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Food Protective Effect of Acaricidal Components Isolated from Anise Seeds against the Stored Food Mite, *Tyrophagus putrescentiae* (Schrank)

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ABSTRACT

The acaricidal activity of anise seed–isolated anisaldehyde and commercially available components of anise seed was examined against *Tyrophagus putrescentiae* adults and compared with those of synthetic acaricides, benzyl benzoate, dibutyl phthalate, and *N,N*-diethyl-*m*-toluamide (DEET). On the basis of LD_{50} (50% lethal dose) values, the compound most toxic to *T. putrescentiae* adults was anisaldehyde (LD_{50}, 0.96 μg/cm^2), followed by benzyl benzoate (LD_{50}, 11.3 μg/cm^2), anethole (LD_{50}, 12.3 μg/cm^2), dibutyl phthalate (LD_{50}, 13.3 μg/cm^2), DEET (LD_{50}, 13.5 μg/cm^2), estragole (LD_{50}, 17.4 μg/cm^2), and myrcene (LD_{50}, 56.2 μg/cm^2). Anisaldehyde was about 11.8 and 14 times more toxic than benzyl benzoate and DEET against *T. putrescentiae* adults, respectively. The results suggested that anisaldehyde, anethole, estragole, and myrcene derived from anise seeds are useful as a lead compound to development new agents for selective control of the stored food mite.

The most important sources of food allergens worldwide are stored food mites, mainly *Tyrophagus putrescentiae* (Schrank). The stored food mite is a cosmopolitan species frequently found in a wide variety of stored foods and grains. It is particularly common in foods with high levels of fat and protein, such as dried eggs, ham, herring meal, cheese, and different kinds of nuts *(3).* It is a significant cause of allergic asthma and allergic rhinitis among farmers, grain handlers, bakers, and food industrial workers because it occurs in large numbers, e.g., in farm buildings and in the food and textile industry, but it is now also being recognized as an important contributor to the allergen content in dust *(1, 5, 10, 19).* Commonly, the stored food mites live on the external surface of these products, but sometimes, they penetrate inside, causing serious economic losses *(20).* Furthermore, the stored food mites are responsible for acute enteritis and severe systemic reactions or anaphylaxis, as a result of the consumption of food that was infested with these mites *(11).* Recently, there has been growing interest in research concerning the possible use of plant extracts and phytochemicals as alternatives to synthetic mite-control agents against *T. putrescentiae (8, 16, 17).*

Plant extracts and phytochemicals may be an alternative to currently used mite-control agents for the control of the stored food mite, because they constitute a rich source of bioactive chemicals *(7, 8, 16–18).* These types of agents are often active against a limited number of specific target species and biodegrade to nontoxic products, and they are potentially suitable for use in integrated management programs. In this regard, they could lead to the development of new classes of possibly safer mite-control agents. Therefore, much effort has been focused on plant extracts and phytochemicals as a potential source of commercial mite-control agents *(8, 15–17).* We extend these studies by investigating the effect of several naturally derived anise seed control agents on *T. putrescentiae,* a stored food mite. In addition, their effects were compared with the effects that synthetic food mite-control agents have against *T. putrescentiae.*

MATERIALS AND METHODS

Chemicals. Benzyl benzoate, dibutyl phthalate, and *N,N*-diethyl-*m*-toluamide (DEET) were purchased from Aldrich (Milwaukee, Wis.). Anethole, anisaldehyde, 3-carene, α-caryophyllene, *p*-cymene, estragole, myrcene, α-phellandrene, α-pinene, and γ-terpinene were supplied by Sigma (St. Louis, Mo.). The purity of the compounds tested was greater than 98%.

