides remains yet to be realized. Due to the paucity of information on the acaricidal effect of these disinfectants in killing house dust mites, this study was undertaken to determine the contact and topical effects of two selected household disinfectants containing chloroxylenol and benzyl chlorophenol, against two species of house dust mites, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* in laboratory settings assay.

In this study, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* mites were obtained from colonies established since 1960s in the Acarology Unit, Institute for Medical Research, Malaysia. The colonies were reared in small glass bottles and fed with ground rat chow. All colonies are maintained at an average temperature of 25°C ± 2°C and 75% relative humidity. Two household disinfectants evaluated in this study were bought from local stores. The chloroxylenol disinfectant is containing <5% chloroxylenol (Reckitt Benckiser Sdn Bhd, Malaysia) and benzyl chlorophenol disinfectant is containing <5% benzyl chlorophenol (Sc Johnson & Son Malaysia Sdn. Bhd.). Serial dilutions (50.0%, 25.0%, 12.5%, 6.3 %, 3.1% v/v) for both disinfectants were prepared from original commercial concentration in distilled water.

The acaricidal effects for both household disinfectants were evaluated by contact and topical activity bioassay in laboratory. Contact activity was performed by an impregnated filter paper method. Square pieces of no. 1 Whatman filter paper (9.5 cm x 9.5 cm) were soaked in 8 mL of diluted solution for 30 minutes. Control papers were similarly treated with distilled water. After drying for two hours, each piece of treated filter paper was attached to the bottom of a round Petri dish (14 cm^2) using double sided tape. Vaseline was applied at the immediate edges of the filter paper to prevent mites from escaping. Thirty random adult mites were placed onto each treated filter paper. Treatment and control Petri dishes were held at 25°C and 75% relative humidity inside a closed glass chamber. Mortalities were determined under a binocular microscope 24 hrs. post-treatment. Mites that were motionless when probed gently with a sharpened wooden application stick were considered dead. All treatments were replicated 12 times.

The topical activity bioassay was conducted as set up in contact activity except that the filter papers were untreated. Thirty adult mites were placed on each filter paper. Two ml of diluted disinfectant solutions were applied onto each mite. Distilled water was used as control. Treated and control mites were held in Petri dishes and were kept at 75% RH at 25°C. Mortalities were assessed 24 hrs. after treatment. Mites were considered dead as per contact activity bioassay. All treatments were also replicated 12 times. The test results obtained from both bioassays were pooled and analysed using probit analysis via SPSS ver. 17.0 to calculate the lethal concentration values (Finney, 1971).

The significance of results was determined with the Mann-Whitney U test as most of the data are not normally distributed. Significant level was set at 0.05.

Generally, mortalities for *D. pteronyssinus* and *D. farinae* mites declined with decreasing of disinfectants concentration in both bioassays. No mortality of control mites occurred throughout the study. Similar percentage of mortality (91 ± 6.49%) was produced by contact activity of chloroxylenol disinfectant on *D. pteronyssinus* and *D. farinae* mites at 50.0% concentration (Figure 1). At the lowest concentration (3.1%), chloroxylenol disinfectant produced mortalities of 13 ± 8.82% for *D. pteronyssinus* and 11 ± 7.59% for *D. farinae*, respectively. On the other hand, benzyl chlorophenol disinfectant exhibited less percent of mortalities in both species of mites. Mortalities of 9 ± 10.25% and 2 ± 1.57% were recorded at the final dilution of benzyl chlorophenol disinfectant (3.1%) for *D. pteronyssinus* and *D. farinae* respectively. Chloroxylenol disinfectant revealed significantly greater than benzyl chlorophenol disinfectant to kill *D. pteronyssinus* at each concentration ($p<0.05$) except for its lowest concentration (3.1%) with LC_{50} at 11.2% and 44.1%, respectively (Table 1). It was shown significantly effective than benzyl chlorophenol disinfectant to kill *D. farinae* in all concentrations ($p<0.05$) with LC_{50} at 12.3% and 103.5%, respectively. Meanwhile, the topical mortalities for both species of mites were much lower compared to contact mortalities (Figure 2). The chloroxylenol disinfectant was indicated higher efficacy in killing *D. pteronyssinus* and *D. farinae* mites (LC_{50} at 48.6%; 60.9%) compared to benzyl chlorophenol disinfectant (LC_{50} at 160.5%; 145.8%) in all concentrations ($p<0.05$) excluding the lowest one (3.1%).

