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Caffeine, a naturally occurring acaricide

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Since caffeine is a plant alkaloid that has been described as a naturally occurring insecticide, its acaricidal effect on Dermatophagoides pteronyssinus (Dp) was investigated. Twelve cultures were established by adding 30 Dp to 200 mg of Tetramin fish food and brewer's yeast (8:2 ratio); six cultures were treated with 20 mg of finely ground caffeine. All 12 cultures were incubated at 75% relative humidity, 25° C, and observed during 8 weeks. Live mites were then counted under a stereoscope, cultures were extracted, and supernatants were analyzed for Der p I and Der f I allergen content with a two-site monoclonal RIA. Live mite counts in untreated cultures varied from 146 to 274 (215 ± 47.1), and in caffeine-treated cultures from 0 to 3 (1 ± 1.2 ; $p \leq 0.0001$). Der p I concentrations in untreated cultures varied from 588 to 9000 ng/gm (3138.3 ± 2990.8 ng/gm), and in caffeine-treated cultures from 52 to 117 ng/gm (78 ± 23.8 ng/gm; $p \leq 0.01$). Der p I was not detected in the food media or caffeine; Der f I was not detected in any of the cultures. Results demonstrate that caffeine inhibits mite growth and allergen production. (J ALLERGY CLIN IMMUNOL 1991;87:107-10.)

Although house dust-mite allergens commonly exacerbate symptoms in subjects with asthma, allergic rhinitis, and/or eczema,^{1, 2} environmental control measures to reduce symptoms in mite-allergic individuals are inconclusive. Some investigators observed improvement in asthma symptoms and bronchial hyperreactivity among allergic patients after removal from mite-infested environments.^{3, 4} Other investigators failed to detect improvement after intensive clean-

Abbreviations used

Dp: *Dermatophagoides pteronyssinus*
Df: *Dermatophagoides farinae*
Der p I: Main allergen of *D. pteronyssinus*
Der f I: Main allergen of *D. farinae*
MAb: Monoclonal antibody

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ing and encasing mattresses and pillows in plastic covers.⁵⁻¹³ None of these studies combined dust-avoidance measures with use of an effective acaricide. These studies suggest that effective control measures should incorporate both the removal of the allergen pool of fecal material and dead mites and the reduction of the mite population.¹⁴

Pirimiphos methyl (I.C.I. Plant Protection Division, Kent, U.K.), a mosquito insecticide with acaricidal activity, is used to protect grain against storage mites but is not commercially available for home

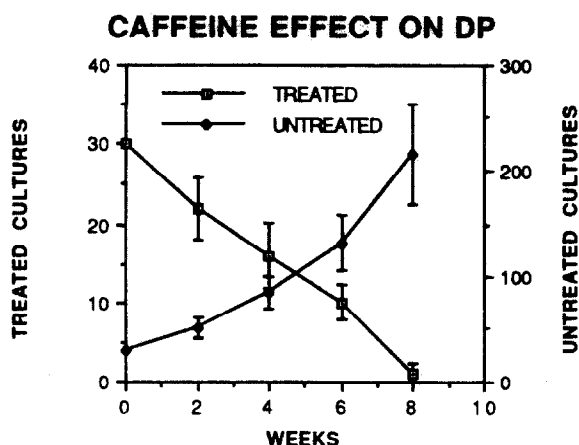


Fig. 1. Growth curves for six *Dp* cultures treated with caffeine, 20 mg per culture, compared to six untreated cultures. The left Y axis corresponds to the treated cultures, and the right Y axis corresponds to the untreated cultures.

use.¹⁵ A compound of benzoic acid esters combined with acrylate polymers is used as a household acaricide in the United States and Europe.¹⁶ A benzyl tannate complex solution, an acaricide with an allergen-reducing agent, is marketed in Australia (Allersearch, Granville, Australia).¹⁷ A 3% tannic acid solution is also available in the United States to denature mite allergens (Allergy Control Products, Ridgefield, Conn.).^{18, 19} The fungicide, natamycin, prevents proliferation of mites by inhibiting reproduction but is not being used commercially.^{20, 21} Liquid nitrogen has been successfully used in controlling mite populations, but its commercial use is not practical.¹⁴ The use of electrical heating blankets is effective in reducing the relative humidity and the number of house dust mites in the surface of mattresses.²²

Nathanson²³ demonstrated that caffeine and other methylxanthines interfered with feeding and reproduction of certain insects. He observed pestistatic and pesticidal effects on the larval form of mosquitoes, tobacco hornworms, mealworms, and butterflies, as well as in milkweed bug nymphs and adult flour beetles.