Stored food mites. *T. putrescentiae* cultures were maintained in the laboratory for 5 years without exposure to any known mite-control agents. The mites were maintained on fry feed no. 1 and dried yeast (1:1 by weight) and reared in plastic containers (15 by 12 by 6 cm) containing 32 g of a sterilized diet. The rearing cages were kept in an incubator at 26 ± 1°C and 76% relative humidity in darkness, within a plastic tray (18 by 18 by 17 cm) that contained a saturated solution of potassium chloride to prevent the mites from escaping and to maintain high relative humidity. The fry feed was purchased from Korea Special Feed Meal Co. Ltd. (Chonju, Korea).

Extraction and identification. Anise seeds, *Pimpinella anisum* L., were collected in July 2003 in Chonju (South Korea) and dried at room temperature. A voucher specimen was authenticated by Professor Sang-Hyun Lee and deposited in the herbarium at the Department of Forestry, College of Agriculture, Chonbuk National University. The samples were washed three times with 500

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ml of distilled water, dried in an oven at 40°C for 3 days, and then finely powdered. Oil (yield, 8.9%) from the anise seeds (1 kg) was extracted by steam distillation as previously described *(10).* The steam distillate (15 g × 3) was chromatographed on a silica gel column (230 to 400 mesh, 500 g, 3.0 by 200 cm; Merck, Darmstadt, Germany), eluted with a stepwise gradient of hexane: ethyl acetate (10:2 to 0:1), giving seven fractions (A1 to A7) (200 ml each). The A3 fraction (2.9 g) was purified by preparative high-pressure liquid chromatography (Spectra System P2000, Thermo Separation Products, San Jose, Calif.) for separation of the biologically active constituents. The column was μPorasil (20-mm inside diameter by 500 mm, Waters, Milford, Mass.) using hexane:ethyl acetate (8:2) as the eluant at a flow rate of 1.5 ml/min, and the eluates were detected at 260 nm. In this step, six fractions (A31 to A36) were obtained and bioassayed at 40 μg/cm^2 as described below. The active A33 fraction (162 mg, 100% mortality) was rechromatographed under the same conditions. Finally, an active compound (137 mg) with a retention time of 34.1 min was isolated. The structure of the active isolate was determined by spectroscopic analyses. ^{1}H and ^{13}C nuclear magnetic resonance spectra were recorded in deuterochloroform with a JNM-LA 400F7 spectrometer (JEOL, Tokyo, Japan) at 400 and 100 MHz, respectively. UV spectra were obtained in methanol with a Jasco V-550 spectrometer (Jasco Corporation, Tokyo, Japan), and the electron impact-mass spectrometry spectra on a JEOL GSX 400 spectrometer (JEOL).

Mite-control activity. An impregnated fabric disk bioassay was used to determine the mite-control effect of the test materials. Ethanol solutions containing various concentrations (80, 60, 50, 40, 30, 20, 10, 5, 2.5, 1.25, 1.0, 0.75, and 0.5 μg/cm^2) of essential oils were applied to the disks of black cotton fabric (5 cm in diameter). Ethanol was applied as a control at the same dose to fabric disks (received 20 μl of ethanol). After air drying in a fume hood for 30 s, each piece was placed in the bottom of a petri dish (5 by 1.2 cm). Then, 20 *T. putrescentiae* (7 to 10 days old) individuals (×3) were separately placed in each petri dish and covered with a lid. The treated and control mites were held at 26 ± 1°C and 76% relative humidity in darkness. Mortalities were determined 24 h after treatment under a stereomicroscope (×20). The stored food mite-control activity was evaluated by determining the mortality of the mites. The mites were considered dead if their appendages did not move when stimulated with a fine pin. Each treatment was replicated three times. All assays were performed in a constant temperature and humidity apparatus at 26 ± 1°C, 76% relative humidity, and in dark conditions. The LD_{50} (50% lethal dose) values were calculated by probit analysis *(2).* The percent mortality was determined and transformed to arcsine square-root values for analysis of variance. Treatment means were compared and separated by Scheffe's test at $P = 0.05$ (SAS Institute) *(14).*

