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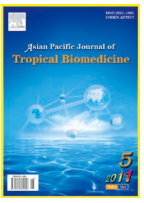

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Acaricidal activity of *Cymbopogon citratus* and *Azadirachta indica* against house dust mites

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ABSTRACT

Objective: To examine the acaricidal effects of the essential oil of *Cymbopogon citratus* leaf extract (lemongrass) and ethanolic *Azadirachta indica* leaf extract (neem) against house dust mites *Dermatophagoides farinae* (*D. farinae*) and *Dermatophagoides pteronyssinus* (*D. pteronyssinus*). **Methods:** Twenty-five adults mites were placed onto treated filter paper that is soaked with plant extract and been tested at different concentrations (50.00%, 25.00%, 12.50%, 6.25% and 3.13%) and exposure times (24hrs, 48hrs, 72hrs and 96 hrs). All treatments were replicated 7 times, and the experiment repeated once. The topical and contact activities of the two herbs were investigated. **Results:** Mortalities from lemongrass extract were higher than neem for both topical and contact activities. At 50 % concentration, both 24 hrs topical and contact exposures to lemongrass resulted in more than 91% mortalities for both species of mites. At the same concentration and exposure time, neem resulted in topical mortalities of 40.3% and 15.7% against *D. pteronyssinus* and *D. farinae* respectively; contact mortalities were 8.0% and 8.9% against the 2 mites, respectively. There was no difference in topical mortalities of *D. pteronyssinus* from exposure to concentrations of lemongrass and neem up to 12.50%; lemongrass was more effective than neem at the higher concentrations. **Conclusions:** Generally, topical mortalities of *D. farinae* due to lemongrass are higher than that due to neem. Contact mortalities of lemongrass are always higher that neem against both species of mites.

1. Introduction

Dermatophagoides farinae (*D. farinae*) and *Dermatophagoides pteronyssinus* (*D. pteronyssinus*) are important pyroglyphid mites because of their cosmopolitan occurrence and abundance in homes, major sources of multiple potent allergens and their causal association with sudden infant death syndrome[1]. Acaricide is a pesticide designed to control harmful species of mites (Acari)[2]. Repeated use of these acaricides has resulted in resistance and undesirable effects on non-target organism and fosters serious human health concerns[3]. Plant extracts may be an alternative source for chemical control of dust mites. Much effort has focused on phytochemicals as potential sources of commercial control agents or as lead compounds [3]. Plants extracts have good potential as control agents of dust mite because many are selective and have few or no harmful effects on non-target organisms or the environment[4]. Various natural bioactive products with acaricidal activity (botanical and microbial pesticides, essential oils, horticultural spray oils, mycopesticides) have become important alternatives to synthesize acaricides [5,6]. Numerous plant-derived substances have demonstrated physiological and behavioral activity against insect pests, and they can provide new sources for the development of natural pesticides[7, 8]. Products with botanical origin have shown a wide range of biological activities including toxicity, repellence, antifeedant, and growth regulatory properties[9-11]. It is reported that many plant extracts and essential oils are known to possess acaricidal activity against house dust mites[12]. Naturally existant acaricidal compounds against house dust mites include O-anisaldehyde citronellal and perillaldehyde derived from perilla oil[13], as well as isosericenine, aryophyllene oxide and α-cadinol from essential oil of the leaves from *Neolitsea sericea* Blume[14].

Fumigants from natural products have potential as house dust mites control agents too. The constituents of

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Paeonia suffruticosa root bark, identified as paeonol and benzoic acid, are useful fumigants against *D. farinae* and *D. pteronyssinus*[3].