The purpose of this study was to evaluate the effects of caffeine on the common pyroglyphid house dust mite, *Dermatophagoides pteronyssinus*, and production of its main allergen, *Der p* I.

MATERIAL AND METHODS

Mite cultures

Cultures were established by adding 30 *Dp* to 200 mg of Tetramin fish food (TetraWerke; Ulrich Baensch GmbH, Melle, Germany) and brewer's yeast (Greer Laboratories,

Inc., Lenoir, N.C.) (8:2 wt/wt) in each of 12 65 cm² Petri dishes. Dose-finding experiments were previously conducted by adding 5, 10, and 20 mg of pure caffeine to mite cultures (unpublished data). Maximum growth inhibition was observed at 20 mg per culture. Therefore, 20 mg of finely ground caffeine (Eastman Kodak Co., Rochester, N.Y.) was added to six of these cultures; the remaining six cultures were kept as controls. All cultures were incubated for 8 weeks at 75% relative humidity and 25°C. Live mites were counted with a stereoscope at weeks 2, 4, 6, and 8. Counting grids were placed underneath the plates, and a fine needle was used to uncover mites hidden in the food medium. A single incubator was cleaned at the beginning of the experiment and used for all cultures. The external surfaces of culture lids were monitored for mite escape at weeks 2, 4, 6, and 8. Other samples collected with cotton swabs from the incubator at weeks 1 and 4 revealed that diminished counts in treated cultures were not due to escape of mites.

Measurement of *Der p* I and *Der f* I allergen concentrations

At week 8, mite cultures were extracted in 6 cc of 200 mmol/L of ammonium bicarbonate for 15 hours at 4°C. The extract was centrifuged, and the supernatants were frozen at -20°C. Mite-allergen concentrations were determined with a two-site MAb RIA as previously described.^{24, 25} Immulon II microtiter plates (Dynatech, Alexandria, Va.) were coated with 1 µg per well of MAbs, 5H8 (*Der p* I assay), or 6A8 (*Der f* I assay). Mite-reference allergens, *Dp* and *Df* from Hollister-Stier Laboratories, Spokane, Wash., calibrated as 117 and 115 µg of *Der p* I equivalents, were used in these assays. For the development of a reference curve, 50 µl of the mite standards at various concentrations were added to the wells along with 50 µl of diluting buffer. One hundred microliters of diluted samples of the culture and media extracts was added in the other wells. The plates were incubated overnight and washed, and then 30 ng of radioiodinated 4C1 MAb in 100 µl were added to each well. After overnight incubation, the wells were washed, dried, and counted in a gamma counter. To control for preexisting contamination, unused caffeine and food media were also extracted, and the concentrations of *Der p* I and *Der f* I were determined.

Statistical analysis

Power calculations were based on the hypothesis that at least 75% of the caffeine-treated cultures and no more than 5% of the control cultures would exhibit inhibition of growth, with $\alpha = 0.05$ and $2\beta = 0.20$, resulting in a minimum sample size of six treated and six control cultures. Data were analyzed with the Statview program (BrainPower, Inc., Calabasas, Calif.) on the Macintosh SE computer (Apple Computer, Inc., Cupertino, Calif.). Analyses included calculations of the mean and standard deviations of mite counts and *Der p* I allergen concentrations in extracts of treated versus untreated cultures and determination of their

TABLE I. Mite counts and *Der p* I allergen concentrations in caffeine-treated versus control cultures

Cultures	Mites*	Range	<i>Der p</i> I†	Range
Treated	1 ± 1.26	0-3	78 ± 23.8	52-117
Control	215 ± 47	146-274	3138 ± 2991	588-9000

*Mites per culture.