RESULTS AND DISCUSSION

When the essential oil extracted from anise seeds was bioassayed by the dry film method, acaricidal activity was observed against *T. putrescentiae* adults (Table 1). Anise seed oil showed a clear dose-response relationship against the stored food mite. Concentrations of 30 μg/cm^2 or greater caused complete mortality against *T. putrescentiae.* The acaricidal activity of anise seed oil was compared with those of benzyl benzoate, dibutyl phthalate, and DEET against *T. putrescentiae* adults (Table 1). The commonly used synthetic acaricides, benzyl benzoate, dibutyl phthalate, and DEET, served as positive controls for acaricidal activity. The LD_{50} value of anise seed oil was 12.8 μg/cm^2 against *T. putrescentiae* adults. On the basis of LD_{50} values, anise seed oil was comparable to those of benzyl benzoate, dibutyl phthalate, and DEET against *T. putrescentiae* adults. There was no mortality in the untreated controls. Although the acaricidal function of *P. anisum* seed–derived components against house dust mites, *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus,* was studied in our previous work *(8),* this study is, to our knowledge, the first to report the acaricidal function of anise seed oil against stored food mite adults.

TABLE 1. *Acaricidal activity of anisaldehyde and commercially available constituents derived from anise seed extracts and synthetic acaricides against* Tyrophagus putrescentiae *adults*[a]

Compound[b]	Slope (±SE)[c]	LD_{50} (μg/cm^2)	95% confidence limit	RT[d]
Anise seed oil	6.34 ± 0.97	12.8	12.19–13.53	1.1
Anisaldehyde	4.98 ± 0.69	0.96	0.89–1.01	14.0
Anethole	4.87 ± 0.66	12.3	11.72–12.92	1.1
Estragole	5.79 ± 0.95	17.4	16.82–18.05	0.8
Myrcene	8.23 ± 1.21	56.2	55.23–57.18	0.2
Benzyl benzoate	3.94 ± 0.88	11.3	10.04–12.62	1.2
DEET	7.21 ± 0.59	13.5	12.26–14.05	1.0
Dibutyl phthalate	6.87 ± 0.81	13.3	12.81–13.79	1.0

[a] Twenty *T. putrescentiae* (7 to 10 days old) individuals (×3) were separately placed in each petri dish (5 by 1.2 cm). Exposed time: 24 h.

[b] Anethole, estragole, and myrcene were derived from anise seeds. Benzyl benzoate, dibutyl phthalate, and DEET as synthetic compounds were purchased from Aldrich and Sigma. α-Caryophyllene, α-pinene, α-phellandrene, γ-terpinene, *p*-cymene, and 3-carene having no activity at the maximum concentration tested (80 μg/cm^2) are not shown.

[c] The percent mortality was determined and transformed to arcsine square-root values for analysis of variance. SE, standard error. Treatment means were compared and separated by Scheffe's test at $P = 0.05$ (SAS Institute).

[d] Relative toxicity = LD_{50} value of the DEET/LD_{50} value of each chemical.

Bioassay-guided fractionation of anise seed oil afforded an active constituent identified by spectroscopic analyses, including electron impact-mass spectometry and nuclear magnetic resonance, and by direct comparison with an authentic sample. The active constituent was identified as anisaldehyde. Spectral analyses of anisaldehyde isolated from anise seed oil are identical to those of anisaldehyde isolated from anise seeds *(8, 18).* In 2004, Lee *(8)* reported that the main constituents of anise seed oils were anethole (80.3%), estragole (8.8%), anisaldehyde (2.9%), α-caryophyllene (1.1%), α-pinene (1.1%), α-phellandrene (0.6%), γ-terpinene (0.6%), *p*-cymene (0.5%), 3-carene (0.3%), and myrcene (0.3%). In this study, the acaricidal activity of the commercially available components identified in anise seed oil against *T. putrescentiae* adults is presented and compared with those of benzyl benzoate, dibutyl phthalate, and DEET in Table 1. On the basis of LD_{50} values, the compound most toxic to *T. putrescentiae* adults was anisaldehyde (LD_{50}, 0.96 μg/cm^2), followed by benzyl benzoate