The biocidal activity of many plants such as *Heracleum sosnowskyi*, *Artemisia vulgaris*, *Tanacetum vulgare*, *Artemisia absinthium*, *Allium sativum*, *Piper nigrum*, *Juniperus communis*, *Cymbopogon nardus* (*C. nardus*)[15], and *Azadirachta indica* (*A. indica*)[16] have been studied against *Sarcoptes scabiei*. *C. nardus* which is a close relative of lemongrass, belongs to the family of Poaceae (Graminae) and yields essential oils which are mainly used in the spice and essential oil industry. *C. nardus* essential oils have been used to control mosquitoes and houseflies[17]. The essential oil of *C. nardus* has been proved to have repellent activity against *Tribolium castaneum*, *Sitotroga cerealella*, *Callosobruchus chinensis*, *Callosobruchus maculatus* and many other stored grain insect pests. The oil extracted from seeds of *Azadirachta Indica* (*A. indica*) is found to have biocidal activity against nearly 200 medical and veterinary arthropods, without any adverse effects toward most non-target organisms[18, 19]. Previous research by Du YH, *et al*[20] demonstrated that neem oil possesses potent acaricidal activity against the scabies mite, *Sarcoptes scabiei* (*S. scabiei*) var. cuniculi. The petroleum ether extract of neem oil is found to be more effective than crude neem oil against the larvae of *S. scabiei* var. cuniculi *in vitro*[21]. The *in vitro* toxicity of neem seed oil was tested against the larvae of three-host tick *Amblyomma variegatum* parasitic to cattle commonly found in Nigeria. Undiluted neem oil (100% concentration) was found to kill all (100% mortality) the larvae after 48 hrs[22]. The purpose of this study is to determine the toxicity of lemongrass and neem against *D. farinae* and *D. pteronyssinus*.

2. Materials and methods

2.1. Plant crude extract

Authenticated lemongrass and neem were purchased from Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor, and crude leaf extracts were prepared using standard methodologies by the Herbal Medicine Research Unit of the Institute for Medical Research. Serial dilutions of neem and lemongrass were prepared in ethanol and ethyl acetate, respectively, and applied to filter papers; ethyl acetate and ethanol were used for controls. Preliminary investigations found no significant differences in mortalities induced by 50% and 100% concentrations of both extracts ($P>0.05$). Due to limited materials, 50% was the highest concentration of extracts used for further detailed investigations.

2.2. Mites

D. farinae and *D. pteronyssinus* were obtained from laboratory colonies maintained in the Acarology Unit, Institute for Medical Research. Those colonies were established since 1970's and have not been exposed to any acaricide.

2.3. Bioassay for contact activity

Square pieces of Whatman No. 1 filter paper (9.8 cm^2) were used for the test. Each paper was soaked in 13 mL of diluted extract for 1 hr. After drying for two hours, each filter paper was attached to the bottom of a round Petri dish (14 cm^2) using double sided cellophane tape. Vaseline was applied at the immediate edges of the filter paper to prevent escape of mites. Twenty-five adult mites (mixed males and females) were placed onto each treated filter paper. Treatment and control Petri dish were held at 75% RH and 25℃ inside a closed air-tight glass chamber. Mortalities were determined 24 hours post-treatment. Mites were considered dead if their appendages do not move when prodded with a pin. All treatments were replicated 7 times, and the experiment repeated once.

2.4. Bioassay for topical activity

The setup was the same as that used for contact activity above except that the filter papers were untreated. Twenty-five adult mites (mixed males and females) were placed on each filter paper. Two L of diluted extracts were applied to each mite. Treated and control Petri dishes were held at 75% RH and 25℃. Mortalities were determined 24 hours after treatment. As above, all treatments were replicated 7 times, and the experiment repeated once.

2.5. Statistical analysis

Results were analyzed by independent sample T-test and one-way ANOVA at 95% confidence level using SPSS ver 13.

3. Results

Generally, mortalities increased with increasing concentration of both extracts of lemongrass and neem. Highest mortalities (>90% for lemongrass, <50% for neem) for both topical and contact activities were at 50% concentration.

3.1. Effect of Lemongrass on D. pteronyssinus and D. falinae

Increasing the exposure period at 25% concentration, significantly increased the topical mortalities of *D. pteronyssinus* ($P<0.01$); at 50% concentration, the mortalities were similar ($P=0.96$). There was no significant increase in contact mortalities at each concentration with increasing exposure times ($P>0.05$) (Table 1). There was significant

Table 1
Mortalities of lemongrass against *D. pteronyssinus* and *D. farinae* (Mean±SD)