†*Der p* I nanograms per gram.

respective 95% confidence intervals. Statistical significance was evaluated with the two-tailed, unpaired Student's *t* test.

RESULTS

There was significant inhibition in the growth of all six treated cultures compared to control cultures. By the end of 8 weeks, mite counts in untreated cultures varied from 146 to 274 live mites ($n = 6$; mean, 215 ± 47.1 ; 95% confidence limits, 166 to 265), whereas mite counts in treated cultures varied from 0 to 3 live mites ($n = 6$; mean, 1 ± 1.2 ; $p \leq 0.0001$; 95% confidence limits, -0.32 to 2.328) (Fig. 1).

Der p I allergen concentrations in untreated cultures ranged from 588 to 9000 ng/gm ($n = 6$; mean, 3138.3 ± 2990.8 ng/g). In the caffeine-treated plates, *Der p* I values ranged from 52 to 117 ng/g ($n = 6$; mean, 78 ± 23.8 ; $p \leq 0.01$) (Table I). *Der p* I was not detected in either the unused caffeine or unused food. *Der f* I levels were below the lower limit of detection of the assay in both treatment and control groups, confirming the purity of the *Dp* cultures.

DISCUSSION

House dust-mite allergy is an important clinical problem in many areas of the world.² Positive skin tests and bronchial reactivity with mite extracts have been detected in 48% to 80% of individuals with asthma.²⁶ Physicians have long advised their patients with house-dust sensitivity to modify their bedrooms to reduce exposure to dust, since some clinical improvement accompanies the removal from the environment of any identifiable offending allergen. Reduction of allergen exposure can be effective at both the initial step of sensitization and at subsequent exposures that provoke symptoms.²⁷ Despite the long presumption of the benefit of these measures and their common use, the decrease in mite-allergen concentrations required to achieve clinical improvement has not yet been quantitated.

Various acaricides have been used to control mite populations and their allergens in the human environment.^{28, 29} The use of acaricides in human dwellings,

however, includes potential problems of toxicity to humans and pets, damage to household contents, and expense.

Exposure to methylxanthines diminishes feeding activity in certain insect larvae and feeding and reproduction in two species of flour beetles.²³ The caffeine-treated cultures also exhibited inhibition of *Dp* growth and a reduction in *Der p* I allergen concentration. Nathanson²³ demonstrated a methylxanthine-induced inhibition of phosphodiesterase activity and a resultant increase of cyclic adenosine monophosphate levels in certain larval tissues studied. Nathanson²³ concluded that these biochemical changes were responsible for the observed interruption of feeding, although the lethal effect of the methylxanthines was not understood. The mechanisms responsible for the acaricidal effect on *Dp* have not been studied. A reduction in *Dp* mite counts by caffeine also results in a decrease of *Der p* I allergen concentration. The same results should be expected with other close-related mite species, such as *Df* and *Euroglyphus maynei*. Future applications of these observations may include the use of caffeine or a related compound to treat carpets, mattresses, and pillow stuffings. For a greater action on mite allergens, caffeine may also be combined with tannic acid, an antigen-denaturing agent.

Adverse reactions to inhalation of, or topical exposure to, caffeine powder have not been reported, although there have not been any systematic studies of its potential toxicity. Cushley et al.³⁰ performed bronchial challenges on 17 subjects with asthma with several aerosolized theophylline preparations and found a brief bronchodilatory effect from all four preparations. Cough and unpleasant taste were the only reported side effects. Kaplan et al.³¹ found a beneficial effect of topically applied caffeine in combination with hydrocortisone on affected skin of patients with atopic dermatitis. There were no adverse effects reported.

Additional studies of caffeine as an acaricide are warranted. It will be necessary to use various con-

centrations and preparations of this chemical in controlled studies in the home environment to determine efficacy and safety.

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