(LD_{50}, 11.3 μg/cm^2), anethole (LD_{50}, 12.3 μg/cm^2), dibutyl phthalate (LD_{50}, 13.3 μg/cm^2), DEET (LD_{50}, 13.5 μg/cm^2), estragole (LD_{50}, 17.4 μg/cm^2), and myrcene (LD_{50}, 56.2 μg/cm^2). However, no activity was observed for α-caryophyllene, α-pinene, α-phellandrene, γ-terpinene, *p*-cymene, or 3-carene at the maximum concentration tested of 80 μg/cm^2 (data not shown). In a previous study of *P. anisum* seed oils against house dust mites *(8),* the reported naturally occurring acaricidal compounds included anisaldehyde (LD_{50}, 1.11 μg/cm^2), 3-carene (LD_{50}, 42.10 μg/cm^2), and estragole (LD_{50}, 43.23 μg/cm^2) against *D. farinae,* but anethole and myrcene had no acaricidal activity against *D. farinae* and *D. pteronyssinus.* Our results indicate that the acaricidal activity of anise seed oil against *T. putrescentiae* adults can be mostly attributed to anethole and anisaldehyde. Although anisaldehyde was about 12.8 times more toxic than anethole against *T. putrescentiae* adults, anethole is likely more important than anisaldehyde, because anethole is 27.7 times more abundant than anisaldehyde *(8).* When compared with commonly used synthetic acaricides, anisaldehyde was about 11.8 and 14 times more toxic than benzyl benzoate and DEET, respectively, against *T. putrescentiae* adults. Anethole and anisaldehyde merit further study as stored food mite control agents or as lead compounds. The oral LD_{50} of anisaldehyde for rat and mice varies from 1,580 to >1,859 mg/kg *(13).*

Plant products are potential sources for agents that control the stored food mite because many of them are selective against these pests, have a minimal impact on nontarget organisms and the environment *(4),* and may be applied to dried eggs, ham, herring meal, cheese, and different kinds of nuts in the same way as other conventional stored food mite control agents *(11, 16, 17).* Many plant extracts and phytochemicals are known to possess acaricidal activity against the stored food mites *(11, 16, 17).* Reported naturally occurring acaricidal compounds against dust mites include cinnamaldehyde, cinnamyl alcohol, 1,8-cineole, limonene, linalool, linalyl acetate, fenchone, menthone, eucalyptol, menthol, and salicylaldehyde from the leaf oil of *Cinnamomum cassia, Eucalyptus globules, Lavandula angustifolia, Lavandula stoechas, Mentha piperita,* and *Pinus pinea (6, 9, 12).* It has been reported that the susceptibility to some components such as 1,8-cineole and limonene was greater in *T. putrescentiae* adults than in *D. farinae* and *D. pteronyssinus* adults *(8, 9).* In our results and the previous study *(8), D. farinae* and *D. pteronyssinus* adults were more tolerant to anethole and myrcene than were *T. putrescentiae* adults, but *T. putrescentiae* adults were more tolerant to 3-carene than were *D. farinae* and *D. pteronyssinus* adults. These results indicate that anethole, 3-carene, and myrcene have a different mode of action for the stored food mite as opposed to the house dust mite.

For the practical use of anise seed oil–derived anethole and anisaldehyde as control agents for the stored food mite, further research should be performed with regard to human health and safety issues for these compounds, acaricidal mode of action, and formulations that improve acaricidal potency and stability.