Mite	Bioassay	Concentration (%)	Mortality			
			24 h	48 h	72 h	96 h
D. pteronyssinus	Topical	3.13	18.6 ± 9.5	18.6 ± 9.5	22.9 ± 6.7	30.0 ± 7.5
		6.25	20.9 ± 5.0	28.6 ± 7.5	29.1 ± 7.6	33.4 ± 9.6
		12.50	29.1 ± 7.8	32.6 ± 9.4	40.6 ±13.8	48.6 ± 15.1
		25.00	48.0 ± 6.7	50.1 ± 7.0	57.1 ± 7.3	73.7 ± 10.4
		50.00	92.6 ±10.7	92.6 ± 10.7	92.6 ± 10.7	94.3 ± 9.1
	Contact	3.13	72.6 ± 15.5	74.9 ± 14.1	76.3 ±13.4	80.9 ± 11.2
		6.25	84.6 ± 9.9	86.3 ± 10.1	86.3 ± 10.1	90.6 ± 7.3
		12.50	84.3 ± 8.2	87.4 ± 7.5	88.9 ± 5.7	90.6 ± 5.3
		25.00	90.0 ± 5.4	92.3 ± 5.5	93.4 ± 5.4	94.6 ± 5.1
		50.00	92.3 ± 7.8	93.5± 7.5	94.0 ± 6.8	94.9 ± 6.7
D. farinae	Topical	3.13	11.4 ± 5.8	17.1 ± 8.2	20.0 ± 8.3	22.9 ± 11.4
		6.25	12.9 ± 8.5	18.6 ± 9.2	20.0 ± 9.2	30.3 ± 10.5
		12.50	16.6 ± 5.6	20.3 ± 7.4	28.3 ± 9.3	34.3 ± 9.5
		25.00	17.1 ± 7.3	24.9 ± 7.0	35.1 ± 8.6	41.1 ± 10.5
		50.00	94.3 ± 5.4	94.6 ± 5.6	95.1 ± 5.5	95.4 ± 5.4
	Contact	3.13	65.1 ± 9.1	66.0 ± 8.8	71.1 ± 9.3	71.1 ± 9.3
		6.25	76.0 ± 14.6	77.2 ± 15.2	80.0 ± 14.3	80.6 ± 14.1
		12.50	82.0 ± 13.9	82.3 ± 13.9	86.6 ± 10.4	87.1 ± 9.6
		25.00	84.0 ± 14.0	86.2 ± 14.0	88.0 ± 12.7	88.3 ± 12.5
		50.00	91.7 ± 7.8	91.7 ± 7.8	92.9 ± 7.6	93.1 ± 7.4

Table 2
Mortalities of neem against *D. pteronyssinus* and *D. farinae* (Mean±SD)

Mite	Bioassay	Concentration (%)	Mortality			
			24 h	48 h	72 h	96 h
D. pteronyssinus	Topical	3.13	19.1 ± 7.2	23.7 ± 7.4	29.4 ± 10.1	32.9 ± 11.6
		6.25	23.1 ± 10.1	25.4 ± 11.8	30.0 ± 11.1	34.9 ± 8.4
		12.50	24.9 ± 8.2	27.7 ± 9.7	31.7 ± 11.1	35.7 ± 10.1
		25.00	28.3 ± 13.0	36.3 ± 12.7	38.9 ± 9.4	43.7 ± 9.9
		50.00	40.3 ± 7.1	42.9 ± 6.2	46.0 ± 8.6	52.3 ± 8.8
	Contact	3.13	1.7 ± 2.6	2.9 ± 3.3	9.1 ± 4.8	13.1 ± 5.3
		6.25	3.1 ± 3.9	3.7 ± 4.0	9.4 ± 7.1	13.4 ± 7.1
		12.50	4.6 ± 4.4	5.1 ± 4.3	14.6 ± 6.2	16.9 ± 6.3
		25.00	6.0 ± 4.9	7.1 ± 4.5	16.6 ± 6.4	18.3 ± 6.0
		50.00	8.0 ± 6.3	10.6 ± 6.0	19.4 ± 8.0	19.7 ± 7.9
D. farinae	Topical	3.13	6.6 ± 6.9	7.1 ± 6.5	8.9 ± 6.9	10.9 ± 6.9
		6.25	6.9 ± 5.7	9.7 ± 3.8	11.4 ± 4.9	13.1 ± 4.8
		12.50	8.9 ± 7.0	10.0 ± 6.2	12.3 ± 6.2	14.3 ± 8.4
		25.00	12.3 ± 6.4	16.0 ± 7.5	16.6 ± 7.8	18.3 ± 7.5
		50.00	15.7 ± 7.8	20.0 ± 8.3	23.4 ± 9.0	24.4 ± 8.5
	Contact	3.13	2.6 ± 3.4	8.0 ± 7.7	8.6 ± 7.7	9.4 ± 7.1
		6.25	4.3 ± 4.6	9.1 ± 6.7	10.3 ± 6.4	12.9 ± 6.1
		12.50	5.7 ± 6.8	10.3 ± 7.0	11.4 ± 6.8	14.6 ± 6.6
		25.00	7.4 ± 6.8	10.6 ± 7.9	12.3 ± 7.6	16.0 ± 7.5
		50.00	8.6 ± 8.1	12.6 ± 7.5	14.3 ± 7.6	17.4 ± 6.8

increase in topical mortalities for each exposure period with increasing concentrations ($P<0.01$); it was similar for contact mortalities ($P<0.01$). At all exposure times, the contact mortalities were significantly higher than the topical mortalities for all concentrations ($P<0.01$) except at 50% where there was no significant difference ($P>0.05$).