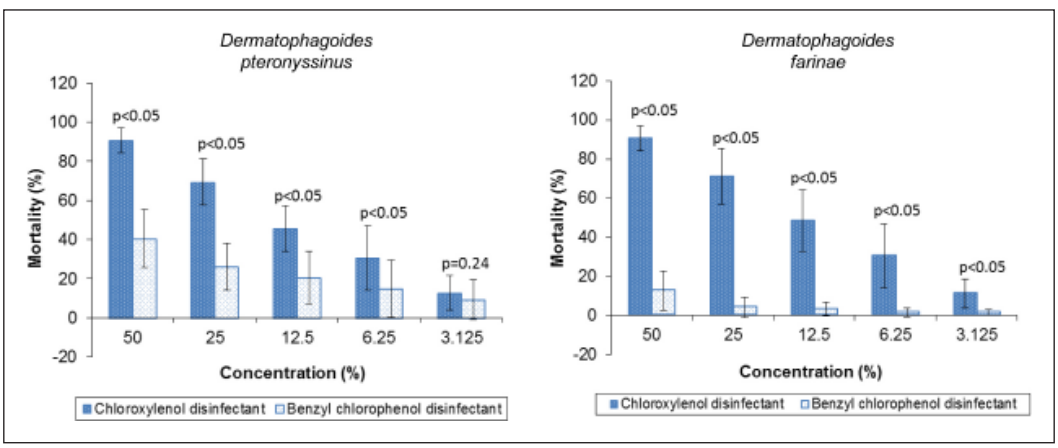

Figure 1. Contact mortalities (mean % mortality ± SD) of *D. pteronyssinus* (left) and *D. farinae* (right) when exposed to chloroxylenol disinfectant and benzyl chlorophenol for 24 hours. Significant difference between chloroxylenol disinfectant and benzyl chlorophenol at $p<0.05$.

Table 1. Contact and topical lethal concentrations of two disinfectants against *D. pteronyssinus* and *D. farinae* mites (95% confidence limits expressed in parentheses)

Species of mite	Disinfectant	Contact activity bioasssay (LC_{50})	Topical activity bioassay (LC_{50})
Dermatophagoides pteronyssinus	Chloroxylenol	11.2 (10.1–15.7)	48.6 (45.8–52.0)
	Benzylchlorophenol	44.2 (42.1–46.5)	160.5 (114.2–313.8)
Dermatophagoides farinae	Chloroxylenol	12.3 (11.4–13.3)	60.9 (46.7–89.1)
	Benzylchlorophenol	103.5 (86.5–134.7)	145.8 (100.7–388.6)

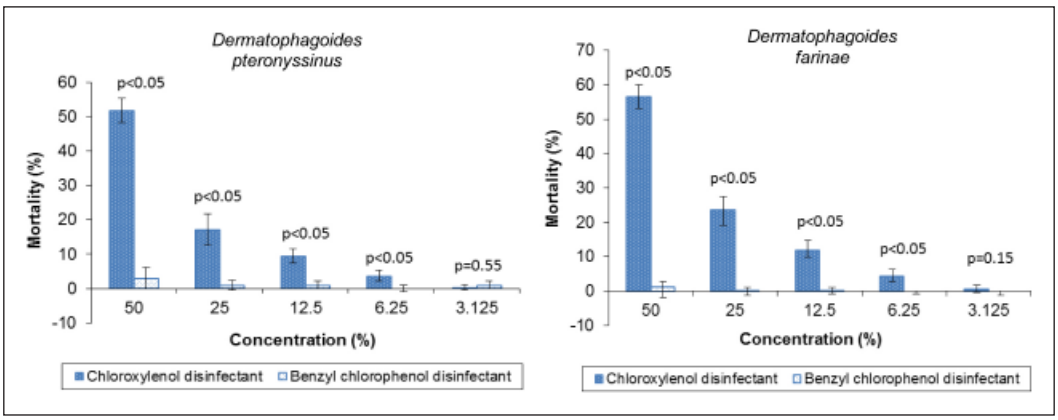

Figure 2. Topical mortalities (mean % mortality ± SD) of *D. pteronyssinus* (left) and *D. farinae* (right) when exposed to chloroxylenol disinfectant and benzyl chlorophenol for 24 hours disinfectants for 24 hours. Significant difference between chloroxylenol disinfectant and benzyl chlorophenol at $p<0.05$.