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REFERENCES

1. Blainey, A. D., M. D. Topping, S. Ollier, and R. J. Davies. 1989. Allergic respiratory disease in grain workers: the role of storage mites. *J. Allergy Clin. Immunol.* 84:296–303.
2. Finney, D. J. (ed.). 1971. Probit analysis. Cambridge University Press, London.
3. Hughes, A. M. 1976. The mites of stored food and houses. Technical Bulletin of the Ministry of Agriculture, Fisheries and Food, London.
4. Isman, M. B. 2000. Plant essential oils for pest and disease management. *Crop Prot.* 19:603–608.
5. Iversen, M., and R. Dahl. 1990. Allergy to storage mites in asthmatic patients and its relation to damp housing conditions. *Allergy* 45:81–85.
6. Kim, H. K., J. R. Kim, and Y. J. Ahn. 2004. Acaricidal activity of cinnamaldehyde and its congeners against *Tyrophagus putrescentiae* (Acari: Acaridae). *J. Stored Prod. Res.* 40:55–63.
7. Kim, M. K., and H. S. Lee. 2002. Growth responses of various flower extracts toward human intestinal microflora. *Food Sci. Biotechnol.* 11:444–447.
8. Lee, H. S. *p*-Anisaldehyde: acaricidal component of *Pimpinella anisum* L. seed oil against the house dust mites *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus. Planta Med.* 70:279–281.
9. Macchioni, F., P. L. Cioni, G. Flamini, I. Morelli, S. Perrucci, A. Franceschi, G. Macchioni, and L. Ceccarini. 2002. Acaricidal activity of pine essential oils and their main components against *Tyrophagus putrescentiae,* a stored food mite. *J. Agric. Food Chem.* 50: 4586–4588.
10. Marx, J. J., Jr., J. T. Twiggs, B. J. Ault, J. A. Merchant, and E. Fernandez-Caldas. 1993. Inhaled aeroallergen and storage mite reactivity in a Wisconsin farmer nested case-control study. *Am. Rev. Respir. Dis.* 147:354–358.
11. Matsumoto, T., T. Hisano, M. Hamaguchi, and T. Miike. 1996. Systemic anaphylaxis after eating storage-mite-contaminated food. *Int. Arch. Allergy Immunol.* 109:197–200.
12. Perrucci, S. 1995. Acaricidal activity of some essential oils and their constituents against *Tyrophagus longior,* a mite of stored food. *J. Food Prot.* 58:560–563.
13. Pickering Laboratories. 2000. Material safety data sheet, section IV—toxicity data. Pickering Laboratories, Inc., Mountain View, Calif.
14. SAS Institute. 1990. SAS/STAT User's Guide, version 6. Statistical Analysis Systems Institute, Cary, N.C.
15. Sung, B. K., J. H. Cho, M. Y. Lim, M. K. Kim, and H. S. Lee. 2003. Acaricidal activity of aromatic medicinal plants against *Dermatophagoides* spp. (Acari: Pyroglyphidae). *Agric. Chem. Biotechnol.* 46: 110–113.
16. Sung, B. K., M. K. Kim, S. H. Lee, J. S. Son, and H. S. Lee. 2004. Acaricidal activity of essential oils derived from 10 Cupressaceae species against stored food and house dust mites. *Food Sci. Biotechnol.* 13:376–380.
17. Sung, B. K., C. H. Lee, C. H. Kim, J. S. Son, and H. S. Lee. 2004. Antimite effect of essential oils derived from 24 Rosaceae and Umbelliferae species against stored food mite. *Food Sci. Biotechnol.* 13: 512–515.
18. Taha, A. A. 2000. Acetylens and dichloroanisoles from *Psathyrella scobinacea. Phytochemistry* 55:921–926.
19. Terho, E. O., K. Husman, I. Vohlonen, M. Rautalahti, and H. Tukiainen. 1985. Allergy to storage mites or cow dander as a cause of rhinitis among Finnish dairy farmers. *Allergy* 40:23–26.
20. Zdarkova, E. 1991. Stored product acarology, p. 211–218. *In* F. Dusbabek, and V. Eukva (ed.), Modern acarology, vol. 1. Academia, Prague.