Topical mortalities of *D. farinae* increased significantly with increasing exposure times for all concentrations ($P<0.01$)

except for 50% where the differences were not significant (P=0.94). Contact mortalities on the other hand were not significantly different at all concentrations with increasing exposure times (P>0.05). At each exposure time, there was significant differences in topical mortalities between the various concentrations (P<0.01); it was similar for contact mortalities (P<0.01). At each exposure time, the contact mortalities were greater than topical mortalities between all concentrations (P<0.01) except for 50% where the differences were not significant (P>0.05).

3.2. Effect of neem on D. pteronyssinus and D. farinae

The topical and contact mortalities of *D. pteronyssinus* at all exposure times were significantly different between concentrations (P<0.05), and 50% concentration caused the highest mortalities (Table 2). Both topical and contact mortalities at all concentrations, increased with increasing exposure times (P<0.05). Topical mortalities at all concentrations and all exposure times were significantly higher than the corresponding contact mortalities (P<0.01).

Topical mortality of *D. farinae* at each concentration,increased with increasing exposure time, however, the difference were not significant for 3.13%, 12.50%, and 25.00% concentrations (p>0.05). Contact mortality at each concentration increased significantly with increasing exposure time (P<0.05). At each exposure time, topical mortality increased with increasing concentrations (P<0.01). The increase in contact mortality at each exposure time with increasing concentration was not significant (P>0.05) except at 96 hours (P<0.05). Generally, topical and contact mortalities at each concentration and exposure time were similar.

4. Discussion

The results demonstrated that lemongrass generally have similar topical and contact effects on both *D. pteronyssinus* and *D. farinae*; there were only a few instances where mortalities in *D. pteronyssinus* were higher. Mortality from topical exposure to neem was higher in *D. pteronyssinus* compared to *D. farinae*. On the other hand for each herbal extract, contact mortalities between the 2 species were generally similar.

Comparing lemongrass and neem, it appears that there was no difference in topical mortalities of *D. pteronyssinus* from exposure to the concentrations of lemongrass and neem up to 12.50%; lemongrass was more effective than neem at the higher concentrations. Generally, topical mortalities of *D. farinae* due to lemongrass were higher than that due to neem. Contact mortalities of lemongrass were always higher than neem against both species of mites.

Lemongrass has both repellant and toxic effects against arthropods. A methanol-leaf extract of lemongrass shows various degree of repellency and larvicidal effect against a malaria vector, *Anopheles arabiensis*. Karunamoorthi K, *et al*[23-25] also reported the use of essential oil of lemongrass as a repellent. It can provide protection against bites of *Anopheles darlingi* and *Mansonia* spp. Morsy TA, *et al*[26] found solvent extracts of lemongrass have larvicidal activity against third instar larvae of *Chrysomyia albiceps*. Lemongrass extract is found to reduce a cattle tick, *Boophilus microplus*, infestation on naturally infested Holstein cows[1]. Pushpanathan T, *et al*[27] reported distilled oils extracted from lemongrass had larvicidal and ovicidal activity against the mosquito *Culex quinquefasciatus*. Jarongsak P, *et al*[28,29] reported that essential oil of lemongrass at the rate of 75 $\mu g/cm^3$ has the highest inhibitory effect, resulting in 97.3± 4.7 mortality. The findings of this survey demonstrated that lemongrass has the potential to be a chemical control agent of dust mites.

The neem tree produces highly active pesticides mainly in the seeds[30]. A commercial neem seed extract known as Tre-san, is available for the control of dust mites[31]; Another formulation of the same seed extract is available as Mitestop, which is for the control of mites and ticks. The leaf extract of neem is well known for its medicinal properties. The finding in this study shows that an ethanol-extract of the neem leaf is toxic against the 2 species of dust mite and may merit further investigations. Singh B, *et al*[32] reported on the development of a pesticide delivery system based on neem leaf powder and the presence of neem can enhance the pesiticide activity of the system.

Conflict of interest statement

We declare that we have no conflict of interest.

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