Various chemical agents have been used in killing mites (Stara *et al.*, 2010); however, some of these products are often causing allergic reactions and somewhat expensive (Anne-Marie *et al.*, 2015). Hence, available household disinfectants product which contains powerful antimicrobial agents also can be proposed as a method to kill and control dust mites at home. Chloroxylenol and benzyl chlorophenol formulated disinfectants are the two most common household products available in Malaysian market. Both disinfectants are phenolic compounds for killing bacteria, algae, fungi and virus; and are widely used in hospitals and household for disinfection and sanitation (Rutala, 1995). In this study, chloroxylenol disinfectant kills higher number of dust mites. The toxicity of chloroxylenol disinfectant to *D. pteronyssinus* and *D. farinae* mites was confirmed by contact and topical activity bioassays. However, LC_{50} values were slightly higher for the later compared to the former. On the other hand, benzyl chlorophenol disinfectant appeared to have less toxic effect via contact and topical activity. The benzyl chlorophenol disinfectant was less effective where a higher concentration is required to kill both species of mites compared to chloroxylenol disinfectant.

Findings on the effectiveness of chloroxylenol disinfectant in killing pyroglyphidae mites by direct topical application in this study were slightly better compared to those obtained by Schober *et al.* (1987) as lower concentration was used in our study to kill mites. However, the result for benzyl chlorophenol disinfectant was not as satisfactory as theirs. The possible reason for this might be their usage of benzyl chlorophenol disinfectant that was stated to contain other additional active ingredients and detergents, whereas the present study evaluated only a single active ingredient of disinfectant. Their assay was by dipping dust mites in the disinfectant which is similar to the topical bioassay in this study. Contact activity assay however was not performed by Schober *et al.* (1987).

Current finding of chloroxylenol disinfectant was not as profound as Leysen *et al.* (1974). Their study found the chloroxylenol disinfectant killed approximately 70% pyroglyphidae mites in 2.5 % concentration of solution prepared from original stock solution through direct spray method against the mite culture. In our topical activity study, we required higher concentration of chloroxylenol disinfectant to kill 50% of mites. The direct spray method used by Leysen *et al.* (1974) was comparable to the topical assay in our research. It should be noted that their study was carried out by spraying disinfectant directly against mites that are in a culture medium, whilst in our study we used topical application onto individual mites that are separated from the

culture medium. It is probable that a high mortality rate stated in their result might be due to the presence of previously absorbed disinfectant in the culture medium which exposed the mites to the already toxic food culture. All our bioassays were conducted against each mite were devoid of any foods or materials which could interfere with the results. All tested disinfectants resulted mites being motionless with slight shrinking of body as well as forelegs extended forwards. None of the mites were discovered undergoing knockdown prior to their death. Dead mites also did not show any change in cuticle glossiness when observed under binocular microscope 24 hrs. post-treatment.

There are various types of bioassays that have been established and standardized to evaluate the effectiveness of different formulated acaricides to eradicate house dust mites (Ju-Hyun *et al*., 2010). The contact activity bioassay basically resembles the application of disinfectants during cleaning of beddings or wiping clean of a surface area. Meanwhile the topical activity bioassay is designed to simulate spray application. The findings of this study suggest chloroxylenol disinfectant is a potential disinfectant to reduce natural mite populations especially in treating beddings such as bed liners, linens and pillow cases. The use of disinfectants alone is unlikely to sufficiently reduce dust mite populations to a level that can lend to clinical improvement in atopic individuals. Disinfectants should therefore be used in an integrated approach together with other mite and allergen control methods. This present study provides beneficial information for health practitioners dealing with disinfections and sanitations works. Our study also highlights the potential use of disinfectant which contains chloroxylenol as household acaricide.

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